FIELD ANESTHESIA OF FREE RANGING MOUNTAIN GORILLAS (*Gorilla gorilla beringei*) FROM THE VIRUNGA VOLCANO REGION, CENTRAL AFRICA

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Abstract

The Morris Animal Foundation’s Mountain Gorilla Veterinary Center (formerly the Volcano Veterinary Center) was established in 1986. Since its inception, 26 field anesthesias of mountain gorillas have been performed for either life-threatening problems or severe human-induced disease. The majority of anesthesias (i.e., 18 of 26, or 69%) were performed to remove rope or wire snares from a limb, or to treat snare induced wounds.

Table 1 summarizes the anesthetic data available from each procedure. For animals with an actual recorded weight the mean induction dosage of ketamine was 7.1 mg/kg (range: 5.9-8.0 mg/kg; n = 4), a dosage similar to those recommended for western lowland gorillas (*Gorilla gorilla gorilla)*.\(^1\) The mean induction time for anesthesias requiring a single initial dose of ketamine was 5.2 min (range: 3-11 min; n = 13). All induction agents were delivered by remote injection using the Telinject® system, and occasionally multiple darting attempts were necessary. Every attempt was made to dart the animal unawares and thus minimize disturbance to the gorillas. All interventions required a team approach with considerable cooperation from the researchers of the Dian Fossey Gorilla Fund’s Karisoke Research Center, the Mountain Gorilla Project and the International Gorilla Conservation Programme. Fundamental to the success of the interventions was the assistance of the park staff and gorilla trackers from the Office Rwandais pour le Tourisme et les Parc Nationaux, the Institut Congolais pour la Conservation de la Nature (formerly the Institut Zairois pour la Conservation de la Nature) and the Karisoke Research Center. In addition, porters were hired to carry the equipment within the forest.

The procedures were performed within or in close proximity to the rest of the group of gorillas. Thus, once the animal was anesthetized, a team was assigned to keep the other gorillas away from the patient and create a visual barrier. Only one human injury (a bite wound) has occurred as a result of an anesthesia. More injuries were reported as a result of the physical restraint of gorillas. Occasionally there was moderate disruption to the group with some reaction from other gorillas, in particular, the silverback males but no long term behavioral effects have been noted. No intraspecific aggression was noted during or immediately after the procedures. The intervention team would remain with the gorilla until it was fully recovered and able to rejoin its group. For
gorillas given a single dose of ketamine the mean recovery time was 42 min (range: 5-80 min; \( n = 9 \)). Four animals did not recover from anesthesia due to the severe nature of their medical conditions. One animal was physically removed from the park and anesthetized at a local human hospital. This individual was not included in this study.

**LITERATURE CITED**

Table 1. Summary of the anesthetic data from the immobilizations of free-ranging mountain gorillas from the Virunga Volcano Region, Central Africa

<table>
<thead>
<tr>
<th>Date</th>
<th>Name</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Drug and dose (mg)</th>
<th>Induction time (min)</th>
<th>Supplemental drugs</th>
<th>Time to recovery (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb 87</td>
<td>Gihonda</td>
<td>16</td>
<td>M</td>
<td>140 est</td>
<td>Telazol 3007</td>
<td>12</td>
<td>None</td>
<td>104</td>
</tr>
<tr>
<td>Apr 87</td>
<td>Tiger</td>
<td>Adult</td>
<td>M</td>
<td>Telazol?</td>
<td></td>
<td></td>
<td>Slow recovery, died 36 hr later</td>
<td></td>
</tr>
<tr>
<td>Apr 88</td>
<td>Katio</td>
<td>3</td>
<td>F</td>
<td>Ket 70</td>
<td>3</td>
<td>30 est Ket 40 est</td>
<td>Did not recover</td>
<td></td>
</tr>
<tr>
<td>May 88</td>
<td>Mukanza</td>
<td>5</td>
<td>F</td>
<td>Ket 50</td>
<td>5</td>
<td>30 est Ket 40 est</td>
<td>Recovered, time not recorded</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30 est Ket 40 est</td>
<td>Did not recover, died the next day</td>
<td></td>
</tr>
<tr>
<td>May 88</td>
<td>Gahuma</td>
<td>Adult</td>
<td>F</td>
<td>Ket 225</td>
<td>5</td>
<td>200 Ket 110 Ket 90</td>
<td>Recovered, time not recorded</td>
<td></td>
</tr>
<tr>
<td>May 88</td>
<td>Peanuts</td>
<td>28 est</td>
<td>M</td>
<td>Telazol 325</td>
<td>4</td>
<td>12 Ket 150 Ket 150</td>
<td>47 Ket 150</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47 Ket 150</td>
<td>75 Ket 150</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>75 Ket 150</td>
<td>90 Ket 150</td>
<td></td>
</tr>
<tr>
<td>Oct 88</td>
<td>Joxi</td>
<td>18</td>
<td>F</td>
<td>Ket 110</td>
<td>Sedated but not immobilized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct 88</td>
<td>Joxi</td>
<td>18</td>
<td>F</td>
<td>40 est Ket 200</td>
<td>10</td>
<td>10 Ket 70</td>
<td>Recovered, time not recorded</td>
<td></td>
</tr>
<tr>
<td>Oct 88</td>
<td>Mutego</td>
<td>4 est</td>
<td>M</td>
<td>22 est Ket 70</td>
<td>3</td>
<td>None</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Apr 89</td>
<td>Peanuts</td>
<td>29 est</td>
<td>M</td>
<td>Ket 525 Ket 150 Ket 150</td>
<td>3 after last dose Ket 150</td>
<td>Az 8 (for excessive salivation)</td>
<td>Did not recover</td>
<td></td>
</tr>
<tr>
<td>Jul 89</td>
<td>Bahati</td>
<td>4 est</td>
<td>F</td>
<td>13 est Ket 70</td>
<td>7</td>
<td>None</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Jan 90</td>
<td>Buddy</td>
<td>7-8 est</td>
<td>M</td>
<td>Ket 500</td>
<td>3</td>
<td>None</td>
<td>Recovered, time not recorded</td>
<td></td>
</tr>
<tr>
<td>Mar 91</td>
<td>Icharwe</td>
<td>5</td>
<td>F</td>
<td>Ket 25</td>
<td>200</td>
<td>None</td>
<td>20</td>
<td></td>
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</tbody>
</table>
Table 1. (continued)

<table>
<thead>
<tr>
<th>Date</th>
<th>Name</th>
<th>Age</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Drug and dose (mg)</th>
<th>Induction time (min)</th>
<th>Supplemental drugs Time (min)</th>
<th>Drug Dose (mg)</th>
<th>Time to recovery (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr 91</td>
<td>Inshuti</td>
<td>3</td>
<td>M</td>
<td>20</td>
<td>Ket. 150</td>
<td>3</td>
<td>None</td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>Dec 91</td>
<td>Gashungu</td>
<td>6</td>
<td>M</td>
<td>300</td>
<td>Ket. 150</td>
<td>3</td>
<td>None</td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>Jan 93</td>
<td>Un-named</td>
<td>F</td>
<td>21</td>
<td>Ket. 200</td>
<td>Wydase 5ml</td>
<td>4</td>
<td>None</td>
<td></td>
<td>60-75 est</td>
</tr>
<tr>
<td>Feb 95</td>
<td>Bahati2</td>
<td>3-4</td>
<td>Est</td>
<td>Telazol 25</td>
<td>Ket. 100</td>
<td>4 after last dose</td>
<td>None</td>
<td></td>
<td>66</td>
</tr>
<tr>
<td>Mar 95</td>
<td>Inyuma</td>
<td>F</td>
<td></td>
<td>Ket?</td>
<td>Repeated doses of</td>
<td></td>
<td>Recovered, time not recorded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jun 95</td>
<td>Roho</td>
<td>27</td>
<td>F</td>
<td>80 est Ket.</td>
<td>500</td>
<td>5</td>
<td>None</td>
<td></td>
<td>Recovered, time not recorded</td>
</tr>
<tr>
<td>Jun 95</td>
<td>Impanga</td>
<td>4</td>
<td>F</td>
<td>30 est Ket.</td>
<td>175</td>
<td>5</td>
<td>None</td>
<td></td>
<td>80</td>
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<tr>
<td>Sep 95</td>
<td>Mawingu</td>
<td>13</td>
<td>F</td>
<td>100 est Telazol</td>
<td>200</td>
<td>4</td>
<td>None</td>
<td></td>
<td>79</td>
</tr>
<tr>
<td>Nov 95</td>
<td>Impanga</td>
<td>4</td>
<td>F</td>
<td>45 est Telazol</td>
<td>90</td>
<td>3</td>
<td>None</td>
<td></td>
<td>52</td>
</tr>
<tr>
<td>Dec 95</td>
<td>Nyaruzizi</td>
<td>4</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td>Recovered, time not recorded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan 97</td>
<td>Shyamba</td>
<td>Adult</td>
<td>F</td>
<td>75 est Ket.</td>
<td>600</td>
<td>4</td>
<td>None</td>
<td></td>
<td>56</td>
</tr>
<tr>
<td>Jan 97</td>
<td>Akago</td>
<td>3</td>
<td>M</td>
<td>17 Ket. 100</td>
<td>6</td>
<td>None</td>
<td></td>
<td>40</td>
<td></td>
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<tr>
<td>Apr 97</td>
<td>Rukundo</td>
<td>3</td>
<td>M</td>
<td>14.5 Ket. 100</td>
<td>11</td>
<td>None</td>
<td></td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Oct 97</td>
<td>Mwirakazi</td>
<td>4.5</td>
<td>Est</td>
<td>20 est Ket.</td>
<td>125</td>
<td>5 est</td>
<td>22 Ket. 50</td>
<td>23 Ket. 50</td>
<td>82</td>
</tr>
</tbody>
</table>

Key to Table 1:

Induction time = time from initial injection to recumbency.
Supplemental drug administration time is listed as time from the initial injection.
Time to recovery = time from initial injection to ambulation.
M = Male, F = Female.
est = estimate.
Ket. = Ketamine, Telazol = mixture of tiletamine and zolazepam, Atrop = atropine, Wydase = hyaluronidase.
? = information may be inaccurate.
Blank space indicates information missing or not recorded.
EVALUATING PUBLISHED IMMOBILIZATION AND ANESTHESIA INFORMATION

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Abstract

Multiple factors must be considered when deciding on a chemical immobilization/anesthetic technique. Novel situations and rare species require technique and drug dosage extrapolation. Critical factors for evaluating published techniques are: individual characteristics of the animal such as age, body composition, level of excitation, and health status. Published dosages must also be interpreted prior to use. This includes determining if the published dosages represent an average of widely variable or precise drug effects. Route of administration, site of injection and the associated time-line are necessary for evaluation of the published technique. Use of validated sources and multiple references coupled with knowledge of the species will result in safe and effective immobilization techniques.

Introduction

The challenge to find, evaluate and modify immobilization/anesthesia techniques is frequently encountered in zoological medicine. In many cases, finding a reference that matches the proposed capture situation or target species can be difficult. If found, the reference usually is missing pertinent information making it necessary to interpret the procedures used. Additionally, immobilization/anesthesia information will often not be the focus of the article and thus the technique presented will constitute a minor part of the citation. As such, immobilization procedures may not be reviewed for their clarity or content and may even be limited to anecdotal comments. The authors can frequently provide personal experiences that are helpful, providing they can be contacted for advise. These limitations often result in a decision dilemma to either modify a published technique or develop a new immobilization procedure (including drugs and dosages).

The goals of immobilization are consistent across species, institutions, clinicians and situations. To reach these goals, the correct drug, dosage, route and delivery technique must be chosen. An understanding of the species and knowledge of the individual animal is important. Currently it is estimated that there are 9-10 million species of animals on the earth of which under 2 million have been characterized. Familial, breed, geographic and genetic differences further increase the variability among animals. When evaluating a recommended immobilization technique, the following factors should be used: individual animal variation, effect of emotional status, dosage recommendation and calculations, conditions of administration, site of injection and pharmacokinetics. This paper will discuss these issues and present approaches to interpretation of immobilization/anesthesia references.
Animal Variation: Physiologic Factors that Affect Animal Response to Anesthetic Drugs

Published anesthetic procedures should include information on age, environment, physical condition and excitement level. The response to anesthetic drugs varies with age. Younger animals, in general, require higher dosages. A number of physiologic factors have been associated with this observation. Young animals have a higher metabolic rate. Thus, they absorb, redistribute, excrete and eliminate drugs in a shorter time period. Establishing and maintaining an effective blood concentration requires higher dosages and more frequent redosing.

Multiple factors result in older animals requiring lower dosages of anesthetics when compared to young animals. These include lower metabolic activity, a higher percentage of body weight as fat and a lower capacity to distribute drugs out of the CNS and blood. Adipose tissue has a low blood supply in comparison to muscle. Thus, anesthetics are slow to redistribute to this tissue resulting in more drug remaining in the blood. In addition, drugs have a slower elimination from fat storage causing the recovery phase to be prolonged in the older animal. Also keep in mind that very young (newborn) and geriatric animals require lower dosages than other age groups.

The season of the year will affect the dosage of the immobilization drug. This is related to the physical activity of the animal, nutritional status and reproductive activities. Wild animals have seasonal variations in the amount of exercise. Spring or fall migrations are associated with marked increases in energy utilization and muscle activity. Nutritional status changes in relationship to food availability and food type. Physical condition has also been shown to affect animals ability to handle stress. In one study, sedentary sheep were less tolerant to heat stress than were physically fit animals. Many captive and non-predatory species are not highly fit. The presence of infections or infestations will also lower animal tolerance to handling or capture. Bears with heavy layers of fat prior to hibernation must be treated differently than animals emerging from their den in the spring. Reproductive activities affect the mental status as well as the nutritional plane of the animal. Breeding and sparring in some species such as moose, leave the dominant bull nutritionally drained by the end of the rut. Appropriate immobilization at this time will require greater precision in selecting the correct dosage and providing necessary support than would be required in the late summer. Evaluate immobilization sources to identify factors such as the hair coat thickness, body fat depth to determine the appropriate dart needle length and site of injection.

Environmental factors can also play an important role in the clinically observed effect of CNS depressants. Ambient temperature and weather conditions can have an important effect on drug dosing. Low environmental temperatures will have an additive effect with the immobilization drugs. Hypothermia has been extensively studied in conjunction with general anesthesia. A slight drop in core body temperature (2 °C) markedly depresses the CNS and enhances the effects of anesthetic drugs. Recovery will also be slowed if the animal has a body temperature lower than normal. Hyperthermia on the other hand will increase metabolic rate and decrease the effectiveness of CNS depressants. As the body temperature increases the demand for oxygen by the tissues increases and the risk for tissue hypoxia also increases. Drug dosages reported from hot climates may be
excessive when used on the same animal that is hypothermic.

The activity level of the animal will change based on their normal diurnal behaviors. Animals that are active at night have an increased sensitivity to anesthetics when administered during the day. Studies have demonstrated that the effectiveness of opioids appears to vary according to time of day. This may be partially due to variations in the intensity of pain at different times of the day. Drug dosages should be selected that have been validated for the time of day when the immobilization has been scheduled.

Published techniques must provide information on the emotional status of the animals for which the dosages are recommended. Tame or calm animals require lower dosages. These animals have usually been raised by and have daily contact with people. Close proximity allows for less traumatic drug administration and lower catecholamine levels. If the animal to be immobilized has been conditioned to people, little to no excitement will occur during the induction phase. Minimal or no flight distance is associated with this group and hand injection or pole syringes can often be used. Animals that are accustomed to people but will not allow physical contact are considered semi-tame. They will have higher dosage requirements than fully tamed animals. The flight distance will be greater and will require remote delivery equipment. The risk of physically restraining these animals is much greater than with tame animals. Familiarity with people can be used to minimize the stress on the animal and improves the predictability of the immobilization procedures.

Wild animals consider people to be a threat. Detection of a person results in an autonomic response with high levels of fear and anxiety. Because of the animals apprehension, there will be larger flight distances. Unless immobilizing drugs can be administered without activating an alarm reaction, the dosage will be much higher than the tame or semi-tame animals. Dosages are frequently doubled if the animal is excited. Tranquilizers and mild sedatives will not immobilize animals which fall into this category.

**Making Sense of Published Dosages: What Do the Numbers Mean?**

Reported dosage ranges can provide insight into how the information was collected. If the dosage range is narrow this usually means that accurate body weights were known and a specific dose was selected for the immobilization procedure. Typically the dosage will have a 1.5- to 2-fold range. If the reported range is quite wide (5- to 10-fold), it indicates that inaccurate body weight estimates were used or a single dose was used for all animals and the dose per kilogram was calculated after immobilization when a body weight could be obtained.

Dosages are often reported as a single dose/kg and at a level well beyond the usable accuracy of clinical practice. This usually indicates a calculated dosage based on multiple immobilizations and published as an average dose per kilogram. Examples of this would be a dose of 0.123 mg/kg. If standard error of the mean or standard deviations are published a range can be estimated for clinical use. Accuracy of drug administration is limited by the syringes we use, the drug concentration, the remote delivery equipment and the accuracy of the animal’s body weight estimate. Extrapolation
of the dose from published sources should be rounded to a usable decimal place and a range established from which a dose can be selected. Well established dosages are almost always rounded to a whole number.

Conversion of working dosages from mg/lb to mg/kg is usually apparent. Dosages of 2.2 mg/kg were actually converted from 1 mg/lb. Errors in weight conversions should be considered when evaluating these dosages. One common practice is to estimate the animal's weight in pounds and then divide by 2 for the estimated weight in kilograms. This process adds a 10% overestimation of the weight in the calculation and thus an increased dose of drug. Evaluation of the recommended dose should be judged with these inaccuracies in mind. Errors in estimation of animal body weight are probably the largest single concern. Knowledge of normal weights for the species, gender, age and the geographic area are important in minimizing the estimation error. In situations where estimation of animal size in not possible prior to dosage calculation, a good policy is to prepare three different doses for a range of animal sizes. Prepare a high dose, medium dose and a low dose, then when the animal is observed the dose closest to the animals ideal dose can be used.

Additional information should be determined from the information source. The conditions for administration can critically affect the efficacy of the dosage. Was the animal excited, was it chased, what was the site of injection, what type of remote delivery system was used? Site of administration will determine several aspects of the drug effectiveness. Injection into muscle is recommended because absorption is predictable while injections into fat will be slowly absorbed to the degree that effective blood levels may not even be reached. Inadvertent intravenous injections can occur from remote injections procedures, rapid deep anesthesia will occur if this happens. Ventilatory and circulatory support and administration of reversal agents should be provided if needed.

The basic question is, how similar is the published immobilization procedure(s) to the immobilization which is being planned?

Evaluation of the suitability of the published procedures also requires information on the time-line associated with the immobilization technique. Most times will be an average of a number of immobilizations and will provide an indication of the variability to be expected with the technique. The times from successful drug administration to the loss of mobility and the loss of consciousness are important. Detailed information on the signs of drug onset will help to stage the immobilization and aid in the decision to supplement with additional drug. The duration of full immobilization enables evaluation of the applicability to the upcoming procedure. Evaluation of the technique should include analysis of all the drugs administered to determine if recovery was dependent on metabolism and excretion or if the recovery was controlled by administration of specific receptor antagonists. Each situation requires preparation for animal recovery. In controlled environments, slow recoveries may be acceptable whereas in wildlife situations, full recovery will be important as soon as feasible.

**Where to Find Anesthesia and Immobilization Data and Dosages**
Whenever possible finding published techniques for immobilizing the species you are planning to anesthetize is ideal. Many sources are now available including books, periodicals, and meeting proceedings.

Additional sources include collections of techniques at facilities such as zoological gardens, animal sanctuaries, universities, animal parks, game farms, etc. These are more difficult to access unless a contact person is known and available to search the records. Finding the “expert” on a given species is often the most satisfying method of species-specific immobilization data. However at this time, a list of recognized species experts is not available. The International Species Information System (ISIS) maintains an inventory of animals at nearly 500 zoological institution members, from 54 countries, (http://www.worldzoo.org). Accessing a list of locations for species in captivity may be a pointing device for finding a knowledgeable individual.

In the future, data libraries will be available to assist with communication between experts and individuals needing species-specific immobilization/anesthetic techniques. MedArks and the Animal Capture and Anesthesia Database (ACAD 1.0) are examples of databases that provide comprehensive information compiled from experts. Increasing access to these sources is needed to improve immobilization procedures and avoid repeating techniques that fail to meet the goals of safe and effective anesthesia.

**What to Do When “Validatable” Reliable Species Specific Data Are Not Available**

If specific techniques are not available, extrapolation from related species is the usual approach. Species cross-references can be obtained through taxonomy sources. All of the considerations presented in the first part of this article must be included in the development of new species techniques. Temperament of the individual and the species will be one of the most critical considerations. Since the reference species may be either less or more excitable, adjustments in the immobilization procedures should be considered based on the observed excitement level of the animal to be immobilized.

**Summary and Conclusions**

A single source for species-specific immobilization information is not always available. In most cases, multiple sources are needed to provide all the necessary information to develop a immobilization technique that can be used with confidence. Critical consideration of the variations within the individual such as age, health status, time of year, and emotional status coupled with the ability to interpret published dosages and conditions of drug delivery are essential in developing a safe and rational approach to zoological animal anesthesia and immobilization. Integrating the published sources of information with key references, experts, and personal experience provide the optimal approach to novel species chemical restraint.
THE USE OF SUPPLEMENTAL PROPOFOL IN NARCOTIC ANESTHETIZED NON-DOMESTIC EQUIDS

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Abstract

This study evaluated the safety and efficacy of propofol when used to deepen anesthesia and provide muscle relaxation in narcotic anesthetized non-domestic equids. Intramuscular carfentanil or etorphine was used either alone or in combination with sedatives to immobilize 23 non-domestic equids representing five species. Each animal received at least one intravenous bolus of propofol. Physiologic data collected during anesthesia included heart and respiratory rate, pulse oximetry and rectal temperature at various time intervals. No significant difference ($P < 0.05$) was noted in any parameter when each time interval was compared with the time 0 measurement (completion of propofol administration). Analysis of results indicate intravenous propofol given at 1-1.5 mg/kg over 1-2 min provided 8-10 min of relaxation in these animals without causing noteworthy changes in physiologic status. If given slowly and titrated to desired effect, intravenous propofol is a safe and effective method of increasing the quality of anesthesia in narcotic anesthetized non-domestic equids.

Introduction

Narcotics are considered the drug of choice for anesthesia in many non-domestic hoofstock species because of their potency, rapid onset of action and reversibility. Respiratory depression in the patient and accidental human narcotic exposure are noteworthy concerns. The safe use of narcotics and other anesthetic agents in non-domestic equids has grown over the past decade due to the appearance of safe and effective protocols in the literature.

The need for supplemental drugs to achieve muscle relaxation in narcotic anesthetized equid species is common due to the muscle rigidity and fasciculation often seen during such immobilizations. Side effects of muscular rigidity may include hyperthermia and myopathy. Furthermore, if the patient is experiencing muscular rigidity or fasciculation it is difficult, if not impossible, to adequately monitor the animal’s clinical status. Vital patient information at risk include accurate auscultation of the rate, rhythm and character of the heart and lung sounds, electrocardiography, and oxygen saturation via pulse oximetry. Since narcotics are well-known respiratory depressants, it would be logical and prudent to include these devices for the safe monitoring of these patients.

Propofol (Diprivan, Zeneca Pharmaceuticals, Wilmington, Delaware 19850 USA) is a relatively new sedative-hypnotic intravenous induction anesthetic agent used in human and veterinary medicine.
This drug is also used to maintain or deepen anesthesia in man and is considered a choice agent for short outpatient surgical procedures. Its use and popularity has grown over the past few years in non-domestic species. Benefits of this drug include rapid metabolism and clearance, short duration of action, titratable effects, and safety in compromised patients. Reported undesirable aspects include dose dependent respiratory depression, cost, and storage requirements. The use of propofol is described in the domestic equid but there are no reports in non-domestic equid species.

Due to its purported benefits, the use of propofol as a supplemental anesthetic agent was investigated. The objective of this study was to improve the quality and/or safety of anesthesia in the narcotic anesthetized non-domestic equid by the administration of propofol.

Methods

Animals: Five species of non-domestic equids were anesthetized for routine procedures at the San Diego Wild Animal Park between 1993 and 1997: Przewalski’s horse (Equus przewalskii), Hartmann’s mountain zebra (Equus zebra hartmannae), kiang (Equus kiang holdereri), Grevy’s zebra (Equus grevyi), and Persian onager (Equus hemionus onager). Nineteen animals were used in this study of 23 anesthetic events. See Table 1 for a review of species, sex, age and weights of animals.

To enter this study, all anesthetic events had to fulfill certain criteria. All animals were clinically normal and were anesthetized only by this author (JRZ) for routine procedures. Narcotic was used as the primary induction agent and all animals had satisfactory dart placement deep into the caudal thigh muscles. Body weight was obtained during the procedure for accurate drug dose calculation. Propofol was administered only if necessary to improve quality of anesthesia.

Induction of anesthesia: All animals received induction and premedication anesthetic agents via remote dart injection (Vario dart, Telinject USA, Saugus, California 91350 USA). All procedures occurred “in the field” away from hospital facilities. See Table 2 for a review of narcotic induction agents used in this study.

Twelve anesthetic events were recorded in 10 Przewalski’s horses. In nine of the 12 anesthetic events carfentanil (Wildlife Laboratories, Fort Collins, Colorado 80524 USA) was used as the sole induction agent. The remaining three animals were premedicated with 10-15 mg intramuscular detomidine (Dormosedan, Pfizer, Inc., West Chester, Pennsylvania 19380 USA) alone or combined with 10 mg intramuscular butorphanol (Torbugesic, Fort Dodge Laboratories, Fort Dodge, Iowa 50501 USA) 15-20 min prior to carfentanil induction. Mild sedation was achieved in all cases.

A total of six anesthetic events in four Hartmann’s mountain zebras were recorded using only carfentanil for anesthetic induction. Three kiang and one Persian onager received carfentanil as the sole induction agent. One Grevy’s zebra received a combination of detomidine (10 mg) and etorphine (M99-Ten [10 mg/ml], Wildlife Laboratories, Fort Collins, Colorado 80524 USA) in the
same dart for induction.

Supplemental anesthesia: All 23 animals received at least one jugular vein infusion of propofol to deepen anesthesia, improve muscle relaxation and increase procedure safety. Prior to anesthesia an estimated weight was obtained on the patient which allowed us to have a pre-calculated amount of propofol ready for initial delivery if needed. The amount of propofol placed in the syringe was approximately 2 mg/kg. A total of 31 propofol events occurred during the 23 anesthetic events; eight animals received more than one dose of propofol. The initial dose of propofol served as time 0 in animals receiving more than one bolus of this drug. Each propofol event was critiqued for safety and efficacy. See Table 2 for a review of mean propofol dosage and administration rate.

Supportive pharmacologic agents: Four horses received intravenous doxapram (Dopram, Fort Dodge Laboratories, Fort Dodge, Iowa 50501 USA) (200-300 mg total dose) 5-8 min following propofol administration for decreased respiratory rate, low oximetry readings and/or pale mucous membrane color. One horse received doxapram prior to the administration of propofol for poor respiratory rate.

Narcotic reversal: Narcotic agent was reversed by single administration of intravenous nalmefene (Anesta Corp., Salt Lake City, Utah 84103 USA) or naltrexone (Trexonil, Wildlife Laboratories, Fort Collins, Colorado 80524 USA) at 50 or 100 times the mg dose of induction narcotic. No renarcotizations were noted.

Patient monitoring: Physiologic parameters monitored included rectal temperature, respiratory rate, heart rate, and indirect oxygen saturation via pulse oximetry. Body weight was obtained during the procedure. Attempts were made to collect data within the following time intervals immediately after administration of propofol: 0-1, 2-3, 4-5, 6-7, 8-10, 11-13, 14-16, and 17-20 min (Table 3). Data were analyzed by one-way ANOVA; P < 0.05 was considered statistically significant. Note that data were not available at certain time intervals so the total number (n) of animals may be different at each interval. All animals were maintained in lateral recumbency and received supplemental intranasal oxygen at 15 L/min.

Anesthetic rating: Each anesthetic event received a subjective rating for quality of anesthesia, muscle relaxation and recovery. Quality of anesthesia was evaluated by the efficacy and safety of propofol administration by the following scoring system: 1 = good, 2 = satisfactory, and 3 = poor. Muscle relaxation due to propofol was scored as 1 = good, 2 = slight, and 3 = poor. Recovery was scored as 1 = good with no instability, 2 = satisfactory with slight instability, and 3 = poor due to instability and potential danger to patient.

Results

Mean anesthesia time (dart to reversal) for the 23 animals was 28.1 min (± 6.5). The mean time at which propofol was first administered was 12.0 min (± 3.9).

Heart and respiratory rates, oximetry readings and rectal temperature did not change significantly
over the anesthetic procedure. No animal became apneic due to the administration of propofol at the dosage and administration rate in this study. All animals had increased muscle relaxation by the end of propofol administration with a mean duration of action of 8.7 min (± 2.9).

The mean rating score for quality of anesthesia was 1.4 (± 0.5); for muscle relaxation 1.3 (± 0.5); and for recovery 1.1 (± 0.3).

Discussion

Propofol provided good muscle relaxation and increased quality of anesthesia in all 23 narcotic anesthetized non-domestic Equidae in this study. No statistically significant changes were noted in the four physiologic parameters measured in this study by comparing each time interval with the initial measurement at time 0 (completion of propofol administration). No individual animal appeared to react adversely to the amount of propofol administered in this study.

Propofol was administered based on pre-procedure estimated body weight and desired level of anesthesia to achieve muscle relaxation. The dose and rate of administration must be considered and titrated to effect to provide muscle relaxation while avoiding significant cardiorespiratory changes. Respiratory depression and apnea are important side effects of propofol but were not seen here likely due to the slow administration and low dose used in these animals. Four animals received doxapram to stimulate respiration following propofol administration; only two of four responded with a slight change in respiratory rate. Since narcotic induction agents cause respiratory depression this could explain the generally low respiratory rate and pulse oximetry readings when propofol was first administered. Respiratory rate and oximetry readings did not change significantly following the administration of propofol. Doxapram is routinely used, sometimes prophylactically, in our practice when equids are immobilized with narcotic agents alone.

Rectal temperature was not expected to change since procedure length was short, however, it has been reported to decrease temperature in dogs due to decreased skeletal muscle tone, vasodilation and impaired thermoregulatory mechanism.16 Pulse oximetry readings were low, but not significantly different over time, for all animals in this study reflecting the respiratory depressive effect of narcotic induction agents. Providing all narcotic anesthetized patients with supplemental oxygen, ideal positioning, respiratory stimulant drugs and short anesthetic times are techniques used to increase patient safety.

Propofol proved to be an effective and safe supplemental anesthetic agent in these study animals. This drug offers predictable results in a variety of other species as well. Many other equid, primate, carnivore, avian and exotic ungulate species have received intravenous propofol to increase quality of anesthesia without complication. This author (JRZ) now considers propofol a routine supplemental agent to improve muscle relaxation and deepen anesthesia in difficult anesthetic events such as rhinoceros immobilizations. This study only included healthy animals but our practice has used propofol safely in clinically ill animals needing to be anesthetized for diagnostic procedures or treatment with similar results. Paramount, of course, is the recognition and avoidance of the
potential side effects of this drug by titrating to effect while diligently monitoring the physiologic status of the patient.

If considering the use of propofol to supplement narcotic anesthesia in non-domestic Equidae the author recommends preparing a precalculated dose of propofol, based on an educated estimation of patient body weight, ready for administration if needed. From this study, the slow bolus administration of propofol given over 1-2 min at 1.0-1.5 mg/kg provided 8-10 min of improved muscle relaxation and anesthesia in healthy non-domestic equids without causing obvious physiologic change in the patient.

LITERATURE CITED


Table 1. Species list and bio-data of 23 non-domestic equids in propofol study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number (M/F)a</th>
<th>Age in yr (range)</th>
<th>Weight in kg (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Przewalski’s horse</td>
<td>9 (5M,4F)</td>
<td>6.6 (1.2 - 26)</td>
<td>294.1 (226.4 - 367.3)</td>
</tr>
<tr>
<td>Przewalski’s horse, with pre-medb</td>
<td>3 (2M,1F)</td>
<td>11.3 (4.8 - 19)</td>
<td>405.9 (345.5 - 451.4)</td>
</tr>
<tr>
<td>Hartmann’s mountain zebra</td>
<td>6 (2M,4F)</td>
<td>5.8 (0.75 - 20)</td>
<td>317.1 (212.7 - 371.4)</td>
</tr>
<tr>
<td>Eastern kiang</td>
<td>3 (3M)</td>
<td>6.8 (3.5 - 9.3)</td>
<td>288.5 (278.2 - 300)</td>
</tr>
<tr>
<td>Persian onager</td>
<td>1 (1F)</td>
<td>4.6</td>
<td>304.5</td>
</tr>
<tr>
<td>Grevy’s zebra</td>
<td>1 (1F)</td>
<td>3.5</td>
<td>425.9</td>
</tr>
</tbody>
</table>

Data are reported as mean age and weight, (range)

a(Male/Female)
bSee Methods

Table 2. Dosage and administration rate of narcotic induction agent and propofol used in 23 non-domestic equids.
<table>
<thead>
<tr>
<th>Species</th>
<th>Induction Agent (mg/kg)a</th>
<th>Supplemental Propofolb</th>
<th>Dosage (mg/kg)</th>
<th>Duration of Administration (sec)</th>
<th>Administration Rate (mg/kg/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Przewalski’s horse</td>
<td>0.0227 Carf c (9)</td>
<td>1.53 ± 0.64 (13)d</td>
<td>60.4 ± 36.6</td>
<td>0.03 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Przewalski’s horse, with pre-med e</td>
<td>0.0209 Carf (3)</td>
<td>0.93 ± 0.30 (4)</td>
<td>63.7 ± 7.50</td>
<td>0.014 ± 0.004</td>
<td></td>
</tr>
<tr>
<td>Hartmann’s mountain zebra</td>
<td>0.0133 Carf (6)</td>
<td>1.03 ± 0.22 (7)</td>
<td>100 ± 34.6</td>
<td>0.013 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Eastern kiang</td>
<td>0.0441 Carf (3)</td>
<td>2.20 ± 0.48 (3)</td>
<td>120 ± 60.0</td>
<td>0.02 ± 0.007</td>
<td></td>
</tr>
<tr>
<td>Persian onager</td>
<td>0.0525 Carf (1)</td>
<td>1.64 ± 0.0 (1)</td>
<td>30 ± 0.0</td>
<td>0.05 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Grevy’s zebra</td>
<td>0.0176 Etor f (1)</td>
<td>0.76 ± 0.42 (2)</td>
<td>150 ± 42.4</td>
<td>0.005 ± 0.001</td>
<td></td>
</tr>
</tbody>
</table>

aInduction agent data reported as mean dosage in mg/kg, (number of animals)
bAll propofol data represent mean ± standard deviation
cCarf = carfentanil
dTotal number of supplemental propofol doses used in each species
eSee Methods
fEtor = etorphine
Table 3. Cardiorespiratory and body temperature measurements in 23 narcotic anesthetized non-domestic equids following propofol administration.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0-1</th>
<th>2-3</th>
<th>4-5</th>
<th>6-7</th>
<th>8-10</th>
<th>11-13</th>
<th>14-16</th>
<th>17-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resp. Rate</td>
<td>9.7 ± 5.1 (22)</td>
<td>9.2 ± 4.0 (19)</td>
<td>9.7 ± 4.0 (21)</td>
<td>9.7 ± 4.4 (16)</td>
<td>11.1 ± 5.3 (21)</td>
<td>11.2 ± 5.1 (18)</td>
<td>10.2 ± 4.0 (13)</td>
<td>12.7 ± 4.7 (6)</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>84.8 ± 31 (23)</td>
<td>85.2 ± 37 (18)</td>
<td>82.3 ± 34.8 (20)</td>
<td>85.3 ± 37.2 (16)</td>
<td>88.8 ± 35.7 (22)</td>
<td>94.1 ± 40.8 (18)</td>
<td>84.1 ± 33.5 (13)</td>
<td>82.4 ± 47.1 (7)</td>
</tr>
<tr>
<td>Oximetry</td>
<td>82.5 ± 9.9 (12)</td>
<td>83.1 ± 11.0 (10)</td>
<td>87.1 ± 5.4 (9)</td>
<td>86.6 ± 6.1 (9)</td>
<td>88.3 ± 5.7 (10)</td>
<td>87.5 ± 4.7 (11)</td>
<td>87.6 ± 5.5 (6)</td>
<td>87.2 ± 4.2 (5)</td>
</tr>
<tr>
<td>Temperature</td>
<td>102.4 ± 2.0 (15)</td>
<td>102.4 ± 2.3 (8)</td>
<td>103.0 ± 2.8 (8)</td>
<td>103.5 ± 2.3 (7)</td>
<td>102.8 ± 2.2 (10)</td>
<td>101.7 ± 3.1 (4)</td>
<td>102.6 ± 1.7 (3)</td>
<td>104.0 ± 1.6 (2)</td>
</tr>
</tbody>
</table>

All data represent mean ± standard deviation, (number of animals).
Time intervals represent minutes following administration of propofol.
*No statistical significance ($P < 0.05$) was noted in any parameter when compared to initial measurement.
KETAMINE/MEDETOMIDINE IMMOBILIZATION AND ATIPAMEZOLE REVERSAL OF CAPTIVE AND FREE-RANGING IMPALA (*Aepyceros melampus*) IN THE KRUGER NATIONAL PARK, SOUTH AFRICA

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Abstract

Investigation of new chemical immobilization agents or current agents in novel combination or new species is important in improved safety and effectiveness in exotic ungulate species. The majority of investigation of immobilization utilizing alpha-2-adrenoreceptor agonists in a variety of species, including ungulates, was conducted by Harry Jalanka. The goal of this study was to determine if the alpha-2-adrenoreceptor agonist medetomidine (MET) combined with ketamine HCl (KET) and antagonized with the alpha-2-adrenoreceptor antagonist atipamezole (ATI) would serve as a safe and effective anesthetic alternative to the narcotic chemical immobilization agents typically used in ungulate species, particularly bovids. The impala was selected due to its abundance, access, and importance as a sentinel species in disease studies in ungulates in the Kruger National Park, South Africa. Also, it is representative of bovid species difficult to anesthetize with currently available narcotic agents.

In April of 1996, 64 anesthetic procedures were performed on 27 individual impala. Preliminary procedures were conducted on recently captured, boma-acclimated male impala to determine an effective and safe MET/KET dose for remote-delivery anesthesia. After determining an effective dose, free-ranging impala were anesthetized; these animals were placed in bomas to permit additional anesthetic procedures for comparison within individuals. The data collected during each anesthetic procedure included: 1) induction time (time of darting to time of recumbency) recorded in minutes (min) and level of activity during this period; 2) level of sedation attained; 3) physiologic parameters of respiration, heart rate, muscle relaxation, blood pressure, blood oxygen saturation, arterial pH, pO₂, pCO₂, during immobilization; 4) response to manipulative procedures of physical examination, transport, tracheal intubation, blood collection; 5) time from injection of antagonist to standing; and 6) degree of anesthetic reversal attained after antagonism. Each anesthetic procedure was given an overall rating of poor, fair, or good assessing the general plane of anesthesia, response to manipulation or touch, and ability to collect data. The established protocol called for darting, permitting the animal to achieve voluntary recumbency (no handling before 15 min after darting), initial physiologic assessment and weighing, tracheal intubation, transport to veterinary laboratories, data collection, transport to the bomas, and reversal of anesthesia. The same size plastic dart and same needle gauge and length was employed in all animals.
There were four anesthetic groups, based upon the dose of MET and/or KET actually received (based upon known body weight), with MET ranging from 150-400 µg/kg and KET ranging from 1-8 mg/kg. Two additional anesthesia groups included animals receiving MET alone or KET alone. Variables included: field versus pen immobilization, hyaluronidase (HYL) added to the MET/KET dose to enhance absorption of the anesthetic agents, particularly in field anesthetics (HYL dose of 7500 IU consistent in all cases), sex, age, dose of MET and/or KET. In all procedures where the level of sedation was sufficient to obtain repeated samples, the following data points were collected every 5 min for eight data points, beginning 15-30 min after down time: 1) respiratory rate, 2) heart rate (auscultation and palpable pulse), 3) rectal temperature, 4) blood oxygen saturation via pulse oximetry, 5) indirect systolic and diastolic blood pressure (non-invasive cuff on front limb), 6) presence of ocular reflexive responses (palpebral, iris diameter, globe position, 7) reflex of ear to touch, 8) muscle tension in limbs (relaxed or tense/ability to flex manually), 9) pain response to needle prick to limbs, perineal area, and 10) muscle jaw tone. Simultaneous arterial and venous blood samples were taken every 10 min during data collection period (goal of four arterial and venous samples per animal) for blood gas analysis. EDTA and whole blood samples were collected at the first observation point for CBC and serum chemistry analysis.

Antagonism of MET was achieved utilizing atipamezole, administered intramuscularly in two sites, at three doses ranging from 200-1500 µg/kg; the mean time from injection to standing was 6.1 min in all animals receiving MET/KET, 2.9 min in animals receiving MET alone.

**Observations**

1. KET alone, at 8 mg/kg body weight, did not result in recumbency in any animal and only minimal visible effect.
2. MET alone in the dose range of 250-400 µg/kg body weight resulted in recumbency and a relaxed animal, but arousal could be induced with loud noise or physical manipulation.
3. MET at 300-400 µg/kg and KET at 5-8 mg/kg produced the shortest anesthesia induction time (mean 4 min) and best relaxation. This was followed by MET at 200-300 µg/kg and KET at 3-6 mg/kg with a mean induction time of 6.5 min.
4. The impala less stressed prior to darting, represented by the field anesthetized animals, had shorter induction times and more consistent and sustained relaxation compared to boma-anesthetized impala.
5. 7500 I.U. of hyaluronidase mixed in the dart with the anesthetic drugs resulted in shorter induction times and more consistent and sustained relaxation in almost all cases.
6. Atipamezole produced a rapid, smooth reversal/recovery from anesthesia when given intramuscularly. Intravenous antagonism resulted in a rapid reversal with hyperexcitation. The most effective dose rate was 400-600 µg/kg body weight (ATI:MET ratio of 2.3:1).
7. Atipamezole did not fully antagonize MET in any impala as evidenced by post-reversal sedation that was observed as mild sedation for ≥ 4 hr post reversal. The animals were easily aroused with visualization of humans or disturbance by other impala. This sedation would make the animal susceptible to predators in free-ranging conditions ans susceptible to aggression from other impala.
8. MET/KET is not a reliable field combination due to the post-reversal sedation and the need to wait 15 min post-darting to achieve adequate drug effect for physical manipulation. This combination does have applicability in captive situations.

LITERATURE CITED

COMPARISON OF THE CARDIOPULMONARY EFFECTS OF MEDETOMIDINE-KETAMINE AND MEDETOMIDINE-TELAZOL INDUCTION ON MAINTENANCE ISOFLURANE ANESTHESIA IN THE CHIMPANZEE (Pan troglodytes)

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Abstract

Alpha2-agonists produce profound sedation and analgesia by virtue of their ability to modulate neurotransmitter release in noradrenergic and serotonergic pathways of the brain and spinal cord. The most potent and highly selective of the alpha2-agonists, medetomidine, has recently become available for use in veterinary medicine in the United States. Dexmedetomidine, the D-enantiomer of medetomidine, is currently being investigated for use in human medicine. Medetomidine alone can be used effectively as a sedative-analgesic in a variety of species, but does not provide complete anesthesia. This has lead to experimentation with a variety of combinations of medetomidine and other classes of injectable agents, including tranquilizers, opioids, and dissociative anesthetics.

The combinations of medetomidine (30-100 μg/kg) and ketamine (1-8 mg/kg depending on species), and medetomidine (6-50 μg/kg) and telazol (0.5-6 mg/kg) have been reported to be quite effective in a variety of nondomestic mammals. Little is known, however, about the cardiopulmonary effects of these combinations, especially in nonhuman primates.

The purpose of our study was to compare the cardiopulmonary effects of combinations of medetomidine (30-40 μg/kg) and either ketamine (2 mg/kg) or telazol (1.25 mg/kg) on maintenance isoflurane anesthesia in the chimpanzee. In a previous report, we showed in both chimpanzees and gorillas that medetomidine-ketamine provided a rapid induction and smooth transition to a 1-hr period of isoflurane anesthesia. Blood pressure, heart rate, hemoglobin saturation, and end-tidal CO2 remained within normal limits throughout this period, and reversal with atipamezole provided rapid and smooth recovery. As the use of medetomidine-telazol is becoming more popular, we chose to extend our study by evaluating medetomidine-telazol under the same conditions.

Thirteen chimpanzees (four juveniles, nine adults) undergoing routine annual (1997 and 1998) physical exams at the North Carolina Zoological Park were included in the study. Medetomidine (10 and 20 mg/ml) and ketamine (200 mg/ml) were purchased separately (Wildlife Laboratories, 1401 Duff Drive, Fort Collins, CO 80542). Telazol (Fort Dodge Laboratories, Fort Dodge, IA 50501) was reconstituted in H2O (100 mg/ml). The drug combinations were mixed in the same syringe prior to being administered either by hand syringe or by dart (Telinject USA Inc., 9316 Soledad Canyon Rd., Saugus, CA 91350). The combinations were allowed at least 5-10 min to take effect. Animals were then masked with 2-3% isoflurane (IsoFlo, Abbott Laboratories, N. Chicago, IL 60064) in 100%
oxygen to permit endotracheal intubation. Following intubation, animals were maintained with 0.5-2.0% isoflurane for the duration of the exam (in most cases 50-60 min) using a non-rebreathing anesthetic circuit. Lactated ringers solution was administered at a rate of 10 ml/kg/hr. To assess the cardiovascular effects of the anesthetic protocols, indirect arterial blood pressure and heart rate were measured oscillometrically (Dinamap Model 8300, Critikon, 4710 Eisenhower Blvd., Tampa, FL 33614) at 3-min intervals beginning at the point where animals became recumbent. To assess respiratory effects, hemoglobin saturation was measured indirectly by pulse oximetry (Model N20, Nellcor, 25495 Whitesell St., Hayward, CA 94545), and end-tidal CO2 and respiratory rate were measured by mainstream capnography (Model 1260, Novametrix, 3 Sterling Dr., Wallingford, CT 06492). Arterial blood gases were measured with a pH and blood gas analyzer (StatPal SP2-A, PPG Ind., 11077 N. Torrey Pines Rd., La Jolla, CA 92037). At the end of the procedure, isoflurane was discontinued, the animals were placed in a recovery cage, and 2 mg/kg atipamezole (Antisedan, Pfizer, Exton, PA 19341) was immediately given i.m.

Medetomidine-ketamine (M-K) provided sedation within 2-5 min (3.0 ± 1.6 min) and light anesthesia within 3-15 min (juveniles 3.7 ± 1.25 min; adults 11 ± 3.8 min). We found it to be very important that animals be left alone for the initial 10 min, as attempts to move them prior to this could result in rapid arousal. Medetomidine-telazol (M-TZ) also provided sedation within 2-5 min (3 ± 1.2 min), but a deeper plane of anesthesia was reached more rapidly (7.25 ± 2.25 min). Animals induced with M-TZ were not easily aroused. Once the M-K or M-TZ had taken effect, only 3-5 min of 2-3% isoflurane by mask was required for intubation. Once intubated, an adequate plane of anesthesia (determined as the minimum concentration required to prevent purposeful movement in response to ultrasonic teeth cleaning) was maintained with 1.2 ± 0.7% isoflurane in M-K animals and 0.8 ± 0.2% in M-TZ animals.

Both M-K and M-TZ animals had elevated blood pressures immediately following induction. Average mean arterial pressures were 121 ± 8 mm Hg for M-K animals and 119 ± 11 for M-TZ animals. Blood pressure decreased steadily over the first 10-15 min and then stabilized. At 50 min, M-K animals had, on average, higher mean arterial pressures (89 ± 15 mm Hg) than did M-TZ animals (73 ± 7 mm Hg). Pulse pressure (the difference between systolic and diastolic pressure, a function of stroke volume and vessel compliance) remained essentially constant throughout the procedures and were nearly identical for both groups (at 50 min: M-K, 58 ± 8 mm Hg; M-TZ, 59 ± 11 mm Hg). Heart rates immediately after induction were similar in both groups (M-K, 78 ± 11 beats/min; M-TZ, 83 ± 12 beats/min), and declined gradually over time (at 50 min: M-K, 67±7 beats/min; M-TZ, 68 ± 14 beat/min). Spontaneous respiratory rates remained elevated (at 50 min: M-K, 31 ± 8; M-TZ, 24 ± 5) and constant throughout the procedures. End-tidal CO2 levels were on average between 37 and 44 mm Hg for both groups and hemoglobin saturation levels were consistently between 93-100%. Arterial blood gas values were within normal limits for those animals tested (four chimpanzees).

Speed and quality of recovery differed markedly between M-K and M-TZ animals. First signs of recovery in both groups occurred within 8-10 min following atipamezole injection. Extubation occurred at this time. M-K animals were fully recovered (standing, alert, vocalizing, climbing)
within 10-13 min. M-TZ animals, in contrast, took much longer (3 ± 2 hr) to reach the same end point, and showed signs of extreme drowsiness, dizziness, ataxia, and GI disturbance (vomiting, lack of appetite). Flumazenil (0.025 mg/kg i.v.) was administered to five M-TZ chimpanzees, and though it did transiently increase alertness, it did not significantly enhance the speed or quality of recovery.

Our results provide some insight into the cardiopulmonary consequences of the use of medetomidine-ketamine-isoflurane and medetomidine-telazol-isoflurane anesthesia in the chimpanzee. Most notably, even at high doses in the chimpanzee, medetomidine, when combined with either ketamine or telazol, does not appear to provoke the extreme bradycardia that is characteristic of its use in other species. Thus, both M-K and M-TZ given intramuscularly provide a rapid and safe induction in chimpanzees, and allow for a smooth transition to inhalation anesthesia. The cardiovascular data obtained (similar heart rates and pulse pressures in both groups, but significantly lower blood pressures in the MTZ-isoflurane anesthetized animals) suggested that the different drug combinations have similar effects on cardiac output but different effects on systemic vascular resistance. M-TZ appears to potentiate the vasodilatory effects of isoflurane. Our data also suggest that M-TZ has a more potent effect on reducing the MAC (minimum alveolar concentration) value for isoflurane. Thus, though both combinations may be considered safe, M-TZ-isoflurane anesthesia may put animals at risk of severe hypotension if close attention is not paid to the maintenance concentration of isoflurane. This is especially true if changes in posture (such as those required for chest films) occur while the animals are anesthetized. By far, the most dramatic difference between the two anesthetic regimes is the quality of the recovery. M-K recoveries are typically complete within 15-20 min and are without adverse side effects. M-TZ recoveries, on the other hand, may be quite prolonged and accompanied by adverse CNS and GI effects.

ACKNOWLEDGMENTS

We thank Wildlife Laboratories for providing concentrated medetomidine, and Dr. W. Karesh and Rochel Laboratories for supplying flumazenil.

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ANESTHESIA OF FREE-RANGING FLORIDA PANTHERS (Puma concolor coryi), 1981-1998

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Abstract

The Florida panther (Puma concolor coryi) is one of the most endangered mammals in the world. The free-ranging population is estimated to be between 30-50 adult animals. Historically, this species of mountain lion ranged from eastern Texas or western Louisiana and the lower Mississippi River Valley east through the southeastern United States, including Arkansas, Louisiana, Mississippi, Alabama, Georgia, Florida and parts of Tennessee and South Carolina. Up until 1966, they were hunted to protect livestock and for sport. South Florida landscape has undergone significant changes including habitat loss from human development, changes in land use to housing and citrus groves, fragmentation by roads, and introduction of exotic plants and animals. The Florida Game & Fresh Water Fish Commission began studying the panther in 1972 and it was listed by the United States Fish & Wildlife Service as an endangered species in 1981. Panthers are now only known to inhabit south Florida and a subset of the population has been studied using radio telemetry since 1981.

Between 1981 and 1998, 72 panthers have been anesthetized multiple times (1-10 times per cat) for a total of 183 capture events. Panthers have ranged from 6-mo- to 16-yr-old. Direct or indirect capture related mortality has only occurred in 3 (0.016 %) of the 183 captures. These mortalities included: A cat that died less than 8 min after being darted and was most likely a result of either a negative anesthetic reaction or a dose miscalculation; A cat died of a cellulitis and toxemia which resulted from a dart that penetrated the abdomen; And a cat that died approximately 3 days post handling but was autolytic to evaluate further.

The field capture event involved a core capture team composed of a hound man, veterinarian, and two biologists. The first phase involved the hound man who, with two to six hounds, located the felid scent and pursued the panther until it “treed.” Actual chases were relatively short and usually ranged from 5-10 min. The second phase involved rapidly assessing the cat’s physical condition, determining the appropriate anesthetic drugs and dose, and the preparation of a 3-cc dart with 1.5 x 30 mm uncollared needle. A CO2 powered rifle with scope (Teleinject, Saugus, California USA) was used to deliver most darts. The third phase usually involved catching the anaesthetized panther in a net as it fell from the tree. If the fall distance was greater than about 5 m, a portable wildlife cushion was used. Occasionally an anaesthetized cat would remained in the tree and a biologist had
to climb up and lower the cat to the ground with a rope. The fourth phase involved biomedical monitoring and research and involved: Physical examination and collection of blood, hair, feces, urine, and external parasites; Full thickness skin punch biopsies were taken. Panthers were vaccinated for rabies, panleukopenia, calicivirus and rhinopneumonitis. Anthelmintics were usually administered. Panthers may also have received long acting penicillin, vitamins, and iron. Intravenous and/or subcutaneous saline was usually administered. Panthers were then fitted with radio collars (Telonics, Inc., Mesa, Arizona USA). These collars are equipped with both an activity switch and a mortality sensor. The cats were usually monitored 3 days/wk through aerial telemetry. For additional permanent identification the cat’s ears were tattooed and a subcutaneous transponder chip was implanted. Body measurements were taken and the animal was weighed. Special studies such as semen evaluation by electro ejaculation may periodically have been conducted. Handling time to complete these tasks has ranged from 12 min-3 hr.

Since 1981, anesthesia on the panthers has been conducted by one biologist and eight veterinarians. Not all records have complete information and the brand of the specific drug may not have been listed. Anesthetic drugs used on free-ranging Florida panthers have included acepromazine (10 mg/ml), ketamine (100 mg/ml or 200 mg/ml) (Ketaset, Fort Dodge Laboratories Inc., Fort Doge, Iowa USA), tiletamine hydrochloride/zolazepam hydrochloride (100 mg/ml) (Telazol, Fort Dodge Laboratories Inc., Fort Doge, Iowa USA), diazepam (5 mg/ml), midazolam (5 mg/ml) (Versed, Roche Laboratories, Mutley, New Jersey USA), and xylazine hydrochloride (100 mg/ml). Drugs were reconstituted with sterile water as necessary.

**Ketamine & acepromazine**

Ketamine (5.5-11.3 mg/kg) and acepromazine (0.01-0.1 mg/kg) were used on the first eight panther captures. Usage of this combination was discontinued when a panther died approximately 6-8 min post dart injection. Necropsy of this animal did not reveal any significant lesions that would have otherwise explained this mortality, and thus it was assumed to be from either a dose miscalculation or an unusual reaction.

**Ketamine**

Ketamine at was administered for initial dart in 48 panthers. Doses ranged from 6.1-12.9 mg/kg. The initial dose and/or only one supplemental dose provided satisfactory results in 15 (31%) panthers. However, the initial dose and multiple supplements (two to nine) were necessary in 33 (69%) panthers to collect biomedical samples. Seizures were occasionally observed to occur. Biologists frequently reported the panthers had prolonged recovery and were still at or within 200 yards of the capture site for 1-7 days post capture. Three juvenile panthers were orphaned or temporarily orphaned due to their prolonged recovery for the anesthesia causing their inability to keep up with their mothers.

**Ketamine & midazolam**

Combinations of ketamine (9.5-14 mg/kg) and midazolam (0.02-0.06 mg/kg) were administered to five panthers. All cats required multiple supplements (two to four).
**Ketamine & diazepam**  
Combinations of ketamine (7.3-12.6 mg/kg) and diazepam (0.05-0.25 mg/kg) were used on four captures. No supplements were needed. However, according to the biologist, extremely prolonged recovery times occurred.

**Ketamine & xylazine hydrochloride**  
Combination of ketamine (6.4 mg/kg) and xylazine hydrochloride (0.71 mg/kg) was used on one panther. Multiple supplements (3) were needed.

**Tiletamine hydrochloride/zolazopam hydrochloride**  
Tiletamine hydrochloride/zolazopam hydrochloride (4.8-7.8 mg/kg) alone was used on 14 panthers. This was predominantly administered to male panthers to undergo electro ejaculation. The initial dose and or only one supplemental dose provided satisfactory results in 10 (71%) of these panthers. The initial dose and multiple supplements (two to five) were necessary in four (29%) panthers to collect biomedical samples.

**Ketamine & tiletamine hydrochloride/zolazopam hydrochloride**  
Ketamine (15.5-4.0 mg/kg) and tiletamine hydrochloride/zolazopam hydrochloride (2.5-0.6 mg/kg) were used in combination for 86 panther captures. Induction time was usually within 4-8 min. Anesthetic times ranged from 12 min to several hours. The cats are usually sternal and trying to get on their feet at 45 min-1 hr. Records note that 63 (73%) of these panthers were adequately anaesthetized with the initial dose or the initial dose and one supplemental dose. The initial dose and multiple supplements (two to six) were necessary in 23 (27%) of these panthers. However, most of these repeated supplements were needed before experience allowed appropriate dose adjustments.

The combination of ketamine (7.0 mg/kg) and tiletamine hydrochloride/zolazopam hydrochloride (0.9 mg/kg) has been used successfully on more than 40 captures and is currently being used. If the cat is high up in a tree, the dose of tiletamine hydrochloride/zolazopam hydrochloride is slightly decreased. This has resulted in less of a fall to some cats as they are able to hang by their front feet and lowering themselves 5-6 feet closer to the net. This provides approximately 45 min of handling time. In addition, to routine biomedical procedures this dosage is adequate for minor surgical procedures such as suturing wound. No juveniles have been orphaned with this combination and the resulting shorter anesthesia time. Biologists report that the cats have usually move 0.5 mile or more by the next telemetry flight.

**ACKNOWLEDGMENTS**

We acknowledge additional telemetry work, capture efforts, and data collection of D. S. Maehr, J. W. McCown, J. C. Roof, S. Bass, D. Jansen, M. Dunbar, and R. C. Belden. We also thank houndsman and marksman R. McBride for his expertise. D. Boon assisted with the database. Funding for this study was provided through the Florida Panther Research and Management Trust Fund, Florida Nongame Wildlife Trust Fund, and the Federal Endangered Species Project E-1.

**LITERATURE CITED**
Transport of Ten Western Lowland Gorillas (*Gorilla gorilla gorilla*) from the Netherlands to Australia, and Their Subsequent Anaesthesia and Health Assessment

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Abstract

In December 1996, a family group of ten western lowland gorillas (*Gorilla gorilla gorilla*) was transported by air from Apenheul Primate Park, Apeldoorn, The Netherlands to Taronga Zoo, Sydney, Australia. The group was comprised of a 19-yr-old silverback (“Kibabu”), a 24-yr-old female (“Mouila”), its 3-yr-old male (“Haoko”) and 7-wk-old male (“Shabani”) offspring, a 17-yr-old female (“Kriba”), its 3-yr-old female (“Kijivu”) and 3-mo-old female (“Safiri”) offspring, a 15-yr-old female (“Frala”), its 5-yr-old female (“Shinda”) and 2-yr-old female (“Anguka”) offspring. An eleventh animal in the group, the 7-yr-old female daughter of “Kriba” (“Joas”) was crated at the same time as the others, but was transported to Basel Zoo, Switzerland. Although many gorillas have been transported around the world, by sea, air and land, there is no record in the readily available literature of the transportation of ten gorillas by air over such a great distance.

With this in mind, a great deal of planning and preparation was put into the shipment. Planning for the crating procedure focused on a strategy aimed at not using chemical restraint. This involved the construction of a “U” shaped race with an offshoot to which the crate was attached. The order of crating was also significant and was based on hierarchy, personalities and family units within the group. In some cases manipulation of lighting was used to encourage gorillas to move from one area to another.² Only the last animal, the 3-yr-old female, required immobilization with ketamine at 10 mg/kg for crating.

Five days prior to transport the group was started on a course of the neuroleptic drug, zuclopenthixol (Cisordinol, Lundbeck, Denmark) to reduce anxiety. This was continued throughout the journey and ceased four days after arrival. Dose rates used ranged from 0.10-0.36 mg/kg p.o., b.i.d. and was varied according to individual behavior and stage of the journey (Table 3).

Zuclopenthixol is a potent neuroleptic of the thioxathene series with general properties similar to the phenothiazine tranquillizers.³ It is available as an oral formulation in Europe only and an injectable (Clopixol-acuphase, Clopixol depot, Lundbeck) elsewhere.

Apart from some anxiety during the crating procedure, the animals were calm and traveled well, maintaining good appetites, particularly during periods that one would expect to be disturbing for the animals (e.g., loading and unloading the airplane, take-offs and landings).
Careful attention was payed to crate design. The group traveled in four crates, with the silverback on its own, the females in crates with a division to separate mother with baby from its juvenile offspring. The animals traveled in the cargo section of a Boeing 747-400, 7 pallet. Three keepers and a veterinarian accompanied and attended the animals during the flight.

After arrival in Sydney, the animals were placed in quarantine for 30 days. In order to comply with Australian Quarantine Inspection Service (AQIS) requirements for tuberculosis testing, six animals were anaesthetized 10 days after arrival. Exemption from testing was sought for the two females with babies, as it was considered that the procedure would put the babies at risk during and after the anaesthesia of their mothers. This was granted on the basis that Apenheul Primate Park had never had a case of tuberculosis and that the other six gorillas tested negative for tuberculosis.

The six gorillas were anaesthetized in one morning. This was achieved with two teams of veterinarians working concurrently. Each animal was anaesthetized using 4 mg/kg of tiletamine and zolazepam (Zoletil 100, Virbac, Australia) administered by remote injection (Telinject, Germany). Anaesthesia was maintained using isoflurane (Forthane, Abbott, Australia) in oxygen via an endotracheal tube. Electrocardiogram, heart rate, respiratory rate, blood pressure, and oxygen saturation of hemoglobin were monitored and recorded (Dinamap plus, Critikon, Australia).

A detailed health screen was carried out on each animal and included physical examination, comparative intra-dermal tuberculin test, gamma interferon assay for tuberculosis, thoracic radiographs, hematology, biochemistry, immune testing, viral serology and fecal parasitology and bacterial culture (Tables 1, and 3 through 7). Each animal was vaccinated against tetanus (Tet-Tox, CSL, Australia) and implanted with an identification transponder (Trovan, AEG/Telefunken, Germany).

Most results fell within or close to published reference ranges for gorillas (where available). The 15-yr-old female and its daughter were mildly anaemic (normocytic, normochromic). The 3-yr-old male, 3-yr-old female, and the 5-yr-old female had elevated serum alkaline phosphatase, most likely due to bone growth in these young animals. The 19-yr-old silverback had slight thrombocytopenia and mildly elevated immunoglobulin. No reference ranges were available for lymphocyte phenotyping in gorillas and most results fell outside those reported for humans.

All six gorillas were positive for cytomegalovirus, which is common in many primate species and always asymptomatic unless the animal is immunosuppressed. Four of the six gorillas were positive for Herpes simplex virus. In serological surveys of gorillas, the prevalence of herpesvirus antibodies is over 60%. Clinical disease is rare in gorillas but is similar to humans with facial and oral ulcers and chickenpox. Genital lesions have not been reported.

Three gorillas had positive readings to avian PPD after 72 hr. As all were negative to bovine PPD, and showed no immunity to mycobacterial antigens on gamma interferon, it is most likely that this was a non-specific reaction, perhaps to atypical mycobacteria in their gastrointestinal tracts, rather than to tuberculous mycobacteria.
Although the gamma interferon assay used at the time of testing (Quanti FERON-TB, CSL, Australia) had not been validated for use in nonhuman primates, the results in these gorillas were consistent with those seen in people that do not have any form of tuberculosis. The assay has now been validated for use in nonhuman primates and is marketed as Primagam (CSL Veterinary, Australia). None of the gorillas showed evidence of immunity to mycobacterial antigens in vitro, which is strong evidence for freedom from infection with either *Mycobacterium tuberculosis*, *M. avium* or *M. bovis*. The zero reaction to the positive control (mitogen) for “Anguka” merely indicated a poor immune response in vitro, which may be due to the animal’s age (2 yr) and therefore an immature immune system.

ACKNOWLEDGMENTS

The author thanks Apenheul Primate Park, Drs Hulst, Higgins, Woods, Ralph, Keogh, Ms Libby Kartzoff and Mr Glenn Smith, the Institute of Laboratory medicine, St Vincent’s Hospital, NSW Red Cross Blood Transfusion service, Westmead Institute of Clinical Pathology and Medical Research, and NSW Agriculture Regional Veterinary Laboratory for their assistance with this work, and Vanessa Di Giglio for preparation of this manuscript.

LITERATURE CITED

### Table 1. Viral serology for six western lowland gorillas at Taronga Zoo, Australia.

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**EMC** - Encephalomyocarditis virus detected by virus neutralization test  
**HBsAg** - Hepatitis B surface antigen detected by EIA/ELISA  
**HCV** - Hepatitis C Virus tested by 3rd generation EIA  
**HTLV-1** - Human T cell leukemic virus, detected by EIA/ELISA  
**HIV-1/HIV-2** - Human immunodeficiency virus 1 and 2 detected by EIA/ELISA  
**HBc** - Hepatitis B core antigen detected by MEIA  
**HBs** - Hepatitis B surface antibody detected by MEIA  
**CMV** - Cytomegalovirus detected by particle agglutination  
**HAV** - Hepatitis A virus detected by MEIA  
**HIV-1/HIV-2 WB** - Human immunodeficiency virus 1 and 2 detected by Western blot  
**HSV IgG** - Herpes simplex virus - Complement fixation test for total antibody titre  
**Rubella IgG** - Complement fixation test for total antibody titre  
**Q fever Chase 2 titre** - Complement fixation test for total antibody titre
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Day 7 to Day 9 = period of crating and transport.
* estimated weights
Table 3. Hematology and serum biochemistry of six western lowland gorillas anaesthetized with tiletamine and zolazepam at Taronga Zoo, Australia.

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<td>Urea mmol/L</td>
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<td>4.2</td>
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</tr>
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<td>Creat. mmol/L</td>
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<td>0.11</td>
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<td>0.07</td>
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<td>Protein g/L</td>
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<td>67</td>
<td>63</td>
<td>64</td>
<td>66</td>
<td>73.0 ± 7.0</td>
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<td>Albumin g/L</td>
<td>42</td>
<td>43</td>
<td>37</td>
<td>39</td>
<td>41</td>
<td>37</td>
<td>37.0 ± 4</td>
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<tr>
<td>Globulin g/L</td>
<td>39</td>
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<td>30</td>
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<tr>
<td>A-G ratio</td>
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<td>T-Bili μmol/L</td>
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<td>4.7</td>
<td>8.6 ± 5.1</td>
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<td>Alk Phos U/L</td>
<td>178</td>
<td>295</td>
<td>642</td>
<td>574</td>
<td>922</td>
<td>750</td>
<td>425 ± 353</td>
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<tr>
<td>AST U/L</td>
<td>36</td>
<td>38</td>
<td>38</td>
<td>35</td>
<td>26</td>
<td>17</td>
<td>33 ± 19</td>
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<tr>
<td>ALT U/L</td>
<td>34</td>
<td>43</td>
<td>26</td>
<td>26</td>
<td>23</td>
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<td>Gamma GT U/L</td>
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<td>7</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>31 ± 37</td>
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<tr>
<td>CK U/L</td>
<td>366</td>
<td>193</td>
<td>203</td>
<td>198</td>
<td>191</td>
<td>113</td>
<td>398 ± 466</td>
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<tr>
<td>Cholesterol mmol/L</td>
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<td>5.8</td>
<td>8.3</td>
<td>8.4</td>
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<tr>
<td>Mg mmol/L</td>
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<td>Calcium mmol/L</td>
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<td>1.0</td>
<td>1.4 ± 0.3</td>
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<tr>
<td>Ca:P ratio</td>
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<td>Sodium mmol/L</td>
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<td>140</td>
<td>137</td>
<td>131</td>
<td>137</td>
<td>135</td>
<td>137 ± 3</td>
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<td>Potassium mmol/L</td>
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<td>3.9</td>
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<td>3.8</td>
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<td>Chloride mmol/L</td>
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<td>102</td>
<td>99</td>
<td>101</td>
<td>104</td>
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<td>Bicarb mmol/L</td>
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<td>23.7</td>
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<td>25.9</td>
<td>28.0</td>
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<tr>
<td>AG mmol/L</td>
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<td>17.3</td>
<td>16.9</td>
<td>11.8</td>
<td>18.9</td>
<td>6.8</td>
<td>-</td>
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<tr>
<td>Uric acid</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.09 ± 0.3</td>
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<td>Lipase U/L</td>
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<td>165</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>13 ± 24</td>
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<tr>
<td>Amylase U/L</td>
<td>56</td>
<td>99</td>
<td>24</td>
<td>58</td>
<td>50</td>
<td>22</td>
<td>29 ± 24</td>
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<td>Na:K ratio</td>
<td>35.1</td>
<td>35.0</td>
<td>35.1</td>
<td>32.8</td>
<td>36.1</td>
<td>35.5</td>
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Table 4. Immunology testing of six western lowland gorillas at Taronga Zoo, Australia.

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<tr>
<th></th>
<th>KIBABU</th>
<th>FRALA</th>
<th>ANGUKA</th>
<th>HAOKO</th>
<th>SHINDA</th>
<th>KIJIVU</th>
<th>Reference Range (human)</th>
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<tbody>
<tr>
<td><strong>Serum EPG</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Total Protein g/L</td>
<td>85</td>
<td>68</td>
<td>69</td>
<td>62</td>
<td>66</td>
<td>67</td>
<td>64 - 80</td>
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<tr>
<td>Albumin g/L</td>
<td>37</td>
<td>36</td>
<td>32</td>
<td>31</td>
<td>34</td>
<td>34</td>
<td>35 - 48</td>
</tr>
<tr>
<td>Alpha-1 g/L</td>
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<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>2.0</td>
<td>3.0</td>
<td>2 - 5</td>
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<td>Alpha-2 g/L</td>
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<td>5.0</td>
<td>8.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>5 - 9</td>
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<td>Beta g/L</td>
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<td>13.0</td>
<td>12.0</td>
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<tr>
<td>Gamma g/L</td>
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<td>11.0</td>
<td>13.0</td>
<td>10.0</td>
<td>11.0</td>
<td>12.0</td>
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<tr>
<td><strong>Serum Immunoglobulins</strong></td>
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<tr>
<td>IgG g/L</td>
<td>23.0</td>
<td>10.5</td>
<td>13.3</td>
<td>9.3</td>
<td>9.8</td>
<td>10.4</td>
<td>2.6 - 15.4</td>
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<td>IgA g/L</td>
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<td>2.0</td>
<td>1.7</td>
<td>1.8</td>
<td>1.8</td>
<td>2.1</td>
<td>0.1 - 3.4</td>
</tr>
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<td>IgM g/L</td>
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<td>0.6</td>
<td>0.7</td>
<td>0.4</td>
<td>0.6</td>
<td>0.9</td>
<td>0.1 - 2.4</td>
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<td><strong>Complement</strong></td>
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<td>C3 g/L</td>
<td>1.36</td>
<td>1.04</td>
<td>1.21</td>
<td>1.05</td>
<td>0.96</td>
<td>1.04</td>
<td>0.82 - 1.45</td>
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<tr>
<td>C4 g/L</td>
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<td>0.24</td>
<td>0.28</td>
<td>0.30</td>
<td>0.23</td>
<td>0.29</td>
<td>0.15 - 0.45</td>
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<td><strong>Antinuclear Antibody</strong></td>
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<td>-</td>
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<tr>
<td><strong>Autoantibodies</strong></td>
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<tr>
<td>Smooth muscle</td>
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<td>-</td>
<td>-</td>
<td>&lt; 1/10</td>
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<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&lt; 1/10</td>
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<td>-</td>
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<td>-</td>
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<td>&lt; 1/10</td>
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<td><strong>Lymphocyte Phenotyping</strong></td>
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<td>Lymphocytes x 10^6/L</td>
<td>1500</td>
<td>1000</td>
<td>1300</td>
<td>1100</td>
<td>1000</td>
<td>600</td>
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<tr>
<td>CD2 + %</td>
<td>81</td>
<td>77</td>
<td>37</td>
<td>69</td>
<td>42</td>
<td>49</td>
<td>73 - 94</td>
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<tr>
<td>E rosette x 10^6/L</td>
<td>1215</td>
<td>770</td>
<td>481</td>
<td>759</td>
<td>420</td>
<td>294</td>
<td>1310 - 2080</td>
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<tr>
<td>CD3 + %</td>
<td>67</td>
<td>68</td>
<td>29</td>
<td>61</td>
<td>34</td>
<td>47</td>
<td>59 - 83</td>
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<tr>
<td>Total T Cells x 10^6/L</td>
<td>1005</td>
<td>680</td>
<td>377</td>
<td>671</td>
<td>340</td>
<td>282</td>
<td>700 - 2600</td>
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<tr>
<td>CD3 + CD4+ %</td>
<td>25</td>
<td>29</td>
<td>18</td>
<td>38</td>
<td>22</td>
<td>32</td>
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<td>Helper T cells x 10^6/L</td>
<td>375</td>
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<td>234</td>
<td>418</td>
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<td>192</td>
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<tr>
<td>CD3 + CD8 + %</td>
<td>38</td>
<td>37</td>
<td>9</td>
<td>23</td>
<td>11</td>
<td>12</td>
<td>14 - 35</td>
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<td>Cytotoxic T cells x 10^6/L</td>
<td>570</td>
<td>370</td>
<td>117</td>
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<td>110</td>
<td>72</td>
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<td>CD3+ HLADR+ %</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<td>00</td>
<td>00</td>
<td>11</td>
<td>00</td>
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<tr>
<td>CD 16+ %</td>
<td>14</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>11</td>
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<td>NK cells x 10^6/L</td>
<td>210</td>
<td>90</td>
<td>91</td>
<td>77</td>
<td>110</td>
<td>48</td>
<td>70 - 280</td>
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<tr>
<td>CD 19+ %</td>
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<td>65</td>
<td>29</td>
<td>58</td>
<td>47</td>
<td>5 - 20</td>
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<tr>
<td>Total B cells x 10^6/L</td>
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<td>230</td>
<td>845</td>
<td>319</td>
<td>580</td>
<td>282</td>
<td>80 - 300</td>
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<tr>
<td>CD20 + %</td>
<td>11</td>
<td>14</td>
<td>55</td>
<td>27</td>
<td>52</td>
<td>42</td>
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<tr>
<td>B cells x 10^6/L</td>
<td>165</td>
<td>140</td>
<td>715</td>
<td>297</td>
<td>520</td>
<td>252</td>
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<tr>
<td>HLA-DR+ %</td>
<td>19</td>
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<td>65</td>
<td>33</td>
<td>56</td>
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<tr>
<td>Class II MHC x 10^6/L</td>
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<td>220</td>
<td>845</td>
<td>363</td>
<td>560</td>
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Table 5. Tuberculosis testing and thoracic radiographs of six western lowland gorillas at Taronga Zoo, Australia.

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<th></th>
<th>KIBABU</th>
<th>FRALA</th>
<th>ANGUKA</th>
<th>HAOKO</th>
<th>SHINDA</th>
<th>KIJIVU</th>
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<tbody>
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<tr>
<td>Avian</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Radiograph</td>
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</table>

TB TEST:
Bovine: 0.1 ml Bovine PPD (1 mg/ml) (CSL, Australia) intradermally into the left upper eyelid. Read at 72 hr.
Avian: 0.1 ml Avian PPD (25 000 u/ml) (CSL, Australia) intradermally into right upper eyelid. Read at 72 hr.

Table 6. Gamma interferon assay (QuantiFERON-TB, CSL, Australia) for tuberculosis in six western lowland gorillas at Taronga Zoo, Australia.

<table>
<thead>
<tr>
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<th>KIBABU</th>
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<th>ANGUKA</th>
<th>HAOKO</th>
<th>SHINDA</th>
<th>KIJIVU</th>
</tr>
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<tbody>
<tr>
<td>Nil Ag</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>HuPPD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>AvPPD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BoPPD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>Mitogen</td>
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<td>0</td>
<td>5.0</td>
<td>13.9</td>
<td>10.8</td>
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</table>

HuPPD = *Mycobacterium tuberculosis*
AvPPD = *M. avium*
BoPPD = *M. bovis*
Nil Ag = negative control
Mitogen = positive control

Table 7. Fecal parasitology and culture results for six western lowland gorillas from feces collected at time of anaesthesia.

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<th>FRALA</th>
<th>ANGUKA</th>
<th>HAOKO</th>
<th>SHINDA</th>
<th>KIJIVU</th>
</tr>
</thead>
<tbody>
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<td>KIBABU</td>
<td><em>Entamoeba coli</em> / <em>Iodamoeba</em> sp. / <em>Campylobacter</em> sp. isolated</td>
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<tr>
<td>FRALA</td>
<td><em>Trichomonas</em> sp. / <em>Entamoeba coli</em> / <em>Entamoeba histolytica</em></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHINDA</td>
<td><em>Trichomonas</em> sp. / <em>Entamoeba histolytica</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANGUKA</td>
<td><em>Campylobacter</em> sp. isolated</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>KIJIVU</td>
<td><em>Entamoeba coli</em> / <em>Campylobacter</em> sp. isolated</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HAOKO</td>
<td><em>Entamoeba coli</em> / <em>Campylobacter</em> sp. / <em>Shigella flexneri</em></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

In three subsequent cultures of group fecal samples, neither the *Campylobacter* nor *Shigella* were isolated.
BEHAVIORAL AND PHYSIOLOGIC RESPONSE TO AN INTERMEDIATE-ACTING TRANQUILIZER, ZUCLOPENTHIXOL, IN CAPTIVE NILE LECHWE (Kobus megaceros)

Tracy L. Clippinger, DVM,1,2* Scott B. Citino, DVM, Dipl ACZM,2 and Scotty Wade2

1Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 USA; 2White Oak Conservation Center, 3823 Owens Road, Yulee, FL 32097 USA

Abstract

Both free-ranging and captive (zoo) wild animals experience stress during periods of adaptation to new or changed surroundings and conditions.1 Though stress has no distinct cause or precise biologic measurement, evaluation of changes in behavioral and physiologic coping mechanisms allow some indication of stress.2 Behavioral coping mechanisms include movement from a threat to a more favorable location, vocalization, increased locomotion, aggression, and stereotypic behaviors. Observation of changes in behavior,3 heart rate,4-6 catecholamine, and corticosteroid5-7 levels may reflect “stress status” in animals.

Zuclopenthixol, a thioxanthene derivative, is a mid-duration neuroleptic, with pharmacologic effects within 1-12 hr of administration and lasting 72-96 hr in previously evaluated wild animals.8-10 Noticeable effects have included attitude modification toward surroundings and other captive animals (manifested as indifference), eating and drinking without inhibition, loss of instinctive fear of approaching humans, and relative calm adaptation to new situations.11

The objective of this study was to investigate the response of captive Nile lechwe (Kobus megaceros) to conditions surrounding typical shipment procedures while under the influence of a mid-duration neuroleptic, zuclopenthixol acetate (Clopixol-acuphase® also known as Cisordinol-acutard®, 50 mg/ml, H. Lundbeck A/S, Copenhagen-Valby, Denmark). The core study group consisted of five male and one female lechwe (two adults weighing 91-104 kg, four yearlings weighing 44-63 kg) with an additional two male sub-adults (estimated weight 86 kg) in trial #1 only from a herd of seven male adult, five female adult, and four undetermined-gender Nile lechwe. Animals were assigned randomly to one of two study groups while accounting for an even mix of ages. The study design provided for cross-over of the test (drug) and the control group for trial #1 and trial #2, which occurred 1 mo apart, allowing an interim return to the herd and rest period.

One month prior to trials, animals in the core study group were anesthetized for collection of baseline physiologic data, implantation of heart rate transmitters, and distinctive marking of individuals. Animals were anesthetized with carfentanil (dosage range: 17-24 μg/kg, Carfentanil, 3 mg/ml, Wildlife Laboratories, Fort Collins, Colorado USA) and xylazine (0.19-0.36 mg/kg, Rompun®, Bayer Corporation, Shawnee Mission, Kansas USA) i.m. by telinject dart, supplemented with ketamine (0.80-1.13 mg/kg, Ketaset®, Fort Dodge Laboratories, Fort Dodge, Iowa USA) i.v. upon initial handling to facilitate transport, intubated, and maintained on isoflurane (Aerrane®,

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Anaquest, Madison, Wisconsin USA). The narcotic and sedative were antagonized with naltrexone (100x carfentanil dose divided 50% i.v. and 50% s.c., Trexanil®, Wildlife Laboratories, Fort Collins, Colorado USA) and yohimbine (0.12-0.17 mg/kg, i.v., Antagonil®, Wildlife Laboratories, Fort Collins, Colorado USA), respectively, prior to release into the herd. Heart rate transmitters (VHF-C-1 with 5000 Mah lithium batteries, Mini-mitter Co., Sunriver, Oregon USA) were surgically implanted on the left dorsolateral thorax following an established technique with minor modification in lead placement. Heart beat signals were monitored with a telemetry receiver (TR-2 fitted with the RA2AK flexible antenna, Telonics, Mesa, Arizona USA). Dependent upon natural coloration, swatches of hair were bleached blonde or dyed black to assist in remote identification of subjects. Prior to the zuclopenthixol trials, baseline observations were collected for general behavior, resting heart rates, and fecal cortisol level for the subjects.

Lechwe were sorted into study groups and placed into adjacent paddocks (21 × 13 m area with 2.5 m wooden walls) for each 4-day trial. Behavioral and physiologic parameters were monitored by observation (direct and video camera for 12 continuous daylight hours), remote biotelemetric monitoring (intermittent), and laboratory testing of feces and blood. On day 1, Zuclopenthixol was delivered into the biceps femoris muscle by Telinject® dart (5 cc vario syringe, 2.0 × 30 mm needle) to the test group at 1 mg/kg (45-105 mg) for trial #1 and 1.5 mg/kg (95-140 mg) for trial #2. Behavior and heart rate were monitored before, during, and after the application of various stressors. A fecal sample from each subject was collected daily for cortisol assay. The “manstand” stressor, characterized by 3 min of keeper presence within the paddock, was applied daily. Six hours were allowed to elapse after tranquilizer administration before the first “manstand” stressor was applied. The “chute” stressor, which involved herding the lechwe in each group singly from a raceway into a hydraulic squeeze chute for 10-15 min where the subjects were restrained for examination, phlebotomy, cervical tuberculin testing, and per rectum fecal collection, was applied on day 2. Venous blood was collected for CBC, serum biochemistry evaluation, plasma lactic acid assay, serum cortisol assay, and plasma catecholamine assay. Arterial blood was collected from an auricular artery for blood gas evaluation. The “novel item” stressor, characterized by release of subjects into a paddock containing a previously unseen 47 cm black trash can for 5 min, was applied on days 3 and 4. The subjects were returned to the main herd on day 4, greater than 74 hr after zuclopenthixol administration. General behavior was observed, random heart rates were recorded, and feces was collected for cortisol assay on days 5 through 7.

No undesirable extrapyramidal side effects or problems associated with zuclopenthixol were seen. Technical difficulties occurred with several transmitters, which did not allow collection of heart rate data for one subject in each trial and hampered precise consistent collection of heart rate data for two other subjects.

Differences were noted between the test (drug) and control group in several categories. Animals in the control group exhibited more pacing along fence lines, whereas animals in the test group were observed to spend more time eating and resting in sternal recumbency, particularly within the first 4-36 hr. Heart rates rose in all subjects during application of all stressors; heart rates remained elevated at a higher percentage of resting rate in control subjects in general throughout the chute...
restraint time period. Metabolic acidosis was observed on arterial blood gas evaluation for control subjects. Values for creatine phosphokinase and total bilirubin were elevated to a greater degree in control subjects. The administration of zuclopenthixol may serve as an adjunctive tool in stress reduction in some antelope species for 2-3 days.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the advice and assistance of Joe Vaughn, Steve Shurter, Dr. Lin Klein, Dr. Sue McDonnell, Dr. Bill Lance, Dr. Bengt Roken, and John Lukas. The hoofstock crew, veterinary preceptors, and certified veterinary technicians at the White Oak Conservation Center provided invaluable aid in handling of animals and samples. Monetary support for this project was provided by White Oak Conservation Center in support of the Graham-Gilman Residency in Wildlife and Zoological Medicine.

LITERATURE CITED

CARDIOPULMONARY EFFECTS AND UTILITY OF A BUTORPHANOL / XYLAZINE /KETAMINE ANESTHETIC PROTOCOL FOR IMMOBILIZATION OF FREE-RANGING BAIRD’S TAPIRS (Tapirus bairdii) IN COSTA RICA

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Abstract

In the past, immobilizations of free-ranging tapirs have utilized an etorphine/acepromazine combination (Immobilon®, Reckitt and Colman, Hull, England). 2,3 Problems with this protocol include the human safety hazard present when using etorphine, especially during night captures. The objective of this project was to devise an alternative anesthetic protocol that: 1) would be safe for the animals, 2) would be safer for the researchers 3) would provide rapid induction 4) would provide adequate immobilization 5) was reversible and 6) was relatively inexpensive. A butorphanol/xylazine combination was chosen because it fulfilled the above objectives and has been extensively applied and researched in the tapir’s close relative, the horse. The butorphanol/xylazine protocol afforded limited muscle relaxation; therefore, ketamine, another drug commonly used in the horse, was added to the protocol. This pilot study fulfilled two purposes: 1) to study the efficacy of the protocol for field situations and 2) initiate an in-depth look at the cardiopulmonary effects of the anesthetic protocol.

Twenty immobilizations of sixteen animals, using either butorphanol/xylazine or a butorphanol/xylazine/ketamine (Torbugesic®, Fort Dodge, Iowa, Rompun®, Bayer, Kansas, and Ketaset®, Fort Dodge, Iowa) protocol, were performed between March 1996 and February 1998. Due to field work logistics, actual body weights were only obtained on three subjects and thus weights were estimated. The purpose of the immobilizations was to radio collar the tapirs in order to record their habitat use, movement patterns and basic ecology. Biologic samples including ectoparasites, blood, skin biopsies, as well as rectal, vaginal and preputial cultures were collected at the time of immobilization. The tapirs were attracted to a capture area by using ripe bananas as bait. Tree platforms were constructed 30-40 feet above the capture area. Immediately after a tapir entered a capture area and began eating bananas, bananas were thrown from the tree platform, to maintain the tapir’s interest and continued to be thrown after the animal was darted. Animals were darted with a CO₂ rifle (DanInject® Wildlife Pharmaceuticals, Colorado) from the tree platforms. Darts (“P” type, Pneu-Dart Inc., Williamsport, Philadelphia) with 1.5 inch barbed or gelatin collared needles were used. The initial dart contained a mixture of butorphanol/xylazine. The doses of butorphanol ranged from 42-74 mg/animal, and the dose of xylazine ranged from 84-150 mg. In those cases where ketamine was utilized, it was delivered either intramuscular or intravenous via hand syringe following immobilization. Ketamine was used at 50-700 mg/animal. Intravenous
ketamine was administered via a 20-22 g catheter in the auricular vein. The use and dose of ketamine was judged subjectively based upon the degree of sedation desired and the length of time needed for immobilization. Time from dart impact to first visible effect (ataxia, inability to prehend bananas), time to sternal recumbency and total immobilization times were recorded. Yohimbine (Yobine®, Lloyd Laboratories, Iowa) or tolazolene (Tolazine®, Lloyd laboratories, Iowa) was used to reverse the alpha-2 adrenergic agonistic effects of xylazine. Yohimbine was used at a dose range of 31-35 mg/animal. Tolazolene was used at 1120-1200 mg/animal. Naltrexone (Trexonil®, Wildlife Laboratories, Colorado) was used to reverse the opioid agonistic effects of butorphanol. It was used at 1120-1200 mg/animal. The reversals agents were administered intramuscular by hand syringe (Table 1). Time to return to sternal recumbency and time to standing were recorded.

Heart rate, oxygen saturation as determined by pulse oximetry, indirect oscillometric arterial blood pressure, lead II electrocardiogram, and body temperature were measured using a portable bedside monitor (NPB-4000, Nellcor Puritan Bennett Inc., California). Pulse oximetry was applied to labia, prepuce, nasal planum or, most often, tongue. A cuff bladder width to forelimb circumference ratio of 40% was used for indirect arterial blood pressure determination. Respiratory rate was counted by direct observation of respiratory excursions. Arterial blood was obtained from the facial artery. Blood gas analysis was done with the use of a portable clinical analyzer (i-STAT, Sensor Devices Inc., Wisconsin). Blood gas analysis also included electrolytes, hematocrit and hemoglobin measurements. Percent cell volume was measured immediately after the captures as part of the blood processing. Each immobilization was rated with a subjective score based on induction, recovery and muscle relaxation.

Time from dart impact to first visible effect (ataxia, inability to prehend bananas) varied from 1-10 min. Time to sternal recumbency ranged from 4-24 min. The total time the tapirs were immobilized ranged from 13-60 min. Once the tapirs were given the reversal agents, the time to return to sternal recumbency ranged between 0-11 min and the time to stand ranged between 0-15 min (Table 2). Heart rates ranged between 28-108 beats/min. Respiratory rates measured between 8-21 breaths/min. Pulse oximeter readings varied between 54-100%. Indirect blood pressure measurements were between 101-202 mm Hg systolic and 66-127 mm Hg mean arterial pressure. Body temperatures remained within 35.5-40° C (Table 3). No irregular rhythms or arrhythmias were detected on electrocardiograms. Blood gas analysis findings on four individuals are summarized in Table 4. Induction, recovery and muscle relaxation ratings are summarized in Table 5.

The butorphanol/xylazine combination proved to be an effective method of immobilizing free-ranging Baird’s tapirs. Induction was relatively rapid; however, the use of bananas as bait was found to be crucial to the success of the induction period. Bananas ensured that the animal was less distracted by the dart and remained in the area until the drugs took effect. The butorphanol/xylazine combination provided sufficient sedation for radio collaring of animals; however, it was not of sufficient quality and duration to allow the extensive data and biologic sample collection. Loud noises, movement, pain and other stimuli caused premature arousal. This is typical for low doses of an agonist/antagonist narcotic and an alpha2-agonist combination in horses. The analgesic effects of xylazine do not typically last more than 45 min in horses. Close inspection of the data in Tables
2 and 5 illustrates that the use of ketamine 1) can be used to increase the immobilization period, and 2) provides a deeper sedation without changing the quality of the recovery described. Premature arousals were noted in animals on which either no ketamine was used, or the ketamine was administered at low doses i.m. The dose range of Ketamine given i.v. to the last six animals immobilized ranged between 0.33 and 0.83 mg/kg (100-250 mg/animal). This dose was initially based on i.v. equine doses and subsequently on the subjective evaluation of the depth of sedation needed to manipulate the immobilized animals. The lower dose was sufficient for minor manipulations such as rolling the animal to lateral recumbency. Additional Ketamine was utilized for major manipulations such as pushing or pulling the animal during measurements or sample gathering. Ketamine i.v. provided a more predictable outcome than ketamine delivered i.m. and did not reduce the quality of the recovery. Reversal of butorphanol/xylazine was achieved with either yohimbine/naltrexone or tolazolene/naltrexone. In our experience, no difference in reversal time was noted with tolazolene versus yohimbine, and thus, due to cost, tolazolene is preferred.

The cardiopulmonary effects of this protocol are reviewed in Tables 3 and 4. Due to the use of different dosing protocols, data cannot be grouped. Tapir normal heart rates are reported to be 45 beats/min. The apparent decrease illustrated by some individuals can be attributed to the bradycardia caused by a reduction in central sympathetic tone caused by xylazine. Subjectively, one can note that heart rates were higher in those animals in which ketamine was used, which might be due to ketamine’s sympathomimetic effects. To our knowledge, respiratory rates of tapirs have not been reported; however, observed respiratory rates were within expected range for adult resting horses. Still, arterial partial pressure of carbon dioxide levels, when measured, were slightly elevated indicating hypoventilation. Indirect percent oxygen saturation as measured by pulse oximetry was less than the ideal 95% in several cases. This is probably due to the combined respiratory depressant effects of both xylazine and butorphanol, as well as the effect of recumbency on ventilation-perfusion matching. The tapirs in this project were kept lateral for more than half of their immobilization periods to accurately acquire morphometric measurements and have access to the medial saphenous vein. In those animals in which blood gas analysis was performed, the arterial oxygen saturation proved to be consistent with the pulse oximeter readings. Hypoxemia is defined in domestic mammals by an arterial partial pressure of 60 mm Hg. Although no animal reached this state, based on a limited number of blood gas measurements, some animals approached hypoxemic states. Blood pressure readings indicated that the mean arterial pressure was probably adequate for organ perfusion. Hematocrits, hemoglobin concentrations and electrolytes as measured by the portable clinical analyzer were within the normal ranges for Baird’s tapirs. Percent cell volumes were repeated immediately following the captures and correlated with the hematocrits provided by the clinical analyzer. Body temperatures also remained within normal values reported for tapirs.

In summary, a butorphanol/xylazine combination can be a safe protocol for the immobilization of free-ranging tapirs. It must be taken into account that it has the potential to produce a hypoxemic state and nasal insufflation of oxygen is recommended and planned for our future captures. Due to the short sedation period afforded by this protocol, the use of butorphanol/xylazine alone should only be utilized in immobilizations lasting less than 30 min. Ketamine is a safe agent that can be used to lengthen the immobilization period and aid in the depth of sedation. Since ketamine is not...
reversible and may produce undesirable side effects, one should allow enough time for ketamine to be redistributed prior to reversing the butorphanol/xylazine. An important side note is that this protocol has been utilized in animals that are relatively calm and focused on feeding. Alternative methodology may be necessary when tapirs are in a state of excitement.

ACKNOWLEDGMENTS

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LITERATURE CITED

Table 1. Individual anesthetic and recovery times.

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<th>NAME</th>
<th>EST. WEIGHT</th>
<th>BUTORPH.</th>
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<th>KETAM.</th>
<th>YOHIM.</th>
<th>TOLAZ.</th>
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<td>300 mg</td>
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Key: Butorph.= butorphanol, Xylaz.= xylazine, Ketam.= ketamine, Yohim. = yohimbine, Tolaz.= tolazoline, Naltrex.= naltrexone.

*Denotes actual body weight determined after anesthetic period.

Table 2. Individual immobilization and reversal drug doses.

<table>
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<th>NAME</th>
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<th>T:</th>
<th>STERNAL</th>
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*Denotes animals that received ketamine.

### Table 3. Individual cardiopulmonary data.

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<th>NAME</th>
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<td>12-16</td>
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<td>N/A</td>
<td>N/A</td>
<td>36.8-37.0</td>
</tr>
<tr>
<td>*BIG MAMA</td>
<td>32-40</td>
<td>12</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>36.1</td>
</tr>
<tr>
<td>RODEO</td>
<td>36-40</td>
<td>20</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>37.4-37.8</td>
</tr>
<tr>
<td>*ROBERTA</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>ROBERTA2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>JUNIOR</td>
<td>45-57</td>
<td>18</td>
<td>75-93%</td>
<td>N</td>
<td>202/72</td>
<td>35.6-35.9</td>
</tr>
<tr>
<td>LEFTIE</td>
<td>41</td>
<td>18</td>
<td>96-97%</td>
<td>N</td>
<td>134/66-136/47</td>
<td>35.5-36.2</td>
</tr>
<tr>
<td>SPOOK</td>
<td>40</td>
<td>18</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>36.9</td>
</tr>
<tr>
<td>MAXINE</td>
<td>35-40</td>
<td>9-12</td>
<td>54-80%*</td>
<td>N/A</td>
<td>N/A</td>
<td>38.6</td>
</tr>
<tr>
<td>*BIG MAMA2</td>
<td>34-39</td>
<td>16-50</td>
<td>88-90%</td>
<td>N</td>
<td>N/A</td>
<td>36.9-37.2</td>
</tr>
<tr>
<td>*FLASH2</td>
<td>40-63</td>
<td>16-20</td>
<td>76-94%</td>
<td>N</td>
<td>148/100-109/75</td>
<td>37.0-37.2</td>
</tr>
<tr>
<td>*RODEO2</td>
<td>56-66</td>
<td>16-20</td>
<td>93-95%</td>
<td>N</td>
<td>154/46-130/56</td>
<td>35.9-36.9</td>
</tr>
<tr>
<td>*LECHE</td>
<td>43-75</td>
<td>14-21</td>
<td>86-97%</td>
<td>N</td>
<td>101/47</td>
<td>37.5-38.1</td>
</tr>
<tr>
<td>*CAFE</td>
<td>75-89</td>
<td>12-20</td>
<td>90-94%</td>
<td>N</td>
<td>155/102</td>
<td>37.2</td>
</tr>
<tr>
<td>*SCARLET</td>
<td>37-45</td>
<td>14-19</td>
<td>92-100%</td>
<td>N</td>
<td>(93)-(115)*</td>
<td>N/A</td>
</tr>
<tr>
<td>*PLAYA</td>
<td>91-108</td>
<td>14-20</td>
<td>82-91%</td>
<td>N</td>
<td>(88)-(93)</td>
<td>36.0-36.4</td>
</tr>
<tr>
<td>*TRAPPER</td>
<td>43-49</td>
<td>8-12</td>
<td>89-95%</td>
<td>N</td>
<td>(100)-(127)</td>
<td>37.1-37.2</td>
</tr>
<tr>
<td>*RIO</td>
<td>84-103</td>
<td>8-12</td>
<td>88-94%</td>
<td>N/A</td>
<td>(66)-(123)</td>
<td>36.3-36.6</td>
</tr>
<tr>
<td>*LUNA</td>
<td>39-42</td>
<td>12-18</td>
<td>90-92%</td>
<td>N</td>
<td>(79)-(83)</td>
<td>36.0-36.6</td>
</tr>
<tr>
<td>*SOL</td>
<td>35-40</td>
<td>8-12</td>
<td>91-93%</td>
<td>N</td>
<td>(75)-(81)</td>
<td>36.5-36.7</td>
</tr>
</tbody>
</table>

Key: HRT = heart, RESP = respiratory, ECG = electrocardiogram, P = pressure, Temp. = temperature.

*Denotes animals that received ketamine.

### Table 4. Individual blood gases data.

<table>
<thead>
<tr>
<th>BLOOD GASES</th>
<th>*CAFE</th>
<th>*PLAYA</th>
<th>*TRAPPER</th>
<th>*RIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial PH</td>
<td>N/A</td>
<td>7.386</td>
<td>7.366-7.416</td>
<td>7.347</td>
</tr>
<tr>
<td>Arterial PCO2</td>
<td>N/A</td>
<td>46</td>
<td>48.3-50.5</td>
<td>49.1</td>
</tr>
</tbody>
</table>
Table 5. Individual recovery scores.

<table>
<thead>
<tr>
<th>NAME</th>
<th>INDUCTION</th>
<th>RECOVERY</th>
<th>M. RELAXATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLASH</td>
<td>EXCT</td>
<td>EXCT</td>
<td>EXCT</td>
</tr>
<tr>
<td>*BIG MAMA</td>
<td>POOR</td>
<td>GOOD</td>
<td>FAIR</td>
</tr>
<tr>
<td>RODEO</td>
<td>EXCT</td>
<td>GOOD</td>
<td>GOOD</td>
</tr>
</tbody>
</table>

Key: PCO2 = partial pressure of carbon dioxide, PO2 = partial pressure of oxygen, HCT = hematocrit, HBG = hemoglobin, BE = base excess, TCO2 = total carbon dioxide, SO2 = saturation of oxygen. *Denotes animals that received ketamine.
<table>
<thead>
<tr>
<th>Animal</th>
<th>Induction Rating</th>
<th>Recovery Rating</th>
<th>Muscle Relaxation Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>*ROBERTA</td>
<td>POOR</td>
<td>POOR</td>
<td>POOR</td>
</tr>
<tr>
<td>ROBERTA2</td>
<td>GOOD</td>
<td>POOR</td>
<td>POOR</td>
</tr>
<tr>
<td>JUNIOR</td>
<td>GOOD</td>
<td>EXCT</td>
<td>GOOD</td>
</tr>
<tr>
<td>LEFTIE</td>
<td>GOOD</td>
<td>EXCT</td>
<td>GOOD</td>
</tr>
<tr>
<td>SPOOK</td>
<td>POOR</td>
<td>GOOD</td>
<td>FAIR</td>
</tr>
<tr>
<td>MAXINE</td>
<td>EXCT</td>
<td>POOR</td>
<td>POOR</td>
</tr>
<tr>
<td>*BIG MAMA2</td>
<td>EXCT</td>
<td>EXCT</td>
<td>EXCT</td>
</tr>
<tr>
<td>*FLASH2</td>
<td>GOOD</td>
<td>EXCT</td>
<td>GOOD</td>
</tr>
<tr>
<td>*RODEO2</td>
<td>EXCT</td>
<td>EXCT</td>
<td>GOOD</td>
</tr>
<tr>
<td>*LECHE</td>
<td>EXCT</td>
<td>POOR</td>
<td>POOR</td>
</tr>
<tr>
<td>*CAFE</td>
<td>EXCT</td>
<td>POOR</td>
<td>POOR</td>
</tr>
<tr>
<td>*SCARLET</td>
<td>EXCT</td>
<td>GOOD</td>
<td>EXCT</td>
</tr>
<tr>
<td>*PLAYA</td>
<td>EXCT</td>
<td>EXCT</td>
<td>EXCT</td>
</tr>
<tr>
<td>*TRAPPER</td>
<td>EXCT</td>
<td>EXCT</td>
<td>EXCT</td>
</tr>
<tr>
<td>*LUNA</td>
<td>EXCT</td>
<td>EXCT</td>
<td>EXCT</td>
</tr>
<tr>
<td>*SOL</td>
<td>EXCT</td>
<td>EXCT</td>
<td>EXCT</td>
</tr>
</tbody>
</table>

**Key:**

**Induction rating:**
- **Poor** = animal walked away after having felt drug effects.
- **Fair** = animal remained in bait area but acted nervous.
- **Good** = animal remained in capture area until became immobilized but was aware of capture team.
- **Excellent** = same as a Good and animal continued to eat until sternal unaware of capture team.

**Recovery Rating:**
- **Poor** = animal was sitting up/standing when reversal was administered.
- **Fair** = animal aroused prematurely, but did not sit or stand up prior to reversal.
- **Good** = animal was not aroused prematurely but when reversed acted frightened and walked away.
- **Excellent** = animal was not aroused prematurely and upon reversal remained in bait area and resumed eating.

**Muscle Relaxation Rating:**
- **Poor** = animal sat or stood up prior to reversal.
- **Fair** = animal voluntarily moved limbs or head, but did not sit or stand up prior to reversal.
- **Good** = animal moved ears, mouth or vocalized but did not sit/stand up prior to reversal.
- **Excellent** = animal did not voluntarily move prior to reversal.

*Denotes animals that received ketamine.
MEDETOMIDINE-KETAMINE/ATIPAMEZOLE ANESTHESIA/REVERSAL IN WILD ARCTIC FOX PUPS IN SWEDISH LAPLAND

A. Alonso Aguirre, DVM, MS, PhD,1* B. Zimmerman, MS,2 Magnus Tannerfeldt, PhD,3 Anders Angerbjorn, PhD,1 and Torsten Morner, DVM, PhD4

1Department of Wildlife, National Veterinary Institute, POB 7073, S750 07, Uppsala, Sweden, Present address: National Marine Fisheries Service, Honolulu Laboratory, 2570 Dole St., Honolulu, HI 96822 USA; 2Wildlife Pharmaceuticals, 1401 Duff Drive Suite 600, Fort Collins, CO 80524 USA; 3Department of Zoology, Stockholm University, S-106 91, Stockholm, Sweden; 4Department of Wildlife, National Veterinary Institute, POB 7073, S750 07, Uppsala, Sweden

Abstract

The arctic fox (Alopex lagopus) inhabits the tundra regions of the northern hemisphere. It is circumpolar and breeds north above the treeline. In Sweden, the population is considered endangered of extinction, comprising approximately 40-80 adult individuals during population phases of low density. Free-ranging arctic fox pups (21) were captured during health assessment studies using metal cage live traps. Dens were continuously observed for 24 hr and when a cub was captured, it was immediately removed from the trap and brought to the field camp (100-200 m away). Six pups, 7 wk of age, weighing 1826 ± 47 g (range: 1715-2080 g) were anesthetized with a combination of medetomidine (Zalopine, Orion-Farmos, Turku, Finland, 1 mg/ml, 0.05 mg/kg) associated with ketamine (Ketaset, 100 mg/ml, 2.5 mg/kg). The intramuscular injection rapidly resulted in complete anesthesia with excellent skeletal myorelaxation, lateral recumbency, and loss of pedal, swallowing, palpebral and corneal reflexes in 92 sec (range: 58-150 sec). Pups were immobilized for a mean time of 18 ± 5 min (range: 13-25 min). Rectal temperature decreased and respiratory and cardiac rates increased during anesthesia. Intramuscular administration of 0.25 mg/kg of atipamezole (Atiprin, Orion-Farmos, 5 mg/ml) associated with ketamine (Ketaset, 100 mg/ml, 2.5 mg/kg). The intramuscular injection rapidly resulted in complete anesthesia with excellent skeletal myorelaxation, lateral recumbency, and loss of pedal, swallowing, palpebral and corneal reflexes in 92 sec (range: 58-150 sec). Pups were immobilized for a mean time of 18 ± 5 min (range: 13-25 min). Rectal temperature decreased and respiratory and cardiac rates increased during anesthesia. Intramuscular administration of 0.25 mg/kg of atipamezole (Atiprin, Orion-Farmos, 5 mg/ml) effectively reversed the effects of this anesthetic combination. All pups were standing within 12 ± 7 min (range: 5-24 min) after reversal. Induction to full recovery lasted 27 ± 5 min (range: 19-36 min). Continuous real time monitoring of pulse rate and percent oxygen saturation of hemoglobin (SpO2) trends were measured with a handheld pulse oximeter N-20PA (Nellcor Puritan Bennett, Pleasanton, CA). All six pups presented stable pulse oximetry profiles. The average of SpO2 values was 89 ± 2% (87-92%). Pulse oximetry and standard anesthetic monitoring procedures indicated no adverse physiologic effects to this drug combination. Following reversal to standing, all pups behaved normally and were returned to their den after full recovery following careful clinical evaluation. All pups were monitored 1 hr, 12 hr, 24 hr and 2 wk post-anesthesia. Although the sample size is small, this reversible neuroleptanalgesic combination proves to be safe and effective in young canids.
CAPTURE OF WILD SANDHILL CRANES WITH ALPHA-CHLORALOSE: TECHNIQUES AND PHYSIOLOGIC EFFECTS

Julia A. Langenberg, VMD,1* Nancy K. Businga, RVT, MS,1 and Heather E. Nevill, DVM2

1International Crane Foundation, Box 447, Baraboo, WI 53913-0447 USA; 2College of Veterinary Medicine, Cornell University, Ithaca, NY 14850 USA

Abstract

Alpha-chloralose, a chloral derivative of glucose which depresses the cortical centers of the brain,1 is regularly used orally in bait to capture wild cranes.2,4 The physiologic effects of alpha-chloralose and this method of capture are not well described. Capture myopathy is not uncommonly seen in cranes,3,5 and has been diagnosed in cranes dying after capture with alpha-chloralose. The goals of this study are (1) to document the physiologic effects of alpha-chloralose capture of wild cranes, and (2) to investigate whether there are clinico-pathologic parameters measurable at the time of capture which can predict which birds are at highest risk to develop capture myopathy.

From 1996-1997, 44 greater sandhill cranes (Grus canadensis tabida) were captured in central Wisconsin using alpha-chloralose for ecologic and disease research. Individual families were habituated to regularly come to whole corn bait stations, and then alpha-chloralose was mixed in to the bait at 0.37-0.43g/cup of corn (approximately 0.16-0.21 g/crane), depending on the ambient temperature. Cranes could generally be approached and restrained within 1-2 hr after they began feeding. Hoods and body wraps were used during transport and sample collection and banding procedures, which on average required 1 hr. The cranes were held in a small pen in the field during recovery, and were released from 8-22 hr after capture. In 1996, capture myopathy was diagnosed in one of the captured cranes who was unable to stand the day after capture; it was rehabilitated and successfully released.

In 1997, physiologic monitoring was done on 18 adult sandhill cranes at the time of capture, at the time of banding (1 hr later), 8 hr after capture, and at the time of release (22 hr after capture). Core body temperature, heart rate, respiratory rate were monitored along with subjective scoring for depth of sedation. Venous blood gas parameters (pH, pCO₂, pO₂, Na, K, iCa, PCV, Hb, base excess, HCO₃, tCO₂, sO₂) were serially measured using a hand-held analyzer (i-STAT, Sensor Devices, Inc., Waukasha, WI 53188 USA). Selected serum enzymes and electrolytes were tested 1 hr after capture and at the time of release. Tables 1 and 2 present the results.

When compared with reference ranges established in captive sandhill cranes, these results do not suggest that the metabolic acidosis associated with capture myopathy is occurring. The highest serum creatine phosphokinase (CK) levels measured 1 hr and 22 hr after capture were in cranes judged to be lightly sedated; the few more heavily sedated cranes had low CK values.
LITERATURE CITED

Table 1. Physiologic data following alpha-chloralose capture of wild adult sandhill cranes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Capture (Number tested)</th>
<th>1 hr post-capture</th>
<th>8 hr post-capture</th>
<th>Release time (22 hr)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(° F)</td>
<td>mean (range)</td>
<td>mean (range)</td>
<td>mean (range)</td>
<td>mean (range)</td>
</tr>
<tr>
<td>cloacal temp.</td>
<td>104.0 (101.3-105.7)</td>
<td>104.2 (101.7-107.3)</td>
<td>105.1 (103.2-107.8)</td>
<td>105.6 (102.0-107.4)</td>
</tr>
<tr>
<td>heart rate (beats/min)</td>
<td>150.4 (96-280)</td>
<td>131.6 (84-200)</td>
<td>140.4 (80-200)</td>
<td>154.2 (80-210)</td>
</tr>
<tr>
<td>pH</td>
<td>7.44 (7.38-7.49)</td>
<td>7.44 (7.34-7.49)</td>
<td>7.42 (7.35-7.50)</td>
<td>7.40 (7.30-7.48)</td>
</tr>
<tr>
<td>pCO₂ (mmHg)</td>
<td>35.4 (27.4-43.9)</td>
<td>36.6 (30.6-49.3)</td>
<td>32.9 (23.1-45.5)</td>
<td>31.9 (22.3-38.9)</td>
</tr>
<tr>
<td>pO₂ (mmHg)</td>
<td>59.6 (50-74)</td>
<td>62.7 (46-78)</td>
<td>63.7 (50-77)</td>
<td>63.2 (53-81)</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>140.7 (138-143)</td>
<td>139.4 (135-143)</td>
<td>145.1 (141-148)</td>
<td>147.3 (142-151)</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>4.2 (3.6-4.9)</td>
<td>4.0 (3.4-5.0)</td>
<td>3.8 (2.9-5.1)</td>
<td>3.6 (3.1-4.3)</td>
</tr>
<tr>
<td>iCa (mmol/L)</td>
<td>1.19 (1.10-1.29)</td>
<td>1.16 (1.04-1.28)</td>
<td>1.23 (1.18-1.31)</td>
<td>1.24 (1.13-1.32)</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>37.7 (31-42)</td>
<td>38.1 (32-43)</td>
<td>34.1 (29-38)</td>
<td>35.4 (27-39)</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.9 (11-14)</td>
<td>13.1 (11-15)</td>
<td>11.7 (10-13)</td>
<td>12.2 (9-13)</td>
</tr>
<tr>
<td>base excess (mmol/L)</td>
<td>0.3 (5.0-4.0)</td>
<td>0.5 (2.0-3.0)</td>
<td>3.3 (9.0-3.0)</td>
<td>4.9 (13.0-1.0)</td>
</tr>
<tr>
<td>HCO₃ (mmol/L)</td>
<td>23.8 (20-29)</td>
<td>24.7 (21-27)</td>
<td>21.2 (15-27)</td>
<td>20.0 (14-25)</td>
</tr>
<tr>
<td>tCO₂ (mmol/L)</td>
<td>25.0 (21-30)</td>
<td>25.7 (22-28)</td>
<td>22.3 (16-28)</td>
<td>20.9 (14-26)</td>
</tr>
<tr>
<td>sO₂ (%)</td>
<td>90.6 (94-96)</td>
<td>91.5 (83-96)</td>
<td>91.5 (84-97)</td>
<td>91.5 (85-97)</td>
</tr>
</tbody>
</table>

*Two cranes were released at 8 hr post-capture
<table>
<thead>
<tr>
<th>Parameter</th>
<th>(Number tested)</th>
<th>1 hr post-capture mean (range)</th>
<th>Release time (22 hr)* mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose (mg/dl)</td>
<td>(18)</td>
<td>251.3 (205-312)</td>
<td>no data</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>(18)</td>
<td>284.2 (208-416)</td>
<td>547.7 (12-1,161)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>(18)</td>
<td>44.6 (25-73)</td>
<td>no data</td>
</tr>
<tr>
<td>AP (U/L)</td>
<td>(18)</td>
<td>161.0 (13-605)</td>
<td>no data</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>(18)</td>
<td>1,111.1 (84-2,630)</td>
<td>3,903.2 (94-12,348)</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>(18)</td>
<td>399.4 (202-933)</td>
<td>410.4 (45-1,400)</td>
</tr>
<tr>
<td>cholesterol (mg/dl)</td>
<td>(18)</td>
<td>146.8 (93-195)</td>
<td>no data</td>
</tr>
<tr>
<td>total protein (g/dl)</td>
<td>(18)</td>
<td>3.4 (2.9-4.5)</td>
<td>no data</td>
</tr>
<tr>
<td>phosphorus (mg/dl)</td>
<td>(18)</td>
<td>2.0 (1.0-4.4)</td>
<td>no data</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>(18)</td>
<td>9.0 (8.0-10.0)</td>
<td>no data</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>(18)</td>
<td>140.8 (136-146)</td>
<td>no data</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>(18)</td>
<td>4.3 (2.1-9.4)</td>
<td>no data</td>
</tr>
<tr>
<td>Cl (mmol/L)</td>
<td>(18)</td>
<td>103.3 (100-107)</td>
<td>no data</td>
</tr>
<tr>
<td>bicarbonate (mmol/L)</td>
<td>(18)</td>
<td>29.7 (25-35)</td>
<td>no data</td>
</tr>
<tr>
<td>uric acid (mg/dl)</td>
<td>(18)</td>
<td>4.3 (2.7-7.3)</td>
<td>no data</td>
</tr>
<tr>
<td>anion gap (mmol/L)</td>
<td>(18)</td>
<td>12.1 (6-17)</td>
<td>no data</td>
</tr>
</tbody>
</table>

*Two cranes were released at 8 hr post-capture
FIELD ANESTHESIA OF CAMELS (*Camelus dromedarius*) AND THE USE OF MEDETOMIDINE/KETAMINE WITH ATIPAMEZOLE REVERSAL

**Thomas W.J. deMaar, DVM,**<sup>1*</sup> **Hester van Bolhuis, DVM,**<sup>2</sup> and **Muriithi J. Mugo, AHT**<sup>1</sup>

<sup>1</sup>*Ol Jogi, Ltd., PO Box 259, Nanyuki, Kenya;*<sup>2</sup>*Faculty of Veterinary Medicine, University of Utrecht, PO Box 80163, 3508 TD Utrecht, The Netherlands*

**Abstract**

Over 50 camels were anesthetized for a variety of reasons including physical examination, suturing, eye enucleation, uterine prolapse and castration. All procedures were undertaken as normal veterinary management of a 250 head Turkana and Somali type camel herd in central Kenya during 1996 and 1997. Xylazine, medetomidine, ketamine and etorphine were compared as anesthetic agents (Table 1). Anesthetic reversals were accomplished with yohimbine, tolazoline, atipamezole and diprenorphine. Initial doses were given using manual restraint or in a camel restraint chute and the animals were released to observe pharmacologic effects. Second doses were administered by hand with manual restraint. Camels can demonstrate a wide range of attitudes from extremely calm to absolutely wild. Anesthetic doses should be interpreted with this fact in mind.

Xylazine is a commonly used and effective sedative and anaesthetic agent for camels. A wide dosage range, from 0.25-2.0 mg/kg, is published for immobilization.<sup>1-6,8</sup> My experience with semi calm camels is that 0.25 mg/kg i.v. was insufficient except for very calm or depressed animals (*n* = 2). Dosages of 0.4-0.7 mg/kg i.m. or i.v. were effective to produce sedate animals (*n* = 15) for examination and further pharmacologic manipulation. In some individuals, 0.7 mg/kg i.v. induced recumbency (*n* = 2). Heart rates ranged from 30-66 beats/min and respiratory rates from 4-28 breaths/min.

Ketamine has been reported at doses of 1-2 mg/kg i.v. when given after xylazine to induce recumbency with sufficient anesthesia for minor surgical procedures.<sup>3,5,8</sup> I have found that ketamine dosages as low as 0.6 mg/kg i.v. (range: 0.6-1.0 mg/kg) will induce recumbency when given after xylazine; 2-30 min after i.v. xylazine (*n* = 7) and 15-35 min after intramuscular xylazine (*n* = 6). Caution with xylazine is warranted as incidence of unexplained anesthetic deaths have been reported.<sup>2</sup>

Reversal of xylazine with yohimbine at 0.4 mg/kg i.v. (*n* = 2) is incomplete with animals remaining ataxic for up to 1 hr after injection, with salivation and drooping lips evident. Reversal with tolazoline i.v. at 1 mg/kg (double a published dose<sup>5</sup>) allowed for immediate standing (*n* = 10) however half of these animals returned to lateral recumbency multiple times after administration. Tolazoline at 4 mg/kg i.v. (labeled equine dose) allowed sustained standing (*n* = 3) however residual effects of salivation, drooping lips, and soft stool immediately following the procedure.
Etorphine has been reported at 0.0055-0.011 mg/kg up to a 4 mg maximum for anesthesia. This was attempted in two animals at 0.011 mg/kg combined with 20 mg xylazine and 12 mg acepromazine i.m. Animals became recumbent however demonstrated muscle rigidity, tremors, and severe opisthotonos. Average physiologic parameters were: heart rate, 60 beats/min (range: 48-79); respiratory rate, 26 breaths/min (range: 18-36); and the time to recumbency was 6-7 min. Combined with its inherent danger to humans, etorphine is not an ideal drug for camels.

Medetomidine/ketamine combinations gave remarkably calm inductions and reversal with atipamezole allowed for fast, smooth recoveries in dromedaries (n = 20). Three trials were performed: A) medetomidine i.m. + ketamine i.v., B) medetomidine i.v. + ketamine i.v., and C) medetomidine + ketamine given together i.m. to simulate a projectile syringe dosage. When the two drugs were given separately the two injections were 7-32 min apart.

An ideal dose appears to be medetomidine at 0.05 mg/kg either i.v. or i.m. followed by ketamine at 0.6 mg/kg i.v. or i.m. When both drugs were given i.m. the ketamine was increased to 1.0 mg/kg (n = 6). Medetomidine doses of less that 0.04 mg/kg were insufficient and required doses of ketamine of over 0.6 mg/kg (n = 2). Using more than 0.6 mg/kg ketamine i.v. (n = 2) or 1.0 mg/kg i.m. (n = 2) increased the incidence of side effects such as apnea, tachycardia and post reversal ataxia especially during short procedures (n = 6). Reversal was achieved with 0.15 mg/kg (range: 0.135-0.25) atipamezole i.v. (n = 20) and regardless of the route of medetomidine administration, animals stood within 2 min (range: < 1-3 min). Atipamezole is also effective when given i.m. (n = 2) and for completely reversing the effects of xylazine after the tolazoline failure.

In order to achieve complete analgesia for painful procedures such as castration, local use of lidocaine is recommended. There is an excellent reference on regional and local nerve blocks in camels. Other anesthetic protocols have been reported using phenothiazines, detomidine, diazepam, chloral hydrate, magnesium sulfate, guaifensin, barbiturates, and halothane.

Regurgitation is a distinct possibility (n = 2) and an enforced fasting time of more than 12 hr will reduce its incidence. Camels are susceptible to ruminal tympany and the other deleterious effects of long term lateral recumbency like other herbivores.

The live weights of these camels ranged from 217-355 kg and were calculated from body measurements. The formula used was: Estimated live weight (kg) = shoulder height (m) × thoracic girth (m) × abdominal girth (m) × 50 (or 48 for young animals). Another method of weight estimation was attempted using a formula designed by the Indian Army Veterinary Corps. Thoracic girth and length measurement are taken in cm and the weight derived from a chart. To this weight, a “hump factor,” which accounts for the size of the hump, must be added. In this study, the body measurements derived were too large to fit the Indian scheme and this second method is probably more suited to more compact camels than the taller, slender East African varieties.

APPENDIX: DRUGS USED
Atipamezole: Antisedan, 5 mg/ml, Orion Farmos, Espoo, Finland; etorphine: Large Animal Immobilon, 2.45 mg/ml with acepromazine 10 mg/ml, C-Vet Veterinary Products, Leyland, Lanc. PR5 3QN, UK; ketamine: Ketaset, 100 mg/ml, Fort Dodge La Cb., Inc., Fort Dodge, Iowa 50501 USA or Ketamine hydrochloride injection 50 mg/ml, Rotexmedica GBMH, Trittau, Germany; medetomidine: Zalopine, 10 mg/ml, Orion Farmos, Espoo, Finland; tolazoline: Tolazine, 100 mg/ml, Lloyd Labs., Shenandoah, IA, 51601 USA; xylazine: Rompun, 20 mg/ml, Bayer, Leverkusen, Germany or Xylazine-100 Injectable, 100 mg/ml, The Butler Company, Columbus, OH 43228 USA; and yohimbine: Antagonil, 5 mg/ml, Wildlife Pharmaceuticals, Inc., Fort Collins, CO 80524 USA or Yobine Injection, 2 mg/ml, Lloyd Labs., Shenandoah, IA 51601 USA.

ACKNOWLEDGMENTS

We thank the owners, and our co-workers, particularly the camel crew, of Ol Jogi for their support. Many of the procedures were performed with veterinary students from the University of Nairobi.

LITERATURE CITED


Table 1. Trial of three methods of medetomidine/ketamine/atipamezole administration in camels.

<table>
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<tr>
<th>Trial</th>
<th>A</th>
<th>B</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>Trial B</td>
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<tr>
<td>Trial C</td>
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56 1998 PROCEEDINGS AAZV AND AAWV JOINT CONFERENCE
<table>
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<tr>
<th></th>
<th>Medetomidine i.m. + ketamine i.v. ($n = 6$)</th>
<th>Medetomidine i.v. + ketamine i.v. ($n = 6$)</th>
<th>Medetomidine i.m. + ketamine i.m. ($n = 6$)</th>
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<td>(1-2)</td>
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<td><strong>(Range)</strong></td>
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<td><strong>Respiratory rate (breaths/min)</strong></td>
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<td><strong>Atipamezole dose (mg/kg)</strong></td>
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PATHOLOGIC FINDINGS IN IDIOPATHIC HEMORRHAGIC VASCULOPATHY SYNDROME (IVHS) OF CAPTIVE BLACK RHINOCEROSSES

Richard J. Montali, DVM,1* Suzan Murray, DVM,2 Nancy P. Lung, VMD, MS,2 Thomas Alvarado, DVM,3 John F. Timoney, DVM, PhD,4 and Donald E. Paglia, MD5

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Abstract

Since 1995 a previously unrecognized condition now called idiopathic hemorrhagic vasculopathy syndrome (IVHS) emerged in the North American captive black rhinoceros population. As of April 1998, seven cases have been identified, (Table 1) six of which occurred in animals from Texas. The syndrome can be acute or chronic, with recurrences, and is potentially fatal. The course of the disease has ranged from several months to well over one year.

The syndrome is characterized by swelling of the shoulders, neck and limbs, lameness, laminitis, oral ulceration and a profound non-hemolytic anemia. The swelling is due to proliferation and inflammation of peripheral blood vessels with resulting subcutaneous and intermuscular pooling of blood. In some cases, laminitis has led to sloughing of the hoof nail with regrowth in the survivors. Packed-cell-volumes were decreased to 12% and lower with a gradual return too normal in the five survivors. The most severely affected animals had epistaxis and hemorrhages associated with proliferative oral ulcerative lesions. Leukograms were variable, with no consistent pattern of white blood cell counts among cases. Pertinent serum chemical changes included decreased phosphorus and albumin levels with elevated muscle enzymes. Liver enzymes and bilirubin typically remained within normal limits. Animals for which clotting profiles were available showed normal clotting parameters throughout disease course.

Biopsies were performed on six of seven of the affected animals at various stages of the disease including the recovery period as with animal #1 (Table 1). In the active stages, skin biopsies over swollen neck and limb areas revealed a spectrum of changes from neutrophilic vasculitis in the dermis to inflammatory lesions with vascular proliferation in the skin and oral mucosa. Deep subcutaneous biopsies were characterized by extensive vascular proliferation. In addition, both animals that died had remarkable pulmonary thrombosis possibly arising as emboli in the affected limbs, and suppurative pneumonia. The pneumonia in rhinoceros #6 was partly a complication of an ingested foreign body that penetrated the diaphragm. The other fatal case, #5 had extensive proliferative vascular changes and thrombosis within the thoracic limb areas. These consisted of redundant, dilated vascular channels (peliosis-like) that were space occupying and compressed the thoracic limb musculature. This resulted in a compartment-like syndrome with muscle atrophy and degeneration.
As of April 1998, no infectious, immunologic, nutritional, or toxicologic etiology has been identified as a specific cause of IVHS. However, the epidemiology and pathologic changes in this disease are suggestive of an infectious or immunopathologic process. The disease resembled some of the clinical aspects of equine purpura hemorrhagica (EPH), however, an immune-complex component as occurs in EPH has not been identified in IVHS, and vascular proliferation does not occur in the equine disease. Serologic screening for exposure to known ungulate viruses including equine herpes, equine arteritis, equine infectious anemia, equine encephalomyelitis, blue tongue, epizootic hemorrhagic fever of deer and the vesicular viruses have been negative or considered noncontributory.

Aspects of the vasculopathy in some of the rhinoceroses resembled proliferative vascular lesions in immunosuppressed humans associated with several bartonella species (the cause of bacillary angiomatosis), and human herpesvirus 8 (implicated in Kaposi’s lesions). A search for similar organisms including bartonella, ehrlichia, the Rocky Mountain spotted fever group of rickettsia and herpesviruses thus far have been all negative by culture, serologic testing, electron microscopy, and polymerase chain reaction procedures. The mucocutaneous manifestations of the vasculopathy differ from a necrotizing skin and oral mucosal disease commonly seen in captive black rhinoceroses.

Various treatments used among the seven cases included broad-spectrum antibiotics and, non-steroidal antiinflammatory drugs. Aggressive supportive care was also administered with the use of fluids, electrolytes, foot and nail care, and attention to pressure sores and draining areas. Some animals received treatment with systemic steroidal antiinflammatory drugs, antifungal medications and targeted nutritional supplements such as vitamin C and fatty acids. An allegedly vitamin C-responsive hemorrhagic condition with some similar clinical manifestations to IVHS was reported in a black rhinoceros in Berlin.

In January 1998, a task force initiated by the International Rhino Foundation was assembled at the Fort Worth and Dallas Zoos to collate clinical information and coordinate further research priorities. The purpose of this was to establish the cause and prevention of this new disease of black rhinoceroses which was named IVHS.

ACKNOWLEDGMENTS

This work was supported in part by the International Rhino Foundation. Black rhinoceros facilities and staff also contributing material for this study include: Dr. Kathryn Gamble, Dallas Zoo, Dr. David Kenny, Denver Zoo, Dr. Robin Radcliffe, Fossil Rim, Dr. Michele Miller, Disney’s Animal Kingdom, Dr. Eric Miller, St. Louis Zoo, and Dr. Scott Citino, White Oak. We thank the following facilities or individuals for special technical and laboratory assistance: The Texas Veterinary Diagnostic Labs, Dr. Ellen Dierenfeld, NY Conservation Society, Dr. Melissa Kennedy, University of Tennessee, Dr. Lisa Tatum, University of Miami, Dr. James Childs, Centers for Disease Control, Drs. Laura Richman and John Strandberg, Johns Hopkins University, Drs. Jon Palmer and Michael Goldschmidt, University of Pennsylvania, Vera Bonshock, National Zoo and John Jenkins, Armed Forces Institute of Pathology.

LITERATURE CITED

### Table 1. Study group of idiopathic hemorrhagic vasculopathy syndrome (IVHS) in black rhinoceroses in the United States.

<table>
<thead>
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<td>Denver</td>
<td>M</td>
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<td>Normal</td>
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<td>Recovered</td>
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<td>Ft. Worth</td>
<td>F</td>
<td>12/95</td>
<td>01/96</td>
<td>Vasculopathy</td>
<td>Healing</td>
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<td>#3 Sinan-pamde</td>
<td>Fossil Rim</td>
<td>F</td>
<td>01/96</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
<td>Recovered</td>
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<td>#4 Indy</td>
<td>Dallas</td>
<td>M</td>
<td>02/97</td>
<td>02/97</td>
<td>Vasculopathy</td>
<td>Oral, S/Q</td>
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<td>#5 Zambezi</td>
<td>Dallas</td>
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<td>Disney</td>
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<td>11/97</td>
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<td>↓ Sampling</td>
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**Clostridium perfringens ENTEROTOXICOSIS IN RECENTLY CAPTURED NORTH AMERICAN RIVER OTTERS (Lontra canadensis)**

George V. Kollias, DVM, PhD,1* Pat McDonough, PhD,2 Beth Valentine, DVM,1 Barry Hartup, MS, DVM,1 Noha Abou-Madi, MS, DVM,1 Kevin Kimber, BS, MS,1 and Edward Gentz, MS, DVM.1,4

1Wildlife Health Laboratory, 2New York State Veterinary Diagnostic Laboratory, 3Department of Pathology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401 USA; 4Wildlife Center of Virginia, Waynesboro, VA 22980 USA

**Abstract**

Members of the genus *Clostridium* are widely recognized as enteric pathogens of humans, domestic animals, and wildlife. Their array of proven and putative virulence attributes is impressive, and infections take a variety of forms in mammalian and avian hosts. *C. perfringens* may be the most widely occurring pathogenic bacterium and is certainly the most important cause of clostridial enteric disease in domestic animals.3 Some types of *C. perfringens* (mainly type A) are consistently recovered both from the intestinal tracts of animals and from the environment, while others (Types B, C, D, and E) are less common in the intestinal tracts of animals.2 As many as 17 exotoxins of *C. perfringens* have been described in the literature. A definitive role in the induction of disease in humans and animals has only been demonstrated for a few of these toxins.4

A clinical syndrome, similar to *Clostridium perfringens* enterotoxicosis (CPE) described in other species, has been identified in approximately 15% of recently captured North American river otters (*Lontra canadensis*) involved in an ongoing population restoration project in New York state. Otters with CPE die peracutely (< 6 hr post capture), acutely (< 60 hr post capture), or develop soft to watery mucoid diarrhea (± blood), and or marked reduction in normal grooming behavior, hypophagia and hypodipsia, profound hypothermia, and in some cases coma at 24-72 hr post capture.

Otters administered a regimen of oral metronidazole, oral or parenteral electrolyte solution, equine hyperimmune antiserum, and general supportive care (e.g., correcting hypothermia, hand feeding) following recognition of the clinical signs/problems described above, have a high rate of recovery, and no recurrence of this problem over a 14-21 day period.1

River otters that die have mild to severe intestinal lesions consistent with CPE, including superficial mucosal necrosis and vascular congestion. In some otters, *C. perfringens* toxin-induced autolysis was hypothesized to have “masked” typical microscopic lesions observed in intestinal sections post mortem.

In all cases, CPE is confirmed by anaerobic culture of fresh feces, *in vitro* detection of Type A (putative) *Clostridium perfringens* exotoxin, clinical signs, and gross necropsy and histopathologic findings.
CPE is currently thought to be minimized by adhering to the treatment protocol (with modifications), described above, to all recently captured otters involved in this population restoration project.

LITERATURE CITED

EVALUATION OF HEMOSIDEROSIS IN CAPTIVE CALLITHRICIDAE

Nicole Gottdenker, DVM, MS,1* Tracey McNamara, DVM, Dipl ACVP,1 and W. Emmett Braselton, PhD2

1Department of Pathology, Wildlife Conservation Society, 185th St. and Southern Blvd, Bronx, NY 10460 USA; 2Animal Health Diagnostic Laboratory, C-302 Veterinary Medical Center, Michigan State University, East Lansing, MI 48824-1316 USA

Abstract

Hemosiderosis, the intracellular deposition of iron, is a manifestation of systemic and/or local iron overload. Because naturally occurring hemosiderosis is a common, yet often disregarded, histologic finding in captive Callithricidae, the purpose of this study is to provide a detailed survey of hemosiderosis in a captive callitrichid population. Livers from nine species of callitrichids (Callimico goeldii, Callithrix argentata, Callithrix jaccus, Callithrix pygmea, Saguinus fuscicollis, Saguinus geoffrei, Saguinus midas, Saguinus mystax, Saguinus oedipus) were evaluated for tissue iron concentration, distribution and intensity of intracellular hemosiderin deposition, and degree of fibrosis. The prevalence of hepatic hemosiderosis was 94.4% (n = 232), with a high prevalence (97.64%, n = 127 neonates examined) of neonatal hepatic hemosiderosis. Mean hepatic iron concentration (HIC) was 4378.2 ± 4459.8 ppm (n = 94), ranged from 94.4-18,500 ppm, and correlated positively with the degree of total iron deposition (Spearman rank coefficient of correlation ST, rho = 0.811, n = 94, P < 10^-2), hepatocyte iron deposition (ST, rho = 0.76, n = 94, P < 10^-2), and sinusoidal iron deposition (ST, rho = 0.73, n = 94, P < 10^-2). Histologic patterns of hepatic hemosiderosis and dietary analysis strongly suggest that a primary factor influencing hemosiderosis in captive Callithricidae is dietary iron overload. Varying amounts of extrahepatic hemosiderin present in the heart, kidney, lung, adrenal, spleen, pancreas, lymph nodes, intestines, thyroid, testes, and ovary of many animals are likely the result of iron overload. These findings show a high prevalence and severity of hemosiderosis in captive Callithricidae that may be caused by a combination of dietary iron overload, increased bioavailability of iron, and/or a genetic affinity for iron absorption.

Introduction

Hemosiderosis and/or hemochromatosis are common pathologic findings in many captive wild mammals and birds, such as common marmosets,23 captive lemurs,10 Egyptian fruit bats,6 and mynah birds.15 A local or systemic overload of iron may cause hemosiderosis, the deposition of hemosiderin within cells, and/or hemochromatosis.5,12,16 Hemochromatosis is tissue iron deposition associated with hepatic hemosiderin deposition, necrosis, fibrosis, nodular regeneration, and damage to other organs, such as the pancreas and heart.5,12 Spelman et al.22 hypothesize that the clinical disease associated with hemosiderosis in captive lemurs is caused by excessive dietary iron, high dietary ascorbic acid (a factor increasing iron absorption), and low amounts of tannins (a factor decreasing iron absorption). Hemochromatosis associated with excessive dietary iron and ascorbic
acid intake is reported as a cause of death in captive Egyptian fruit bats.\textsuperscript{6} Experimental parenteral systemic iron overload in gerbils causes hepatic and cardiac lesions consistent with hemochromatosis.\textsuperscript{3}

While there have been many reports of hemosiderosis in captive callitrichids,\textsuperscript{17,18,23} authors differ in their interpretation of its significance and cause. A recent experimental study shows that excessive dietary iron caused common marmosets (\textit{Callithrix jaccus}) to develop significantly increased liver iron content.\textsuperscript{17} In the aforementioned study, marmosets on a high iron diet had a significantly higher mortality rate than control animals. These results suggest that dietary excess of iron may be a significant cause of disease and/or death of captive callitrichids that is commonly overlooked.\textsuperscript{17} The purpose of the present study is to evaluate naturally occurring hemosiderosis in captive Callithricidae. The results of this study may be applied to the assessment and improvement of captive management and to the study of diseases in both captive and free-ranging Callithricidae.

**Methods**

This was a retrospective cross-sectional survey of nine species of captive Callithricidae (\textit{Callimico goeldii, Callithrix argentata, Callithrix jaccus, Callithrix pygmea, Saguinus fuscicollis, Saguinus geoffrei, Saguinus midas, Saguinus mystax, Saguinus oedipus}) that died at the Bronx Zoo between 1978 and 1997. Samples of formalin fixed livers of 94 callitrichids were sent to Michigan State Diagnostic laboratory for complete mineral analysis, including liver iron levels. The livers of 232 callitrichids were stained with Perl’s Prussian blue and examined for hemosiderin deposition by two pathologists using a scoring method described by Deugnier et al.\textsuperscript{8} that allowed for the study of the degree of zonal deposition (zones 1, 2, and 3 of Rappaport) of hemosiderin (Prussian blue positive granules) within hepatocytes and mesenchymal tissue (sinusoidal endothelium, macrophages, biliary epithelium, vessels, and connective tissue). According to Rappaport’s definition of the hepatic acinus, zone 1 consists of periportal hepatocytes, zone 2 consists of midzonal hepatocytes, and zone 3 consists of periacinar hepatocytes.\textsuperscript{14} Hepatocyte iron score (HIS) is the degree of hemosiderin deposition within hepatocytes. Sinusoidal iron score (SIS) is the amount of hemosiderin within sinusoids. Portal iron score (PIS) is the degree of hemosiderin deposition within portal vessels, connective tissue, and bile duct epithelium. Total iron score (TIS) is calculated as: \( TIS = \text{HIS} + \text{SIS} + \text{PIS} \). Two hundred and nine livers were stained with Masson’s trichrome for detection of hepatic fibrosis. The degree of portal fibrosis was examined by a grading scheme of 0-3, defined as: no portal fibrosis (0), non extensive portal fibrosis (1), extensive portal fibrosis (2), and diffuse hepatic fibrosis with macronodular regeneration (3).\textsuperscript{8} Other histopathologic findings were also noted within livers examined (i.e., triaditis, necrosis, lipidosis). In addition, tissues of selected cases (heart, lung, spleen, adrenal, pancreas, intestinal tract, lymph nodes, thyroid) were examined for hemosiderin deposition. Descriptive and analytic statistical tests were performed using Microsoft Excel for Windows 95 (Version 7.0) and Sigma Stat 2.0 (Jandel Scientific).

**Results**
**Hepatocyte Iron Concentration**

Mean HIC was 4378.2 ± 4459.8 ppm (n = 94) and ranged from 94.4-18,500 ppm. The mean HIC (ppm) for each species was as follows: *C. argentata*, 5552.5 ± 3546.8 (n = 8); *C. goeldii* 773.54 ± 714.36 (n = 9); *C. jacchus*, 8423.2 ± 6811.2 (n = 15); *C. pygmea*, 2106.7 ± 1981.8 (n = 4); *S. fuscicollis*, 2388.9 ± 2460.2 (n = 12); *S. geoffrei*, 1710.7 ± 1765.1 (n = 7); *S. midas*, 6036.2 ± 3053.0 (n = 8); *S. mystax*, 5771.9 ± 4833.1 (n = 11); *S. oedipus*, 3893.2 ± 3018.7 (n = 17). Among the species sampled, *C. goeldii* had the lowest mean HIC, 773.54 ± 714.36, and *S. midas* had the highest mean HIC, 6036.2 ± 3053. HIC differed significantly between *Callimico goeldii* and the three following species (*P* < 0.05; all pairwise multiple comparison test, Dunn’s method): *S. midas*, *C. jacchus*, and *C. argentata*. There was no significant difference in HIC (ppm) between sex (*P* = 0.084, Mann-Whitney Rank Sum Test) and age class (neonate vs. adult; *P* = 0.33, Mann-Whitney Rank Sum Test).

**Histologic Assessment of Hepatic Hemosiderin Deposition**

Of callitrichid livers examined, 94.4% (219/232) had some degree of hemosiderin deposition within hepatocytes and/or mesenchymal tissue, ranging from minimal (sparse basophilic dusting) to severe (coalescing masses of hemosiderin). Of callitrichids examined, 5.6% (13/232) had no hemosiderin within liver sections. There was also a high prevalence (97.64%, *n* = 127 neonates examined) of neonatal hepatic hemosiderin deposition. Of callitrichids examined, 17.7% (41/232) had no detectable to mild hepatic hemosiderin deposition (TIS 0-11), and 82.3% (191/232) had moderate to severe hepatic hemosiderin deposition (TIS 12-44). The hepatic iron concentration of animals with no detectable to mild hemosiderin deposition ranged from 95-1,880 ppm and the hepatic iron concentration of animals with moderate to severe hemosiderosis ranged from 94.4-18,500.

Mean TIS was 21.9 ± 10.9 (range: 0-44; *n* = 232). TIS correlated positively with hepatic iron concentration (Spearman rank coefficient of correlation ST, rho = 0.811, *n* = 94, *P* < 10^-2). Mean HIS was 16.94 ± 8.86 (range: 0-36; *n* = 232) and mean SIS was 3.8 ± 2.8, *n* = 232). Overall, mean parenchymal iron and sinusoidal hemosiderin deposition was greatest in zone 1, with a decreasing gradient throughout the lobule from zone 1 to zone 3. Both HIS (ST, rho = 0.76, *n* = 94, *P* < 10^-2), and SIS (ST, rho = 0.73, *n* = 94, *P* < 10^-2), correlated positively with HIC (ppm). There was a slight positive correlation between liver iron concentration and the liver sinusoidal gradient across zones 1 and 3 (ST, rho = 0.32, *n* = 94, *P* < 10^-2), with a higher concentration of sinusoidal hemosiderin in zone 1 as liver iron concentration increased. There was no significant correlation between age and HIC, TIS, or HIS. Sinusoidal iron deposition, (ST, rho = 0.35, *n* = 209, *P* < 10^-2) correlated positively with age.

There was a significant difference in total hepatocyte hemosiderin deposition and total sinusoidal hemosiderin deposition between adults/subadults (> 2 mo of age) and neonates (< 2 wk of age) (Mann-Whitney Rank Sum Test, *P* < 0.001). Adults/subadults had higher mean SIS (4.8 ± 3.5) than did neonates (3.1 ± 2.2). Neonates had higher mean total HIS (18.3 ± 7.2) than did adults/subadults (14.7 ± 11.0). There was no significant difference in total liver (hepatocyte and mesenchymal)
hemosiderin deposition and overall mesenchymal hemosiderin deposition between adults/subadults and neonates.

**Hemosiderin deposition in other tissues and associated lesions**

There were varying amounts of hemosiderin in the heart, kidney, lung, adrenal, spleen, pancreas, lymph nodes, intestines, thyroid, testes, and ovary. Hemosiderin was present in 19 of 33 (56%) hearts examined. The HIC of animals with intracardiac hemosiderosis ranged from 1,290-18,500 ppm. The HIC of animals with no hemosiderin within their hearts ranged from 434-13,600 ppm. Fourteen of 21 adults/subadults had mild to moderate hemosiderin deposition, primarily within myocytes. Cardiac hemosiderin deposits were found within the sarcoplasm of myocytes, cytoplasm of fibroblasts, and endothelial cells, predominantly in the subepicardial region. Five of 12 neonates examined had mild amounts of cardiac hemosiderin deposition.

Hemosiderin deposition within renal tubular cells and/or renal interstitium was present in 15 of 33 (45%) animals. HIC of animals with renal hemosiderin deposition ranged from 2450-18,500 ppm. Renal hemosiderin deposition was present in 14 of 19 (74%) of adults/subadults examined. Although one neonate out of 14 examined was positive for renal hemosiderin deposition, histologic artifact cannot be ruled out as a cause of renal hemosiderin deposition in this case. Splenic hemosiderin deposition was present within splenic macrophages in 21 of 23 (93%) spleens examined. Adrenal cortical hemosiderin deposition was present within cortical cells and connective tissue in 13 out of 19 adrenals examined. Pancreatic hemosiderin deposition was present, primarily within pancreatic interstitium, in five of eleven animals examined. Although no neonates exhibited intestinal hemosiderin deposition, hemosiderin was present within submucosal macrophages of the intestines or colon in 7 of 10 animals. Callitrichids with extrahepatic hemosiderin deposition in three or more organs had a relatively high mean HIC of 8217.7 ± 4717.5 ppm (range: 2,320-18,500 ppm; n = 22 callitrichids examined).

Of callitrichids examined, 42.2% (98/232) had hepatic fibrosis ranging from non extensive portal fibrosis to bridging portal fibrosis. No cases of hepatic cirrhosis were seen within callitrichids examined. Hepatic fibrosis correlated positively with age (ST, rho = 0.36, P < 0.001, n = 209) and total hepatic hemosiderin deposition (ST, rho = 0.20, P < 0.01, n = 232). Although there was no significant correlation between hepatocyte and portal hemosiderin deposition and degree of fibrosis, sinusoidal iron deposition correlated positively with the degree of portal fibrosis (ST, rho = 0.36, P < 0.001). The degree of portal fibrosis was significantly higher in adults and juveniles than in neonates (Wilcoxon Signed Rank Test, P < 0.05). Mild to severe triaditis was found in 37.1% (86/232) of callitrichids. The HIS of callitrichids with triaditis ranged from 95-18,500 ppm. In the sample population, 14.7% (34/232) had multifocal to diffuse hepatic lipidosis.

**Discussion**

The prevalence, distribution, and severity of hepatic hemosiderosis correlated with hepatic iron concentration in this study strongly suggests that iron overload is a significant pathologic finding
in callitrichids that should not be interpreted as innocuous. If large amounts of hepatic iron deposition were normal, then one would expect iron concentrations to be relatively high, with little variation. The large range of hepatic iron concentrations (from 94.4-18,500 ppm) suggests that excessive hepatic iron concentration is abnormal in callitrichids.

The results of this study parallel in many ways studies of human genetic hemochromatosis, as well as experimental animal models of iron overload. Similar to a retrospective study of human genetic hemochromatosis, this study shows a strong correlation between hepatic iron concentration and total hepatic hemosiderin deposition. In addition, there is a predominance of hepatocellular hemosiderin in both neonatal and adult Callithricidae. Studies show initial parenchymal overload when excessive amounts of iron are absorbed from the gut. In humans with hemochromatosis caused by excessive iron absorption (i.e., genetic hemochromatosis), hemosiderin deposits primarily within parenchymal cells of the liver. In patients with genetic hemochromatosis, Deugnier et al. (1992) state that the existence of a decreasing hepatic hemosiderin gradient from zone 1 to zone 3 "strongly points to an intestinal hyperabsorption of iron." Similarly, in this study, iron tended to accumulate in a zonal gradient throughout the hepatic lobule, with highest average hemosiderin deposition in periportal parenchymal cells (zone 1). Sinusoidal iron deposition in callitrichids correlates positively with age and hepatic iron concentration. In experimental rat models, hepatic sinusoidal iron increases with exposure time to dietary overload. Thus, the predominant intrahepatocytic hemosiderin deposition, zonal gradient of hepatic hemosiderin, and age-related increase in sinusoidal hemosiderin deposition suggest that hemosiderosis in callitrichids at the Bronx Zoo is primarily due to enteric iron absorption.

In addition, the majority of neonatal callitrichids surveyed had moderate to severe hemosiderin deposition, primarily within hepatic parenchyma. In human neonates, moderate to severe hemosiderosis, similar to intensities seen in this study, is considered abnormal because most iron stores should be mobilized for hematopoiesis (Dr. Baergen, Cornell Medical College, personal communication). The high prevalence of neonatal hemosiderosis may be due to transfer of excess circulating iron from the maternal plasma to the placental trophoblast, where fetal transferrin binds and carries iron to fetal tissues.

In humans there appears to be a threshold for hepatic iron storage which, when exceeded, results in iron deposition in extrahepatic sites such as the pancreas, heart, kidney, reproductive and endocrine tissue. Hemosiderin is also present in extrahepatic callitrichid tissues, including the heart, adrenal, pancreas, intestines, testes, and ovaries. Callitrichids with extrahepatic hemosiderin deposition in three or more tissues have a high average HIC (8217.7 ± 4717.5 ppm), suggesting that thresholds for hepatic iron storage were exceeded. In humans with systemic iron overload, cardiac failure with associated myocardial hemosiderin deposition is among the most significant causes of death. However, the clinical pathology of extrahepatic hemosiderin deposition in callitrichids in this study is not known.

Regarding associated pathology, the degree of periportal and sinusoidal fibrosis was positively correlated with hepatic iron concentration. The extensive hepatic fibrosis often seen in human
patients with iron storage disease\textsuperscript{6,14,16} and gerbils with parenteral iron overload\textsuperscript{3} is not present in callitrichids at the Bronx Zoo. The lack of cirrhosis in callitrichids with severe hepatic hemosiderosis should not be interpreted as non-pathologic, because cytosiderosis can cause severe and toxic ultrastructural alterations within hepatocytes.\textsuperscript{12,13}

Iron overload may also cause immunosuppression by interfering with polymorphonuclear cell superoxide anion generation, phagocytosis, and killing, and by decreased proportions of CD4+ lymphocytes.\textsuperscript{7} Infections with \textit{Klebsiella pneumonia}, \textit{E. coli}, \textit{Pasteurella multocida}, and \textit{Clostridium perfringens} have been associated with increased available iron.\textsuperscript{24} Although the present study was not designed to investigate the relationship between hemosiderosis and cause of callitrichid mortality, many deaths in this study sample, as well as in more recent cases, have been attributed to the aforementioned bacterial infections.

Proximate and ultimate factors may account for the high prevalence and intensity of hepatic hemosiderosis in this study. Proximate factors explaining increased hepatic iron storage in callitrichids include dietary iron overload, high dietary vitamin C levels, hypotransferrinemia, and increased intestinal absorption and/or bioavailability of iron. Ascorbic acid in fruits given to the callitrichids may increase bioavailability of iron.\textsuperscript{22} Estimates of the daily iron concentrations in marmoset and tamarin diets at the Bronx Zoo range from 191.2-238.2 mg/kg and 191.9-305.6 mg/kg, respectively, excluding additional mineral supplementation. Indeed, the diets of captive marmosets and tamarins in this facility contain an iron concentration that exceeds the 180 mg/kg RDA for primates.\textsuperscript{20} Furthermore, these iron requirements are based on experimental studies in the rhesus monkey, and may be too high for callitrichids.\textsuperscript{20}

Ultimately, callitrichids may have an increased genetic affinity for iron absorption because they have evolved to avidly absorb iron due to living in environments where dietary iron is limited or where supplies of dietary iron are temporally or spatially unpredictable. Interestingly, \textit{Callimico goeldii} has a significantly lower mean HIC than three species of callitrichids in this study. Perhaps the ecology of Goeldii’s monkeys is such that their foraging strategies include higher dietary iron availability than other callitrichids.

In conclusion, this study’s results show that captive Callithricidae have a high prevalence of hepatic and extrahepatic hemosiderin deposition, supportive of systemic iron overload. Additional captive prospective feeding trial studies, information of mineral intake of free-ranging callitrichids, serum transferrin levels, and ultrastructural studies are necessary to further understand the pathology of hemosiderosis in captive callitrichids.

\textbf{ACKNOWLEDGMENTS}

Thanks to A. Ngbokoli, M. Shvarstur, and J. Budde for technical support. We also thank Dr. Baergen, The New York Hospital, Dr. Shlisky, Albert Einstein College of Medicine, Dr. Voelkerding, U. of Wisconsin Hospital, Dr. Benirschke, San Diego Zoological Park, Dr. Trupkiewicz, Philadelphia Zoological Garden, and Dr. Linn, Wildlife Conservation Society, for their professional assistance.
LITERATURE CITED

SEVEN NEW CASES OF INTRANUCLEAR COCCIDIOSIS IN TORTOISES: AN EMERGING DISEASE?

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Abstract

Intranuclear coccidiosis in tortoises is apparently rare. Only one published account exists, documenting the disease in two captive-bred juvenile radiated tortoises (Geochelone radiata) from St. Catherine’s Island, Georgia USA. 1 Of 93 chelonians submitted to Northwest ZooPath from 1994-1998, only two cases (2.0%) were documented, in a wild-caught adult male impressed tortoise (Manouria impressa) (tortoise 1) and a captive-bred 7-mo-old Leopard tortoise (Geochelone pardalis) (tortoise 2). At the Wildlife Conservation Society, two additional cases were documented from adult radiated tortoises from St. Catherine’s Island (tortoises 3-4), and in three Travancore tortoises (Indotestudo forstenii) from an illegal shipment originating in Celebes, that was confiscated by the United States Fish and Wildlife Service (tortoises 5-7). Clinical signs and treatments are summarized in Table 1. Gross and microscopic lesions, distribution of intranuclear protozoa, ultrastructural features and concurrent disease processes are summarized in Table 2. A confirmed ante-mortem diagnosis of intranuclear coccidiosis was not available for any of the tortoises, so specific anti-coccidial drugs were not always administered. Although tortoises were treated with a variety of drugs, a treatment affect was not apparent in necropsy tissues. Systemic infection with intranuclear coccidia was fatal or contributed significantly to death in all cases. Tortoise #1 was unique because intracytoplasmic sporulated stages were detected in some hepatic cells that resembled the sporulated stages of Goussia-like oocysts described in Nile crocodiles (Crocodylus niloticus). 2 Tortoise #2 was unique because the coccidia were only detected in the epithelium lining the inner ear and Eustachian tube. Morphologic and ultrastructural features of the parasites indicate that in all cases the organism is a coccidian parasite. These features are similar in tortoises 2-7, and suggest the same species caused infection in these cases. The presence of intracytoplasmic sporulated stages of the parasite in tortoise #1 suggest that this may be a related but different parasite, or that the parasite has a different host relationship in the impressed tortoise. Treatment inconsistencies and lack of ante-mortem diagnoses precluded accurate assessment of a treatment response. The source and route of infection, and predisposing factors for infection have not been determined. Concurrent infectious disease seen in some of the turtles, and evidence of wasting and lymphoid depletion is some tortoises suggest that stress, malnutrition and
immunosuppression may be predisposing factors in the development of clinical disease.

**LITERATURE CITED**


**Table 1. Clinical signs and treatment protocols in tortoises with intranuclear coccidiosis.**

<table>
<thead>
<tr>
<th>Tortoise</th>
<th>Treatments</th>
<th>Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>fenbendazole¹, metronidazole², Albon³, cefazamide⁴, chloramphenicol⁵</td>
<td>anorexia, wasting, several months</td>
</tr>
<tr>
<td>2</td>
<td>fenbendazole⁶, metronidazole⁷, enrofloxacin⁸</td>
<td>lethargy, ocular/nasal discharge, 4 wk</td>
</tr>
<tr>
<td>3</td>
<td>cefazadime⁹, chloramphenicol¹⁰, itraconazole¹¹, fenbendazole¹²</td>
<td>lethargy, ocular/nasal discharge, 2 days</td>
</tr>
<tr>
<td>4</td>
<td>enrofloxacin¹³</td>
<td>lethargy, ocular/nasal discharge, 3 wk</td>
</tr>
<tr>
<td>5</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>6</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>7</td>
<td>riare</td>
<td>none</td>
</tr>
</tbody>
</table>

1. Panacur, Hoechst-Roussel, Somerville, New Jersey 08876 USA; 50 mg p. o., once, 3 mo prior to death.
2. Flagenase, Laboratorios Lismont, Mexico; 50 mg p.o., 3 , 2.5, and 1.5 mo prior to death.
3. Albon Liquid, SmithKline Beecham, West Chester, Pennsylvania, 19380 USA; 25 mg p.o., once, 2.5 mo prior to death.
4. Fortaz injectable, Glaxo Pharmaceuticals, Research Triangle Park, North Carolina, 27709 USA; 14 mg i.m., s.i.d, 3 to 1 wk prior to death.
5. Chloro Drops, Steris Laboratories, Phoenix, Arizona 85043 USA; s.i.d for 7 days, 2 wk prior to death.
6. Panacur, Hoechst-Roussel, Somerville, New Jersey 08876 USA; 10mg p.o., once, repeated in 2 wk.
7. Flagenase, Laboratorios Lismont, Mexico; 20 mg p.o., repeated in 2 wk.
8. Baytril, Bayer Co. Shawnee Mission, Kansas 66201 USA 5 mg/kg, p.o., s.i.d for 7 days, 3 wk prior to death.
9. 2.5% dextrose + 0.5% NaCl, 10cc; s.c., s.i.d for 6 days, 3 wk prior to death.
10. Panacur, Hoechst-Roussel, Somerville, New Jersey 08876 USA; 50 mg p.o., once, prior to onset of clinical illness (for oxyurids).
11. Flagenase, Laboratorios Lismont, Mexico; 200 mg/kg p.o., duration unknown.
12. Baytril, Bayer Co. Shawnee Mission, Kansas 66201 USA 2.5 mg, i.m., s.i.d for 10 days.
13. Fortaz injectable, Glaxo Pharmaceuticals, Research Triangle Park, North Carolina, 27709 USA; 20 mg/kg i.m., q 72 h, for 11 days, until death.
14. Chloro Drops, Steris Laboratories, Phoenix, Arizona 85043 USA; b.i.d for 11 days, until death.
15. Sporonox, Janssen Pharmaceuticals, Titusville, New Jersey 08560 USA; 100 mg q 48 h, until death.
<table>
<thead>
<tr>
<th>Tortoise</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross</td>
<td>emaciation, coelomic effusion</td>
<td>none</td>
<td>reddened lungs mucus in trachea yellow kidneys</td>
<td>reddened lungs mucus in eyes and nasal cavity</td>
<td>hepatic mass coelomic effusion</td>
<td>no fat, coelomic effusion frothy lungs</td>
<td>mottled liver membranous typhilitis</td>
</tr>
<tr>
<td>Distribution</td>
<td>hepatocytes, renal tubular epithelium splenic/int. macrophages Also, intra-cytoplasmic in liver.</td>
<td>epithelium of middle ear, Eustachian tube</td>
<td>epithelium of renal tubules, nasal mucosa intestinal macrophages</td>
<td>epithelium of intestine, lung kidney</td>
<td>epithelium of intestine, lung stomach, pancreas</td>
<td>epithelium of pancreas, liver, kidney, lung, intestine colon, stomach</td>
<td></td>
</tr>
<tr>
<td>Ultrastructure</td>
<td>merozoites microgametocytes macrogametocytes</td>
<td>trophozoites microgametocyte trophozoite</td>
<td>microgametocyte macrogametocytes schizont</td>
<td>not done</td>
<td>not done</td>
<td>not done</td>
<td>not done</td>
</tr>
<tr>
<td>Other diseases</td>
<td>none.</td>
<td>bacterial sepsis Pseudomonas pneumonia</td>
<td>none</td>
<td>amoebiasis fungal gastritis bacterial hepatitis</td>
<td>fungal rhinitis</td>
<td>amoebiasis fungal gastritis</td>
<td></td>
</tr>
</tbody>
</table>
ANEMIA, MYOPATHY, AND/OR STEATITIS IN NEW WORLD MONKEYS - VITAMIN E AND/OR SELENIUM DEFICIENCY?

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Abstract

Vitamin E and selenium are micronutrients with a prominent involvement in diseases of animals, including fish, birds, reptiles, and mammals.1,5,6,8-10,12-16,18 In primates, there are reports of spontaneous and experimental hemolytic anemia, myopathy, and/or steatitis associated with vitamin E deficiency and/or positive response to vitamin E/selenium therapy.1,4,5,10-14 The purpose of this communication is to describe the clinicopathologic findings of suspected vitamin E/selenium deficiency in three common marmosets (Callithrix jacchus) and two squirrel monkeys (Saimiri sciureus) that presented either for clinical examination or necropsy.

Case no. 1, an underweight (260 g) 2-yr-old male common marmoset, was submitted for necropsy with a prolonged course of non-specific signs. Gross pathology was unremarkable except for muscle atrophy and fecal retention. Pyogranulomatous and fibrosing steatitis of abdominal fat was the most prominent microscopic finding; macrophages surrounding adipocytes had a PAS- and ZN-positive, finely granular cytoplasm, consistent with ceroid. Other lesions were: degenerative myopathy of the diaphragm; myocardial fibrosis; histiocytosis and increased erythrophagocytosis in lymph nodes; hemosiderosis of the reticuloendothelial system and hepatocytes; nephrocalcinosis; and serous atrophy of fat.

Case no. 2, a 3-yr-old male common marmoset, presented with anorexia and alopecia of the tail and forelimbs. The diet consisted of a commercial fruit mix, insects and occasionally yogurt, cooked meat and fish. Calcium and a multivitamin supplement that lacks vitamin E and selenium were used. Alopecia improved in association with protein supplementation over a 2-mo period, with body weight ranging 300-340 g. Six months later, this marmoset presented with a 2-wk history of anorexia, weight loss (268 g) and hindlimb paresis. Alopecia was still present in the left forelimb. CBCs revealed leukopenia with neutrophilia, eosinophilia and lymphopenia, and thrombocytopenia. CPK Value was 465 IU/l. The animal was given ivermectin (Ivomec,® Merck Sharp & Dohme B.V., Haarlem, The Netherlands; 200 μg/kg p.o.) and vitamin E (225 IU/kg) with sodium selenite (1.15 mg/kg) (Toco-Selenio®, Calier, Barcelona, Spain; p.o., once every 3 days for 1 mo). Cessation of paresis and return to normal activity and weight was noted after 1-mo therapy, what was considered significant as therapy did not begin until 2 wk after presentation and clinical signs remained unchanged during that time; however, a temporal coincidence cannot be ruled out. A diagnosis of vitamin E/selenium-responsive myopathy and/or neuropathy was made. Although CPK was not
elevated, nutritional myopathy is usually episodic and CPK is short-lived; thus myopathy cannot be ruled out on the basis of normal CPK value alone.

Case no. 3 was a 3-yr-old female common marmoset with a 2-mo history of weight loss and alopecia. The diet consisted of commercial cereal and fruit mixes with honey, insects, fruits and vegetables, and multivitamin and mineral supplements that lacked vitamin E and selenium. Clinical examination revealed emaciation (220 g), marked alopecia of the tail and head, and enlarged, dark inguinal lymph nodes. A soft mass was palpated just caudoventral to the liver; radiographically, it was moderately and uniformly radiopaque. The animal was given ivermectin as above. CBCs revealed marked anemia, leukopenia with neutrophilia and lymphopenia, and thrombocytopenia. There was mild hypoproteinemia with marked hypoalbuminemia and hyperbeta
globulinemia. Protein supplements and arabic gum were used, and the owner was informed about the possibility of hypoglycemic episodes. Two weeks later the owner reported increasing appetite and body weight (238 g). At that time, CPK, LDH and AST were markedly elevated, and anemia was characterized as regenerative with < 1% of Heinz bodies. A presumptive diagnosis of vitamin E/selenium deficiency was made. The owner used wheat germ oil instead of the recommended vitamin E-selenium product. Three weeks after presentation the marmoset was found comatose; the owner recovered it by heating and giving juice with sugar. The animal died 2 mo after presentation and was not submitted for necropsy.

Case no. 4 corresponded to two young-adult squirrel monkeys with a progressively deteriorating body condition. Both animals had been fed dog food and home-made meals for 6 mo, and did not have access to sunlight. The male presented with weight loss (520 g), impaired locomotion, and diffuse moderate alopecia. CBCs revealed moderate anemia, hypoproteinemia, and hypoalbuminemia, and marked hyperbeta
globulinemia. ALP was elevated and calcium low. CPK and AST values were normal. At necropsy, the female was emaciated and had diarrheic feces. The gastrointestinal contents was liquid. Major microscopic findings were fibrosing and degenerative cardiomyopathy with lipofuscinosis and cardiomyocyte atrophy, and necrosis and saponification of pericardial, mediastinal and perirenal fat. Mild degenerative myopathy was also present. In the kidneys, a granular eosinophilic material was present in numerous convoluted tubules. Other lesions were: diffuse hepatic fatty change; hemosiderosis of the reticuloendothelial system; disseminated intravascular coagulation; and focal suppurative gastritis. A presumptive diagnosis of nutritional osteomalacia and vitamin E/selenium deficiency was made, and treatment with vitamin E (3.75 IU/kg) and vitamin D<sub>3</sub> (5,000 IU/kg) (Veterín Vit.® A+D<sub>3</sub>+E; p.o., once weekly) was initiated in the male. However, it was lost for followups.

Although inconclusive since vitamin E/selenium analyses were not performed, the clinicopathologic findings are suggestive of vitamin E/selenium deficiency. Enhanced peroxidative hemolysis has been induced in vitamin E-deficient common marmosets<sup>1</sup> and is one of the features of spontaneous hemolytic anemia of owl monkeys (<i>Aotus trivirgatus</i>), that is associated with vitamin E deficiency and myopathy.<sup>13</sup> Similar syndromes have been documented as major mortality factors in colonies of moustached tamarins (<i>Saguinus labiatus</i>)<sup>1</sup> and common marmosets.<sup>2</sup> Excessive Heinz body counts
help diagnose hemolytic anemia in these species. Marmoset no. 2 had marked regenerative anemia with normal Heinz body counts, associated with elevations of enzymes consistent with muscular damage. Excessive Heinz body counts were a common but not constant finding in a common marmoset colony with a high incidence of hemolytic anemia, myopathy and undetectable vitamin E levels in some of the affected animals.4

Degenerative myopathy, that was present in the two necropsy cases, may support a diagnosis of vitamin E deficiency since it was a common finding in vitamin E-deficient moustached tamarins, common marmosets and owl monkeys, and has been experimentally induced in several primate species by feeding vitamin E-deficient diets. Fibrosing and degenerative cardiomyopathy was a prominent finding in the female squirrel monkey and has been reported in vitamin E-deficient gelada baboons (Theropithecus gelada)10 and mountain gorillas.12 Experimental selenium deficiency has been induced in squirrel monkeys fed a diet adequate as for its vitamin E contents; alopecia and degenerative myopathy were also observed.14 In rhesus monkeys, experimental selenium deficiency induced cardiomyopathy only in association with dietary protein deficiency.3

Steatitis, a prominent finding in marmoset No. 1, has been reported in vitamin E-deficient moustached tamarins,1 and in fish-eating crocodilians and birds, mink, and cats suspected or known to be vitamin E-deficient,8,15 steatitis has also been experimentally induced in vitamin E-deficient animals such as rats.5 Steatitis is a sensitive indicator of vitamin E deficiency in animals fed on diets rich in polyunsaturated fatty acids; adequate vitamin E status depends on the type of dietary fats.5

Fat malabsorption is another possible cause of vitamin E deficiency. Fat malabsorption, with subsequent vitamin E deficiency, altered cholesterol metabolism and red blood cell membrane lipid composition, has been suggested as the underlying problem in owl monkeys with hemolytic anemia and myopathy.13 Fat malabsorption mostly occurs in exocrine pancreatic insufficiency (EPI); dogs with EPI usually have low vitamin E levels.20 In human beings, cystic fibrosis is a cause of EPI; one of its complications is hemolytic anemia in vitamin E-deficient infants.19 The most common cause of EPI is pancreatic acinar atrophy (PAA).20 In primates, PAA and impaired exocrine pancreatic function have been experimentally induced in rhesus monkeys (Macaca mulatta), with concurrent myopathy,4 and patas monkeys (Erythrocebus patas)7 by protein-deficient diets. PAA and fibrosis were prominent in marmosets with wasting marmoset syndrome (WMS), hindlimb paresis and paralysis and degenerative myopathy suspected to be associated with protein deficiency.2 Marmoset No. 3 and the male squirrel monkey had hypoproteinemia and/or hypoalbuminemia with no biochemical evidence of renal or hepatic damage, thus suggesting dietary protein deficiency and/or enteric loss. Interestingly, pancreas disease of salmonids, characterized by PAA and fibrosis, is associated with hypoproteinemia, hypoalbuminemia, degenerative myopathy and vitamin E/selenium deficiency.6 Another possible cause of EPI is chronic pancreatitis,20 that has been found in marmosets with the pancreatic worm Trichosporura leptostoma and WMS.17

Pancreatic lesions were not found in the necropsy cases of this report, but protein deficiency and any other cause of EPI should be considered as possible concurrent or even primary factors in the pathogenesis of WMS in callitrichids and any syndrome associated with anemia, myopathy and/or
steatitis and suspected vitamin E/selenium deficiency in primates.

Callitrichids have high protein requirements, so some home-made diets may be protein-deficient for these species. Most marmosets attended at our institution have similar diets and usually present with WMS. Some of these animals seem to respond to protein supplementation.

The possibility of vitamin E/selenium and/or protein deficiency-induced WMS in callitrichids should be carefully investigated through intensive antemortem testing (CBCs with characterization of anemias; total proteins and protein profile; serum chemistries including CPK, LDH, AST, ALT, amylase, lipase, and cholesterol; circulating vitamin E and selenium levels; and, if indicated and possible, exocrine pancreatic function tests and lipoprotein profile), together with nutritional analyses, and postmortem studies (pathology and tissue vitamin E and selenium levels).

LITERATURE CITED

ACUTE HIND LIMB ATAXIA - PARESIS IN CHEETAH (Acinonyx jubatus) CUBS

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Abstract

Within the European Endangered Species Program (EEP) cheetah (Acinonyx jubatus) population, numerous cases of hind limb paresis have occurred in cheetah cubs over the past years. This paper describes a nervous disease of cheetah cubs in three different clusters during the period from 1995-1997. This disease is clinically characterized by an acute onset of hind limb ataxia-paresis. On histopathology, mild to severe demyelination, predominantly of the dorsal spinocerebellar tracts and the sulcomarginal tracts, was found throughout all levels of the spinal cord. The onset of ataxia was regularly in chronological relation to sneezing, ocular and nasal discharge. Serum samples, conjunctival and nasal swabs confirmed the presence of Feline Herpes Virus 1 (FHV1). The authors suggest a possible viral etiology. Ataxia progression was inhibited by the application of the anti herpes drug Acyclovir and Prednisolone. A primary-latent oro-nasal FHV-1 infection may, under stressful conditions, trigger an immunologic process leading to ataxia with myelin destruction.

Introduction

Within the European Endangered Species Program (EEP) cheetah (Acinonyx jubatus) population, numerous cases of hind limb paresis have occurred in cheetah cubs over the past years. In reports made to the International Cheetah Studbook, various facilities acknowledge hind limb paresis as a cause of death in related juvenile animals. These cases have occurred in certain focal clusters. Taking the data obtained by an EEP questionnaire amongst the cheetah holders, eight locations in Europe, one in Namibia, and one in Dubai were identified (Walzer, 1997 unpublished data). Hind limb paresis in cubs is at the present one of the most important limiting factors in the growth of the EEP cheetah population and the major veterinary concern of the cheetah EEP and European Feline Taxon Advisory Group (TAG).

Material and Methods

This paper describes spontaneous ataxia in four litters in three separate clusters (Nuremberg Germany, Salzburg Austria, Fota Ireland) within the Cheetah EEP population. A very detailed description of the clinical and serologic findings has been published previously.

In Nürnberg and in both litters in Salzburg it was noted that the ataxia occurs spontaneously (within
minutes to hours). In two litters (Nürnberg and Salzburg #1) ataxia occurred following a stressful experience for the individual or the litter. In all three clusters ataxia was preceded by ocular and/or nasal discharge, as is commonly seen in clinical FHV-1 infection.

From the two litters in Salzburg serologic evidence of a FHV-1 infection was determined. FHV-1 was isolated in cell culture from nasal and ocular swabs. PCR methods were established using primers selected on the basis of FHV-1 sequences in the GenBank database. The amplified products of the PCRs were sequenced and compared with the FHV-1 sequences in the GenBank showing a 99% identity compared to the published sequences. A cross-neutralization test using FHV-1 isolates from a domestic cat and a cheetah, as well as the corresponding antisera, yielded no significant titre differences. These results conclusively demonstrate that the isolates from the cheetah and the domestic cat are very closely related.

Complete histopathology has to date been carried out on six ataxic cubs in the three clusters. Though the degree of the lesions varies with the time lapse between ataxia onset and histopathology it is by and large identical in all cubs. Neuropathology of brain, spinal cord, sciatic nerves and brachial plexus revealed only lesions of the spinal cord. A severe demyelination, predominantly of the dorsal spinocerebellar (ascending) tracts and the sulcomarginal (descending tracts of the extrapyramidal system) tracts, was found throughout all levels of the spinal cord. The demyelination was associated with severe astrogliosis, some degree of axonal swelling and focal small non-suppurative inflammatory infiltrates in the white matter and the leptomeninges. Intriguingly, the ascending tracts of the dorsal funiculus (gracile and cuneate fascicle) were consistently unchanged. Brain and peripheral nerves showed no pathologic changes.

CNS tissue (cerebrum, cerebellum, hippocampus and brain stem) from cub FD90 were examined with the tmidinkinase - PCR. All the tissue samples proved positive. Negative controls were all negative. However, when inoculating these same organ samples on CRFK cell cultures, no CPE was observed.

Ataxia in litter 2 in Salzburg appears to have resolved upon therapy consisting of Acyclovir (Zovirax®, Glaxo-Wellcome GmbH Vienna, Austria) and Prednisolone (Solu-Dacortin®, E. Merck, Darmstadt, Germany).

Of the five animals in the Nürnberg litter, one cub was hand raised and therefore had no contact to the dam and other littermates, this animal never developed any clinical ataxia and no CNS lesions could be identified at necropsy.

Discussion

This report describes a nervous disease of cheetah cubs which is characterized by acute onset hind limb paresis and ataxia. Literature reports on this subject are very scarce. Ataxia in cheetah cubs is mentioned only by Brand,1 Zwart,12 and Hafner.2 These papers discuss ataxia as a result of a copper deficiency and one paper compared it to enzootic ataxia of goats.12 The demyelinating lesions
described in this paper are confined to the spinal cord, which is in contrast to lesions in enzootic ataxia in sheep and goats, where neurons in the brainstem and spinal cord are also affected. Furthermore on neurologic examination the sheep and goats affected with enzootic ataxia demonstrate flaccid paresis, hypotonia and hyporeflexia (Lower Motor Neuron Disease) which is in clear contrast to the considered Upper Motor Neuron Disease established in cubs in litter #1 Salzburg. Considering the non-responsiveness of the ataxia to aggressive copper therapy as carried out by Gaukler in the Nürnberg Zoo (A. Gaukler, personal communication), and similar experiences encountered in Fota (L. Guerin, personal communication), we believe that copper deficiency can be discounted as a primary etiology in this nosological entity.

In adult cheetahs there are reports on animals with a spinal demyelinating disease located between T6 and L3. A hereditary myelopathy with a late onset was suggested as a possible cause. The main difference between this description in adult cheetahs and the ataxia in the cubs is in the distribution of the lesions in the spinal cord and the progressive onset in the adult animals. Furthermore, as the cubs in the three clusters were born of different parents, a hereditary degenerative disease is unlikely.

The pattern of the neuropathologic lesions and the absence of distinct inflammatory changes seem to exclude a viral etiology. On the other hand, various facts collected in the three clusters suggest an infectious agent as the cause of the ataxia. In Nürnberg four parent-raised cubs developed ataxia, whereas, the hand raised littermate did not. The onset of ataxia was regularly in chronologic relation to sneezing, ocular and nasal discharge. The inhibition of ataxia in litter 2 Salzburg, with the application of the anti herpes drug—Acyclovir—suggests a viral agent as etiology.

Some interesting parallels seem to exist between cheetah cub ataxia and Multiple Sclerosis (MS), the most common human demyelinating disease. Although the diseases have significant morphologic differences and cannot be compared per se, the symptoms of MS result from recurrent inflammatory attacks on the CNS. Litter 2 in Salzburg experienced two separate onsets of ataxia. Similar to the spontaneous clinical onset in the cheetah, over 50% of the MS patients report an abrupt onset of the neurologic symptoms; this although, the destruction of the myelin is a complex process that most likely takes several days to complete. As in the cheetah ataxia, MS occurs in clusters. One assumes that an environmental factor is responsible for the geographic link. MS may begin when the immune system is activated by some environmental event, a virus or other infection. The difficulties encountered in trying to reconcile the myelin destruction observed in MS with primary involvement of CNS antigens has led to a theory of possible cross reacting infectious antigens. Recently it has been demonstrated that T-cells cloned from MS patients recognize not only the CNS antigen Myelin Basic Protein (MBP) but also numerous antigens from various infectious agents such as herpes simplex, influenza and Epstein-Barr virus.

It can be speculated that a primary-latent oro-nasal FHV-1 infection may trigger an immunologic process similar to that in MS, leading to ataxia with myelin destruction.

At the present, in-situ hybridization work and immunohistochemistry is ongoing to attempt to explain
the immuno-pathologic process involved in cheetah cub ataxia.

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LITERATURE CITED

INTESTINAL SPIROCHETOSIS OF NORTH AMERICAN OPOSSUMS (Didelphis virginiana): A POTENTIAL BIOLOGIC VECTOR FOR PATHOGENIC SPIROCHETES

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Abstract

Intestinal spirochetosis (IS) is a disease of humans and animals that has potential public health significance. Two spirochetes have been proposed as etiologic agents of IS, Serpulina pilosicoli and Brachyspira aalborgi. The purpose of this study was to determine the identity of spirochetes present in North American opossums (Didelphis virginiana) with IS. Histopathologic changes indicative of IS were present in the ceca of 17 opossums obtained from California (n = 4), Connecticut (n = 9), Nebraska (n = 2), and New York (n = 2). Ultrastructural examination of cecal tissue obtained from ten opossums revealed spirochetes only in six specimens and spirochetes together with flagellated bacteria in four others. Spirochetes were isolated in pure culture from ten specimens, and total DNA from nine isolates was amplified using 16S ribosomal RNA (rRNA) polymerase chain reaction (PCR) assays specific for either B. aalborgi or S. pilosicoli. Seven isolates yielded products with both 16S rRNA PCR assays, whereas two isolates were negative for B. aalborgi, but positive for S. pilosicoli using the 16S rRNA PCR and a 23S rRNA PCR. The presence of IS in opossums obtained from eastern, central and western states suggested that the disease may be widespread in the United States. These findings help define the epidemiology of IS and provide evidence that the opossum is a natural reservoir for the agent(s) and potentially act as a biologic vector for transmission to mammals and birds.

Introduction

Serpulina pilosicoli is the etiologic agent of a diarrheal disease of humans, non-human primates, swine, dogs, and birds that has been variously referred to as intestinal spirochetosis (IS), colonic spirochetosis, colo-rectal spirochetosis, rectal spirochetosis or cecal spirochetosis.3,5,8,10,11,13 Spirochetes of unknown taxonomic classification also have been seen in the cecum and colon of opossums and laboratory guinea pigs.3,12 The disease caused by S. pilosicoli is characterized by intimate attachment of spirochetes to the apical surface of the cecal and colonic enterocytes with or without invasion into the gut wall.1,3,5,8,12,13 Also, spirochetes structurally and phenotypically different from S. pilosicoli, and called Brachyspira aalborgi, as well as some unclassified flagellated bacteria have been seen attached to the cecal and colonic enterocytes of humans and rhesus macaques.1,3,4,6 The purpose of this study was to determine the identity of intestinal spirochetes of
North American opossums (Didelphis virginiana) with IS. To ascertain the presence of B. aalborgi and S. pilosicoli in IS of opossums, pure cultures of intestinal spirochetes were isolated from opossums affected with the disease. Then the spirochetes were examined for their growth characteristics on artificial medium and for the presence of spirochete-specific 16S ribosomal RNA (rRNA) and 23S rRNA gene sequences by polymerase chain reaction (PCR) amplification assays.

Methods

Sample collection. Cecal tissues and contents from 17 mature opossums obtained from four states were processed for microbiologic, light and ultrastructural examinations (Table 1). Opossums from California were captured alive as part of epidemiologic studies at two dairy farms in San Bernardino (no. 1, 2, and 3) and an aviary near Concord (no. 4). Opossums no. 6 and no. 8 were found dead on roads at various times after being hit by cars in Connecticut. Opossum no. 14 and no. 15 were found dead in residential areas in Lincoln, Nebraska, one with heavy parasitism and septicemic salmonellosis, and the other after being hit by a car, respectively. The remaining opossums were caught live in traps either by wildlife control servicemen in residential areas in Connecticut, or by a commercial wildlife supplier in New York. Live opossums were euthanatized by administration of an overdose of barbiturate.

Pathologic examination. Blocks of cecal tissue were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, cut at 5 µm, and stained with hematoxylin and eosin (HE) and Warthin-Starry (WS) stain. Additional formalin-fixed deparaffinized sections were processed for staining of spirochetes by immunohistochemistry using the Serpulina spp. periplasmic flagellar FlaB-specific mouse monoclonal antibody 7G2 and avidin biotin complex alkaline phosphatase (Dako, Carpinteria, CA). For transmission electron microscopy, fresh (no. 6-13), formalin-fixed (no. 14-17), or formalin-fixed deparaffinized blocks (no. 1-5) were immersed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2), embedded in araldite, processed for ultrathin sectioning, stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (Philips 201, Eindhoven, The Netherlands).

Bacteriologic examination. Primary isolation of spirochetes was done by streaking cecal mucosal scrapings onto colistin, vancomycin, and spectinomycin selective agar medium and incubation at 37°C in a commercial anaerobic culture system (Gas Pak Anaerobic System, BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD) as previously described. Pure cultures of spirochetes were propagated on trypticase soy agar with 5% citrated sheep blood (TSA). Isolates no. 14 and 15 were examined for growth on TSA incubated at either 37°C or 42°C.

PCR assays. Total DNA from pure cultures of spirochetes was isolated using a previously described method. Serpulina pilosicoli identification was done by PCR amplification with a 16S rRNA sequence-specific forward primer (5'-AGAGGAAAGTTTTTTCGCTTC-3') and a conserved Serpulina spp. 16S rRNA reverse primer (5'-TCCGCTACTCACCTTTTAC-3') as previously described. For PCR amplification of B. aalborgi, a 16S rRNA-specific forward primer (5'-GCCTGTTGCTGAGATAAAAG-3') was used in combination with the conserved Serpulina spp.
16S rRNA reverse primer as previously described. The total DNA from each spirochete isolate was examined further using a S. pilosicoli 23S rRNA gene-specific forward (5'-AGGTGATGGTTATCCTC-3') and reverse (5'-AACCTTAGGAATTATTTCTAA-3') primer set and PCR amplification. Positive and negative controls consisted of S. pilosicoli porcine strain P43/6/78T DNA and no template DNA, respectively. The amplified products were visualized after electrophoresis in 1.5% agarose gels and staining with ethidium bromide.

Results

Light microscopic examination of HE-stained sections of ceca revealed a diffuse basophilic 4 µm layer on the brush border of enterocytes along the surface and extending about half-way into the crypts in 16 of 17 opossums. Opossum no. 14 had large numbers of spirochetes in the mucin on the surface and in the crypt lumena with rare foci of surface attachment. The basophilic layer was dark brown to black with WS stain. Immunohistochemical staining confirmed the presence of a dense layer of spirochetes arranged in parallel arrays on the apical surface of cecal enterocytes in all of the opossums. Associated inflammatory infiltrates or exudates were minimal or non-existent. Ultrastructural examination revealed diffuse colonization by spirochetes attached perpendicularly by one pole to the apical membrane of enterocytes causing effacement of microvilli and disruption of the terminal web microfilaments (Table 1). Vesicles that contained electron-dense granular material were present in the apical cytoplasm of colonized cells. In some specimens, spirochetes were seen between epithelial cells or free in the lamina propria, and in opossum no. 11, few spirochetes also were seen free in the cytoplasm of a goblet cell. In addition to the spirochetes, flagellated bacteria were attached perpendicularly by one pole to the apical membrane of enterocytes in the ceca of opossums from California and New York.

Spirochetes were isolated from ten out of 17 intestinal specimens with IS; spirochetes were not isolated from specimen nos. 1, 2 and 3 which had been frozen, whereas specimens 6, 11, 12, and 13 had spirochetes in wet mounts of cecal contents examined by dark field microscopy that were overgrown by contaminants. All spirochete isolates grew very slowly taking up to 14 days to form a visible non hemolytic surface film that became weakly β-hemolytic after 21 days of incubation at 37°C. When examined for growth at 42°C, isolates no. 14 and no. 15 did not show any surface growth or hemolysis after 21 days. Nine of the isolates were examined using the 16S rRNA PCR assays, and with the exception of no. 9 and no. 10, all of the isolates yielded 314-bp B. aalborgi-specific and 361-bp S. pilosicoli-specific products (Table 1). The same isolates were negative for S. pilosicoli-specific 23S rRNA, whereas isolates no. 9 and no. 10 yielded S. pilosicoli-specific products with the 16S and the 23S rRNA gene sequence-specific PCR assays, and no products with the B. aalborgi 16S rRNA PCR assay.

Discussion

Intestinal spirochetosis of wild opossums was first reported by Turek and Meyers; five opossums from Illinois had adherent spirochetes that caused disruption of the brush border of cecal enterocytes.
in a pattern similar to that seen in the present report. The spirochetes isolated from these opossums grew as a thin haze on the surface of blood agar, producing very slight or no hemolysis when incubated anaerobically at 37°C. Transmission electron microscopic examination of the cultured spirochetes revealed four to five subterminally inserted periplasmic flagella at each end of the protoplasmic cylinder. These morphologic and cultural characteristics were consistent with those of *B. aalborgi*, a spirochete that was later described in human beings with IS.\(^6\)

Based on the pathology and microbiology findings, a diagnosis of IS was made in all 17 opossums. The ultrastructural data also indicated that flagellated bacteria often were present together with the spirochetes; a disease association similar to that seen in human beings and non-human primates,\(^1,3,4\) but different from swine, dogs, and birds where flagellated bacteria have not been seen.\(^3,5,8,12\) Although there was evidence of massive mucosal involvement, the contents of the ceca were normal in consistency. Therefore the clinical significance and pathogenetic mechanisms of IS in these opossums remains unknown. However, the data presented extended the observations made by Turek and Meyers,\(^12\) and further indicated that IS was widespread among opossums in the United States.

With the advent of DNA-based typing methods, it is assumed that the identity of culturable intestinal spirochetes can be determine with some confidence. However, on the basis of PCR amplification of 16S rRNA gene sequences, the majority of the isolates gave positive amplification products with both PCR assays. Because the growth characteristics of *S. pilosicoli* are different from *B. aalborgi*; growth occurs within days and produces a clear zone of ß-hemolysis on blood agar plates incubated at 37°C and 42°C,\(^6,8,11\) the results of the *S. pilosicoli* 16S rRNA PCR assays might not be specific. This is supported by the absence of growth of two of these isolates at 42°C and the absence of specific products with the *S. pilosicoli* 23S rRNA PCR assay. Since not all of the isolates were examined for growth at 42°C, the possibility that some of the cultures contained *S. pilosicoli* and *B. aalborgi* cannot be ruled out completely. Conversely, the results of 16S and 23S rRNA PCR assays suggested that opossums no. 9 and no. 10 had only *S. pilosicoli*. Although additional work is needed to improve the specificity of DNA-based typing methods, spirochetes are genetically diverse, and it is possible that the opossum spirochetes belong to a group that shares characteristics of *S. pilosicoli* and *B. aalborgi*.

The data presented indicated that the North American opossum is a natural reservoir for IS. Because IS was found in opossums living in both rural and residential areas, it raised the possibility that the North American opossum may serve as a biologic vector for transmission of the disease to livestocks and urban dwellers.

ACKNOWLEDGMENTS

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LITERATURE CITED

Table 1. Laboratory findings in wild-caught North American opossums with intestinal spirochetosis.

<table>
<thead>
<tr>
<th>Origin/Case no.</th>
<th>TEM†</th>
<th>Spirochete Flagellates</th>
<th>PCR§</th>
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<th>Ba</th>
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</table>

†Presence (+) or absence (-) of spirochetes or flagellated bacteria attached to the enterocyte brush border of the cecum, as determined by transmission electron microscopic (TEM) examination; ND = not determined.

‡Spirochete isolated (+), not isolated (-), or overgrown (over.) by contaminants after anaerobic incubation of cecal scrapings on selective agar medium for intestinal spirochetes at 37°C for 21 days.

§Presence (+) or absence (-) of products after amplification of purified DNA from pure cultures using polymerase chain reaction (PCR) of 16S rRNA gene sequences of *Serpulina pilosicoli* (*Sp*) or *Brachyspira aalborgi* (*Ba*); NA = Not available; ND = Not determined.
PATHOLOGY AND EPIDEMIOLOGY OF NEOPLASIA IN THE CAPTIVE POPULATION OF BLACK-FOOTED FERRETS (Mustela nigripes)

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Abstract

The capture of the last 18 known wild black-footed ferrets (Mustela nigripes) in 1986-1987 marked the beginning of one of the most successful captive propagation efforts in the history of endangered species conservation. Since then, close to 2000 black-footed ferrets have been born in the seven institutions involved in this breeding program. Neoplasia is an important cause of morbidity and mortality in ferrets after they reach adult age. In order to better characterize these neoplastic conditions, and to evaluate their significance in the management of this species, we conducted a retrospective study of the cases of neoplasia diagnosed in the captive population between 1988 and 1998. Prior to April 1, 1998, a total of 206 adult black-footed ferrets (at least 1-yr-old) have died in captivity. At this time, postmortem reports and embedded tissues were recovered from 180 of these adult ferrets (102 males and 78 females). Pertinent information was also recovered from the studbook maintained at the USFWS National Black-Footed Ferret Conservation Center. Each tumor was examined by the same pathologist, and classified according to its histologic features. A total of 172 neoplasms was diagnosed in 97 of the 180 adult ferrets (53.9%) included in this study. Multiple neoplasia were common; thirty-five, ten, four, and two ferrets being affected by respectively two, three, four, and five different types of tumors each. The prevalence of most of the tumors increased with the age at death (Fig. 1). With the exception of two cases, all ferrets with tumors were older than 4 yr. Neoplasia was believed to have contributed to the death of 59 ferrets (32.8%), all but two being over 4-yr-old (Fig. 1). The most common tumors observed were, in decreasing order of frequency, renal tubular neoplasms (prevalence: 21.7%); sweat gland adenomas/adenocarcinomas (prevalence: 19.4%); biliary cystadenomas/cystadenocarcinomas (prevalence: 14.4%); mammary adenocarcinomas (prevalence in females: 15.4%); adenomas/adenocarcinomas of the perianal glands (prevalence: 8.3%); adenomas/adenocarcinomas of the preputial glands (prevalence in males: 5.9%), and squamous cell carcinomas (prevalence: 4.4%). At least 14 other types of tumors were also present.

Renal tubular neoplasms were commonly multiple and bilateral. These usually protruded from the renal capsule and ranged from small microscopic aggregates of neoplastic tubular cells to large locally invasive masses replacing most of the renal parenchyma. Based on their cellular morphology and invasive behavior, most of these tumors were classified as renal carcinomas, but distant metastases were found in only one of the 39 cases.
Biliary cystadenocarcinomas were usually fast growing, markedly invasive malignancies, frequently associated with extensive abdominal carcinomatosis. An interesting morphologic feature of these tumors was their usual affiliation with large biliary cysts. Neoplastic papilliform growths could usually be seen originating from the epithelium lining these cysts. Biliary cysts were very common in the liver of adult black-footed ferrets (65.6% of the animals examined). These cystic dilations of bile ducts seem to increase in size and in number with age. Their etiology is at this time unclear.

Most of the sweat gland tumors were cystic, slow growing, and located on the tail. Despite the anaplastic appearance of the cells in most of these tumors, distant metastases were rarely observed. In contrast, adenocarcinomas of the perianal apocrine glands were usually markedly invasive, and frequently metastasized to regional lymph nodes, abdominal cavity and lungs. These tumors were mainly observed in males. Three of the apocrine preputial gland tumors were classified as adenomas and three as adenocarcinomas. Abdominal metastases were present in one of these cases. All the 12 tumors of the mammary glands diagnosed were classified as adenocarcinomas, and distant metastases were detected in four of these cases.

Most squamous cell carcinomas affected the oral cavity, where they were markedly invasive. Pulmonary metastases were detected in one animal.

This study shows that neoplasms, especially of epithelial origin, are very common, and represent an important cause of mortality in captive adult black-footed ferrets. The very high prevalence of neoplasia in this population is intriguing, and suggests a potential genetic predisposition, and/or exposure to carcinogenic factors.

The impact of a high prevalence of neoplasia on the captive propagation of this species is most likely limited, since tumors are encountered almost exclusively in post-reproductive ferrets. The effect on the wild population would also probably be insignificant, since ferrets released into their natural habitat rarely reach the age when these neoplasms occur.

ACKNOWLEDGMENTS

Funding for this project, and the stipend for S.L., was generously provided in full by the Zoological Society of Toronto. We are grateful to clinicians and pathologists involved in the black-footed ferret captive propagation program, including Corrine Brown (Omaha’s Henry Doorly Zoo), Roy Burns (Louisville Zoo), Graham Crawshaw (Toronto Zoo), Mary Duncan (St. Louis Zoological Park), Della Garell (Cheyenne Mountain Zoo), Julie Kreeger (National Black-Footed Ferret Conservation Center), Don Kwiatkowski (formally at the National Black-Footed Ferret Conservation Center), Dick Montali (National Zoological Park), Jon Patterson (Michigan State University), Allan Pessier (National Zoological Park), and Chris Schiller (All Creatures Pathology Service). Our thanks also goes to Bill Russell for studbook keeping, and to Paul Marinari and Astrid Vargas from the USFWS National Black-Footed Ferret Conservation Center.
Figure 1. Age distribution of biliary cysts and various neoplasms in postmortem examination of adult (> 1-yr-old) black-footed ferrets, 1988-1998. n = 171 (age was available for 98 males and 73 females).
AUTOMATING MORBIDITY AND MORTALITY SUMMARIES FOR ZOOS: TRYING TO IMAGINE THE FOREST FROM CROSS-SECTIONS OF INDIVIDUAL TREES

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Abstract

As we approach the new millennium, continued growth of human populations and shrinking wildlife habitats make it seem likely that, at least during the next few decades, zoos will become the last refuges for more rather than fewer species. Long-term survival of small populations of captive wildlife requires intense management that embraces a multitude of scientific disciplines, including genetics, nutrition, ethology and veterinary medicine. The Species Survival Plan (SSP) committee was the response of the American Zoo and Aquarium Association (AZA) to this need to actively manage, as a single population, a species that is often housed in small groups at a number of institutions that are geographically scattered across North America. Under these conditions, seeing the “big picture” is one of the greatest challenges that we face, simply because most of us get to work directly with only a small fraction of the total captive population.

The ISIS Physiological Normals project was one of the first attempts to provide zoo veterinarians with clinically important information derived from multiple institutions. During the first 15 yr of this project, ISIS collected results on more than 27,000 blood samples contributed by 54 different institutions. The reference ranges calculated from these data provided clinicians with the first published set of “normal” hematology and blood chemistry values for a much greater number of wildlife species than was available in the literature. In 1992, the project began to accept records from the MedARKS software package and in the last 6 yr has added almost 50,000 sample records and increased worldwide participation to over 100 institutions. The next publication, scheduled for August 1998, will make more than 2000 pages of reference ranges available to clinicians on a CD-ROM in HTML (World Wide Web page) format.

Using these calculated reference values to automatically mark any test results that are outside the expected range provides the clinician with some diagnostic assistance, but it is still a broad gap between indicating abnormal test results and arriving at a diagnosis. Closing this gap will require much more knowledge about the common diseases and problems that occur in a particular species. Information about the diseases of captive wildlife is mainly recorded in the literature as case reports, in disease reviews, and in some specialized texts. The medical literature has been systematically reviewed and summarized for only a limited number of species and I am aware of only three species where existing medical records from multiple institutions were reviewed in an attempt to more fully categorize the diseases and problems of a species. Both literature surveys and reviews of medical records are labor intensive and time consuming. Thus, it is a fairly rare event for a zoo clinician to have information on the incidence and prevalence of various problems in a particular species and
it is difficult to see how this might change if we continue to rely on manual analysis techniques.

Over the past decade, the MedARKS software program has been adopted as the “de facto” standard for computerized medical records in zoos. Currently there are more than 1.5 million medical records in the MedARKS format, making this the single largest computerized database of medical information on captive wildlife. Unfortunately, it is also a distributed database with those records scattered across 150 institutions in at least a dozen countries and in several languages. If this information could be assembled in one location, it is estimated that it would occupy about 3 gigabytes of disk space. However, it would only be worth undertaking that effort if the information could then be more easily used to advance the field of captive wildlife medicine.

This adoption of a standard format for medical records has allowed experimentation with merging information from a number of institutions into “library” disks that can be accessed through the MedARKS software. Early efforts have included a disk of giraffe immobilization drugs and doses and a disk of carfentanil immobilization records for a variety of species. Attempts to assist SSP medical advisors have also led to limited experiments that consolidate all MedARKS records for an individual species. As an example, the assembled MedARKS records for Malayan tapir (Tapirus indicus) contain 23 animals with problem-oriented medical records (defined medical problems in the format of a “master” problem list). A few minutes work with this disk allows you to tally those problems that are recorded for more than one of these animals (Table 1). While this partially automated analysis of medical records is far easier than the equivalent analysis using paper records, it complete ignores the 54 other tapirs where the MedARKS records were not problem-oriented and for which only text records were available. The question of whether those animals with problem-oriented records accurately reflect the diseases/problems seen in the overall population cannot be answered without a much greater in-depth (and time-consuming) investigation into the medical records.

As we continue to move from paper record systems to computerized medical records, the potential certainly exists for automatically extracting the common causes of morbidity and mortality for a species. There is even the potential to begin to address issues of actual disease incidence and prevalence within a population. The traditional problem-oriented record with a master problem list is well suited for automated analysis, but, as most clinicians have discovered, it is also more difficult and time-consuming to maintain than simple text records. Automated searching of text records for a series of words or phrases is a relatively simple process and can quickly be used to produce a list of records for further review. However, techniques for automatically extracting critical information from text records and summarizing the causes of morbidity and mortality are in their infancy. Developing effective algorithms to extract morbidity and mortality information from text medical records poses the next great challenge.

In summary, as we enter the 21st century, we will need improved access to the experience and knowledge that reside in our medical record files. Theoretically, computers offer a means to more efficiently tap into this knowledge and the widespread acceptance of MedARKS software by the zoo community provides a common format for sharing our medical experience. The problem-oriented
record, with well-defined problems and onset and resolution dates, offers the easiest means to automate the analysis of medical records. Computer assisted extraction of useful information from text records is a much more difficult process. However, the current reliance of many zoo clinicians on text records means that this is a challenge that we will need to face even as we promote the use of the problem-oriented approach to keeping medical records.

<table>
<thead>
<tr>
<th>Medical Problem</th>
<th>Percentage with this problem in medical history*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lameness/foot problems</td>
<td>35%</td>
</tr>
<tr>
<td>Lacerations/abrasions/bite wounds</td>
<td>30%</td>
</tr>
<tr>
<td>Anorexia/partial anorexia</td>
<td>30%</td>
</tr>
<tr>
<td>Dermatitis/dermatomycosis</td>
<td>26%</td>
</tr>
<tr>
<td>Conjunctivitis/ocular discharge</td>
<td>13%</td>
</tr>
<tr>
<td>Parasites</td>
<td>8%</td>
</tr>
<tr>
<td>Self-inflicted trauma</td>
<td>8%</td>
</tr>
</tbody>
</table>

*Problem occurs at least once in the master problem list for an individual.
EXPERT SYSTEMS, BOON OR PITFALL?

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Abstract

Advances in software development tools have made it feasible for the relatively rapid construction of visually complex multimedia tutorial and expert systems with relatively little computer expertise. These significant advances allow true experts in a discipline or clinical technique to generate useful materials with less time investment. However, this ease of development requires that end users carefully evaluate programs for content and data management algorithms when they consider using them in their practice.

Tutorial programs are designed to convey information. Evaluation of the quality of the content should be the most important evaluation consideration. Who is compiling the information? What are their qualifications? How much direct input into the program have they had? This last question can be difficult to assess just from promotional materials or title screens in the program. Look for the level of referencing and degree of internal qualification of information. Can you identify original sources with reasonable effort? Are appropriate caveats presented with speculative information?

A sophisticated interface with high quality visual and audio effects is attractive and can enhance the learning experience. However, not only can it disguise weak content, it can obscure the availability of excellent content. Occasional use of images for purely esthetic purposes is reasonable and to be expected, but the majority of images and sounds should contribute to the understanding of the user. Images should be easily related to textual materials and labels. The interface itself should not be exceptionally complex and should, ideally, allow for alternate ways of navigating the program. A key requirement for programs destined to be used more than once is the ability to rapidly search the information available and move to selected points. Although there are applications for software that is used only once and requires that you move linearly through a single path to each piece of information in sequence, most users prefer to be able to customize their path. When testing new software, try searching on various subjects of interest to you. Not only will this give you a better idea of the content quality, but it will allow you to evaluate the speed and usefulness of the search engines embedded in the program.

Expert systems take the user a step further than tutorial systems. Expert programs can include tutorial subroutines. This may be a major benefit, but this is not a mandatory consideration. Expert systems are designed to take input from the user and help in decision parsing. All of the concerns related to tutorial programs apply to expert systems. The quality of data within the program
determines the quality of the decisions it can make and graphic displays can enhance or obscure the understanding of the basis of those decisions. For veterinarians, most of these programs are focused on disease diagnosis or the generation of differential diagnoses. It is critical that the user be aware of the algorithms being used to generate these decisions. Levels of confidence for the decisions should be readily apparent when using the program. High quality programs should allow the user to select among decision making algorithms and help menus or manuals should clearly explain the mathematic basis of the parsing occurring with each option.

Several of the commonly available algorithms include Factor inventory, Weighted factors, Bayesian methods, and Combination Analysis. Each of these can be done in several different ways but basically each represents an increase in sophistication. Factor inventory simply tallies the number of attributes entered by the user that match those included in the database. It is the least sophisticated approach to ranking diagnoses and has obvious limitations. However, when the data for frequency of occurrence of diagnostic factors or symptoms is weak, this method has the advantage of not being affected. Weighted factors greatly improve the likelihood of accurate parsing of a differential as they take into account the fact that all attributes of a disease are not seen with equal frequency in all cases. Of course, accuracy of these methods is affected by the match between the programmed expected frequencies and reality. Bayesian methods are based on Baye’s Theorem which assigns a quantitative change to the probability of a diagnosis based on absence as well as presence of a factor. The major advantage in these methods is that they incorporate the impact of the absence of a finding in the decision parsing. Combination analysis considers the possibility that multiple diseases may be present at one time and may better explain a set of findings. The complexities of the algorithms involved in assessing combination potentials vary greatly from simple disease attribute addition methods, which are most commonly used to very sophisticated modifications of probability theory.
CITATION DATABASES FOR ZOO AND WILDLIFE VETERINARIANS

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Abstract

Expanding information resources have broadened the options for those seeking information on veterinary topics. Governmental, university, and private organizations offer a variety of mechanisms that can effectively deliver answers to the questions that arise in research or in practice. However, there are many choices to be made when seeking informational services. A personal information-seeking style should be first identified, and then a specific service can be matched to it. In addition, the format of information products must be considered as well. For instance, is gleaning pertinent facts from an abstract an option, or is obtaining a full text article a necessity? Is current or retrospective literature needed? Another issue is cost; those on limited budgets may need to obtain information for free, but if you can afford their services, for-profit institutions can act as your personal librarian. All of these issues come into play when evaluating informational resources.

Information-Seeking Styles

A starting point in finding information sources might be to evaluate personal styles of learning or problem solving. These might be relying on memory, asking questions of other veterinarians, or consulting personal books, journals, computerized and print indexes, and files. Other approaches may require the use of an intermediary, such as a librarian or research firm. The approach that creates the broadest information base for questions that arise in treating multiple animal species requires the use of computerized and print indexes. The trend in veterinary schools is to require course work in bibliographic searching, so many new graduates have learned how to formulate basic search strategies and choose the correct database to search. If the “do it yourself” approach is daunting, academic librarians and other information professionals are often willing to evaluate search strategies or to formulate them using specialized vocabularies. Libraries associated with schools of veterinary medicine also offer a variety of services to veterinarians in their state or region. These services generally include computer bibliographic searches of veterinary databases with little more than a cost-recovery fee. A listing of the veterinary libraries in the United States and Canada and the library services available at each can be found in the American Veterinary Medical Association (AVMA) Membership Directory and Resource Manual.

Citation Databases

Once the topic is defined and the search strategy constructed, a decision about what database to search must be made. The most comprehensive databases have historically been compiled by
governmental agencies. Agricola, Medline, and CAB International are all examples of government-created compilations that contain citations from veterinary journals. These are the computerized versions of the Bibliography of Agriculture, Index Medicus, and Index Veterinarius, respectively. Their dates of coverage span almost four decades making these three databases ideal for retrospective searches. Private database vendors, such as DataStar and Dialog Knight-Ridder, Scientific Technical Network, Ovid Technologies, and SilverPlatter offer access to a multitude of these databases through subscriptions to libraries, individuals, and companies. These databases are most cost-effectively utilized at university or college libraries, because searching is free or available for a nominal charge. The AVMA’s NOAH site offers access to the ISI/NOAH World Veterinary Index and the Veterinary Literature Database.

Because many databases were compiled by the government with taxpayer monies, the trend is to allow free use by the public. The National Library of Medicine provides Internet access to Medline in the form of PubMed and Grateful Med and the National Agricultural Library has plans to make Agricola available in the future. The querying mechanisms that allow searching of these databases have evolved to the point that a novice can successfully structure searches and obtain relevant citations. Other databases such as TOXLINE, CANCERLIT, and the CAB International databases are available for free searches from non-profit or corporate sponsored sites. The Fish and Wildlife Reference Service database consists of more than 25,000 bibliographic citations and covers many State resource agency project reports that are not published in journals. This is a free search database, with a CD-ROM version available from the National Information Services Corporation.

Securing Documents

Once the citation is identified, there are several methods available to obtain the reference. A public library may be a useful starting place. It is usual policy that a public library will attempt to obtain book and journal information through interlibrary loan services for its clients. A point to keep in mind, is that interlibrary requests usually take 4-6 wk to be filled. University or college libraries usually maintain good journal collections, and it may be worth the time to travel to these, and make photocopies of needed materials, minding copyright restrictions. However, if none of these solutions are workable, private companies offer their services as well. Some free-search databases have associated fee based document delivery options, such as Loansome Doc for Medline, and the Fish and Wildlife Reference Service. An Internet-based current awareness service is Uncover. This database contains over 5 million articles from 1989 to the present that are available through an online order system. The database can be scanned for free and users only pay for the articles they request. Uncover’s Reveal Alert Service is a fee-based system which allows subscribers to receive table of contents or results from user-created searches directly to an E-mail address. Other providers of current references and documents are the Institute for Scientific Information (ISI) with Table of Contents information from over 938 journals in the Agriculture, Biology, and Environmental Sciences edition, as well as the BIO-JOURNALS/bionet.journals.contents Table of Contents Archive. Options also exist for obtaining author’s addresses so reprints can be requested with no charges involved. EBSCO Document Service offers a full-service document delivery service in the form of article photocopies, reprints, and conference proceedings. The Canada Institute for
Scientific and Technical Information (CISTI)\textsuperscript{20} is another supplier of scientific, technical and medical information. Clients can use the Internet to search CISTI’s online catalog and order documents from the more than half a million books, reports, and conference proceedings from around the world.

Other information outreach programs exist that can also obtain books, run retrospective literature searches, or put you in touch with an expert in your field. One such organization that does all that in addition to document delivery is the Wisconsin Tech Search,\textsuperscript{21} an information outreach program located at the University of Wisconsin. This organization offers act as a personal librarian and bills on a cost-recovery basis.

\textbf{Resources}

1. DataStar and Dialog Knight-Ridder - http://www.krinfo.com/
3. Ovid Technologies
5. AVMA NOAH - http://www.avma.org/
18. BIO-JOURNALS Table of Contents Archive - http://www.bio.net/BIO-JOURNALS.html
(ACAD 1.0): THE ANIMAL CAPTURE AND ANESTHESIA DATABASE†

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Abstract

Introduction

The success and growth of any discipline is dependent upon communication and interaction between primary researchers and those performing field applications. If communication or information exchange is inefficient, poor techniques are repeated, and growth is slow. If information is readily accessible and exchanged freely, techniques are refined rapidly, and rapid improvement ensues.

While expertise on restraint, immobilization, anesthetic, and analgesic techniques exists for many species, access is limited in our current system of information dissemination. Currently, research on these topics is published in a multitude of periodicals and references, many of which are foreign or specialty journals with limited circulation. Lag time between development of new techniques and publication also occurs. Other techniques are disseminated by personal communication only, without ever being published. Lastly, techniques which are associated with mortality and/or morbidity may never be reported or published.

Additionally, much of the available information is incomplete. Many references provide only dosage information, while information concerning drug concentration, time parameters (induction, working restraint time, recovery time), confinement, and emotional status of the animal are not included. These parameters are often required to determine applicability of the technique, and to assist in making rational anesthetic decisions.

Anyone working with exotic species can attest to the often urgent need for anesthetic information, while locating a source, acquiring original research material, and extracting the needed data can be difficult and time consuming.

Thus, there is a need for a central information source of readily accessible bibliographic information, and rapid access to reliable, detailed species specific anesthetic, immobilization, capture, and analgesic information. The Animal Capture and Anesthesia Database (ACAD 1.0) meets this need.
Objective

The purpose of the ACAD is to expand access to validated anesthetic techniques, and to encourage better data collection and sharing of information among professionals, by organizing and assembling a comprehensive, peer reviewed, central computerized database for animal capture, immobilization, and anesthetic techniques. This database would be readily accessible electronically, affording quick access to pertinent information necessary to make informed choices for anesthetic regimens.

What is the structure of ACAD?

The ACAD is a collection of data records in a computerized relational database. Records consist of synopses of anesthetic/analgesic research, and procedures collected from a variety of sources including peer reviewed references, textbooks, meeting proceedings, and personal communication of field techniques from primary researchers.

How does ACAD function?

Step 1: The opening screen lists all Data Records in alphabetical order by species.

![ACAD Database Screenshot](image-url)
Step 2: Records can be sorted by selecting Retrieval Topics or Keywords.

Retrieval Topics/Keywords Include: Species (Deer, Elk, Tigers, etc.), Family (Cervids, Canids, Camelids, etc.), Drug Used (ketamine, carfentanil, xylazine, telazol etc.), Warnings (complications reported with the use of techniques or medications in various species, documented drug interactions, or studies which documented the LD 50), Pre-Anesthetic Considerations, Pathology (Drugs used in sick and debilitated animals which afforded survival), Clinical Pathology (Anesthetic effects on Clinical Pathology results), Reproduction (Effects in Pregnant Animals, or during obstetric procedures), Oral Medications, Author, and Title.

By selecting a keyword, Data Records associated with the keyword are retrieved and listed. These records are defined for “quick scan” by the Species, Drug Used, Confinement, Author, and Date. In this example, the keyword “Cervids” is selected, and all “Cervids” linked records are displayed.
Step 3: To view a specific Data Record, double mouse click on the selection. This opens the Complete Data Record which contains the following information:

   a. Source of information (journal, book, proceedings, or personal communication)
   b. Species
   c. Scientific Name
   d. Drug Used including Dosage and Concentration
   e. Induction Time (time from medication injection to loss of head lifting response when mechanically stimulated)\(^1\)
   f. Antagonist (if used) including Dosage and Concentration
   g. Total Restraint Time (time from loss of head lifting response to regaining head lifting response.)\(^1\)
   h. Total Time to Recovery (time from loss of head lifting response to standing)\(^1\)
   i. Type of Confinement the animal was in, Degree of Restraint prior to anesthesia, and Method of Drug Delivery.
   j. Number of Animals in the study
   k. Mortality Rate
   l. Any pertinent Comments about the procedure
   m. Warnings
   n. Keywords (all keywords by which the record can be retrieved.)

(Dosages peer determined to be “Preferred Dosages” are indicated by Double Asterisk**)  

In the example below, we double mouse clicked and opened this Complete Data Record on Black Bear.
What other information is readily accessible via the ACAD?

Techniques associated with mortality or morbidity are scattered and difficult to find in the literature. ACAD organizes this information and provides rapid access to Species, Drug, and Drug Interaction Warnings.” Pre-anesthetic considerations, regimens found safe in debilitated animals, and the effects of anesthetics on clinical pathology results and reproduction are also easily retrievable.
How do you obtain access to the Animal Capture and Anesthesia Database?
Access will be by internet, hard copy, and distribution of periodically updated discs.

Conclusions

The Animal Capture and Anesthesia Database (ACAD 1.0):

* Centralizes rapid access to validated, peer reviewed, detailed species specific anesthetic, immobilization, capture, and analgesic information.

* Allows comprehensive species and topic bibliographies to be readily obtained.

* Alerts and warns of anesthetic related mortality or morbidity, and the effects of anesthetic procedures on clinical pathology results or reproduction.

* Encourages better data collection and sharing of information among professionals.

* Enhances the ability to make informed choices on anesthetic regimens in captive and free-ranging species.

ACKNOWLEDGMENTS

This work was supported by Safe-Capture International, Inc. and the Safe-Capture International Foundation—promoting animal welfare by collecting, organizing, and disseminating information on anesthetic regimens and techniques useful in the handling of free ranging and captive species.

LITERATURE CITED


WILDPRO MULTIMEDIA: AN ELECTRONIC MANUAL ON THE HEALTH, MANAGEMENT, AND NATURAL HISTORY OF CAPTIVE AND FREE-RANGING ANIMALS

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Abstract

Professionals concerned about the health and management of wild animals, both free-ranging and captive, often encounter situations involving unfamiliar species or conditions. The breadth and depth of the information resources available to resolve these issues varies widely depending on position and location. This is particularly important in developing countries and remote sites. Paradoxically, in many of these circumstances access to textbooks or libraries are limited, but computers are readily available. To address these needs, the WILDPro project is taking an innovative approach which could redefine the structure of reference materials. In contrast to standard print textbooks and journals, containing prose organized in a linear fashion, WildPro presents facts succinctly, with multiple rapid links to other relevant data. The extensive use of photographs, video and audio components reinforces the material, in a manner unavailable in books. Through this method, information ordinarily contained in a shelf of print volumes can be condensed and distilled into convenient CD-ROM form, as well as accessed through the Internet World Wide Web.

WILDPro’s organizational structure is inspired by the interrelationships of individual animals, disease agents and the environment, which underlie the presence of health or disease in all animals. Information arranged within these groups, is presented in an intuitive and practical manner, using dynamic interconnecting links, and offered in a manner to be accessible to those of various backgrounds and educational levels. The applications of this concept can best be demonstrated by a variety of potential WILDPro applications:

- A biologist/veterinarian working in a conservation area in Africa is asked to assist in the translocation of a group of gazelles from one area of the park to another. To accomplish this task, he/she would need to address the best procedure for animal capture, ranges of body weights, recommended doses of anesthetic drugs, and standards of construction transport containers.

- Port customs officials find improper paperwork on shipment of marmosets, and must delay the cargo until the documents are corrected. They must then determine the best conditions for holding these animals considering their needs for appropriate food, temperature, humidity, etc.
- Patrolling rangers discover 500 dead birds around the edge of a park lake. They must rapidly determine what samples and information are needed so that the cause of this mortality can be determined, and actions taken to reduce further losses.

To assess the viability and relevance of the concept, a WILDPro prototype has been created and distributed to over 300 wildlife professionals (biologists, CITES officials, zoo directors and curators, wildlife managers and veterinarians) in 61 countries for their assessment and comments. Of the 128 respondents, 96% felt that a fully developed version of WILDPro will be useful to wildlife professionals; 82% further indicated that they would be likely to use WILDPro as a reference on a regular basis. Although the prototype is representative of the potential future product, it is not robust enough to manage the profusion of data, and the needs for constant revisions and expansions, therefore the development of a complex database architecture is now underway. Once completed, data input can begin, with the availability of at least one module late in 1999.
FACTORS INFLUENCING INTERPRETATION OF INDIRECT TESTING METHODS FOR TUBERCULOSIS IN ELEPHANTS

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Abstract

Serologic and other laboratory tests (such as BTB, ELISA and gamma interferon) are often used in conjunction with the intradermal tuberculin test to detect tuberculosis (TB) in animals.2 The skin test is considered the “gold standard” in domestic cattle and humans, and the BTB test has been highly rated for use in cervid species. However these indirect methods for TB diagnosis have not been proven valid in most exotic species susceptible to the Mycobacterium tuberculosis complex (which includes M. bovis) infection. In addition, many of the tuberculin skin testing methods used in exotic species are not uniform in terms of tuberculin type(s) and sites used and interpretation of the end points.3

Recently, cases of TB due to M. tuberculosis have occurred in several elephant collections located in the Midwestern and western United States. In these cases, intradermal and serologic tests did not correlate well in elephants that were culture-positive for M. tuberculosis.1 This led to the development of guidelines to control TB in elephants in which culturing the trunk for TB organisms is the key diagnostic procedure (U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Animal Care, Guidelines for the Control of Tuberculosis in Elephants, November 1997). However, intradermal and serologic testing have been encouraged in order to collect further data as a research effort to establish the validity of these ancillary tests in elephants.

In April of 1997 a female Asian elephant (Elephas maximus, elephant #1) at the National Zoological Park had a 3-cm reaction to a ppd-bovis intradermal skin test administered while being clinically evaluated for an intermittent low-grade cough. A BTB test, taken the day of the tuberculin test (4/27/97), was negative. A comparative test 9 days after the initial intradermal test (5/2/97) with balanced ppd-bovis and ppd-avium tuberculins showed a 3:1 avium:bovis reaction indicating the original skin test reaction was most likely from cross-reactivity to atypical mycobacteria. A blood sample taken on the same day as the comparative test (5/2/97), however, came back with a strongly positive ELISA test for M. tuberculosis (0.2-0.5 = weakly positive to strongly positive) in which only M. tuberculosis antigens were used (Fig. 1). Using banked serum samples acquired in 1995, 1996, and 1997, further ELISA testing was performed using antigen from M. tuberculosis and M. avium (Fig. 2). All samples acquired prior to and including the day of the original skin test (4/23/97) were in the ± zone but the sample taken 9 days after tuberculin testing was strongly positive. The responses to M. avium antigen were greater than the responses to M. tuberculosis antigen.
Subsequent blood samples taken from elephant #1 taken at monthly intervals in 1997, showed a continued rise of the ELISA test well into the positive zone over the next month followed by a steady decline towards the negative zone over the following 2 mo (Figs. 3a and 3b). ELISA tests were performed on two other female Asian elephants (elephants #2 and #3) and one female African elephant (*Loxodonta africana*, elephant #4), using stored serum (no tuberculin testing during this period), serum taken the day the animals were given comparative tuberculin skin tests, and, subsequent to the comparative tuberculin testing (elephants # 2 and #3). As with elephant #1, the ELISA readings rose in elephants #2 and #3 after tuberculin testing with elephant #2 approaching the positive zone (data not shown). Elephant #4 was not skin tested and ELISA readings remained in the normal zone (data not shown). In addition, elephants #1 and #4 tested “positive” for *M. bovis* antigen using the BTB test.

Elephant #1 recovered from its vague illness after antibiotic treatment. Trunk cultures performed on the animal three times within a 7-day period were all negative for *Mycobacteria* sp. as were trunk cultures performed on the other three elephants during the course of the indirect testing. All six of the elephant keeper staff had negative tuberculin tests within the same time period.

This experience demonstrated some of the pitfalls in trying to rely on ancillary tests for determining the tuberculosis status of an elephant collection. None of the animals in our herd have had any previous exposures to other elephants for the past 10 yr. The initial reaction to the ppd-bovis in elephant #1 was attributed to cross-reactivity to non-tuberculous mycobacteria as determined by the comparative skin test. The “positive” ELISA and BTB tests performed on serum taken after the skin tests in elephant #1 were attributed to tuberculin exposure. Taken out of context, this finding may have placed the animal in jeopardy if interpreted as positive for tuberculosis.

It is beneficial to perform comparative testing in elephants and even to initiate testing using the comparative procedure. It is also important when using ELISA and other serologic tests to request that both *M. avium* and *M. tuberculosis* (and/or *M. bovis*) antigens be compared in the testing procedure. Elephants are constantly exposed to saprophytic *Mycobacteria* sp. and can be readily colonized by these “atypical” organisms by their habit of trunk “bathing” with water and soil. Most of the ELISA reactivity in the tests shown above (Fig. 3b and not shown), were more intense with the *M. avium* antigens, supporting the cross-reactivity interpretation of the comparative test in elephant #1. In addition, from our temporal studies of stored sera and from subsequent blood sampling before, at the point of, and after skin testing, it was evident that tuberculin exposure influences the ELISA and to some extent the BTB tests and can cause false-positive results in elephants.

As of April 1998 (approximately 1 yr after the initial testing), all elephants are well and the cough in elephant #1 has not recurred. The temporal serologic studies were continued to determine if, and at what point, the ELISA readings return to pre-tuberculin exposure levels.

In this time of heightened activity of testing for TB in elephants, it will be important to continue studies of the indirect tests and correlate the findings with the culture results. This should result in...
establishing more reliable tests for diagnosing TB in the earlier (non-shedding) stages of the disease and for prognostic purposes.

ACKNOWLEDGMENTS

This work was supported in part by the Friends of the National Zoo (FONZ) Kumari Elephant Fund. We thank Dr. Sang Nae-Cho, Colorado State University, National Veterinary Services Laboratories’ mycobacteriology staff, Ames, IA and Dr. Charles Thoen, University of Iowa for laboratory assistance, and the National Zoo’s elephant staff for special assistance while evaluating and testing the elephants.

LITERATURE CITED

ASIAN ELEPHANT #1
(retrospective serum tests before tuberculin exposure)

Fig. 2 ELISA

ASIAN ELEPHANT #1
Antigen: M. tuberculosis Culture Filtrate Protein
(continuation of serum tests after tuberculin exposure)

Fig. 3a ELISA

ASIAN ELEPHANT #1
Antigen: M. avium Culture Filtrate Protein

Fig. 3b ELISA
CLINICAL EXPERIENCE WITH FATTY ACID SUPPLEMENTATION IN A GROUP OF BLACK RHINOCEROS (Diceros bicornis)

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Abstract

Evaluation of fatty acids (FA) in the diet of humans is an area of intense research. Initial indications are that the ratio of linoleic to linolenic acid is more important than the levels of each in the diet of humans. This also appears to be true in some laboratory animals. Numerous physiologic abnormalities are encountered when improper dietary ratios or deficiencies are present. These include increased capillary permeability, phospholipid deficiency, changes in reproduction, mitochondrial “swelling” resulting in altered cellular respiratory and phosphorylating mechanisms, and various dermal abnormalities. These deficiencies are accompanied, in most animals, by significant decreases in serum dienoic and tetraenoic FA levels with concomitant increases in monoenoic and trienoic FA levels.

A recently imported female black rhinoceros (Diceros bicornis michaeli), (“Lucy”), demonstrated two episodes of hyperbilirubinemia, moderate hypophosphatemia, hypercalcemia, elevated alkaline phosphatase, anemia and monocytosis less than 12 mo after arrival. Both episodes coincided with major stressful events. The episodes resolved over the course of 2-3 mo. The animal appeared to improve with supportive care and the supplementation of fresh browse flown in weekly.

Based on a suspicion of FA imbalance contributing to Lucy’s medical problems, serum % relative FA profiles were evaluated in five black rhinoceroses before and after supplementation with a flaxseed based FA supplement (Missing Link, Designing Health, Valencia, California 91355 USA). FA % relative analysis of this product revealed 50% linolenic acid and 18% linoleic acid. The supplement was administered on a daily basis to two 4-yr-old, captive born male rhinoceroses (“Rudy” and “Tucker”), one long-term captive rhinoceros (“Dal49”), and the recently imported 3-yr-old female rhinoceros (“Lucy”). In July of 1997, a 3-yr-old female eastern black rhinoceros (“Luyisa”) was imported from the Addo Elephant Park in South Africa to the Kansas City Zoological Gardens. Initial FA evaluation within 1 mo of arrival demonstrated FA levels comparable to the captive, supplemented rhinoceroses. Therefore, FA supplementation was not initiated. Several FA profiles were evaluated over the course of 4 mo after arrival, whereupon changes consistent with the initial unsupplemented rhinoceroses were noted (Table 1).

Of 37 FA’s profiled, consistent changes were noted in octadecadienoic (linoleic-18:2), octadecatrienoic (linolenic-18:3), gamma linolenic omega 6 (18:3), eicosatrienoic omega 6 (20:3), and eicosatetraenoic omega 6 (arachidonic-20:4) following supplementation. In addition, the ratios
of 20:3/20:4, and 20:3/20:5 “improved” in all five rhinoceroses supplemented (Table 1). In the human and laboratory animal literature, the 20:3/20:4 ratio may predict a FA (linoleic) deficiency or imbalance.\(^1\) Ratios above 0.4 are indicative of an imbalance.\(^1\) The 20:3/20:5 ratio may predict a FA (linolenic) deficiency.\(^1\) Again, ratios above 0.4 are indicative of an imbalance.\(^1\) In every rhinoceros evaluated, these ratios improved after supplementation.

Diets in four of the rhinoceroses were evaluated for FA composition. Dietary analysis on an as fed weight:weight basis without supplementation revealed a linoleic to linolenic acid ratio of 3:1. Diets were composed of alfalfa hay, a ground aspen pelleted feed (Mazuri Moose Maintenance, Purina Mills, Inc. St. Louis, Missouri USA), small amounts of oranges, a commercial salt block, and various browse items (mulberry, *Morus* sp.; *Pyracanthus* sp.; honeysuckle, *Lonicera* sp.; and willow, *Saliaceae* sp.) when available.

In the human and laboratory animal literature, the trienoic acid that accumulates during fat deficiency is eicosatrienoic acid.\(^1\) In each rhinoceros; the eicosatrienoic acid levels decreased or disappeared after supplementation. In the most recently imported rhinoceros (“Luyisa”) eicosatrienoic acid levels gradually increased without supplementation. After supplementation, eicosatrienoic acid levels decreased.

Using the human and laboratory animal results of FA evaluation as a basis for comparison, FA supplementation may be indicated in the black rhinoceros. The only addition to the diets of these rhinoceroses was the FA supplement. Recent research has demonstrated high levels of linolenic acid are contained in fresh browse items favored by the rhinoceroses.\(^7\) In addition, it has been demonstrated that FA levels in fresh-cut browse decrease dramatically over a short period of time.\(^8\) During the winter of 1997, the ill rhinoceros (“Lucy”) was not supplemented with fresh browse items, but continued on the FA supplement. No recurrence of clinical signs has occurred despite additional stressful events.

Changes in the FA profiles were consistent in all five rhinoceroses. Based on initial results, and compared to the human and laboratory animal literature, fatty acid supplementation appears to be a component of Lucy’s improvement. To date, 1 yr after supplementation with FAs, this rhinoceros is clinically normal. Further feeding trials are continuing with analysis of each dietary component for FA determination.

To date, no adverse side effects of supplementation have been noted. Potential adverse effects of long-term supplementation will have to be investigated before recommendations can be made regarding supplementation of this product to rhinoceroses.\(^4\)

**ACKNOWLEDGMENTS**
The authors would like to thank Dr. Mike Nance, Garden City, Kansas, Dr. Bob Collette, Missing Link Products, Designing Health Inc. Valencia, California. Additional appreciation is extended to the Kansas City Zoo’s rhinoceros management team, Dr. Chris Miller, Miami Metrozoo, and Dr. Tom Alvarado, Dallas Zoo, for contributing serum samples from their black rhinoceros for continued evaluation of fatty acids in rhinoceros.

LITERATURE CITED


Table 1. Serum % relative fatty acid profiles in five black rhinoceroses (Diceros bicornis) before and after fatty acid supplementation.

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<td>0.72</td>
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<td>0.21</td>
<td>&lt;0.1</td>
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*Before FA supplementation
**After FA supplementation

INVESTIGATION OF DIAGNOSTIC PARAMETERS AND TREATMENT REGIMENS FOR TUBERCULOSIS IN BONGO ANTELOPE (Tragelaphus eurycerus)
Abstract

The current status of the National Cooperative State-Federal Bovine Tuberculosis Eradication Program has significant implications for the zoo community. A 1997 U.S. Department of Agriculture report states that “the goal of eradication of bovine tuberculosis in the United States is biologically and economically feasible.” Further, the National Tuberculosis Program Review Team of the National Academy of Sciences (NAS) recommends that the “U.S. Department of Agriculture should vigorously pursue eradication of bovine tuberculosis in cattle, bison, elk, deer and other farmed exotic hoofed species…and although improvements in tests used to detect infection appear possible and should be pursued, the committee believes existing diagnostic technology is adequate to support a vision of the total eradication of bovine tuberculosis from all domestic species, or any other species on farms unless it is introduced from another country.” The U.S. Department of Agriculture does, however, acknowledge that zoos and wildlife should be excluded from these definitions until we better understand how to manage and eliminate tuberculosis in these groups.1

It remains questionable whether existing technology is adequate to reliably diagnose tuberculosis antemortem in non-domestic hoofstock species. The lack of correlation between the “gold standard” intradermal test and actual disease has been well documented in a variety of exotic species and has often resulted in unnecessary euthanasia.2-5 While the test and slaughter method has proven effective in domestic species, it is neither desirable nor acceptable for endangered wildlife. Unfortunately, a lack of information on treatment combined with U.S. Department of Agriculture imposed restrictions often make euthanasia the only alternative.

Following the death of an adult male bongo antelope (Tragelaphus eurycerus) due to Mycobacterium bovis infection, an exposed herd of six female bongo was screened diagnostically five times over a 3-yr period. Diagnostic evaluation included intradermal skin testing; thoracic radiography; cytology, culture and Mycobacteria test direct (MTD) of tracheal wash samples; ELISA serology; and the blood tuberculosis test (BTB). Treatment was initiated with isoniazid (Major Pharmaceuticals, Chicago, IL USA), rifabutin (Pharmacia-Upjohn Co., Kalamazoo, MI USA), and ethambutol (Lederle Laboratories, American Cyanamid, Pearl River, NY USA) in order to obtain pharmacokinetic information and evaluate the feasibility of treatment.

Immediately following the death of the male, five females (animals #1-5) were administered a single cervical test (0.1 ml Bovine PPD; U.S. Department of Agriculture, Animal and Plant Health Inspection Service). One young female (animal #6) was not tested due to its age. Four of the five
animals tested had suspect reactions and one was negative. The same five animals were administered comparative cervical tests (CCT) 60 days later. Two animals were considered positive reactors (animals #1 and 2) and three were considered negative (animals #3, 4, 5). The CCT was administered 18 mo post exposure to all six animals and four were considered reactors (animals #1-4), one was negative (animal #5), and the young, previously untested, female (animal #6) was suspect. Skin biopsies of the test sites were compatible with delayed hypersensitivity reactions in the four positive reactors. Animals #5 and #6 both died 6 mo after the second CCT. Neither animal had histologic evidence of tuberculosis and *M. bovis* was not isolated by culture from either animal.

*Mycobacterium bovis* was not isolated from tracheal washes taken from the six bongo females (animals #1-6) submitted at 1, 18, 21 and 23 mo post-exposure although *M. flavescens*, *M. kansasii*, and a scotochromagen were isolated from each of three samples. MTD results were negative on all six animals at 18 mo post-exposure. Cytology of tracheal wash samples was not suggestive of mycobacterial infection (no evidence of foamy macrophages) in any of the samples.

Radiographs taken at 18, 21 and 23 mo post-exposure showed no evidence of tuberculosis. ELISA serology using a five-antigen assay was evaluated at 18, 21 and 23 mo post-exposure. One of the six animals was positive using a formula derived from known positive domestic bovids and all six were considered positive when evaluated using a formula derived from known positive cervids.

Twenty six samples were submitted for BTB testing. Of those, one sample demonstrated evidence of *M. bovis* reactivity, four samples were negative (no reaction to either *M. bovis* or *M. avium*), and 21 of the samples were classified as “no data.” “No data” is reported when the assay controls do not perform to expected levels because of damaged cells or an absence of viable cells. This may be a result of stress to the animal, sedation effects, or cell damage during bleeding. In this study, BTB results differed between samples which were collected during narcotic immobilizations and samples collected during standing sedation. Of the 22 samples collected during narcotic immobilization, one sample showed *M. bovis* reactivity, one was negative and 20 were reported “no data.” Of four samples collected in a restraint chute under haloperidol (McNeil Pharmaceutical, Spring House, PA USA) sedation, three were negative and one was reported “no data.”

Preliminary pharmacokinetic data indicates that blood levels of isoniazid approaching acceptable human levels can be achieved at a dose of 20 mg/kg orally. Rifabutin does not seem to be well absorbed at the doses given. Ethambutol results are unavailable at this time.

**ACKNOWLEDGMENTS**

This project was supported in part by grants form the Institute of Museum and Library Services, The Conservation Endowment Fund of the American Zoo and Aquarium Association and Ortho-McNeil. The authors would like to thank Scott Larson and Dr. Mo Salman of Colorado State University for performing ELISA assays, Lederle labs for donating ethambutol and Pharmacia-Upjohn for donating rifabutin. A special thank-you is extended to the mammal and hoofstock staff at the Audubon Park Zoo for their care and behavioral conditioning of the bongo herd.
LITERATURE CITED

A SURVEY FOR FECAL Clostridium perfringens AND C. difficile TOXINS IN PRIMATE FECES AT THE WILDLIFE CONSERVATION PARK/BRONX ZOO

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Abstract

Clostridia are anaerobic, but aerotolerant, rod shaped, gram-positive bacteria. They are harbored in the gastrointestinal tract of many animals, including humans where the organism is also found in the female genital tract. Both Clostridium perfringens and C. difficile elaborate toxins that produce diarrheal diseases and both form spores that are stable in the environment.

Clostridium perfringens type A causes enterotoxicosis in mink (Mustela vison), dogs, and cheetahs (Acinonyx jubatus jubatus) as well as food poisoning in humans. The cue for toxin production is unclear but may be different in food poisoning versus naturally occurring diarrhea. Clostridium perfringens type A may be spread via spores in food, ingestion of spores from the environment, or fecal-oral transmission.

Clostridium difficile toxin causes enterocolitis, characterized, in its most severe form, as pseudomembranous colitis as seen in hamsters (Cricetus spp.), rabbits (Oryctolagus spp.), guinea pigs (Cavia porcellus), Kodiak bears (Ursus arctos middendorffi), cotton top tamarins (Saguinus oedipus) and humans subsequent to antibiotic administration. Clinical signs in humans range from mild self-limiting diarrhea to life-threatening pseudomembranous colitis. The organism can be isolated from the stools of 3% of healthy human adults who have not previously been treated with antibiotics. In one human study, antibiotic treatment increased the frequency of asymptomatic carriers to 48%. In human infants, C. difficile and its toxins are commonly present in the gastrointestinal system, but do not cause disease. It is hypothesized that neonate intestinal epithelium does not yet have a toxin receptor. Clostridium difficile causes naturally occurring enterocolitis in swine (Sus scrota) and foals (Equus caballus) suggesting that there are circumstances other than antibiotic depression of normal flora which may create conditions allowing C. difficile to proliferate and subsequently produce toxin. There are many factors which contribute to the pathogenicity of C. difficile, of which the most important, and most widely studied is toxin production.

A cross-sectional survey was performed to determine the prevalence of fecal C. perfringens and C. difficile toxins in healthy, asymptomatic, non-human primates at the Wildlife Conservation Park/Branson Zoo. This study was conducted after several clinically ill callitrichids were suspected of having clostridial disease. These animals developed signs of wasting disease, with or without diarrhea, and did not respond to conventional therapy of nutritional supplementation, fluids and broad spectrum antibiotics. Fecal samples from these animals were positive for C. perfringens...
and/or \textit{C. difficile} toxins. The animals demonstrated weight gains and improved attitudes within 3 days of treatment with metronidazole benzoate (Mortar and Pestle Pharmacy, 3701 Beaver Ave, Des Moines, IA 50310) (25-30 mg/kg divided b.i.d.) and tylosin tartate (Tylan, Elanco Animal Health, Indianapolis, IN 46285) (5 mg/kg b.i.d.).

Individuals from one building with 19 enclosures containing approximately 75 individuals of 15 primate species (callitrichids, callimicos, and cebids) were evaluated. Composite fecal samples from each enclosure were submitted for anaerobic bacterial culture, an enzyme linked immunoassay for detection of \textit{C. difficile} toxin, and a reverse passive latex agglutination test for \textit{C. perfringens} toxin.

\textit{Clostridium difficile} and \textit{C. perfringens} toxins were detected together in feces from 12/19 (63.2\%) enclosures, \textit{C. difficile} toxin was detected alone in 5/19 (26.3\%), and no toxins were detected in 2/19 (10.5\%) enclosures. \textit{Clostridium perfringens} was cultured from feces in 4/19 (21\%) enclosures and \textit{C. difficile} was not isolated from any enclosure.

Eleven of 19 (57.9\%) enclosures had animals born within 1 yr preceding the survey and of these, four (36.4\%) had animals born within 2 mo of the start of the survey. At the time of sampling none of the animals were being treated for antibiotics, although animals in five enclosures (26.3\%) had been treated with antibiotics within the preceding year and three of these (60\%) had been treated within the previous month. Surveyed animals were on a rotating bimonthly anthelmintic and antiprotozoal program during the sampling period.

The results of this survey demonstrated that most of the enclosures surveyed were positive for one or both toxins, although the animals contained in them were asymptomatic. There was no correlation between the presence of young animals in the group and the detection of toxins or between a history of recent antibiotic treatment and positive test results. Finally, culture results correlated poorly with the presence of toxin. One enclosure from which \textit{C. perfringens} was cultured was toxin negative and nine enclosures were \textit{C. perfringens} culture negative and \textit{C. perfringens} toxin positive. Bacterial culture is not specific for type A toxin producing \textit{C. perfringens} and this may partially explain the poor correlation between culture and toxin results. \textit{C. difficile} culture is technically difficult and may yield false negative results (P. McDonough, personal communication). In both cases, the toxin test is more sensitive than culture (P. McDonough, personal communication). Based on these findings, it was not possible to determine the role of \textit{C. perfringens} and \textit{C. difficile} toxins in diarrheal or wasting diseases because animals were positive for either or both toxins without clinical illness. However, several cases which stimulated this broader survey demonstrated that animals may respond to treatment for clostridial disease when conventional therapy fails. This evidence and the results of this study, indicate that more research on this topic is needed to understand the significance of positive clostridial toxin screen in primates.

\textbf{ACKNOWLEDGMENTS}
The authors thank Dr. Patrick McDonnough for his support, guidance, and assistance with interpretation of results.

LITERATURE CITED

MANAGEMENT AND TREATMENT OF A *Mycobacterium tuberculosis* POSITIVE ELEPHANT AT THE SAN FRANCISCO ZOO

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Abstract

A newly acquired 31-yr-old female Asian elephant ("Calle"), came under suspicion of being infected with *Mycobacterium tuberculosis* when a former penmate at another institution was diagnosed with tuberculosis on post-mortem examination.

Using guidelines for evaluating tuberculosis suspect elephants provided by the Elephant Tuberculosis Advisory Committee and the National Tuberculosis Working Group for Zoo and Wildlife Species, we evaluated Calle and one other resident Asian elephant female who was considered a contact animal. On 20 June 1997, a trunk wash culture taken from Calle came back positive for *M. tuberculosis*. With this positive culture came the many challenges associated with managing and treating a *M. tuberculosis* positive elephant, a culture negative contact animal, personnel working with those animals and the general public.

These challenges were met by becoming familiar with the disease caused by *M. tuberculosis*, educating the entire zoo staff and addressing their concerns, and educating the public relations department so they could address the concerns of visitors.

Quarantine procedures were established for the elephant barn and a program was established to screen and protect zoo personnel exposed to Calle. Treatment protocols were developed for Calle and the contact elephant. Because Calle refused to take oral medication (isoniazid and rifampin) a rectal suppository dosing regimen was developed. Oral prophylactic treatment was initiated on the contact animal but is becoming increasingly difficult to administer. The animal is undergoing conditioning so that a suppository can be used on it as well.

Both oral and suppository treatment have presented many challenges. At this time, we have achieved acceptable blood drug levels only with isoniazid and work continues with rifampin to achieve a multiple drug treatment protocol. Since starting the current treatment protocol, Calle has been trunk wash culture negative and the other animal has remained negative.

The management of these two elephants has been accomplished by developing a communication network including members of the Elephant Tuberculosis Advisory Committee and Working Group, members of the San Francisco Public Health Department, compounding pharmacists, and other zoos and elephant owners with tuberculosis positive elephants. There has been a significant financial commitment by the San Francisco Zoo and time commitments by zoo personnel involved in the care
and treatment of these animals. A successful, proactive public relations campaign has resulted in an informed public, which has been overwhelmingly supportive of the zoo’s efforts.
PRELIMINARY EVALUATION OF SERUM PROTEIN ELECTROPHORESIS AS A DIAGNOSTIC TOOL IN THE BLACK RHINOCEROS (Diceros bicornis)

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Abstract

Serum protein electrophoresis (SPE) is a valuable tool for the diagnosis of certain diseases in humans and animals.1,3,8,12 This technique could be particularly beneficial when applied to health monitoring in the black rhinoceros (Diceros bicornis), a species which is predisposed to a number of diseases in captivity, the etiologies of which are not yet fully understood.2,14-19 Serum protein fractionation varies widely between species, thus it is necessary to establish a reference range in order to interpret SPE for a given species.10,13,20 This study has two purposes. The first is to determine normal ranges and patterns of serum proteins separated by SPE in the black rhinoceros. The second is to make a preliminary evaluation of the diagnostic potential of SPE for this species.

Serum protein electrophoresis was performed on samples from both clinically healthy (n = 38) and clinically ill (n = 16) black rhinoceroses. Data gathered from healthy individuals were used to establish reference ranges for SPE. The absolute ranges for total protein, albumin, and gamma (γ) globulins in the black rhinoceros are similar to those of domestic mammals, however the albumin to globulin ratio and alpha (α) globulins tend to be lower while the beta (β) globulins tend to be higher in the black rhinoceros than in domestic mammals. Preliminary evaluation of data gathered from ill and subclinically ill individuals indicates that serum protein electrophoresis is helpful in the diagnosis of clinically inapparent disease in the black rhinoceros. Research toward identifying and characterizing electrophoretic patterns associated with subclinical and clinical disease in the black rhinoceros continues.

ACKNOWLEDGMENTS

The authors would like to thank Howard Gilman, John Lukas, Dave Thompson, Cyd Teare, and the staff of White Oak Conservation Center, and Carolyn Cray from the University of Miami, Division of Comparative Pathology, for making this research possible. The authors also thank Eric Miller and the staff of the Saint Louis Zoo, Dave Jessup of the California Department of Fish and Game, Robin Radcliffe and the staff of Fossil Rim Wildlife Center, Mitch Finnegan and the staff of the Oregon Zoo, Tom Alvarado and the staff of the Dallas Zoo, Chris Miller and the staff of the Miami Metrozoo, Peregrine Wolff, Michelle Miller, and the staff of Disney’s Animal Kingdom, Nancy Lung and the staff of the Fort Worth Zoo, Ray Ball and the staff of Busch Gardens, Tampa, Nadine Lamberski and the staff of Riverbanks Zoological Park, and Roberta Wallace and the staff of the Milwaukee County Zoo for their time and effort in collecting and sending samples, Tom Foose of the International Rhino Foundation and The Wilds for providing studbook
information and current locations of black rhinoceroses, and Eric Lumis Shapiro of the Hospital of the University of Pennsylvania, Department of Anesthesiology, for reviewing this manuscript. Special thanks go to Lonnie McCaskill and Tortoise at White Oak Conservation Center for providing the inspiration to begin this work.

LITERATURE CITED

### Table 1. Serum protein electrophoresis: protein fractions and components.\(^9,11\)

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</tbody>
</table>

\(^a\)High density lipoprotein (HDL).
\(^b\)Very low density lipoprotein (VLDL).
\(^c\)Low density lipoprotein (LDL).

### Table 2. Differential diagnoses of serum protein alterations.\(^9,11\)

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Differential diagnoses(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>Increase: Dehydration</td>
</tr>
<tr>
<td></td>
<td>Decrease: Hepatic disease, gastrointestinal disease, renal disease, internal parasites, overhydration, malnutrition, blood or plasma loss</td>
</tr>
<tr>
<td>(\alpha_1) Globulins</td>
<td>Increase: Acute inflammatory disease, pregnancy</td>
</tr>
<tr>
<td></td>
<td>Decrease: Hepatic disease, pulmonary disease, nephrotic syndrome</td>
</tr>
<tr>
<td>(\alpha_2) Globulins</td>
<td>Increase: Acute inflammatory disease, nephrotic syndrome, hepatic disease, diabetes mellitus, hypothyroidism</td>
</tr>
<tr>
<td>(\beta) Globulins</td>
<td>Increase: Acute hepatitis, chronic active hepatitis, nephrotic syndrome, suppurative dermatopathy, anemias</td>
</tr>
<tr>
<td></td>
<td>Decrease: Autoimmune disease</td>
</tr>
<tr>
<td>(\gamma) Globulins</td>
<td>Increase: Chronic inflammatory disease, immune mediated disease, infectious disease, suppurative disease, connective tissue disease, multiple myeloma, lymphosarcoma</td>
</tr>
<tr>
<td></td>
<td>Decrease: Immune deficiency diseases</td>
</tr>
</tbody>
</table>

\(^a\)Evaluation of serum protein status should include assessment of the albumin to globulin ratio (A:G).
Table 3. Reference ranges for SPE in the black rhinoceros ($n = 33$).

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Quantity (g/dl)</th>
<th>Percent of total protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Total protein</td>
<td>6.00-8.40</td>
<td>7.37</td>
</tr>
<tr>
<td>A:G ratio</td>
<td>0.31-0.79</td>
<td>0.53</td>
</tr>
<tr>
<td>$\alpha$ Globulins</td>
<td>1.79-3.41</td>
<td>2.49</td>
</tr>
<tr>
<td>$\alpha_1$ Globulins</td>
<td>0.09-0.27</td>
<td>0.16</td>
</tr>
<tr>
<td>$\alpha_2$ Globulins</td>
<td>0.23-0.68</td>
<td>0.45</td>
</tr>
<tr>
<td>$\beta$ Globulins</td>
<td>1.69-3.33</td>
<td>2.51</td>
</tr>
<tr>
<td>$\gamma$ Globulins</td>
<td>1.08-2.58</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Table 4. SPE reference ranges (g/dl) for the black rhinoceros compared to domestic animals.

<table>
<thead>
<tr>
<th>Protein fraction</th>
<th>Black rhinoceros$^{a,b}$</th>
<th>Horse$^c$</th>
<th>Cow$^c$</th>
<th>Dog$^c$</th>
<th>Cat$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>6.00-8.40</td>
<td>5.20-7.90</td>
<td>6.74-7.46</td>
<td>5.40-7.10</td>
<td>5.40-7.80</td>
</tr>
<tr>
<td>A:G ratio</td>
<td>0.31-0.78</td>
<td>0.62-1.46</td>
<td>0.84-0.94</td>
<td>0.59-1.11</td>
<td>0.45-1.19</td>
</tr>
<tr>
<td>Albumin</td>
<td>1.79-3.02</td>
<td>2.60-3.70</td>
<td>3.03-3.55</td>
<td>2.60-3.30</td>
<td>2.10-3.30</td>
</tr>
<tr>
<td>$\alpha$ Globulins</td>
<td>0.33-1.00</td>
<td>0.37-2.01</td>
<td>0.75-0.88</td>
<td>0.50-1.60</td>
<td>0.50-1.60</td>
</tr>
<tr>
<td>$\alpha_1$ Globulins</td>
<td>0.10-0.32</td>
<td>0.06-0.70</td>
<td>0.20-0.50</td>
<td>0.20-0.50</td>
<td>0.20-0.50</td>
</tr>
<tr>
<td>$\alpha_2$ Globulins</td>
<td>0.23-0.68</td>
<td>0.31-1.31</td>
<td>0.30-1.10</td>
<td>0.30-1.10</td>
<td>0.30-1.10</td>
</tr>
<tr>
<td>$\beta$ Globulins</td>
<td>1.81-3.33</td>
<td>0.69-2.47</td>
<td>0.80-1.12</td>
<td>1.30-2.70</td>
<td>1.30-2.70</td>
</tr>
<tr>
<td>$\gamma$ Globulins</td>
<td>1.08-2.58</td>
<td>0.55-1.90</td>
<td>1.69-2.25</td>
<td>0.90-2.20</td>
<td>1.70-4.40</td>
</tr>
</tbody>
</table>

$^a$Reference ranges for the black rhinoceros are repeated from Table 4 for ease of comparison.

$^b$Rhinoceros values determined by agarose gel SPE; domestic animal values by cellulose acetate SPE.
JOHNE’S DISEASE (PARATUBERCULOSIS) IN ZOO ANIMALS

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Abstract

Johne’s disease (paratuberculosis), a fatal and contagious gastrointestinal disease of ruminants, has been diagnosed by the Johne’s Testing Center by radiometric culture of the causative agent, Mycobacterium paratuberculosis at 5 of 49 zoos over the past 2 yr.

Control of this slowly developing disease can be troublesome in a zoological park. The biology of this disease makes surveillance difficult as clinical and immunologic signs can be subtle and appear late in the disease process. Infection usually occurs in the first few months of life, but markers for diagnosis (e.g., cytokine and antibody production, fecal shedding of the organism) may show up months to years later. The majority of infected animals shed organisms but appear clinically normal. The full impact of herd infection can be masked both by the years that can pass before clinically affected animals are detected and by the dispersion of animals (and the infection) to other collections through breeding exchanges and sale.

Detection

There are a number of methods available for surveillance and detection of animals with Johne’s disease. Application of a number of testing techniques can greatly improve the effectiveness of a Johne’s disease control program. Reliable animal identification systems and accurate record keeping are critical to managing this, as with any, disease control program.

Detection of infected animals or herds may rely on some or all of the following techniques:

1. Clinical assessment by keepers, curators and veterinarians.
2. Necropsy screens of collection and feral animals.
3. Serologic detection (new species-specific and multi-species ELISAs are in development).
4. Bacteriologic culture (biopsy/necropsy tissue, feces, environmental samples).
5. Genetic probe.

Once clinical signs compatible with Johne’s disease are noted in an animal, detection of an immunologic response to infection or detection of M. paratuberculosis itself is necessary to confirm the clinical diagnosis. Clinically affected animals are the most likely to have produced antibody in response to infection and to regularly be shedding the organism. The choice of detection method is influenced by available resources (time as well as money), animal species and Johne’s disease prevalence in the exhibit. For most situations, bacteriologic culture to isolate the organism from
fecal samples is the best choice to confirm clinical suspicions. AGID or ELISA testing may be useful for faster verification of the diagnosis in animals with clinical signs consistent with Johne’s disease.

Should the clinical diagnosis be confirmed, further steps are necessary since Johne’s disease is a herd problem as well as an individual animal problem. Once a diagnosis has been made in a single animal, carefully examine herd-mates, offspring, and the environment for signs and pathways of infection. Colostrum/milk used for hand-rearing infants, exposure of young stock to adult manure, exposure to contaminated water in exhibits and fecal contamination of feed bunks are a few of the routes in which the disease may be transmitted to other animals. Consider annual fecal culture screening of all adult animals in the exhibit. Infected animals should be euthanatized or at least isolated and their offspring should be evaluated as well.

Control

Johne’s disease should always be on the list of differential diagnoses generated for a thin animal. The cost of missing the diagnosis in a herd of exotic hoofstock can be so severe that the expense of ruling it out must be accepted. In exhibits where there is any possibility of Johne’s disease (based on clinical assessment, exposure or test results), early detection of infectious animals is critical. Regular (annual) screening of adult animals over a period of years is necessary to reveal and eliminate a herd infection. For optimal fecal culture screens, three samples should be collected some days or weeks apart. This protocol will improve the likelihood of obtaining samples containing organisms from animals shedding *M. paratuberculosis* intermittently. Picking up samples from a clean stall or pen floor from animals held for a short time or from the ground in the exhibit itself is acceptable for animals that cannot easily be penned and handled.

Control of disease transmission in a herd or exhibit following detection of an infected animal may include:

1. Exhibit sanitation: removal and composting of feces, elimination of standing water, tuberculocidal disinfectant for all tools, boots, etc.
2. Keeper and veterinary staff awareness of clinical signs
3. Necropsy screens (primary tissues of interest include the distal gastrointestinal tract and associated lymph nodes)
4. Milk and colostrum from Johne’s disease-free sources for hand-reared neonates
5. Annual herd testing, testing of animals with compatible clinical signs and culling or isolation of test positive animals

Sale/exchange

In many zoos, three negative fecal cultures collected some weeks apart are required prior to accepting animals into quarantine at the new site. While in quarantine, an additional fecal culture is recommended, preferably on a sample collected immediately after arrival at the new facility, as
it is thought that an animal is more likely to shed the organism when stressed. Testing requirements for export are as specified by the receiving country.

“Children’s Zoo” animals
Many facilities seasonally introduce new ruminants to the more permanent residents of the Children’s Zoo collection. The source herd for these animals should be assessed for Johne’s disease. An optimal arrangement is to contract with the source herd owners and specify the infectious disease testing protocol for the entire source herd to improve the likelihood that individual animals brought to the zoo are not infected. These source herds may then also serve as sources of \textit{M. paratuberculosis}-free milk/colostrum.

Future Trends

\textit{Regulations}
It is expected that the U.S. Department of Agriculture will define paratuberculosis as a “program disease” in the near future. This means that Congress will allocate funds to stimulate control and/or monitoring of Johne’s disease. While focused on agricultural animals, zoological collections may also be affected by new regulations. Regulatory topics of particular concern are mandatory slaughter of animals with positive fecal culture results and an amendment of CFR 80 to halt interstate movement of fecal culture-positive animals.

\textit{Zoonosis}
An association between \textit{M. paratuberculosis} and Crohn’s disease, a human inflammatory bowel disease, is still being explored. The public perception of a zoonotic risk and actual risk are not always in synchrony: deflecting both the perception of and a potentially real zoonotic risk is recommended for the zoo community. This can be done by establishing and documenting control programs for Johne’s disease.

\textit{AAZV guidelines}
Members of AAZV and other individuals with expertise in mycobacterial infections currently are investigating development of guidelines for diagnosis and control of Johne’s disease in zoological collections. These guidelines are expected to support uniform interpretation of test data, pre-shipment test requirements and other aspects of paratuberculosis control of benefit to the zoo community at large.
FATAL PNEUMONIA IN CAPTIVE WAHLBERG’S EPAULETTED FRUIT BATS (Epomorphous wahlbergi) CAUSED BY A Pasteurella-LIKE BACTERIUM†

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†Published as Lubee Journal Series No. 49.

Abstract

An unclassified Pasteurella species was isolated at necropsy from lung and subcutaneous abscesses in two Wahlberg’s epauletted fruit bats (Epomorphous wahlbergi). The bats were part of a group of three Wahlberg’s epauletted fruit bats previously housed at the Oregon Zoo, Portland, Oregon. They were transported to the Lubee Foundation, Florida and became part of a breeding colony consisting of four males and ten females. Physical examination performed at the beginning and end of a 30 day quarantine period revealed no abnormalities.

Five days following the end of quarantine, an adult, gravid female (case 1) died after an attack by a cagemate. Gross and histopathologic examination revealed suppurative pneumonia of the left lung with chronic abscessation and intralesional gram-negative bacterial rods. Bacterial culture of the lung tissue yielded heavy growth of an aerobic gram-negative non-enteric rod. Nine days following the death of case 1, an adult male bat (case 2) was evaluated at the Veterinary Medical Teaching Hospital, University of Florida, with a history of lethargy and anorexia of three days duration. A complete blood count revealed leukopenia (2,700/µl) characterized by monocytosis and neutropenia with a degenerative left shift. This animal was found dead with hemorrhage from the mouth and nares 24 hr following initial presentation. Gross and histopathologic examination revealed diffuse, subacute bronchopneumonia with intralesional gram-negative rods present in both lungs and a 2.5 cm focal abscess adherent to the pericardium and the parietal pleura of the right ribcage. Culture of the lung resulted in heavy growth of an aerobic non-enteric gram-negative rod.

Samples of the aerobic, non-enteric, gram-negative rod obtained from both bats were found to be morphologically and biochemically similar. A sample of the organism from case 2 was submitted to the National Veterinary Services Laboratories, Ames, Iowa and was identified as an aerogenic Pasteurella species. Further characterization of this organism was performed by the Special Bacteriology Reference Laboratory, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia. Although the CDC found the biochemical and cellular fatty acid profiles of this organism
to be consistent with either *Pasteurella* or *Actinobacillus* species, it did not correspond to any known pathogens.

The remaining Wahlberg’s epauletted fruit bats were screened for the presence of this organism by culturing pharyngeal swabs. Aerobic, non-enteric, gram-negative rods, morphologically and biochemically similar to the pathogen isolated from case 2 were identified in 80% (8/10) of bats cultured. This organism appeared sensitive to a wide variety of antimicrobials, as demonstrated by Kirby-Bauer susceptibility testing. The Oregon Zoo, Portland, Oregon, also experienced mortalities due to pneumonia in a collection of Wahlberg’s epauletted fruit bats. Six of the 50 bats in the collection died with necropsy findings consisting primarily of severe, unilateral suppurative pneumonia with abscess formation. Four of five of these animals cultured for bacteria demonstrated heavy growth of a *Pasteurella* sp. organism, species *non-multocida*.

The cause of death in the two *E. wahlbergi* bats discussed in this report was determined to be bacterial pneumonia produced by a newly identified *Pasteurella* or *Actinobacillus* species. Historical data provided by the Oregon Zoo indicates that pneumonia had been occurring in this species prior to their arrival at the Lubee Foundation. Culture screening of the remaining captive *E. wahlbergi* population at the Lubee Foundation suggests that this organism is a component of normal flora. In asymptomatic bats, this organism comprised a smaller percentage of the total bacterial population than in symptomatic bats. This is consistent with *Pasteurella*-associated disease occurring in other mammalian species.

**ACKNOWLEDGMENTS**

The authors would like to acknowledge the assistance of Dana LeBlanc and John Seyjagat in this study.
RISK FACTORS FOR CANINE DISTEMPER VIRUS SEROPOSITIVITY IN ZOO CATS

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1Chicago Zoological Society, Brookfield Zoo, 3300 Golf Rd, Brookfield, IL 60513 USA; 2Great Plains Veterinary Educational Center, Institute of Agriculture and Natural Resources, University of Nebraska, Clay Center, NE 68901 USA; 3College of Veterinary Medicine, University of Illinois, Urbana, IL 61801 USA

Abstract

Canine Distemper Virus (CDV) is a common pathogen in canids, hyaenids, mustelids, procyonids, and viverrids, but, until recently, was not thought to cause disease in felids. CDV outbreaks have devastated lion populations in Serengeti National Park, Africa and have caused deaths of large cats in North American zoos. Dogs and raccoons were believed to be the source of cross-over infections in these cats. In order to understand the transmission of this agent and to develop strategies to protect susceptible captive carnivores, this study examined risk factors for CDV infection in zoo cats.

To determine prevalence of infection with CDV, we used banked and newly collected serum samples from 85 captive felids from Brookfield Zoo in Cook County, IL. These sera had been collected between 1984 and 1998. Sera were also collected from 59 raccoons captured within zoo grounds in 1995 and 1996. Serologic testing was done by Cornell University using the CDV serum neutralizing test. Geographic information systems (GIS; MapInfo® and ArcView®) were used to create maps of Brookfield Zoo, overlay habitat information and link data on the age, sex, location, housing characteristics, and history of each cat. Accessibility of each exhibit to raccoons was also assessed through a survey of the exhibits and interviews with keepers. Risk factors were compared between seropositive and seronegative animals using contingency tables and multivariate logistic regression.

Seventy-three percent of raccoons and 32% of the cats were seropositive for CDV. This level of exposure among cats was considerably higher than that observed in zoo cats in other studies.1 Cats housed outdoors had a twenty-three times greater risk of being seropositive for CDV ($P < 0.01$). Among the smaller felid species, cats housed outdoors had a forty times greater risk of being seropositive than cats housed indoors ($P < 0.01$). Other significant risk factors included species, sex, and age. Large cats were three times more likely to be seropositive for CDV than small cats. Males were four times more likely to be seropositive for CDV than females ($P < 0.05$), and there was a ten percent increase in risk with each increasing year of age for both sexes ($P < 0.01$). The time span during which seroconversion occurred was identified in nine cats. We are currently examining keeper records and animal health records produced during these time periods to look for specific exposure opportunities and we are testing banked wild raccoon serum samples to identify disease status in the wildlife populations during these periods.
Zoos provide a unique opportunity to determine risk factors for disease transmission that are relevant to both captive and wild populations. The development of preventive medicine programs in zoos should balance the protection of individuals from disease with the potential for using exposure and disease data to understand disease risk and transmission.

ACKNOWLEDGMENTS

The authors wish to thank the keeper staff and laboratory personnel who assisted in the gathering of these data and processing the serum samples; Mark Jocelyn, at the Illinois Natural History Survey, for GIS assistance; and the Howard Hughes Medical Institute for its support.

LITERATURE CITED

CARDIOPULMONARY AND ANESTHETIC EFFECTS OF MEDETOMIDINE-KETAMINE-BUTORPHANOL AND ANTAGONISM WITH ATIPAMEZOLE IN SERVALS (*Felis serval*)

Jennifer N. Erdtmann, DVM,1* Juergen Schumacher, Dr. med. vet.,1 Christal Pollock, DVM,1 Susan E. Orosz, PhD, DVM,1 Mike P. Jones, DVM, Dipl ABVP, Avian Practice,1 and Ralph C. Harvey, DVM, Dipl ACVA2

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Abstract

Seven (three male, four female) 4- to 7-yr-old captive servals (*Felis serval*), weighing 13.7 ± 2.3 kg (mean ± SD), were used to evaluate the cardiopulmonary and anesthetic effects of i.m. medetomidine (47.4 ± 10.3 µg/kg), ketamine (1.0 ± 0.2 mg/kg), and butorphanol (0.2 ± 0.03 mg/kg). Inductions were smooth and rapid (11.7 ± 4.3 min) and resulted in good muscle relaxation. A significant (*P* < 0.05) decrease in heart rate (85 ± 12 beats/min) at 10 min and in respiratory rate (27 ± 10 breaths/min) at 5 min was present following induction and continued throughout the immobilization period. No significant changes in rectal body temperature or arterial blood pressure were seen during the anesthetic event. Arterial blood gas analysis, performed at 1, 15, and 30 min after induction, showed PaO$_2$ decreased significantly and PaCO$_2$ increased significantly during immobilization. Changes were within clinically acceptable limits. No periods of hypoxemia (PO$_2$ < 60 mm Hg) were noted. Arterial blood oxygen saturation (SaO$_2$) was greater than 90% at all times. Relative arterial oxygen saturation (SpO$_2$) values, indicated by pulse oximetry, were lower than SaO$_2$ values. All animals could be safely handled while sedated. Administration of the α$_2$-antagonist, atipamezole (236.8 ± 51.2 µg/kg half i.v. and half s.c.), resulted in rapid (4.1 ± 3 min to standing) and smooth recoveries. At the dosage used in this study the combination of medetomidine-ketamine-butorphanol was effective for immobilizing captive servals.
GASTRIC ANALYSES OF COLOBINE PRIMATES

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1Veterinary Services, San Diego Zoo, PO Box 551, San Diego, CA 92112 USA; 2Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616 USA

Abstract

Gastroenterocolitis is a leading cause of morbidity and mortality in Douc langurs (Pygathrix nemaeus) at the San Diego Zoo. A review of necropsy information revealed that gastroenterocolitis was present in 11/52 (21%) animals and was the primary finding in 8/52 (15%) necropsies.4 This abstract discusses the use of gastric sample analysis in colobine primates as an assessment of gastrointestinal function and subsequent health.

The Douc langurs at the San Diego Zoo have had intermittent problems with gastrointestinal disease. Symptoms range from mild to severe diarrhea and vomiting with subsequent metabolic derangement. There is little published information on the gastric physiology of colobine primates. To better characterize the gastric environment, analyses of gastric contents have been performed over the past 3 yr. Because of similarities between ruminants and folivorous forestomach fermenters, the rationale was to utilize the same tests used to evaluate rumen fluid. Gastric samples from Douc langurs, Francois langurs (Trachypithecus francoisi), Kikuyu colobus (Colobus guereza) and Angolan colobus (Colobus angolensis) were examined. Gastric samples were obtained using a large bore (18-28 French) red-rubber tube (Sovereign Feeding Tube and Urethral Catheter, Sherwood Medical, St. Louis, MO 63130 or Robinson Catheter, Davol Inc., 100 Sockanossett Crossroad, Cranston, RI USA) and either a catheter tip syringe or a specimen trap (Sherwood Medical, St Louis, MO 63103 USA) attached to a suction unit. Minimal sample volumes of 10-20 ml were sought.

Testing of each sample included pH measurement, occult blood detection, methylene blue reductase activity assessment, wet-mount examination for protozoans, and cytology to assess the bacterial flora and screen for evidence of inflammation. The pH was measured using pH strips (J.T. Baker Inc., Phillipsburg, NJ 08865 USA). Occult blood was measured using Hematest Reagent Tablets (Miles Inc., Diagnostics Division, Elkhart, IN 46515 USA). Methylene blue reductase activity quantification is a means of assessing anaerobic bacterial fermentation and is used in ruminants to assess rumen microflora health. The redox potential of the bacterial population of the rumen allows for reduction of methylene blue.2,3,5 A 10 ml aliquot of gastric contents was mixed with 0.5 ml of a 0.03% methylene blue solution and the time for the solution to clear recorded. A healthy population of rumen flora is able to reduce methylene blue within 6 min.5

Table 1 summarizes sample distribution for species, number of individuals, number of samples, and health status. A clinically normal animal is defined as one that was examined for a routine procedure
or follow-up on a resolved problem and was not showing clinical signs of illness at the time of the examination. A clinically abnormal animal was defined as one examined for any other reason. The results for pH testing are shown in Table 2. The normal pH range of langur gastric contents has been reported as 5.0-6.7. The pH for the samples presented here was generally higher. This may be due to differences in methodology and sampling location. Contamination of a sample with saliva can artificially elevate the pH. There was little difference in the average pH between clinically normal and abnormal animals. However, the range of pH values was greater for the clinically abnormal group. Most samples were occult blood negative. Positive results can be attributed to gastric hemorrhage, iatrogenic trauma during sample collection, post-surgery, or a false-positive result secondary to reactive non-heme compounds found in gastric contents. The times to reduce methylene blue in clinically normal and abnormal animals is shown in Table 2. Eighty-four percent of clinically normal animals had methylene blue reductase times of 6 min or less whereas only 35% of abnormal animals reduced methylene blue in 6 min or less. Sixty-five percent of clinically abnormal animals compared to 15.8% of clinically normal animals had methylene blue reductase times greater than 6 min. A gastric sample with pH less than 6 and/or a methylene blue reductase greater than 6 min should be considered abnormal and the animal scrutinized for diseases affecting the gastrointestinal system.

The gross appearance of the gastric samples was not objectively evaluated. Subjectively, a gastric sample that was watery, brown, had a fetid odor, or contained large heterogeneous particulate matter was considered abnormal. Normal samples were green and viscous with a homogenous consistency. The wet mount and cytologic data need further analysis but were useful in detecting gastric parasitism (Trichuris sp.) in two Kikuyu colobus.

Tests used for rumen sample analyses are applicable to other foregut fermenters. Gastric content analyses can be used as an additional diagnostic tool for assessing the health of colobine primates. Abnormal results may be indicative of primary gastrointestinal abnormalities or secondary to other diseases. Additional studies evaluating the gastric physiology of colobines, including volatile fatty acid content of gastric contents, is warranted.

ACKNOWLEDGMENTS

The authors wish to thank the Clinical Pathology Laboratory at the Zoological Society of San Diego for their diagnostic support.

LITERATURE CITED

Table 1. Summary of samples based on species, individuals, number of samples, and health status.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number* individuals</th>
<th>Number samples</th>
<th>Clinically normal</th>
<th>Clinically abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Francois’ langur</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Kikuyu colobus</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Angolan colobus</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Douc langur</td>
<td>9</td>
<td>28</td>
<td>8</td>
<td>20</td>
</tr>
</tbody>
</table>

* The number of samples differs for each test because not all tests were performed on all samples.

Table 2. Gastric content pH values for clinically normal versus abnormal animals.

<table>
<thead>
<tr>
<th>pH</th>
<th>Clinically normal ((n = 18)^*)</th>
<th>Clinically abnormal ((n = 23)^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>6.5 - 8.0</td>
<td>4.5 - 8.5</td>
</tr>
<tr>
<td>Average</td>
<td>7.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.41</td>
<td>2.33</td>
</tr>
</tbody>
</table>

* The number of samples differs for each test because not all tests were performed on all samples.
Table 3. Gastric content occult blood results for clinically normal versus abnormal animals.

<table>
<thead>
<tr>
<th>Occult blood</th>
<th>% Clinically normal*</th>
<th>% Clinically abnormal*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>86 % (18/21)</td>
<td>67 % (16/24)</td>
</tr>
<tr>
<td>Positive</td>
<td>14% (3/21)</td>
<td>21 % (5/24)</td>
</tr>
<tr>
<td>Slight positive</td>
<td>0 % (0/21)</td>
<td>12 % (3/24)</td>
</tr>
</tbody>
</table>

* The number of samples differs for each test because not all tests were performed on all samples.

Table 4. Gastric sample methylene blue reductase times for clinically normal versus abnormal animals.

<table>
<thead>
<tr>
<th>Methylene blue reductase</th>
<th>% Clinically normal*</th>
<th>% Clinically abnormal*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3 min</td>
<td>36.8 % (7/19)</td>
<td>20 % (4/20)</td>
</tr>
<tr>
<td>4-6 min</td>
<td>47.4% (9/19)</td>
<td>15% (3/20)</td>
</tr>
<tr>
<td>7-9 min</td>
<td>10.5 % (2/19)</td>
<td>25 % (5/20)</td>
</tr>
<tr>
<td>&gt; 9 min</td>
<td>5.3 % (1/19)</td>
<td>40 % (8/20)</td>
</tr>
</tbody>
</table>

* The number of samples differs for each test because not all tests were performed on all samples.
DISPOSITION OF ENROFLOXACIN (BAYTRIL®) IN RED-TAILED HAWKS (Buteo jamaicensis) AND GREAT HORNED OWLS (Bubo virginianus) FOLLOWING A SINGLE ORAL, INTRAMUSCULAR, OR INTRAVENOUS DOSE

Lisa A. Harrenstien, DVM,1* Lisa A. Tell, DVM,1 Richard Vulliet, PhD, DVM,2 Martha Needham, BA,1 Chris Brandt, BS,2 Angela Brondos, BS,2 and Bret Stedman3

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Abstract

Enrofloxacin was administered as a single dose (dosage of 15 mg/kg) to eight red-tailed hawks (Buteo jamaicensis) and five great horned owls (Bubo virginianus) by the oral (in prey) or intramuscular route, and was administered to eight red-tailed hawks by the intravenous route. The disposition of enrofloxacin was evaluated in serial plasma samples up to 48 hr after administration (before dosing, 0.25, 0.5, 1, 2, 4, 8, 12, 24, and 48 hr after dosing).

Oral administration was accomplished by force-feeding the raptor with a small mouse that had been injected intraperitoneally with an injectable formulation of enrofloxacin (Baytril®, 22.7 mg/ml, Bayer Corp., Shawnee Mission, KS 66201 USA). Oral administration resulted in plasma concentrations of enrofloxacin that peaked at 4-12 hr after dosing. Enrofloxacin levels remained above typical gram-negative bacterial pathogens’ MIC90’s for at least 18 hr after oral administration, although there was an initial lag time of approximately 4 hr for absorption from the gastrointestinal tract.

Intramuscular enrofloxacin was administered into the pectoral musculature, with the dose divided between two sites. Intramuscular administration resulted in plasma concentrations that peaked at 0.5-2 hr after dosing; enrofloxacin levels remained above MIC90’s for at least 12 hr after intramuscular administration.

Enrofloxacin was administered intravenously to eight red-tailed hawks via jugular vein, basilic vein or medial metatarsal vein. In these hawks, enrofloxacin levels remained above MIC90’s for at least 15 hr after intravenous administration.

Two great horned owls were administered enrofloxacin intravenously via basilic vein; these owls showed acute weakness, tachycardia and peripheral vasoconstriction during injection, as an apparent direct effect of enrofloxacin. The owls’ clinical signs resolved by 1-3 hr after injection, after they were treated with intravenous and subcutaneous fluids, atropine, and oxygen.

It appears that oral (in-prey) and intramuscular routes are reliable and effective means of administration of injectable enrofloxacin in red-tailed hawks and great horned owls, using a dosage...
of 15 mg/kg every 24 hr for most susceptible bacterial pathogens. Intravenous administration can be performed with caution in red-tailed hawks, but should not be attempted in great horned owls. The exact reason for great horned owls’ adverse reaction to intravenous enrofloxacin is unknown.

ACKNOWLEDGMENTS

The authors would like to thank the following volunteers for their help in performing this study: Kathleen Creighton, Sean Goodell, Heather Graves, Kristine Jensen, Kevin Kwak, John O’Keefe, Michael Schindel, Rebecca Stanton, Sam Tanng, Mary Beth Wood, and Jennifer Yamamoto.
ITRACONAZOLE LEVELS IN SERUM, SKIN AND FEATHERS OF GOULDIAN FINCHES (Chloebia gouldiae) FOLLOWING IN-SEED MEDICATION

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Abstract

Itraconazole, an azole anti-fungal agent, has been widely used in avian medicine, primarily as a treatment for aspergillosis. Oral administration of itraconazole to birds has proved problematic as the most economical formulation, Sporanox\(^8\) (100 mg capsules, Janssen Cilag Pty. Ltd., 706 Mowbray Road, Lane Cove, NSW 2066 Australia) provides too large a dose for most avian patients (recommended dosage is 100 mg/kg s.i.d. or b.i.d.). Each capsule contains approximately 260 small inert plastic beads spray coated with a thin layer of itraconazole. Itraconazole therapy has previously involved dosing birds according to individual body weight. This is especially difficult in birds of less than 100 g. In addition, individual dosing often necessitates frequent handling of patients. This study investigated the use of itraconazole-medicated seed in a group of Gouldian finches (Chloebia gouldiae), average body weight 16 g.

A group of Gouldian finches housed in a heated outdoor aviary at Melbourne Zoo developed multiple disease problems. Chronic feather loss of the head and neck, associated with hyperkeratosis of skin, beak and feet was seen in many individuals. Histopathology suggested dermatomycosis although fungal elements have not been cultured. Previous studies found that itraconazole beads added to seed achieved above therapeutic levels in plasma of Gouldian finches.\(^1\)

Itraconazole-medicated seed (20 or 100 mg itraconazole per 100 g seed) was offered to Gouldian finches \textit{ad lib} for extended periods of time. Sporanox\(^8\) microbeads (approximately 0.39 mg per bead) were mixed with seed and a little vegetable oil. The mix was offered fresh daily.

Blood was collected from the jugular vein of anesthetized finches and serum was submitted for itraconazole assay.\(^2\) Feathers and plucked skin were collected separately from recently euthanatized finches and submitted for itraconazole assay.\(^3\) Full post-mortem examination was conducted on each dead bird and a range of tissues submitted for histopathologic examination.

Serum from finches fed 100 mg itraconazole per 100 g seed for more than 40 days was pooled to provide one sample, with an itraconazole level of 7040 nmol/L. Therapeutic efficacy is reported to be achieved at levels greater than 350 nmol/L (Janssen Cilag, personal communication).
Serum levels in finches fed 20 mg itraconazole per 100 g seed for more than 80 days averaged 1278 nmol/l (range: 293-2377 nmol/L; n = 7).

Skin itraconazole levels in finches fed 20 mg itraconazole per 100 g seed for more than 40 days averaged 1077 pmol/g (range: 526-1945 pmol/g; n = 5).

Feather itraconazole levels in finches fed 20 mg itraconazole per 100 g seed for more than 40 days were all below 5 pmol/g (n = 5).

Skin itraconazole levels in finches fed 20 mg itraconazole per 100 g seed for more than 100 days averaged 1394 pmol/g (range: 1163-1624 pmol/g; n = 2).

Feather itraconazole levels in finches fed 20 mg itraconazole per 100 g seed for more than 100 days averaged 444 pmol/g (range: 402-486 pmol/g; n = 2).

Histologic examination of post-mortem tissues did not show evidence of pathology attributable to itraconazole toxicity.

Feather regrowth was noted in most, but not all finches following prolonged itraconazole therapy. Histopathology indicated birds with unresolved feather loss were suffering from chronic hyperkeratosis and follicular atrophy. Fungal elements were not detected in histology, or by culture in finches following 100 days of itraconazole treatment.

These studies indicated that medication of seed with Sporanox® microbeads is an effective method of administration of itraconazole to groups or individual seed-eating birds. Serum levels in birds fed itraconazole at 20 mg per 100 g seed averaged well above that reported to be required for therapeutic efficacy. Skin levels were equivalent to serum levels indicating itraconazole should be effective in azole-susceptible avian dermatomycoses. There was significant increase in feather itraconazole levels over time indicating itraconazole may take many months to reach therapeutic levels in feathers.

**LITERATURE CITED**

SUSPECTED FENBENDAZOLE TOXICITY IN BIRDS

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Abstract

Previously,3 we reported on suspected toxicity of albendazole in several species of birds (kea, southern speckled pigeons, pink spotted fruit doves). In this report we present additional evidence of benzimidazole toxicity in birds, this time with fenbendazole (Panacur®, Hoechst Roussel Agri-Vet Comp, Somerville, NJ 08876-1258), affecting solitary lories (Phigys solitarius), rock doves (Columba livia, also known as Birmingham roller pigeons), bare-faced ground doves (Metriopelia ceciliae ceciliae) and Southern picui doves (Columbina picui picui). In all cases a 100 mg/ml suspension of fenbendazole was used.

A group of seven solitary lories (four males, three females, ages 6 mo to 1 yr-9 mo) were treated with fenbendazole (50 mg on food s.i.d., 10 days) for capillariasis, based on examination of a pooled fecal sample. Two days following the cessation of treatment, one bird presented with lethargy. CBC revealed profound leukopenia (no white blood cells seen on smear). Despite supportive care, the bird died a few hours later. The next day another bird died before any blood could be obtained. Subsequently, the remaining five birds were examined (day 3 post fenbendazole). Complete blood counts revealed profound leukopenia in all five birds. The total number of white blood cells seen on the smears ranged from 17-38, with the majority of cells being lymphocytes. No heterophils were detected. Mild anemia was also present in four of the five birds (PCV = 28-30%). Total protein and albumin levels were decreased (total protein < 2.0-3.0 g/dl; albumin 1.2-1.6 g/dl). Individual birds had mild elevations of CPK or LDH. Treatment consisted of supportive care (subcutaneous fluids, forced feeding, housing in incubator) and prophylactic antibiotic and antifungal therapy (enrofloxacin 15 mg/kg i.m., b.i.d., Baytril®, Bayer Corp., Agriculture Division, Animal Health, Shawnee Mission, KS 66201 USA; itraconazole 10 mg/kg p.o., s.i.d., Sporanox®, Janssen Pharmaceutical Inc., Titusville, NJ 08560 USA). A third bird died on day 6 post fenbendazole treatment. The remaining four birds survived, and by day 10-12, the white blood cell counts were beginning to recover (total count 2200-10,000/μl; 35-63 % heterophils, 29-57% lymphocytes, 2-8% monocytes). Necropsy of the three dead birds revealed severe bone marrow hypoplasia, primarily of the myeloid series, and peracute bacteremias (gram-positive cocci). Ulceration of the crop and/or esophagus, with overgrowth of gram-positive cocci, was seen in two birds, while the third had necrosis of the trachea and crop with similar overgrowth of gram-positive cocci. In one bird, there was mild multifocal dilation of small intestinal crypts with attenuation and individual cell necrosis of crypt epithelial cells.
A similar scenario was experienced with a group of ten rock doves (eight males, three females; adults, exact ages unknown). Following diagnosis of capillariasis from examination of fecal samples, the rock doves were gavaged with fenbendazole (50 mg/kg p.o., s.i.d., 5 days). The first day following completion of therapy, one bird was found dead. The second day post treatment, seven birds died and by the end of the fourth day, all 10 birds were dead. Complete blood counts performed on four of the birds revealed profound leukopenia (white blood cells too few to estimate total count), characterized primarily by heteropenia. Packed cell volumes ranged from 51-62%. Other consistent findings included mild elevations of uric acid, AST and CPK. Necropsy on all 10 rock doves showed similar lesions. Bone marrow hypoplasia was severe, with myeloid cell lines the most affected. Damage to mucosal epithelial cells was evident in the intestine (where there was dilation and necrosis of crypts), and in the squamous mucosa of the esophagus, crop and cloaca. At the latter sites, epithelial cell hypertrophy was accompanied by karyomegaly and multinucleation, resulting in a disordered appearance of the epithelium. Other lesions included skeletal muscle degeneration and necrosis (four birds), renal tubular epithelial cell necrosis with mild regeneration (four birds) and acute rod bacteremia (six birds). All of the above changes were acute to subacute. Many birds also had more chronic lesions (non-suppurative enteritis with coccidia, proventricular nematodiasis, migrating nematode larvae, mild portal hepatitis) that were considered subclinical.

The last incident of presumed fenbendazole toxicosis concerned two picui doves and two bare-faced ground doves. Following death of an exhibit mate from ascariasis, these doves were treated with fenbendazole (50 mg/kg p.o., s.i.d., 5 days). The second day following completion of the treatment, one of the bare-faced ground doves presented to the hospital with lethargy. The bird died following blood sampling. Only 96 white blood cells (almost all lymphocytes) were seen on the blood smear. PCV was 8%. Necropsy revealed normal bone marrow cellularity but the population of cells was primarily immature (left shift). Intestinal crypt necrosis and epithelial cell hypertrophy were similar to that seen in the rock doves. The bird also had mild leukocytozoonosis and other incidental lesions. Examination of the three other birds revealed similar lethargy, with profound leukopenia (total numbers of cells on smears was 5-18). AST was elevated (592-1076 U/L). Prophylactic treatment with doxycycline (50 mg/kg p.o., s.i.d.) and itraconazole (10 mg/kg p.o., s.i.d.) was instituted. When rechecked about 1 wk later, white blood cell counts had recovered (8000-14,200/μl) and the birds were released from the hospital soon thereafter.

In summary, the most characteristic finding in all cases was profound leukopenia, with nearly absolute heteropenia. Other common, but variable, clinical findings included hypoproteinemia, hypo-albuminemia, elevated liver and muscle enzymes, uricemia and weight loss. Characteristic post-mortem lesions included bone marrow suppression (especially myeloid), epithelial cell necrosis and regeneration (especially of rapidly dividing cells), and acute bacteremia. Death in most cases was likely due to overwhelming bacterial infection, secondary to the severe immunosuppression. Virus isolation was attempted from liver of one rock dove and was negative. Although a possible viral etiology cannot be totally ruled out, lesions highly suggestive of viral infection were not seen. These clinical and post-mortem observations are similar to those seen in the previously presented cases of suspected albendazole toxicity in birds. In addition, comparable bone marrow toxicity has been described with albendazole administration in mammals. Based on the histories and similarities
in clinical and necropsy findings between all of these cases, we strongly suspect benzimidazole toxicity as the common link. Benzimidazoles are frequently used anthelmintics with a wide range of antiparasitic action, high degree of efficacy and a good margin of safety.\textsuperscript{2} The dosages used in these cases are within the recommendations for avian species\textsuperscript{1} and have been used without apparent ill effect in many species of birds at our institutions. Chemical analysis of one of the lots of fenbendazole used was within acceptable tolerance range. Thus, it may be that there are species-specific susceptibilities to toxicosis, especially within the Columbiformes. Alternatively, there may be as yet undiscovered co-factors in these cases that may have predisposed to toxicity.

\textbf{LITERATURE CITED}

COMPARISON OF ORAL AND PARENTERAL HEAVY METAL CHELATORS FOR THE TREATMENT OF LEAD TOXICOSIS IN COCKATIELS (Nymphicus hollandicus)

Mary C. Denver, DVM,1* Lisa A Tell, DVM,2 and Francis D. Galey, DVM, PhD3

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Abstract

Lead toxicosis is a relatively common and clinically significant medical problem in both companion and wild birds.1,4 The current recommended therapy for lead toxicosis is calcium disodium edetate (CaEDTA), however, parenteral administration is required and the potential for nephrotoxicity has been reported in mammals.2-4 Identification of an oral heavy metal chelator with minimal side effects for the treatment of avian lead toxicosis is, therefore, desirable. In this study, an oral chelating agent, meso 2,3-dimercaptosuccinic acid (DMSA, Chemet®, Bock Pharmacal, PO Box 419056, St. Louis, MO 63141-9056 USA), was evaluated alone at two dosages (40 mg/kg and 80 mg/kg), and in combination with the parenteral chelating agent, CaEDTA at 40 mg/kg (Calcium Versenate®, 3M Pharmaceuticals, Northridge, CA 91324 USA) at both DMSA dosages to evaluate its safety and efficacy at reducing blood lead concentrations in cockatiels. In addition, sodium sulfate salts (Anhydrous Sodium Sulfate, ACS Grade, Fisher Scientific, Fairlawn, NJ 07410 USA) were utilized at a dose of 0.5 g/kg in conjunction with these two chelators to evaluate their effectiveness at reduction of gastrointestinal absorption of lead particles. Results from this study indicated that administration of DMSA resulted in a rapid decline in blood lead concentrations when used alone or in conjunction with CaEDTA, however, DMSA had a low margin of safety at the higher dose. The administration of DMSA at the 80 mg/kg dose in cockatiels without lead toxicosis caused death in greater than 60 % of the cockatiel subjects in that group. Administration of sodium sulfate salts resulted in no significant difference in reduction of blood lead concentrations and the use of these salts would not be recommended. CaEDTA was not found to cause nephrotoxicity in cockatiels when used twice daily for 21 consecutive days. DMSA was not found to cause significant histopathologic changes in the cockatiels that survived treatment.

ACKNOWLEDGMENTS

This work was supported by a grant from the Center for Companion Animal Health, School of Veterinary Medicine, University of California at Davis. We thank John Trupkiewicz, DVM for performing the necropsies and histopathology, Don Priesler for photography, and Kirsten Dahl, Martha Needham, Shan Ikazawa, Leslie Storwick and Todd Hughes for technical support.

LITERATURE CITED


COMPARISON OF THREE MEDIA FOR THE STORAGE OF AVIAN WHOLE BLOOD

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Abstract

Transfusion medicine is a relatively novel facet of avian medicine. In transfusion studies using fresh whole blood, it has been found that heterologous transfusions do not last as long as homologous transfusions.1,2 Storage of avian whole blood could be useful so that hospitals would not have to maintain donor birds of many species. To date, there have been no studies in long-term storage of avian whole blood or its components. The shorter life span and higher metabolic rate of the nucleated avian erythrocyte is currently thought to make storage difficult, yet no controlled studies exist.3

The purpose of this study is to determine the effectiveness of three media: 0.9% citrate, acid-citrate-dextrose (ACD), and citrate-phosphate-dextrose with adenine (CPDA), in maintaining avian erythrocytes for 21 days. Ten milliliters of whole blood were collected from 10 healthy donor macaws (Ara spp.) monthly for 3 mo. Aliquots of 2 ml were collected from each sample on days 0, 7, 14, and 21. Potassium, sodium, pH, glucose, uric acid, and packed cell volume (PCV) were measured at each time point. Subjective measurements of gross hemolysis and red cell morphology were also made at each sample day. Intracellular ATP was measured on days 0 and 7 for the ACD and CPDA samples.

In all media, serum potassium levels became significantly elevated (P = 0.00025) by day 7. Sodium levels decreased in all samples (P = 0.0001), but were not thought to be clinically significant. Similarly, pH levels were lower in the ACD and CPDA samples than in citrate (P < 0.0001) because of the pH of the storage media, but were not thought to be of clinical relevance. Uric acid levels increased in all samples (P = 0.0008). Glucose levels decreased in the citrate samples (P = 0.042), but not in the ACD or CPDA samples (P = 0.024). PCV decreased significantly in all samples. Hemolysis increased over time in all samples. Cell morphology showed variable cell size and shape, irregular cytoplasmic membranes, clumped chromatin, and refractile granules within the cytoplasm. ATP levels significantly decreased (P = 0.011) in CPDA, but were not significantly different between days 0 and 7 in ACD.

The changes suggest a failure of the sodium-potassium exchange system within the erythrocyte during storage in the three media studied. The addition of dextrose and other cellular nutrients did not reverse or forestall this failure. It is thought that avian erythrocytes use lipid and protein degradation products for a significant energy source.4 The addition of these compounds to the
storage media remains to be investigated. Because of the decrease in ATP and large increase in serum potassium, it is suggested that none of the three media maintained viable erythrocytes in a medium which could be safely transfused into a patient.

LITERATURE CITED

Optimization of Deoxyribonucleic Acid Extraction from Mycobacterium avium and M. genavense for Polymerase Chain Reaction Testing

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Abstract

Mycobacteriosis is a bacterial disease that affects many species of birds. This disease predominately affects the gastrointestinal and hepatic systems, although respiratory and skeletal involvement may occur.12 Mycobacterium avium, M. tuberculosis, and more recently M. genavense are the species most commonly isolated from birds.8,9 Ante-mortem clinical diagnostic procedures used for screening avian patients include hematologic, biochemical, and serologic testing.13 In addition, radiographic studies, endoscopic examinations and acid-fast staining of feces and tissue biopsies may be utilized. The disadvantages of these tests are their low specificity, sensitivity, and general inability to provide a definitive etiologic diagnosis. The tuberculin intradermal skin test has been used successfully for identifying infected fowl and domestic poultry flocks, however, this test has been of little use for exotic avian species.1 Mycobacterial cultures and the BACTEC AFB system (Becton Dickinson Diagnostic Instruments, Sparks, MD 21152 USA) are more definitive ways of diagnosing mycobacteriosis.2 The disadvantages to these techniques are that a limited number of laboratories have the capabilities for culturing these organisms, it takes an extended period of time to get a positive diagnosis, and there is a potential for false negatives (the organism may fail to grow).

The newest development in diagnosing mycobacterial infections involves nucleic acid amplification and identification via polymerase chain reaction (PCR). Preliminary work (six cases) has shown PCR to be successful in the identification of M. avium and M. genavense in tissues from necropsied birds.2,3 Mycobacterial deoxyribonucleic acid (DNA) amplification and PCR identification have been described for direct detection in human clinical specimens (sputum and feces),4,5,10,11 however ante-mortem molecular diagnostic testing for birds has been limited.7

In order to develop a sensitive PCR assay for identifying mycobacterial organisms in tissue or fecal samples from birds, it is imperative that an optimal DNA extraction technique be identified. The challenge with mycobacteria is the high concentration of lipid within their cell wall, therefore, traditional DNA extraction procedures do not work or are less than optimal. Reported methods for extracting mycobacterial DNA include boiling,10 sonication,6 heat shock,14 and intensive enzymatic lysis.6,10 To date, only one study has addressed differences between extraction techniques utilizing
M. lepare, M. lepraemurium, and M. bovis.\textsuperscript{14} No work has been done to determine which DNA extraction technique is optimal for \textit{Mycobacterium avium} or \textit{M. genavense}.

The goals of this study were to compare and contrast four different DNA extraction methods (mechanical disruption, boiling, heat shock, enzymatic lysis) utilizing \textit{M. avium} and \textit{M. genavense} derived from synthetic media. \textit{M. fortuitum} (a fast growing ubiquitous mycobacterial species) was also utilized for initial development of the extraction techniques.

ACKNOWLEDGMENTS

This work was supported by a grant from the Center for Food Animal Health, School of Veterinary Medicine, University of California, Davis.

LITERATURE CITED

SUCCESSFUL SURGICAL MANAGEMENT OF CONGENITAL BIFID STERNUM IN TWO AFRICAN GREY PARROTS (Psittacus erithicus)

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Abstract

Congenital bifid sternum (cleft sternum) is an uncommon defect previously reported only in humans.1,2,4,5 Superior, inferior, and complete cleft sterna have been described with inferior cleft considered the most rare.1 Approximately half of the patients presenting with bifid sternum have clinical signs.5 Clinical signs occur as a result of respiratory compromise secondary to the paradoxical movements of the skin and viscera (usually the heart) through the defect during respiration and include cyanosis, dyspnea and recurrent respiratory infections. This can be a life-threatening condition in human patients. In patients without clinical signs, indications for surgical repair include providing protection of the subcutaneously located heart, and cosmetics as the view of the protruding, pulsating heart is rather disconcerting.2,5,6 In humans, surgical management within the first 6 wk of life is recommended in order to close the defect while the chest and lungs are still relatively compliant. In older patients, the use of costal chondrotomies, osteotomies, and artificial implants with Marlex or Teflon mesh, or acrylic plates has been described. This report describes the surgical repair of inferior bifid sternum in two unrelated African grey parrots (Psittacus erithicus) using bilateral myocutaneous pectoralis muscle transfer.

Case 1

A 7-mo-old African grey parrot of unknown gender weighing 370 g was referred from Salem, OR to the Veterinary Medical Teaching Hospital at the University of Florida for surgical treatment of inferior bifid sternum. The bird was sent air cargo and arrived without consequence. On physical examination the bird appeared healthy in all respects except for the presence of an inferior bifid sternum. The keel had fused cranially but was separated about 1 cm caudal to the cranial apex of the sternum. The distance between the halves of the sternum was 2 cm at midsternum and 3 cm at the caudal extent of the sternum. The cardiac movements were clearly visible subcutaneously and the skin over the heart was eroded. Preoperative complete blood count (CBC) and plasma biochemistry values were within reference ranges.

The bird was anesthetized with isoflurane in oxygen delivered by mask. Once anesthesia was induced the patient was intubated and maintained on isoflurane in oxygen. An i.v. catheter was placed in the medial metatarsal vein and LRS was administered at 10 ml/kg/hr. A single dose of cefazolin (20 mg/kg) was administered i.v.
The bird was prepared for aseptic surgery and an incision was made from 1 cm caudal to the cranial apex of the sternum to 2 cm caudal to the defect along the midline. The coelomic viscera (heart and liver) were directly dorsal to (beneath) the skin overlying the defect and were visualized through the incision. The skin was carefully elevated from the pericardium. The insertion of the pectoral muscles was incised along the keel and the muscles were elevated off the keel on each side using a periosteal elevator. Where the sternum was cleft a portion of the keel (perpendicular to the sternum) was present on each side. Since this vertical section of bone would inhibit the muscle transposition it was removed using bone cutters. The pectoral muscles were then elevated off the sternum progressing laterally continuing the dissection over the ribs. The superficial and deep pectoral muscles were not separated but, rather, elevated together as a unit with the skin. The thickness of the combined pectoral muscles was 1.5 cm.

Once the pectoral muscles were adequately mobilized on both sides they were sutured together along the midline using 3-0 polyglactin 910 (Vicryl) in a simple interrupted pattern. Tension on the sutures was minimal. The skin was closed with 3-0 nylon in a simple interrupted pattern.

Recovery was uneventful and the bird was given butorphanol at 1 mg/kg i.m. q 4 hr as a postoperative analgesic for 24 hr. The bird was housed in a small cage to minimize wing activity which might have stressed the pectoral muscle sutures. Skin sutures were removed 2 wk postoperatively and at that time cardiac movements were not visible.

**Case 2**

A 5-yr-old African grey parrot of unknown gender weighing 340 g presented to Sonora Veterinary Surgery and Oncology for surgical repair of an inferior bifid sternum. Several attempts had previously been made to close the sternal cleft; the most recent effort used Marlex mesh in an attempt to cover the defect. Unfortunately, the skin over the mesh became devitalized and the mesh was grossly visible protruding through the skin. The cardiac movements were visible beneath the mesh.

The bird appeared healthy in other respects. Preoperative CBC and plasma biochemistry panel values were within reference ranges. The anesthetic and surgical procedure used in this bird were analogous to that described above. The Marlex mesh was removed following skin incision and the soft tissues surrounding the mesh were debrided.

Recovery was uneventful and the bird’s wings were bandaged in an effort to prevent wing-flapping which would stress the suture line by increasing tension in the pectoral muscles. The bird returned for suture and wing bandage removal 2 wk postoperatively. The incision had healed and the cardiac pulses could no longer be visualized.

**Discussion**
Congenital bifid sternum is an uncommon defect reported in humans with superior (cranial) cleft being the most common and inferior (caudal) cleft sternum being the most uncommon. Embryologically, avian sternal development parallels mammalian development. In humans, the sternal bars develop at the sixth week of gestation. The sternal bars fuse from cranial to caudal with fusion being complete at the ninth week. The manubrium develops differently which may account for the higher incidence of superior cleft sternum in humans.

Respiratory compromise as a result of the paradoxical chest wall movement in the area of the defect accounts for the cyanosis, dyspnea, and recurrent pneumonia in clinically affected individuals. Birds do not rely on a negative intrapleural pressure for movement of air through the respiratory tract and are physiologically unaffected by bifid sternum. Other indications for surgery include cosmetics and to provide protection for the heart. It is unnerving to visualize the bird’s heart beating immediately beneath the skin. The bird in case 1 had skin erosions over the heart creating concern that, left unattended, the heart might eventually migrate through the skin with fatal consequences.

Early repair of bifid sternum is recommended in humans as the chest and lungs are more compliant in neonates. The surgical procedure is much more complicated when the patient is greater than 2 mo of age requiring extensive orthopedic reconstruction and synthetic implants. In both cases reported here, the sternum was ossified and not mobile enough to allow the halves of the keel to be approximated. Additionally, because of the air sac system, if the halves had been approximated, respiratory compromise may have occurred by decreasing the air sac volume. Because the pectoral muscles in birds are large in comparison to mammals, they provided a thick muscle pad to protect the heart from exterior trauma. For this reason, it was determined that a bony plate was not necessary. The keel (vertical portion) had to be removed in the area of the defect to allow the muscles to slide together over the defect to the midline. This section of bone was easily removed without complication.

Meshes have been used to repair bifid sternum in older human patients where it was not possible to reconstruct the sternum due to patient age. Repairs using mesh are more prone to complications and avoidance of the use of implants is a major reason early surgical intervention is recommended in humans. Birds have relatively little subcutaneous tissue to support a synthetic material. This is likely the cause of its failure in case 2.

This report describes a previously unreported congenital defect in birds—inferior bifid sternum—and its successful surgical management. In birds it appears that the defect is not associated with clinical signs and surgery is recommended in order to protect the heart from exterior trauma.

NOTE: Dr. Bennett has recently been contacted about a grey cheeked parakeet (Brotogeris pyrrhopterus) with inferior bifid sternum.

LITERATURE CITED
AVIAN WING BANDAGING AND THERAPEUTIC ULTRASOUND TREATMENT

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Abstract

Simple figure-of-eight bandaging is a widely employed practice following wing injury. Uses of wing bandaging include: immobilization before and after fracture repair, during wound management, and to protect the wing during transient nerve paralysis. Flighted pigeons were used to assess the impact of bandaging on soft and hard tissues in the pigeon wing, and the potential efficacy of ultrasound therapy in reversing these changes.

Methods

In two separate experiments presented here, ultrasound therapy was applied under standardized conditions (Forte US8 ultrasound machine, Chattanooga Group, Inc., Hixson, TN 37343 USA; probe = 2 cm diameter, scan rate = 4 cm/sec, intensity = 0.1 W/cm², session duration = 3 min, duty cycle = 100%, coupling = room temperature ultrasound gel) for the prevention and correction of bandaging induced contractures and reduced range of motion of the carpal and elbow joints in pigeons. In a third experiment, bone loss during bandaging and during ultrasound therapy was evaluated.

Contracture development and reversal was assessed by applying a 0.5 kg weight to the outer two wing primaries with a velcro cuff while the pigeons were in a standard measurement apparatus; angle measurements were taken once the wing was fully relaxed. A goniometer (loaded goniometry) was used to measure the angles of extension of the elbow (forward angle) and carpus (backward angle). Bone loss was determined by standardized serial measurements of metacarpals II-III employing an x-ray scanning bone densitometer.

Results

The results from the first experiment indicated a highly significant ($P \leq 0.0001$) effect of 10 twice weekly ultrasound treatments, for the prevention of both bandaging-induced elbow and carpal rotation loss, starting at four days following the start of bandaging as compared to bandaged control wings. In the second experiment, pigeons that had one wing bandaged for 42 days, randomly assigned to either of two treatment groups, showed that even three twice weekly ultrasound treatments reversed the loss of wing rotation at the carpus significantly as compared to unbandaged controls ($P = 0.0014$); although elbow rotation had not significantly changed with only three...
treatments, a trend was evident ($P = 0.14$). In the final experiment, bone loss over 4 wk of wing bandaging occurred at a rate of 2.96 %/wk, and bone density was significantly reduced ($P = 0.03$) as compared to unbandaged control wings in the same birds; however, as expected, ultrasound therapy had no significant effect on bone loss during this same period.

**Conclusions**

Based on these preliminary investigations, therapeutic ultrasound was useful in the prevention and reversal of wing joint rotational loss seen in response to figure-of-eight wing bandaging, but did not reverse the bone loss trend toward disuse osteoporosis in these same birds. The potential therapeutic application of this treatment modality for a swifter return to normal soft tissue function and wing use after bandaging appears promising.
THE IMPACT OF EL NIÑO ON MARINE MAMMALS

Terry R. Spraker, DVM, PhD; Francis Gulland, DVM, PhD; and Robert DeLong, PhD

Abstract

El Niño is a disruption of the ocean and atmospheric systems that affects weather worldwide. In non-El Niño years trade winds in the Pacific blow towards the west. These winds pile up warm surface water (about 0.5 m) in the western Pacific. The waters in the western Pacific are up to 8°C higher than the eastern Pacific along the South American coast. The upwelling of cold water off the South American coast is associated with nutrient-rich water, supporting high levels of productivity at all levels of the food chain. During El Niño, the trade winds relax in the central and western Pacific leading to a depression of the thermocline in the eastern Pacific and an elevation of the thermocline in the west. During the El Niño of 1982-1983 the thermocline (level where water is below 17°C) dropped from 50-150 m. This reduces the efficiency of upwelling to the surface and cuts off the supply of nutrient rich water to the euphotic zone. The results are a rise in sea surface temperature and a decline in primary productivity, the latter of which adversely affects higher trophic levels of the food chain.

The impact of an El Niño on marine mammals depends on it’s intensity and therefore can be minimal to severe. We have had an opportunity to necropsy California sea lion pups from San Miguel Island during two different El Niños, the first in the summers of 1991 and 1992 and again in January of 1998. Many of the young pups that were found dead and examined in the summers of 1991 and 1992 were emaciated and had severe hookworm and lice infections. Most of the pups found dead in January of 1998 were emaciated and had minimal hookworm infections. El Niño of 1998 had a tremendous impact on rehabilitation centers along the California coast. For example, approximately 500 animals are brought into the Marine Mammal Center, Sausalito, California in an “average year”; however during the El Niño years the number of animals brought into the Center doubles. Also the number of specific species varies. For example, in an “average year” the Center receives approximately four to five northern fur seals and this year alone the Center has received 45 fur seals. Conditions found in these animals brought to the rehabilitation center included emaciation, increased parasitism, lungworm pneumonias, penetrating gastric ulcers with localized peritonitis, and gun shot.
CLINICOPATHOLOGIC FEATURES OF AN EPIZOOTIC IN THE DOUBLE-CRESTED CORMORANT (Phalacrocorax auritus) ALONG THE FLORIDA GULF COAST

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Abstract

Epizootics in the double-crested cormorant (Phalacrocorax auritus) population have been documented along southern Florida’s gulf coast for the past 20 yr. Affected animals exhibited ataxia and disorientation and were frequently captured and brought to local wildlife rehabilitation centers for treatment. C.R.O.W., a rehabilitation center on Sanibel Island, received 227 affected cormorants in 1995 and 161 affected cormorants in 1996. In both years, the majority of debilitated birds were admitted during a period of only a few months. Histopathology on tissues collected post-mortem failed to reveal a common etiology or an explanation of clinical signs. Multi-organ bacterial cultures were non-specific and virus isolation was unproductive.

Between October and December of 1997, C.R.O.W. admitted 55 cormorants. Severe ataxia was the predominant clinical finding in 50 cormorants. The five cormorants in which ataxia was not noted presented for other reasons (hit by car, unknown trauma, and fish hook ingestion). All affected cormorants suffered from cerebellar ataxia characterized by a broad based stance, truncal incoordination, hypermetric gait, and intention tremors of the head. Normal limb strength was preserved. Stimuli often resulted in exaggerated responses and hyperactivity. Neurologic signs were worsened by patient handling. Eighty-four percent of affected cormorants were markedly underweight (<1.3 kg). All of the ataxic cormorants had melena and watery diarrhea. Severe anemia (PCV less than 20%) was present in 28% of affected birds and hypoproteinemia (TS less than 2.0 g/dl) was present in 40% of affected birds. Endoparasitism was a common clinical feature. Contracaecum spp. and Tetramera spp. were frequently identified. Renal trematodiasis was observed in all samples that were examined with light microscopy. Thirty-two percent of affected cormorants responded to supportive care and were released 4-8 wk later.

As in previous years, the histopathologic findings from the 1997 epizootic were non-specific. Pulmonary hemorrhage and congestion were commonly noted but were though to be associated with acute agonal cardiovascular collapse. Other consistent findings included hepatic and splenic hemosiderosis as well as chronic mild cholangitis and nephritis. None of these lesions were interpreted as severe enough to be the primary cause of death. Acute bacterial septicemia and intravascular coagulation were noted in several cases. No brain or brainstem lesions were found.
A brevetoxin immunohistochemical staining technique was used to detect brevetoxin in samples from four affected cormorants. Brevetoxin, produced by the marine dinoflagellate *Gymnodinium breve*, is a neurotoxin known to affect marine animals. Furthermore, blooms of this dinoflagellate, called red tide, are often associated with fish kills. The immunohistochemical test documented uptake of brevetoxin in lymphoid cells of the spleen and macrophages of the spleen and lung in all four cormorants. Coincident with the 1996 and 1997 epizootics in cormorants, West Indian manatees (*Trichechus manatus latirostris*) in this area experienced a mortality event attributed to brevetoxicosis.¹ Local red tide blooms were documented during these time periods also. The cormorant immunohistochemical results showed a pattern of immunostaining similar to the pattern seen in samples taken from the manatees. A controlled study is currently in progress to validate the use of the brevetoxin immunohistochemical test in the double-crested cormorant.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Kim Miller and the staff at the National Wildlife Health Center for graciously providing the double crested cormorant samples necessary to validate the immunohistochemical test.

LITERATURE CITED

UPDATE ON THE ANIMAL MEDICINAL DRUG USE CLARIFICATION ACT OF 1994 REGULATIONS FOR WILDLIFE VETERINARIANS

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Abstract

With the passage and 1996 advent into law of the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA), several questions remain unanswered with regard to the implications for the practice of veterinary medicine involving free-ranging wildlife or zoological species. The practical application of the provisions of this act for wildlife species that are defined as food producing animals are largely left to the discretion of the veterinarian and include acquiring information relative to use of any drugs prescribed or dispensed for extra-label use, determining appropriate dosage, dose frequency, and meat withdrawal times. Therefore consideration must be given to the following questions and/or situations: 1) Does a valid veterinarian-client-patient relationship exist when drugs are used in an extra-label manner by lay personnel; 2) is there sufficient information to justify the extra-label use of a drug (reasonable basis, lack of approved drugs, withdrawal times can be followed, drug metabolism data); 3) is the veterinarian prepared to be responsible for consequences of extra-label use of a drug including adverse reactions and residue problems. Limited data are available for meat withdrawal periods for wildlife species that are considered to be food producing animals. Further information will be forthcoming as the provisions of AMDUCA are tested over time.

Introduction

The practice of veterinary medicine is filled with an ever changing world of animals and owners. When practicing on wildlife species, the excitement and challenges of veterinary medicine are magnified, sometimes to great magnitudes with the variety of wildlife species that could be faced.

The legal and logical use of drugs is one of the most prominent problems that face exotic animal veterinarians. The use of drugs in animals by veterinarians is regulated under the Code of Federal Regulations, specifically Regulation 21 or the Federal Food, Drug and Cosmetic Act (CFR 21 USC 360b(a)) which addresses the manufacturing, distribution, and usage of drugs in animals.

Currently, there are only five drugs that are approved for use in free-ranging wildlife species. Four of these drugs are limited to use in cervids and include xylazine hydrochloride (Cervizine, Wildlife Pharmaceuticals; Rompun, Bayer), yohimbe hydrochloride (Antagonil, Wildlife Pharmaceuticals) for the reversal of xylazine, carfentanil citrate (Wildnil, Wildlife Pharmaceuticals), and naltrexone (Trexonil, Wildlife Pharmaceuticals) for the reversal of carfentanil. The fifth drug is fenbendazole.
(Safe-Guard/Panacur, Hoechst-Roussel) which is approved for use in bears, large cats, feral swine, several species of antelope, and bighorn sheep. The use of all other drugs in exotic animals is considered extra-label at best and illegal at worst.

Because the number of approved drugs is limited, veterinarians and non-veterinarians have often used many drugs in an extra-label manner in animals, especially for wildlife. Extra-label usage is defined as the use of a drug at a dosage, or frequency or in a species other than that which is approved. Extra-label use allowed veterinarians to use approved drugs in non-approved species and non-approved drugs and human label drugs in animals on an as needed basis.

Provisions of AMDUCA

In October 1994, the Animal Drug Use Clarification Act of 1994 (S 340) was passed, and as originally written, AMDUCA codified the extra-label use of drugs in animals by veterinarians. The final version of AMDUCA was approved in November 1996 and became effective on December 9, 1996. The basic provisions of AMDUCA are summarized below. Note that there is a major difference between the requirements for extra-label use of drugs between non-food and food producing animals.

Extra-label use of human or animal drugs is NOT allowed if:
1. the drug is to be used in or on animal feed
2. the Secretary of Health and Human Services prohibits certain uses of animal drugs
3. there is another animal drug with the same ingredients, dosage form and concentration that provides for the intended use
4. the Secretary of Health and Human Services finds that the use of the drug may present a risk to public health
5. the use of the drug results in residues exceeding safe levels as established by the Secretary of Health and Human Services

Extra-label use of animal drugs is allowed in non-food animals if the drugs use are:
1. approved by the Food and Drug Administration for use in animals
2. by or on the lawful written or oral order of a licensed veterinarian
3. within the context of a veterinarian/client/patient relationship
4. in compliance with rules under the Secretary of Health and Human Services

Extra-label use of human drugs is allowed in non-food animals if the drugs used are:
1. approved by the Food and Drug Administration
2. by or on the lawful written or oral order of a licensed veterinarian
3. within the context of a veterinarian/client/patient relationship
4. in compliance with rules under the Secretary of Health and Human Services

Extra-label use of animal and human drugs is allowed in food animals if:
1. there is no approved animal drug labeled for such use (same active ingredient, dosage form and concentration)
2. the approved animal drug is clinically ineffective for its intended use
3. the veterinarian
   a. makes a careful diagnosis and treatment plan
   b. establishes a substantially extended withdrawal time
   c. ensures that treated animal(s) are identified
   d. ensures that withdrawal times are followed
4. such use is in accordance with appropriate medical rationale
5. no human food safety information is available, the animal must not enter the human food supply

Implications of AMDUCA

Now, what do all these regulations mean for veterinarians and wildlife health professionals? First, under AMDUCA, a veterinarian must select, prescribe and/or dispense drugs that are to be used in an extra-label manner within the context of a valid veterinary-patient-client relationship. Second, for extra-label use, AMDUCA requires that only Food and Drug Administration approved drugs be used. Third, AMDUCA requires that animal drugs approved for a particular use be used when they are available. Since there are approved formulations of xylazine, yohimbine, carfentanil, and naltrexone available for use in cervids, these are the forms of these drugs that should be used in deer. Using other products, although they may contain the same active ingredient in the same concentration, in species for which the drug is approved would appear to be contrary to the current requirements of AMDUCA. Fourth, AMDUCA requires that the veterinarian establish a scientifically appropriate withdrawal time for drugs used in an extra-label manner in food-producing animals. Since there are no residue studies available for drugs in wildlife species, the current recommendations are to use an extended withdrawal period for all drugs used in deer, although the extended period is undefined. Fifth, AMDUCA requires specific labeling requirements for drugs that are dispensed or prescribed for extra-label use. Finally, under AMDUCA, the extra-label use of drugs in or on food is illegal, except for treatments directed at individual animals. Drugs that are currently approved for use in this manner are still legal in the species for which they are approved, so, for instance, the use of Strongid-C, the equine pelleted dewormer, in horses is allowed; so is SafeGuard, the pelleted bovine dewormer, in cattle, bears, large cats, feral swine, ruminants in the families Antilopinae, Hippotraginae, and Caprinae, and bighorn sheep. But the use of these products is not legal in species for which they are not approved, even as an extra-label use. Therefore, in food-producing animals, including species of wildlife defined as food-producing animals, feed based drugs used in an extra-label manner should be considered prohibited.

So, given these regulations, what effect do they have on wildlife veterinarians and wildlife health personnel working in the field? Several questions regarding extra-label use of drugs in wildlife remain unanswered.
Under AMDUCA, extra-label usage of drugs should take place under supervision of a veterinarian. What is the definition of supervision within the context of the diversity of people involved in wildlife health? In essence, it becomes the responsibility of the veterinarian to decide if extra-label drug use is appropriate and whether people are capable of using the drugs appropriately and safely. Training of lay personnel is essential!

What sources of information are appropriate from which to obtain data for the establishment of withdrawal times in species in which drugs are used in an extra-label manner? Generally any reasonable source is fine, but again the veterinarian is responsible for the consequences of such use and decisions regarding appropriate withdrawal times.

Can data for appropriate drug dosages and withdrawal times be extrapolated between species? The answer is unclear. The veterinarian is responsible for decisions based on whatever data are available, but what if no data are available for the species in question? How then is a withdrawal time to be established? Table 1 lists some general information on meat withdrawal times for several drugs used in wildlife. The data for deer were based on theoretical use of the drug in a 70 kg deer. Notice that there is a major difference between suggested withdrawal times in cattle and deer, despite the fact that deer have a smaller body size and a higher metabolic rate per unit of body mass than cattle.

What allowances are made within AMDUCA for species in which there are established hunting seasons or for species in which subsistence hunting occurs? Again, the veterinarian is responsible for establishment of a withdrawal times in such situations and will be held responsible for any residue problems that may develop. However, some follow-up questions should immediately come to mind, like should wildlife that is captured, handled, or treated in any fashion prior to or during the hunting season be marked to allow private citizens to identify them prior to harvest? Because the veterinarian has no control over movements of animals, what should be done in the case of animals that move over the course of time? The answer was “to be careful.”

For wildlife, none of these questions have clear answers, but we must be aware of the current situation and the decisions that must be considered prior to the use of drugs in an extra-label manner in wildlife species. Interpretation of AMDUCA is underway and only time and experience will provide better definition to these and other questions.

LITERATURE CITED
1. Food Animal Residue Avoidance Database, United States Department of Agriculture.
<table>
<thead>
<tr>
<th>DRUG</th>
<th>FARAD Deer</th>
<th>USA Cattle</th>
<th>NZ Deer</th>
<th>NZ Cattle</th>
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<td><strong>Fenbendazole</strong></td>
<td>Dose</td>
<td>10 mg/kg p.o. q 24 hr × 3 days</td>
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<td></td>
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<td>Dose</td>
<td>0.2 mg/kg s.c. once</td>
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<td>11 mg/kg i.m. q 48 hr × 5 days</td>
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<td><em>(LA 200)</em></td>
<td>Withdrawal</td>
<td>45 days</td>
<td>28 days</td>
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<td><strong>Penicillin</strong></td>
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<td>30,000 U/kg i.m. q 24 hr × 7 days</td>
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<td><strong>Carfentanil</strong></td>
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<td><strong>Xylazine</strong></td>
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<td><strong>Yohimbine</strong></td>
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<td>no approval</td>
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<sup>a</sup>Withdrawal time for bears, large cats, feral swine, antelope, and bighorn sheep is 14 days for the oral formulation.<br><br> <sup>b</sup>These drugs should not be used in free-ranging cervids within 30 days of the hunting season.
ANIMAL TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES (TSEs) AND THEIR IMPACT ON ANIMAL AND HUMAN HEALTH WORLDWIDE

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Abstract

Bovine Spongiform Encephalopathy (BSE) was first diagnosed in Great Britain in 1986. Between 1986 and May 1998 there have been over 171,000 confirmed cases of BSE reported in Great Britain. In addition, BSE has been diagnosed in native cattle from other countries of the United Kingdom and Europe. The BSE epidemic in the United Kingdom peaked in 1992 and 1993 with approximately 1000 suspect cases reported per week. Currently the number of cases reported per week is 100 or less. This decline is largely a reflection of the control measures, especially certain feed bans which were enacted in 1988 and thereafter. BSE is a member of the family of disease known as the transmissible spongiform encephalopathies (TSEs). Other related animal diseases include scrapie of sheep and goats, chronic wasting disease (CWD) of deer and elk, transmissible mink encephalopathy (TME), feline spongiform encephalopathy (FSE), and TSEs of exotic ruminants.

Many of these diseases are either known to be inter-related or speculation of causative inter-relationships exist. For example, evidence indicates that FSE and the TSE in exotic ruminants was caused by the feeding of BSE contaminated products to these species. There is also evidence that a new variant of Creutzfeldt-Jakob Disease, a human TSE was caused by the BSE agent. It has been postulated that scrapie started the above chain of events being the underlying cause of BSE. Although no case of BSE has been detected in the United States, trade has been impacted by the existence of scrapie, TME, and CWD which are cited as risk factors.

BSE strictly as a disease entity has been reported to have clinically affected less than 200,000 head of cattle worldwide in a 12-yr period of time. The other diseases have been reported at even lower levels. These are relatively small in comparison to deaths and other losses from highly infectious, contagious diseases such as Foot and Mouth Disease (FMD). The primary impacts on the world animal health situation have been in the areas of a likely or speculated human health link, various regulatory controls, trade, consumption, public perception about animal health, research, and economics.

For diseases that have had little impact on actual animal health, millions of dollars have been lost by countries involved in the trade of animals and animal products (even those without BSE). These diseases have also caused the loss of consumer confidence in the food supply and governmental controls in a number of countries.
ON COMMON GROUND: COOPERATIVE PERSPECTIVES OF WILDLIFE VETERINARIANS, WILDLIFE BIOLOGISTS, AND WILDLIFE REHABILITATION VETERINARIANS

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Abstract

A sense of proportion arising from our mutually exclusive backgrounds has separated wildlife veterinarians, wildlife biologists, and wildlife rehabilitation veterinarians by a veil of misconception. A new generation of scientists has begun to understand that the origin, history, characteristics, habits, population dynamics, and health of plants and animals are indivisible, as is the biomedical community. Shared insights of each genre can facilitate rather than handicap our shared goals.

In a world of limited environmental resources and expanding human populations, public interest puts pressure to bear not only on wildlife, but on wildlife stewards. Lay and scientific views of problem solving appear conflicted when we resort to labeling "environmentalist," "animal rights activist," "anthropomorphic," "hunter," etc. with disdain. Although it may be impossible to completely understand one another’s views, it is practical at least to respect the right to those views and worthwhile to attempt to understand their basis. How can those professionals who deal with wildlife populations and those who deal with wildlife individuals be aware of each other’s concerns and address issues cooperatively? By embracing the knowledge of their fellow researcher.

Personally, I am a veterinarian, retired from clinical practice. As a boarded surgeon, with experience in exotics, I gravitated to an avocation of treating wildlife patients. With an agricultural upbringing and as a traditional sportsman, I understand a wide variety of concerns regarding the role of wildlife in today’s changing attitudes toward it’s management. That change is all the more reason for a cooperative presence. In my manner, I seek the wisdom of colleagues. In my experience, for every answer found, there are many new questions. In my decision, “it’s your call,” not because I shirk responsibility, but because I recognize the limits of my proficiency. In government and non-government agencies and private research, there is an overlap of analysis, but the unique perspective of the investigative class enriches rather than diminishes the results. I can provide the wildlife biologist a surgical solution to a moose calf’s leg fracture; one can direct its management, nutrition, and resolution. Wildlife veterinarians alert me as a rehabilitation veterinarian to new disease concerns; I report incidents of disease and suspected foul play in patients taken directly from the wild which could serve to avert an epidemic or apprehend a poacher.

Objective vs. subjective, emotional vs. dispassionate: What’s in a word? Semantics is less important than recognizing our common ground, wildlife welfare. Human dimensions escalate contact with wildlife, both consumptive and non-consumptive, promoting a call for medical care of wildlife
individuals that cannot be ignored. If we recognize that pragmatic compassion can blend with indigenous population management and sustainable biodiversity, we have an efficacious coalition. Candid dialogue is not wasted on the earnest.

If communication is the responsibility of the communicator, can I emphasize my concerns in a straightforward and diplomatic manner? If awareness of the other party’s worry requires my positive regard, can I put aside prejudice? How do I implement my good intentions? Self-determination forces me to be patient in the company of mental midgets, humble in the presence of mental giants, flexible rather than deadlocked, humorous in the face of challenge. There is nothing more satisfying than an equitable solution, unless as my wife claims, it’s morning “sex.”
TOWARDS THE GREATER GOOD: FINDING COMMON GROUND WITH ANIMAL PROTECTION ORGANIZATIONS

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Abstract

“Every living thing has an intrinsic value that derives from creation. It exists by reason of a design and order not of human making. Yet humankind has all too often abused or destroyed other life capriciously and wantonly.”

These are the words which begin to define the statement of principles and beliefs of The Humane Society of the United States (HSUS). It is the primary and motivating concern of The HSUS to prevent cruelty to all living creatures. We believe that humans are responsible for the welfare of those animals which have been domesticated and for those animals upon whose natural environment humans have encroached. We are accountable as a species, for we have neither the right nor the license to exploit or abuse any animal. Therefore, as an animal protection organization, The HSUS endeavors to promote animal welfare and strives to engender a respect for all living things.

As the wildlife veterinarian for The HSUS it is my hope that this is a fundamental common philosophy shared by the wildlife and zoo veterinary community. It is basic to our principles and convictions as veterinarians to be advocates for the animals placed in our care. These principles compel us to seek better animal welfare regulations for wild animals in captivity to minimize stress and to provide behavioral enrichment; to improve federal animal transport regulations; to support the maintenance of such federal policies as the Endangered Species Act, the Marine Mammal Protection Act, and the Migratory Bird Treaty Act; and to promote the enforcement of CITES and other international animal protection agreements. It is also our profound responsibility to oppose the traffic of wild animals particularly for the exotic pet trade, and to discourage the public from keeping wild animals as pets.

On many of these issues, the wildlife/zoo veterinary community and animal protection organizations like The HSUS have found common ground. Public education on the needs of wild animals and their roles in ecosystems must be a common goal. If we strive to put the animals’ welfare foremost in our efforts, then, indeed we will be working towards the greater good.
DISEASES OF CORALS AND OTHER REEF ORGANISMS

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Abstract

Tropical and subtropical marine ecosystems are noted for the diversity of their fauna and flora and particularly for the coral reefs which directly and indirectly provide food, habitat, and other resources in what have been considered to be nutrient-poor waters. The scleractinian corals and coralline algae form the structural and functional basis of the reefs. Thus, the demise of these organisms in many localities has brought concern about their fate and that of the other species they support. Beginning in the mid-1970s, several diseases of corals were recognized. Black-band and red-band diseases result from the formation of mats consisting of consortia of cyanobacteria and other microbes that produce hydrogen sulfide and anoxia to cause the death of the coral. Rapid tissue loss (a few millimeters to centimeters per day) has also been observed in what are known as white-band disease, white plague, and white pox, but the causes of these diseases are still under investigation. Yellow-blotch and yellow-band diseases, in which a patch or margin of yellowish-lightened tissue appears on the surface of some species of corals, have reached epizootic levels on some reefs. Bleaching, loss of the corals’ symbiotic algae or algal pigments, has occurred globally. Coralline algae have succumbed to coralline lethal disease and coralline lethal orange disease. Diseases that are suspected to be caused by pathogens have also affected other reef species, including sponges, gorgonians, giant clams, and echinoderms. The most extensive epizootic known in marine ecosystems reduced populations of the Caribbean long-spined sea urchin by 85-100 percent. As in other organisms, the roles of pathogens and abiotic factors are undoubtedly tightly interlinked. Changes in water quality, such as increased nutrient, chemical, and sediment loading, extremes in temperature and salinity, and increased ultraviolet radiation, are believed to be altering the susceptibility of reef organisms to parasites and pathogens. Continuing research on the causal agents of disease in corals, coralline algae, and other reef invertebrates should improve our understanding of these interactions and suggest potential management options for protecting these important organisms and the coral reef ecosystem.
DISTRIBUTION AND PREVALENCE OF BITTER CRAB SYNDROME IN SNOW (Chionoecetes opilio) AND TANNER (C. bairdi) CRABS OF THE BERING SEA, 1988-1996

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Abstract

Bitter crab syndrome (BCS) is a fatal disease of crustaceans that is caused by a parasitic dinoflagellate of the genus Hematodinium. The causative agent has been reported in crustacean hosts from Australia, Europe, eastern Canada and the United States, western United States and Canada, and Alaska. Upon gaining entry in to a crustacean host, the parasitic dinoflagellate proliferates, eventually replacing all host blood cells in the hemolymph. The mechanism of death is not completely understood, but it is clear that just prior to death, remarkable hemolymph changes do occur. Death resulting from secondary invaders is also likely.

Because infections are fatal and prevalences in some regions have approached 100%, the disease is believed to have significant impact on host populations. In southeast Alaska, areas of high BCS prevalence have been avoided in attempts to minimize spread of the pathogen and because commercial crabbing in these high prevalence areas is not economically profitable; product from these areas is rejected by the processors because of poor quality.

Between 1988 and 1996, hemolymph samples from 14,359 Eastern Bering Sea (EBS) Tanner crabs, Chionoecetes bairdi (n = 5180) and C. opilio (n = 9184) were examined for the presence of a parasitic dinoflagellate, Hematodinium sp., the causative agent of Bitter Crab Syndrome (BCS). For this time period, total prevalences of BCS in C. bairdi and C. opilio were 1.87% and 3.57%, respectively. In C. bairdi, prevalences from yearly random samples ranged from 0% in 1989 and 1994 to 5.68% in 1996. Infections in both males and females were highest in 1996, reaching 9.93% in females and 2.74% in males; however, overall Hematodinium prevalences were only slightly more elevated in females (1.93%) than males (1.65%). For C. opilio, yearly random sample prevalences ranged from 0.30% in 1994 to 8.45% in 1988. Highest Hematodinium prevalences in C. opilio were observed early in the survey; in 1988 during which male and female infection prevalences were 7.62% and 10.00%, respectively. Overall parasitic prevalences in C. opilio were more elevated in females (4.23%) than males (3.23%).

BCS infections in both C. opilio and C. bairdi were most common in the Bering Sea at latitudes above 57N. In general, infection prevalences in C. opilio increased with increase in latitude with
prevalences of 50-80% common in Norton Sound and west of St. Lawrence Island. Despite the fact that prevalences were generally lower in the Chukchi Sea than in Norton Sound and west of St. Lawrence Island, a greater percentage of sampled stations were positive for BCS in the Chukchi Sea. For *C. bairdi*, infections were rare in the Eastern Bering Sea, and increased only slightly along the shelf edge west and north of the Pribilof Islands.

Infections of the parasitic dinoflagellate in *C. opilio* were more common in shell condition-1 crabs (e.g., recently molted but soft shelled crabs) with females (15%) more frequently infected than males (6.7%). In contrast, infections in *C. bairdi* were generally much lower and most frequent in shell condition-2 crabs (e.g., recently molted but hard shelled crabs); overall, male and female prevalences were 1.7 and 2.6%, respectively.

For both *C. opilio* and *C. bairdi*, infections were more common in small crab less than 60mm; after which, prevalences remained low. In *C. bairdi*, the highest infection rates were observed in 20 mm crab attaining levels of 62.5% in males and 65% in females. In *C. opilio*, highest prevalences were observed at 35 mm with little difference in prevalence between males and females.

In summary, our results suggest that: 1) juvenile crabs are more frequently infected than large mature crabs, 2) infections are more prevalent in crabs collected from warm water than cold, 3) infections are more prevalent in recently molted crabs, 4) infection prevalences in *C. opilio* increase with increase in latitude, 5) infections in *C. bairdi* are sporadic and rare, and 6) two species of *Hematodinium* may exist in the Bering Sea. At the present time, sufficient information on the life history of the parasitic dinoflagellate is not available to address the annual differences that the data have presented.
HEALTH STATUS OF MARINE AND ESTUARINE FISH IN THE USA: EMERGING ISSUES

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Abstract

Over the last 20 yr, increasing mass mortality and diseases in aquatic organisms in the United States are being considered as another “sign of declining ecosystem health.” Causal factors typically associated with disease and mortality are poor water quality, toxicants, other environmental perturbations, infectious agents, and genetic susceptibility. Despite the ubiquitous nature of harmful algal blooms in marine environments, the extent to which biotoxins are involved in mortality and disease processes in fish (and other aquatic organisms) has only recently been realized. This paper will discuss a series of fish disease and mortality events in the Gulf of Mexico, eastern USA, and Salton Sea, California, will provide specific examples of the difficulties and challenges associated with current diagnostic approaches, and will hypothesize multifactored causal scenarios.

Harmful algal blooms and biotoxins

Harmful algal blooms (HABs) cause massive fish kills and animal mortalities, shellfish poisonings, and respiratory irritation in humans. For example, at least 37 species of toxic microalgae, including 11 ichthyotoxic species, are known in the Gulf of Mexico coastal waters. The sudden appearance of toxic planktonic blooms (e.g., red tides) that lead to acute, mass mortalities of fish have been historically documented. Traditionally, HABs are known for their lethal, fast-acting effects on aquatic organisms, but recently the potential role of HABs and their associated biotoxins in chronic or sub-lethal disease events has been identified.

In the summer of 1980, winter/spring of 1993/1994 and again in the summer of 1997 (unpublished data), heavy mortalities of tropical reef fish were reported along Florida’s southeast coast and along the Florida Keys. In each case a chronic disease syndrome affected mostly adult herbivores or omnivores. In most cases, diseased fish had lesions or shallow ulcerated body sores, fin or tail rot, and a heavy mucus coating on the body surface. Numerous protistan parasites and bacterial infestations were detected. The widespread nature and distribution of disease amongst different fish species with a commonality in specific feeding strategies suggested that the potential pathogens observed were not the principal cause of the disease syndrome but were secondary invaders of fish whose health had already been compromised. Biotoxins should be considered as a strong possibility in the role of primary stressors leading to chronic toxicity and immunosuppression. Tropical reef fish are potentially exposed to numerous biotoxins through their diet, either through direct consumption of toxic macroalgae such as Caulerpa, or through the consumption of toxic...
microalgae such as cyanobacteria or dinoflagellates that are epiphytic on macroalgae, seagrass, sponges, corals, or sand. Thus, many herbivorous or omnivorous fish species are at risk of dietary exposure to biotoxins that may be a predisposing factor in fish disease either directly or up the food chain. The seasonal dynamics in population abundances of these organisms over a widespread area might correlate with the sporadic and isolated incidences of this tropical reef fish disease syndrome. In many ways, this scenario may be linked to the tropical food poisoning in humans known as ciguatera that is also common in the same areas and may involve the same toxins. Traditionally, ciguatera toxins produced by benthic dinoflagellates such as *Gambierdiscus toxicus* are passed up the food chain without affecting fish health. There are strong indications that this may not necessarily be the case.

From 1980-1989, at least 50% and 69% of fish kills in the Gulf of Mexico and South Atlantic respectively were attributed to low dissolved oxygen. It is possible that many of these kills were associated with harmful algal blooms caused by small, ephemeral dinoflagellates that were not, until recently, recognized as being toxic. Such fish kills would have been attributed to low dissolved oxygen associated with the bloom rather than direct ichthyotoxicity. Recently, a series of fish kills, ulcerated fish disease events, and public health threats have highlighted the enigmatic life strategies of small (< 25 μm), toxic dinoflagellates such as *Heterocapsa*, *Gymnodinium*, *Gyrodinium*, *Pfiesteria* or a new cryptoperidiniopsoid. Ulcerative disease syndrome (UDS) in estuarine fish has been documented from the late 1970s until the present, from New York to Florida. While a clearly defined pathology and the presence of opportunistic pathogens characterize the disease, it was unclear what environmental factors were involved. Only recently has an association between UDS and *P. piscicida* been implicated. When fish are experimentally exposed to *Pfiesteria piscicida*, toxins cause hemorrhaging and sloughing of the skin epithelium. Ulcers are caused by the proliferation of opportunistic pathogenic fungi or bacteria (*e.g.*, *Aeromonas hydrophila*) that invade the damaged external layers of the fish skin. The basic pathology of UDS appears to be similar in all areas surveyed, but the opportunistic pathogens associated with the disease may vary because most invading microbial flora are typical estuarine inhabitants that are usually normal components of fish surface, gills, and intestinal tracts. In the wild, at least 25 species of estuarine fish are affected by UDS. UDS is common in low to moderate salinities but not all fish species in these salinity ranges are affected. Degraded water quality conditions in certain estuarine waters (UDS occurs in inshore areas of low to moderate salinity up to 25 ppt) are thought to be associated with disease outbreaks, but no definitive cause-effect has been shown. There does appear to be strong circumstantial evidence for a correlation between the presence of UDS and *P. piscicida*, but this needs to be verified in the field and investigated with respect to the distribution of other potentially toxic dinoflagellates.

Many dinoflagellates have superficially similar morphologies that can lead to misidentification and inaccurate reporting. For example, we have recently identified as many as 10 potentially toxic dinoflagellate species at fish kill or disease events. The potential for several species of dinoflagellates to co-occur in estuaries makes specific identification of these organisms critical because management strategies may vary depending upon the risk of toxin exposure to animal resources or to the public. Along the eastern seaboard, we have consistently documented the
presence of the cryptoperidiniopsid at sites where fish lesion events are occurring. Along with *Pfiesteria piscicida*, the role of the new cryptoperidiniopsid in the potential initiation of fish lesions needs to be examined.

**Pathogens**

Mass mortalities of a single species of free-ranging marine fish over a broad area would typically be associated with a species-specific infectious pathogen. However, such cases are rarely documented. In the fall of 1995 and summer 1996, a mass mortality of the hardhead catfish, *Arius felis*, was recorded throughout the Gulf of Mexico and Florida Atlantic from Jacksonville, Florida to Galveston, Texas (Landsberg et al., unpublished data). Millions of (mostly) adult fish were estimated to have died. Initial investigations revealed the presence of amoebae and non-specific ciliate protists on the gills of fish. External characteristics of affected fish showed marked, bloody lesions of the mouth, barbels, and nares, as well as petechiae on the body. By light microscopy, intranuclear, eosinophilic rhomboidal or diamond-shaped inclusion bodies were typically noted in 3.5-μm histologic sections of the posterior kidney (*n* = 27) with some inclusion bodies present in the liver and spleen. Transmission electron microscopy (TEM) of infected posterior kidney typically revealed viral arrays (R. Reese, FDEP, unpublished data) in the nucleus of tubular epithelial cells. Viral arrays were detected by TEM in the majority of fish examined from throughout the Gulf of Mexico (*n* = 15). The presence of the virus in dead and moribund fish and its absence in control fish suggests a strong, circumstantial but etiologic relationship with the hardhead catfish mortality. The absence of marine catfish cell lines and limited funds have precluded identification of the virus and further investigation of this event.

In August 1997, a widespread mortality of fish, particularly tilapia, occurred in the Salton Sea, California. Examination of a small sample (*n* = 23) revealed heavy infestations of the parasitic dinoflagellate *Amyloodinium ocellatum* on the gills of 95.6% of the fish. *A. ocellatum* is recognized as a persistent pathogen that causes serious mortalities in aquaculture facilities and in aquaria. Under such conditions, where fish are confined and overcrowded, and apparently also in the Salton Sea where the ecosystem is closed, parasite levels can build up to extremely high levels. Healthy marine fish held in aquaria can die after only 12 hr of exposure to *Amyloodinium*. This parasite is global in distribution and infects a wide range of fish hosts, including over 100 in North America alone. In the wild, however, the number of parasites per fish is typically very low and fish do not usually die from infestation. The life cycle of *Amyloodinium* consists of three stages: a trophont that feeds by attaching to the gills and skin of fish, an encysted tomont that develops after the trophont detaches from the fish, and motile dinospores that are released after the tomont divides. The parasite impairs both respiratory function and osmotic balance, and can suffocate the fish when present in high numbers. Diagnosis is based on finding the attached trophont stage in gill scrapings. Since the life cycle can be completed in less than 1 wk at high temperatures and highly saline conditions, like those present in the Salton Sea, massive and lethal infestations could develop rapidly. Now that the parasite is present in the Salton Sea and apparently is able to reproduce rapidly without control, *Amyloodinium* might be associated with persistent, chronic die-off of fish during the next few years. High salinities are optimal for this parasite, but it does not
tolerate freshwater or low salinity conditions. Along with other environmental stressors and bacterial pathogens, *Amyloodinium* represents a further threat to an ecosystem already in distress.

The above examples highlight some emerging issues with respect to fish health in the United States. Many interactive environmental factors may contribute to disease processes in free-ranging fish species. Environmental conditions or anthropogenic inputs such as nutrients, contaminants, or the results of water management policies may ultimately influence ecologic systems and indirectly lead to epizootics. It is essential to document the interaction of causal factors in the development of disease in fish populations by investigating environmental cofactors or potential initiators of stress.

**LITERATURE CITED**

MARINE BIRDS AS MONITORS OF MARINE ECOSYSTEM HEALTH

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Abstract

Monitoring the health of marine ecosystems is a relatively new concept, and the extent to which mortality events involving different species at various trophic levels may serve as a measure of health is only just now being explored. Marine birds may serve as a monitor of short and longer term disturbances than may reflect the health of the marine environment. Oil spills and other causes of pollution, and local changes in prey abundance, either as a result of fisheries practices or natural cycles, may severely impact local marine bird populations. Changes in ocean temperature and quality, which may be related to human activity within the near shore environment, as well as global climate change, El Niño events and natural ocean cycles may influence prey abundance and/or algal species and abundance, resulting in red tide events. These events and processes may be of various durations, cyclic, or periodically repeated.

Three recent examples of marine bird die-offs we have explored that have implications for the health of the marine environment are:

1) In August of 1997, approximately 400 common murres (Uria aalge) were found dead in a relatively confined area of the southern end of the Monterey Bay National Marine Sanctuary. This is the first report of inhaled brevetoxin killing birds on the Pacific coast, but in retrospect, brevetoxicosis is suspected in several recent common murre die-offs in California, each involving hundreds of murres and each occurring during months with warmer ocean temperatures.

2) Over an approximately 1-wk period in late October of 1997, five hundred marine birds, predominantly western grebes (Aechmophorus occidentalis), common loons (Gavia immer), and surf scoters (Melanitta perspicillata) became fouled with a fish oil. This product caused water saturation, hypothermia, and associated debilitation, but many birds also suffered enteritis and septicemia due to Salmonella. The combination of physical fouling and acute stress due to oil, bacteremia, and migration related debilitation, resulted in relatively high mortality.

3) Over a 3-mo period in the winter of 1997-1998, a significant percentage of the common murre population off of California’s central coast died as a result of oil and tar contamination. Over 500 live birds, 94% of which were murres, and over 650 dead birds were recovered. Although
there was no point source for the petroleum several lines of investigations are being followed. Events of this type have occurred repeatedly in California over the last 5-10 yr, and they may have serious population level effects.
THE IMPORTANCE OF MONITORING FREE-RANGING PINNIPEDS AND HOW IT RELATES TO THE MARINE ECOSYSTEM HEALTH

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Abstract

Since marine mammals are at the top of the food chain, studying them is an extremely important facet of any program to monitor the marine ecosystem. In order to monitor marine mammals one must have them in hand. There are two primary avenues of acquisition of these animals: you can go to them in the wild (field trips to find sick or dead animals) or you let the general public bring them to you (working with rehabilitation centers). Both are important and have advantages and disadvantages, but both avenues should be used to its maximum. Procedures used to monitor marine mammals both within rehabilitation centers and in the wild are similar. A complete physical examination should be done including collection of blood for blood cell counts and serology. Swabs of the nasal cavity and rectum should be taken for routine bacterial cultures. Feces should be collected for routine flotation examination for parasites. If the animal dies a complete necropsy should be done as quickly as possible, preferably immediately after the animal dies. Selected tissues should be collected and frozen and fixed in 10% neutral buffered formalin for histologic studies. Tissues saved for toxicologic analysis for organochlorines, polychlorinated biphenyls and petroleum hydrocarbons should be wrapped in teflon. Tissues saved for trace minerals should be saved in plastic whirl packs. Tissues saved for virus isolation, micronutrients, biotoxins and various enzymes need to be preserved in liquid nitrogen. Liquid nitrogen dewars work well in the field for this task and can be taken on airplanes. Two examples that were found during a monitoring program of Northern fur seals that has a connection with the health of the marine ecosystem include entanglement of seals by orphan fish netting and packing bands and a condition called white muscle syndrome will be discussed.
ASSESSMENT OF POTENTIAL HEALTH EFFECTS FROM ORGANOCHLORINE CONTAMINANTS EXPOSURE IN FREE-RANGING NORTHERN FUR SEALS (Callorhinus ursinus) PUPS FROM ST. GEORGE ISLAND, ALASKA

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Abstract

The northern fur seal (Callorhinus ursinus) population that breeds on St. George Island, Alaska, declined at an annual rate of approximately 6% from 1980-1996. Previous studies found lower than expected return rates after initial post-weaning migrations. In order to examine the possible role of organochlorine contaminant-linked immunosuppression, two cohorts of pups and their dams were examined. Forty-two neonates were captured for blood sampling, vaccinated with tetanus toxoid and re-sampled at 4-6 wk later. In addition, matched dams of 33 were concurrently captured for blood and milk sampling. Organochlorine (OC) compounds were extracted from whole blood and milk then subjected to high-performance liquid chromatography to identify 14 selected individual polychlorinated biphenyl (PCB) congeners and DDT metabolites. Cellular immune function assays along with complete blood cell counts, serum retinol, serum thyroxine levels and tetanus antibody response were used as indicators of health status. The pup’s blood parameters were then compared to their individual and maternal OC congener profiles. PCB congener profiles of pup blood were better correlated to the dam’s milk than blood with variations due to age and other factors. Inter-annual differences in exposure levels and specific congener concentrations were apparent. The milk of young dams (presumably primiparous) and their neonate’s blood had significantly elevated levels of various congeners over that of older dam’s milk and neonate blood. Serum retinol and thyroxine levels, which are known to be decreased as part of the toxic effect of PCBs in laboratory animals, were negatively correlated to increasing toxic equivalency quotients (TEQs) and select congeners in pups. Functional lymphocyte proliferation responses to the mitogen ConA were decreased in correlation with increasing levels PCB congeners. The results of this study demonstrate the utility of using field-cryopreserved blood samples to monitor health status and potential OC contaminant exposure in living, free-ranging fur seal pups.
RETROSPECTIVE EVALUATION OF RENAL DISEASE IN BLACK HOWLER MONKEYS (Alouatta caraya) AT THE RIVERBANKS ZOOLOGICAL PARK AND BOTANICAL GARDENS

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Abstract

Medical records of 15 black howler monkeys (Alouatta caraya) were reviewed. Six of the 15 (40%) records included a diagnosis of renal disease. The six animals were wild caught and included three males and three females. The hematologic and serum values, gross and histopathologic abnormalities, as well as subjective clinical findings were compared and documented. Further comparisons included age of onset of disease, duration of disease and age at death. Average age of onset of azotemia was 14.8 ± 2.9 yr while the average age at subjective notation of clinical signs was 17 ± 4.7 yr. For most of the cases (66.6%), the mean age of onset of azotemia (14.8 ± 2.9 yr of age) was often much earlier that the onset of notation of clinical disease (17.7 ± 4.7 yr of age). Average duration of disease was found to be 2.83 ± 1.6 yr with an average age at euthanasia of 18 ± 4.7 yr. Chronic tubulointerstitial nephritis with secondary glomerular sclerosis was the common histopathologic lesion in all the cases. Further investigations into specific etiologies of chronic tubulointerstitial disease in this species are warranted.

Conclusions

1. A high prevalence (40%) of irreversible, chronic renal disease exists in the black howler monkeys in this study. The etiology of the disease is unknown at this time.
2. Onset of azotemia appears in middle-age to older animals (14.8 ± 2.9 yr).
3. This disease appears to be chronic in nature with duration of disease in 50% of the cases averaging 3-4 yr.
4. In azotemic patients, helpful diagnostic aids to determine extent of renal disease include urinalysis, UP:UC, urine protein electrophoresis, survey and contrast radiology, and ultrasonography. Antemortem investigation into etiologies of renal disease, nephron component involvement, and degree of tissue damage may warrant renal biopsy.
5. Supportive care with oral fluid supplementation, dietary protein restriction, and phosphate binding therapy may have contributed to the slow progression of disease.
6. The decline in quality of life warranting euthanasia corresponded to dramatic progression of azotemia (BUN = 124 ± 58.9 mg/dl, creatinine = 7.8 ± 1.4 mg/dl).
7. Chronic tubulointerstitial nephritis with secondary glomerular sclerosis was the common histopathologic lesion in all the cases.
RENAL OXALOSIS IN A CHEETAH (*Acinonyx jubatus*): PRESUMPTIVE ETHYLENE GLYCOL TOXICITY

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Abstract

Case Report

Lethargy and vomiting were observed in a 4-yr-old female cheetah at the National Zoological Park (NZP) on the morning of 9 November 1996. Later in the day the animal appeared brighter and subsequently ate half of its normal diet. The next day the cheetah was alert and responsive but anorexic. On 11 November its condition had significantly worsened. The cheetah was unable to stand, severely depressed, and polyuric. Physical examination revealed mild dehydration and severe, bilateral renomegaly. Complete blood count and serum chemistry analysis demonstrated mild anemia (hematocrit 37%) and severe azotemia (blood urea nitrogen 230 mg/dl, creatinine 12 mg/dl, phosphorus 14.6 mg/dl). Urinalysis was unremarkable (specific gravity 1.016 g/dl, pH 6.0). Radiology and transabdominal ultrasonography confirmed that both kidneys were markedly enlarged. In addition, there was increased renal cortical echogenicity and loss of corticomedullary definition. A percutaneous renal biopsy was obtained from the left kidney. Pending histopathology, the presumptive diagnosis was toxin-induced acute renal failure.

The cheetah improved clinically in response to initial therapy (intravenous and subcutaneous fluid therapy, cephalothin, ranitidine). The animal continued to produce urine and accepted oral medicines the following day (aluminum hydroxide, omeprazole, cephalexin). However, severe azotemia (BUN 290 mg/dl, creatinine 26 mg/dl) was evident upon repeat examination on 13 November. Biopsy results revealed severe acute tubular degeneration associated with birefringent crystals typical of calcium oxalate. These findings were considered characteristic of ethylene glycol toxicity.1,3,5

Although prognosis was extremely poor, the cheetah was alert and active. Uremic signs were limited to the gastrointestinal tract (vomiting, diarrhea). The animal was hospitalized for continuous fluid therapy and supportive care, including total parenteral nutrition (TPN) and whole blood transfusion. Hemodialysis was considered at this time but the technical demands and potential complications of the procedure were judged to be excessive. Fluid and diuretic therapy over the next several days was successful in promoting diuresis. The animal’s strength improved on TPN, which was designed to limit protein (1.5 g/kg) and to deliver 50% of its daily caloric requirement each as lipid and dextrose. After 5 days the animal regained its appetite and readily consumed small amounts of lamb and turkey (lower protein relative to the commercial horsemeat diet.) However, the next day, the ingestion of even a single piece of meat produced signs of renal encephalopathy.
(e.g., vision loss, stupor, seizures). Hemodialysis was attempted on 20 November but the cheetah died of complications during the procedure.

Necropsy results confirmed the diagnosis of renal oxalosis; there was marked tubular degeneration and regeneration associated with massive numbers of birefringent crystals in the tubular lumen. The crystals were proven to be the monohydrate form of calcium oxalate using infrared microspectroscopy and a laser Raman microprobe.\textsuperscript{6}

**Discussion**

The following were considered potential causes of renal oxalosis in this cheetah: ingestion of 1) food contaminated with ethylene glycol (either inadvertent or malicious), 2) an ethylene glycol-containing chemical (e.g., antifreeze, photographic chemicals, brake fluid), or 3) oxalate containing plants.\textsuperscript{1,3-5,7,8} Chemical exposure was highly unlikely given that there was no vehicle access to the cheetah holding areas or yards. While a sparse number of oxalate-containing plants were growing in the exhibit, no cheetah was ever observed to eat these, or other, plants. Therefore, contaminated food was considered the most likely source. This poisoning was either an act of vandalism (the cheetah was hand-reared and could have accepted the toxin in food from the public), or the commercial horsemeat diet contained the chemical. There is precedent for accidental contamination of zoo felid diets with ethylene glycol.\textsuperscript{4,7,8} In 1978, several felids from various zoos died from renal oxalosis.\textsuperscript{4,8} The source was traced to a faulty machine at the meat processing plant but only after several months of testing multiple batches of diet (M.K. Stoskopf, personal communication).

In domestic dogs and cats, ethylene glycol toxicity is almost exclusively the result of antifreeze ingestion. Unless treated specifically within 24 hr of exposure (4-methylpyrazole or ethanol, sodium bicarbonate) this condition is usually fatal, even if the animal survives the induction phase and enters the maintenance phase of acute renal failure.\textsuperscript{3} At this stage, the prognosis depends largely upon the severity of uremic signs (e.g., anemia, platelet dysfunction, gastritis, encephalopathy) and the success of supportive care, which requires months of therapy to allow the tubules time to regenerate and regain function. Although hemodialysis offers the best chance of survival, it requires special equipment and repeat treatments (2-4 hr of diuresis a day for the first week, then two to three times weekly to maintain acceptable BUN and creatinine levels).\textsuperscript{2} During the session, the patient must remain stationary with the heart maintained at the level of the mechanical kidney in the dialysis machine. In a cheetah this would have required repeated sedation and long-term catheterization.

The source of ethylene glycol in the NZP cheetah case was never identified. Analyses were performed at the Animal Health Diagnostic Laboratory at the Michigan State University College of Veterinary Medicine (PO Box 30076, East Lansing, MI, 48909-7576 USA). Representative samples from the commercial horsemeat-based diet were negative for both ethylene glycol and oxalates. Beet pulp (used as a filler in this product) samples provided by the manufacturer were also analyzed for oxalates and found to contain minimal amounts. Blood and urine from the cheetah were also negative for ethylene glycol. This finding was not surprising given that the samples were obtained at least 72 hr following initial exposure.\textsuperscript{3,5} No illness developed in the cheetah that consumed meat.
from the same package as the affected cheetah. However, once the diagnosis was confirmed, all NZP felids were placed temporarily on a nutritionally complete all beef diet. A new vendor was subsequently identified and all of the felids were switched to the new, nutritionally balanced, horsemeat-based product.

ACKNOWLEDGMENTS

The authors wish to thank Mark Berkowicz, DVM and staff from Veterinary Referral Associates, Gaithersburg, MD for providing the hemodialysis equipment and technical expertise, and Jose A. Centeno, Ph.D. from the Department of Environmental and Toxicologic Pathology, Armed Forces Institute of Pathology, Washington, DC for microspectroscopy.

LITERATURE CITED

SUSPECTED SULFADIMETHOXINE INTOXICATION IN A CAPTIVE GIANT ANTEATER (Myrmecophaga tridactyla)

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Abstract

One male and one female adult captive giant anteaters (Myrmecophaga tridactyla) were treated with a single dose of sulfadimethoxine 60 mg/kg p.o. Of these animals, one showed acute symptoms such as convulsions, apparently due to abdominal pain and vomiting. Treatment and recovery of this animal are described. Clinical examination suggests an impaired liver function or an idiopathic hypersensitivity as possible reasons for the unexpected reaction to the sulfadimethoxine.

Introduction

The giant anteater (Myrmecophaga tridactyla) belongs to the order of the Xenartra. They inhabit the neotropical regions of Central and South America. This order also includes the armadillos and sloths. Due to their exceptional biology and behavior the Xenartra represent a unique challenge to the zoo veterinarian. In comparison to the other Xenartra very few anteaters are kept in zoological gardens and therefore very little is known about their management and reproduction. This lack of knowledge becomes apparent when information is needed, concerning the veterinary care of these animals. The aim of this case is to share the experience of a complication during the deworming of a giant anteater and the therapy used to save the animal.

Case Report

In 1995-1996, one male and one female adult giant anteaters arrived to Zurich Zoo from different zoos. The two animals were housed together during the day and separated during the night. They had an indoor and an outdoor enclosure. Their diet consisted of 1 L of a soup made from dog chow, minced meat, oats, fruits (e.g., kiwi, bananas, pears), and a vitamin and mineral supplement. Since their arrival, the animals appeared healthy. In October 1997 during a routine fecal parasitologic examination it was found the both animals were shedding Capillaria spp. and Eimeria spp. Due to the fact that occasionally both animals had produced loose stool it was decided to treat them against the parasites.

They were first treated with fenbendazole (Panacur® Suspension 10%, Hoechst) 15 mg/kg p.o. for 3 consecutive days. Nine days later each animal was given sulfadimethoxine (Maxulvet®, Veterinaria) 60 mg/kg p.o. Approximately 6 hr later, the female animal showed symptoms of
incoordination, which deteriorated within 1 hr. At the first clinical examination the animal was in lateral recumbency, vomiting, and had strong convulsions, apparently due to abdominal pain.

Differential diagnoses at this point were, in order of probability: an intoxication (sulfadimethoxine), a gastrointestinal obstruction (e.g., foreign body, parasites, enteritis), a reproductive disease, and a septic disease.

For the next 36 hr the animal was treated with diazepam (Valium®, Roche), an anti-inflammatory drug metamizol (Vetalgin®, Veterinaria), vitamin K (Konakion®, Roche), enrofloxacin (Baytril® 10%, Bayer). The main aim of the treatment was to sedate the animal to a point were convulsions would disappear. Body temperature and heart rate were mildly elevated.

On the second day, an intravenous catheter was placed in the cephalic vein to initiate fluid therapy. Blood was collected from the animal for hematology and blood chemistry (Table 1). The most obvious finding was a moderate to strong increase in the serum enzyme activity of AST, ALT, GGT, and LDH. A blood gas analysis was also performed on day 2. It did not reveal any pathologic changes. An x-ray examination revealed a hyperostosis in the thoracolumbar spine. On the basis of these findings, differential diagnoses such as gastrointestinal obstruction, reproductive problem, and an infection were rejected. An intoxication, most likely due to the sulfadimethoxine was considered the diagnosis.

Table 2 gives the chronologic treatment and the course of the disease. The male, which was kept separate at all times showed minor symptoms of incoordination 30 hr after treatment. The symptoms resolved within 2 hr without therapy. Approximately 48 hr after onset of symptoms the animal’s condition started to improve and within another 48 hr it returned to normal behavior. Since then the animal has been in good health.

Discussion

This case reports the symptoms and treatment of a suspected intoxication with sulfadimethoxine in a captive giant anteater. The finding that this drug could lead to an adverse reaction in this animal was surprising. Sulfadimethoxine is a long-acting sulfonamide, which is known for its broad activity against gram positive and negative bacteria as well as coccidia, and its low toxicity. It has been used in a wide range of species including exotic mammals, birds, and reptiles. In most species, sulfadimethoxine is acetylated in the liver to acetylsulfadimethoxine and excreted unchanged in the liver. Sulfadimethoxine’s long elimination half-lives are a result of its appreciable reabsorption in the renal tubules. Sulfadimethoxine is therefore contraindicated in patients with impaired liver and/or kidney function. In humans, adverse effects to sulfonamides include: gastrointestinal disorders (e.g., nausea, vomiting, diarrhea), hypersensitivity reactions, neurotoxicity, allergic and toxic hepatic disease. To the author’s knowledge such symptoms related to sulfonamide therapy have not been previously described in Xenartra. Sulfonamides have been used in captive giant anteaters before without adverse effect (Osmann, personal communication). Possible explanations for the occurrence of this reaction only in one of the two animals, might be an inhibited function of...
the liver or an idiopathic hypersensitivity to sulfadimethoxine. Blood biochemistry results suggested a possible liver disease. In the literature, liver disease, such as liver cell necrosis, have been reported in giant anteaters.\textsuperscript{2,4} A chronic impaired liver function in this animal and therefore a reduced metabolization of the medicament could have been the cause that led to these symptoms. The body weight of this animal was 63 kg, which is considerably higher than body weights of anteaters given in the literature (30-40 kg).\textsuperscript{4} The animal does not make an obese appearance, but some degree of fatty liver degeneration may be present. A conclusive diagnosis of liver disease could have only been possible on the basis of a liver biopsy, which in this case was not available.

The vertebral hyperostosis, which was diagnosed in the radiologic examination, was considered to be of nutritional origin. In anteaters (\textit{Tamandua tetradactyla} and \textit{T. mexicana}), similar changes have been found and it was suggested that they might be caused due to a hypervitaminosis A and/or D.\textsuperscript{1} As a result, an evaluation and improvement of the diet has been initiated. As has been demonstrated with the present case, further clinical and pathologic research is needed to improve the management of giant anteaters in captivity.

ACKNOWLEDGMENTS

The authors thank Heinz Kohler from Zurich Zoo for his assistance.

LITERATURE CITED

Table 1. Blood values of a giant anteater (*Myrmecophaga tridactyla*) with suspected sulfadimethoxine intoxication.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin g/dl</td>
<td>15.0</td>
<td>11.3 - 13.5</td>
</tr>
<tr>
<td>Red blood cell count (10^6/µl)</td>
<td>2.7</td>
<td>2.4 - 3.0</td>
</tr>
<tr>
<td>White cell count (10^3/µl)</td>
<td>9.5</td>
<td>5 - 10</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>43</td>
<td>30 - 50</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>56</td>
<td>46 - 49</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>35</td>
<td>31 - 35</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>159</td>
<td>135 - 137</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>2.7</td>
<td>1.6 - 4.7</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>3.6</td>
<td>3.7 - 5.9</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>107</td>
<td>79 - 106</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>69</td>
<td>52 - 74</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>32</td>
<td>12 - 33</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>1.5</td>
<td>2.2 - 3.2</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>16</td>
<td>74</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>102</td>
<td>19</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>70</td>
<td>33</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>119</td>
<td>13</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>380</td>
<td>334</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>147</td>
<td>152</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>5.0</td>
<td>5.8</td>
</tr>
<tr>
<td>Cl (mmol/L)</td>
<td>108</td>
<td>110</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>2.8</td>
<td>2.1 - 2.5</td>
</tr>
<tr>
<td>P (mmol/L)</td>
<td>1.3</td>
<td>1.8 - 2.0</td>
</tr>
</tbody>
</table>

* Source: Ruempler (1995)
Table 2. Chronologic course of a giant anteater (*Myrmecophaga tridactyla*) with suspected sulfadimethoxine intoxication. (The animal’s weight was 63 kg).

<table>
<thead>
<tr>
<th>Time</th>
<th>Therapy</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.00 h</td>
<td>Diazepam 20 mg i.m.</td>
<td>Onset of symptoms: colic, vomiting,</td>
</tr>
<tr>
<td></td>
<td>Metamizol 1000 mg i.m.</td>
<td>and lateral recumbency.</td>
</tr>
<tr>
<td></td>
<td>Scopolamin 8 mg i.m.</td>
<td>Following medication → calm in lat. recumb.</td>
</tr>
<tr>
<td>21.00 h</td>
<td>Diazepam 20 mg i.m.</td>
<td>Again colic</td>
</tr>
<tr>
<td></td>
<td>Metamizol 1000 mg i.m.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scopolamin 8 mg i.m.</td>
<td></td>
</tr>
<tr>
<td><strong>Day 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>07.00 h</td>
<td>Diazepam 20 mg i.m.</td>
<td>Again colic and vomiting</td>
</tr>
<tr>
<td></td>
<td>Metamizol 2000 mg i.m.</td>
<td></td>
</tr>
<tr>
<td>09.00 h</td>
<td>1 l of Lactated Ringer’s solution</td>
<td>Calm in lat. recumb., placement of i.v.-</td>
</tr>
<tr>
<td></td>
<td>and 250 ml of NaCl/Glucose i.v.</td>
<td>catheter. Collection of blood samples</td>
</tr>
<tr>
<td></td>
<td>over 7 hr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin K 10 mg i.m.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enrofloxacin 300 mg i.m.</td>
<td></td>
</tr>
<tr>
<td>16.00 h</td>
<td>Diazepam 10 mg i.m.</td>
<td>Calm in lat. recumb.</td>
</tr>
<tr>
<td><strong>Day 3</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>07.00 h</td>
<td>First lat. recumb.; wakes up and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>eats 1 dl of yogurt. Starts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>walking around. Then sleeps for</td>
<td></td>
</tr>
<tr>
<td></td>
<td>several hours.</td>
<td></td>
</tr>
<tr>
<td>19.00 h</td>
<td>Active, eats</td>
<td></td>
</tr>
<tr>
<td><strong>Day 4/5</strong></td>
<td></td>
<td>Steady improvement, apparently healthy.</td>
</tr>
</tbody>
</table>
ANESTHETIC APNEA IN TWO BLACK AND WHITE COLOBUS MONKEYS (*Colobus guereza*) POSTULATED TO HAVE RESULTED FROM BUTORPHANOL TARTRATE ADMINISTRATION

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Abstract

A mother:daughter pair of black and white colobus monkeys (*Colobus guereza*) aged 17 and 14 yr, respectively, at the Topeka Zoological Park were immobilized for annual examinations and dental work. Butorphanol tartrate (Fort Dodge 10 mg/ml) was given intramuscularly at a dose of 0.2 mg/kg for pain caused by tooth extraction. Both animals became apneic. The first animal was ventilated until spontaneous respiration had resumed. The second was induced to resume respiring by administration of naltrexone HCl to antagonize the butorphanol.

Case Report

On 6 October 1997, two black and white colobus monkeys were immobilized for physical examination and dental work with 75 and 50 mg Telazol (tiletamine HCl-zolazepam HCl, Fort Dodge Laboratories, Inc. Fort Dodge, Iowa 50501 USA) respectively. They were transported to the Animal Health Center, intubated and maintained on isoflurane gas anesthesia (Iso Flo, Abbott Laboratories, North Chicago, Illinois, 60004). Blood was drawn from the femoral vein for CBC, chemistry panel and serum banking. Whole body and skull radiographs were taken. Treatments in addition to the Telazol included Baytril (Bayer Corporation, Shawnee Mission, Kansas 66201 USA) 4.3 mg/kg, palpebral tuberculosis testing with mammalian tuberculin (Human Isolates Intradermic Coopers Animal Health, Inc., Kansas City, Kansas 66103-1438 USA) 0.1 ml intradermally and Torbugesic (butorphanol tartrate, Fort Dodge Laboratories, Inc. Fort Dodge, Iowa 50501 USA; 10 mg/ml) 0.2 mg/kg i.m.

Both animals have had a history of dental disease of unknown origin. They have both had multiple extractions in the past. The older animal required all of its remaining teeth to be pulled. The younger one was left with five teeth. The daughter was immobilized first. The animal was immobilized with 75 mg Telazol i.m., transported to the zoo’s hospital, intubated and maintained on isoflurane. Additional treatments included 35 mg Baytril, 1.4 mg butorphanol, and a tuberculin test. During the procedure, the animal stopped breathing. Intermittent positive pressure ventilation was initiated and the isoflurane flow rate was decreased and then stopped, maintaining the animal on oxygen alone. The animal was treated numerous times with Dopram (Fort Dodge), soon after which it would take a few breaths and then stop. Even presumed pain would not induce the animal to breathe as there was no respiration associated with the extraction of teeth. The heart rate continued to be stable despite the continued apnea. The procedure was completed, yet the animal...
was still not breathing on its own. IPPV was continued. At one point approximately 2.5 hr later, mucus membranes turned gray, pupils dilated, and the animal appeared to be dying. IPPV was increased to a rapid rate, more Dopram was given and the animal suddenly began breathing and rapidly returned to a normal, stable plane. From that point on, the animal seemed fine. Mucus membrane color became pink and CRT improved to less than 2 sec.

Before sedating the second animal, a quick literature search revealed that Telazol can have some depressive effects on respiration. These effects were mainly post induction apnea and were transient but could be treated if necessary with 5.5 mg/kg Dopram. The decision was made to continue with the second procedure in the hope that the previous incident was an individual idiosyncrasy with regards to the Telazol as both of these animals had been sedated with Telazol in the past.

The second procedure was identical to the first except for a reduced Telazol dose of 50 mg in case the previous apnea had been due to Telazol. Throughout the induction and the initial procedure, the animal continued to breathe normally. Breathing continued normally until the point of giving the additional medications which included 34 mg Baytril, 800 mg Pipracillin (Pipracil, Abbott Laboratories), 1.5 mg butorphanol, and the tuberculin test. Within 1 min, the animal also stopped breathing. Dopram was given and just like the first animal, this animal would take one breath immediately after the administration, then go apneic again. At this point the assumption was made that it was not the Telazol that was causing the apnea but the butorphanol. The animal was treated with 25 mg naltrexone to reverse the effects of the butorphanol. Within a few minutes it resumed breathing and respiratory rate remained normal for the duration of the procedure.

Conclusion

The reversal of the apnea after administration of naltrexone suggests that the butorphanol was the culprit. Other drug combinations such as Telazol followed by isoflurane can probably be ruled out because of the timing involved when the apnea occurred. Butorphanol, on a weight basis, is four to seven times as potent of an analgesic as morphine. Its analgesic action occurs at sites in the limbic system. Overdosage may cause respiratory depression and should be treated with Naloxone and appropriate supportive therapy. A chance for overdosage with this particular product includes mistaking the 10 mg/ml product for the 0.5 mg/ml. Having only one size on hand will eliminate that possible error. I have used butorphanol successfully in other primates at the same published doses. These two individuals may have a genetically related sensitivity to the drug. Whether or not it is dose related is unknown as it has not been used again on these animals.

LITERATURE CITED

SUSPECTED VACCINE-AND/OR DART-ASSOCIATED FIBROSARCOMA IN A TIGER
(Panthera tigris)

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Abstract

Histopathologic changes found in a grade III fibrosarcoma in a 12-yr-old captive-raised tiger (Panthera tigris) resemble those reported in vaccine-associated sarcomas in domestic cats. However, these changes are also compatible with foreign body-associated sarcomas and a broken dart needle was found near the tumor. Because the histopathologic changes are non-specific, it cannot be definitively determined whether this lesion was vaccine-induced or dart-induced; however the changes are characteristic enough to suggest that the vaccinations, the metallic foreign body, or both, contributed to this tumor’s development. Because of the potential risk (albeit low) in developing sarcomas, the frequency of vaccinating exotic felids and the leaving of broken dart needles (or other foreign bodies) in exotic felids, may need to be re-evaluated.

Case Report

A 12-yr-old captive-raised female tiger (Panthera tigris) was anesthetized for examination of a large circular swelling on its right hip just caudal to the right ischial tuberosity. According to zoo personnel, the fluctuant mass had suddenly appeared and had reached its current size over a period of 2-3 days. Using ketamine and midazolam, the tiger was anesthetized for biopsy, culture and sensitivity, and survey radiography of the mass. A fine needle aspirate of the mass revealed clear yellow fluid. Surgical exploration of the mass was performed and revealed deep invasion of the mass into the leg musculature. Removal of the mass was aborted when it became apparent that complete removal would not be possible without irreparable damage to the leg. Additional incisional biopsies were taken; these revealed a hollow, necrotic-appearing core within a hard fibrous mass. Because of the tumor’s size and location, closure could not be accomplished without incorporating the tissue of the mass into the incision line. Consequently, dehiscence was anticipated as a likely complication. The tiger was administered procaine penicillin s.c. Recovery from anesthesia was uneventful and a course of oral amoxicillin was begun the following day.

Histopathologic findings were consistent with fibrosarcoma with up to two mitotic figures per high power field. Multifocal areas of coagulative necrosis were also evident. Culture results revealed an alpha-streptococcal sp. that was responsive to most antibiotics. Radiographs revealed a 3-cm, barbed, tubular metal structure (consistent with a barbed dart needle) to be present in the soft tissues.
caudal and ventral to the left acetabulum. Full delineation of the soft tissue mass could not be determined, however the needle appeared to be 2 cm cranial to the cranial extent of the mass.

The tiger did well postsurgically with no gait abnormalities and minimal drainage from the incision site. Four days after surgery, however, a small 2-cm dehiscence in the ventral aspect of the incision was noted; the following day the incision completely dehisced with exposure of tumor and muscle. Dehiscence was exacerbated by the tiger’s constant licking of the wound. The tiger was re-anesthetized, the tumor was debulked and flushed with 2 L of sterile saline, and the wound was closed again. Suture tension was minimal, however dehiscence was anticipated because of the large size of the tumor and presumptive wound infection.

Two days after the second surgery (7 days following the initial surgery), the incision again completely dehisced. Closure of the incision was not attempted as euthanasia was being strongly considered. The tiger was euthanatized on the following day and a postmortem examination was performed.

At necropsy, the tumor measured 20 × 14 × 8 cm. The tumor was multinodular and extremely firm to palpation. A broken needle from a dart was found within the musculature, 2 cm anterior and dorsal to the neoplasm. The dart was close to, but not directly associated with, the body of the neoplasm. The lungs contained multiple, 2-5 mm diameter, firm, palpable nodules.

Histopathology revealed the primary tumor to be a grade III fibrosarcoma. Histopathologic changes were consistent with those found in vaccine-associated fibrosarcomas in domestic cats. Muscle tissue from around the area of the needle revealed the tumor to infiltrate between muscle bundles in sheets of spindle cells. The lungs revealed lesions of metastatic fibrosarcoma with cells identical to those in the primary lesion. Two, 3-mm diameter lesions were detected with cellular changes consistent with bronchoalveolar carcinoma.

Vaccine-Associated Sarcomas

The neoplastic changes that occurred in this tiger were clinically and histologically comparable to those seen in domestic cats with vaccine-associated sarcoma. Such changes are also consistent with tumors induced by foreign bodies. Over this tiger’s 12-yr lifespan, it was vaccinated at least ten times with several killed vaccine products. Unfortunately, medical records were not specific regarding dart injection sites; however, the hip and thigh muscles are likely sites, as six of the 10 vaccinations were administered via blow-dart.

Associations have been demonstrated between vaccination with killed vaccine and fibrosarcoma development at the vaccination site. Numerous investigators have concluded that in a low number of feline patients (1:10,000), vaccinations can lead to fibrosarcomas at injection sites, particularly when vaccination is repeatedly given at the same location. One study determined that the risk for developing a fibrosarcoma was 50% higher in cats that received a vaccination compared to those that did not; the risk in cats with two vaccinations was 127% higher and 175% higher in cats with...
three to four vaccinations. A single vaccine brand has not been singled out or excluded from suspicion. Presently, vaccination-site sarcoma formation appears to be unique to cats.

Electron probe x-ray microanalysis has revealed that material within many of the vaccine-associated sarcomas is composed of aluminum and oxygen. Whether the aluminum is oncogenic, or merely a marker of the neoplastic reaction, remains to be determined. Histologically, vaccine-associated sarcomas are enveloped in dense, fibrous connective tissue and infiltrated with inflammatory lymphocytes and macrophages. Bluish foreign material has been present in macrophages of many vaccine-associated sarcomas. The tumor in this animal did not have such material, but did have other histologic changes consistent with vaccine-associated sarcomas. Necrosis has been found to be more common in vaccine-induced fibrosarcomas than in non-vaccine induced fibrosarcomas, with 25% of the vaccine-associated tumors having cavitated centres. The tumor in this tiger was highly necrotic with a large cavitated core.

Vaccine-induced sarcomas appear to be fast-growing and very invasive locally with infrequent metastasis; however failure to recognize metastatic lesions may be due to short survival time of animals with these tumors. Postvaccinal sarcomas often appear within 3-9 mo of vaccination; tumors not associated with vaccination are typically slower growing. Vaccine-associated sarcomas occur more frequently in the subcutis, while non-vaccine-associated sarcomas occur more frequently in the dermis. The tumor in this tiger was predominantly in the subcutaneous space with deep invasion of the muscles of the leg.

The histologic similarities of the tiger’s lesion to vaccine-associated sarcomas in domestic cats raise some interesting issues. Considering that there is currently a great deal of research and debate focused on vaccination recommendations in domestic cats, perhaps it would be wise to apply some of the same discussion to vaccination of exotic felids. We recognize that no vaccines are approved in exotic felids, that vaccine-associated sarcomas are relatively rare events (even in domestic cats), and that protection of exotic felids for infectious diseases is an important component of preventative health programs. However, we should examine the following recommendations regarding vaccination of domestic cats and apply them, as appropriate, to exotic felids. Current recommendations hold that none of the killed vaccines should be given in the interscapular space. The location, manufacturer, and serial number should be recorded in the medical record. Previous vaccination sites should be avoided when giving booster vaccinations. Cats should not be unnecessarily vaccinated and vaccination protocols should be dictated by risk of infection and prevalence of disease.

Foreign Body-Associated Sarcomas
The broken dart needle’s close proximity to the tumor site, however, also suggests that the tumor may have been caused by chronic irritation by this foreign-body. In addition to being darted for vaccinations, the tiger was also anesthetized multiple times, with five anesthetia procedures prior to those associated with treatment of the fibrosarcoma. Anesthetic records do not document all sites of anesthetic injection, however an episode in 1989 noted that a dart needle broke off in the right hip area. At that time, the hip was radiographed and the broken needle was found within the soft tissue of the hip. However the object could not be externally palpated and no further treatment was pursued.

Tumors induced by foreign bodies have been studied in animals; physical factors that influence the foreign body response of inflammation and fibrosis appear to influence tumorigenesis and latency. Critical factors defined in the induction of sarcomas include the configuration of the implant (smooth, intact, large foreign bodies are more tumorigenic than roughened, perforated, smaller ones) and a period of latency long enough to allow progression to neoplasia of atypical elements arising within this perturbed environment. In a 1976 report, several tumors were described that were associated with metallic implants. Of the eight animals reported, only one was a felid (domestic cat); this animal developed a fibrosarcoma adjacent to a Jonas intramedullary splint of the right femur. A case report in a human described an aggressive soft tissue sarcoma that was detected in association with an aluminum oxide ceramic total hip implant; this tumor was detected 15 mo after implantation of the device.

This information suggests that careful consideration should be given before any foreign object is left within an animal. Although there are situations in which it is necessary to leave metallic foreign bodies inside of animals (i.e., treatment for a fracture), or where surgical removal of foreign bodies is not a viable option, the chronic presence of these materials may induce abnormal changes in surrounding cells and these changes may promote tumor formation. Aluminum, in particular, causes persistent inflammatory and immunologic reactions that may predispose cats to a derangement of the fibrous connective tissue repair response which may lead to neoplasia.

Discussion

It is unclear whether the tumor in this cat was associated with the previous vaccinations, with the broken dart needle, with both events, or with neither. However, it is hoped that this case will stimulate others to report their experiences with tumors that occur near sites of previous vaccination or adjacent to metallic foreign bodies, to gain a more accurate interpretation of the presence, incidence, and etiology of such tumors. Because of the potential risk (albeit low) in developing sarcomas, the frequency of vaccinating exotic felids and the leaving of broken dart needles (or other foreign bodies) in exotic felids, may need to be re-evaluated.

LITERATURE CITED

CANINE DISTEMPER IN A CAPTIVE INDOCHINESE TIGER (*Panthera tigris corbetti*)

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Abstract

A 10-yr-old captive-born female Indochinese tiger (*Panthera tigris corbetti*) housed at the Cincinnati Zoo presented with mild neurologic signs. The animal was housed with an adult male in an outdoor yard and had access to an indoor area during inclement weather. Initial signs included mildly uncoordinated ambulation and difficulty jumping down from a platform in the indoor area. Eleven days later, marked ataxia and paraparesis were observed, with forelimbs more severely affected than hind limbs. The animal was restrained in a squeeze cage for phlebotomy. Hematology and serum chemistries were unremarkable. Titers for feline leukemia virus, feline infectious peritonitis virus, feline immunodeficiency virus and toxoplasmosis were negative. Primary muscle origin was ruled out based on SGOT and CPK values being within reference ranges.

Neurologic signs were unchanged on day 16. The animal was immobilized with tiletamine and zolezepam (Telazol™, Fort Dodge Laboratories, Incorporated, 800 Fifth Street Northwest, Fort Dodge, IA, 50501 USA) 500 mg i.m. by dart and maintained on isoflurane (AErrane™, Ohmeda Pharmaceutical Products Division Inc., Liberty Corner, NJ 07938-0804 USA). Physical examination revealed normal muscle mass and tone and normal- to hyper-reflexia in all limbs. Urinalysis was unremarkable. Plain radiographs of cervical and thoracic spine showed fractures of dorsal spinous processes of caudal cervical and cranial thoracic vertebrae. Trauma was added to the list of differential diagnoses. Recovery from anesthesia was unremarkable. On day 17 the animal was eating well, but had developed mild head tremors which persisted for two days.

By day 27 the paraparesis had worsened. On day 30 the patient was immobilized a second time, following the same protocol, and a cisternal spinal tap was performed. Analysis of cerebrospinal fluid showed a mild pleocytosis, but was otherwise unremarkable. A cervical and thoracic myelogram showed a possible extradural mass at C2-3. Recovery from anesthesia was prolonged and mild head tremors, which lasted for several days, were again observed.
At day 35 the patient was immobilized again and taken to a local human hospital where a magnetic resonance imaging (MRI) workup of cranium and cervical and thoracic spine was performed. A 1.5 Tesla GE signa scanner was used with standard pulse sequences. A brain MRI was within normal limits. A cervical spine exam was done without contrast. This showed an abnormal cervical spinal cord. Patchy mixed signal extended from the C-1 level to the C5-6 level. This signal was predominately high T2 without any sign of cord expansion. Some minimal intervertebral disc bulges were seen at C2-3 and C3-4, which contacted the anterior cervical spinal cord but did not compress the cord. Incidental old spinous process fractures were noted at thoracic levels 3 and 4. MR Angiography was done in the cervical region and showed normal vertebral and carotid arteries. The abnormal signal in the cervical spinal cord was nonspecific. Tumor and post traumatic changes were considered unlikely. An inflammatory, demyelinating, metabolic or possibly toxic process was considered.

Over the next several days neurologic signs worsened further. Based on the progressive neurologic signs and poor prognosis suggested by MRI images, the tiger was anesthetized a final time on day 38 and euthanatized by intravenous barbiturate overdose (Beuthanasia-D Special™, Schering-Plough Animal Health, Union, NJ, 07083-1982 USA).

Gross postmortem examination revealed healing fractures of dorsal spinous processes of C7, T1, T3 and T4. No other significant lesions were noted. Histopathologic lesions were most pronounced in the brain stem and cervical spinal cord. Within these areas, there was marked nonsuppurative meningitis, gliosis and small regions with neuronal swelling and necrosis of neuropil. Many neurons and astrocytes in the most severely affected areas had eosinophilic intranuclear inclusion bodies that were confirmed to be canine distemper virus by immunohistochemistry. Descending tracts in the spinal cord white matter had demyelinization and axonal swelling. No significant lesions were noted in other tissues.

The source of infection of this tiger is unknown. However, following this case, two wild raccoons showing neurologic signs were captured on zoo grounds. Histologic lesions consistent with canine distemper were identified.
DETECTION OF *Mycobacterium avium* subspecies *avium* IN FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUES OF CAPTIVE EXOTIC BIRDS USING POLYMERASE CHAIN REACTION

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Abstract

A presumptive diagnosis of avian tuberculosis can be made when characteristic histopathologic lesions and acid-fast bacilli are observed in avian tissue samples. However, a definitive diagnosis requires isolation of the causative organism, a process that can take several weeks to complete. The purpose of this study was to determine if formalin-fixed, paraffin-embedded avian tissues could be tested by polymerase chain reaction (PCR) to reliably and rapidly diagnose avian tuberculosis. Ninety-seven samples of formalin-fixed tissues collected over a 14-yr period (1983-1997) from both definitive and presumptive cases of avian tuberculosis in captive exotic birds were examined. The primers used for PCR amplified a 180 base pair fragment of 16S rRNA which is a sequence shared by *Mycobacterium avium* subspecies *avium* and *Mycobacterium avium* subspecies *paratuberculosis*. All positive samples were presumed to be due to the presence of *M. avium* subspecies *avium* rather than *M. avium* subspecies *paratuberculosis*.

PCR testing detected the sequence in 26 of the 97 samples (27%). Of the 17 samples that were culture positive for *M. avium* and were known to have been fixed in formalin for 4 wk or less, 11 tested positive by PCR (65%).

Some of the negative PCR results may have been due to the degradation of nucleic acid in the samples. Nucleic acid degradation may be caused by a variety of factors, including prolonged fixation in formalin. This study demonstrates that PCR can be a rapid indicator of the presence of *M. avium* subspec. *avium* in formalin-fixed, paraffin-embedded tissues, however, the low sensitivity the test demonstrated in this sample set may limit its practical use as a diagnostic tool.
IMMOBILIZATION OF BABIRUSA (Babyrousa babyrussa) WITH XYLAZINE AND TILETAMINE/ZOLAZEPAM AND REVERSAL WITH YOHIMBINE AND FLUMAZENIL

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Abstract

Babirusa (Babyrousa babyrussa) are endangered wild pigs that naturally inhabit Sulawesi. They weigh up to 90 kg in the wild and adult males have four tusks, of which the maxillary pair grow through the dorsal aspect of the muzzle.4 Although they are bred and maintained as exhibit animals in several zoos there is little published information about their veterinary care.

While many different anesthetic protocols, including barbiturate combinations, dissociatives, opioids, alpha-2 agonists, tiletamine/zolazepam, propofol and multiple different combinations, have been used in domestic and nondomestic swine,1,3,6,8 there are limited published reports of anesthesia in babirusa.1,7 One protocol that has been used in babirusa includes a combination of butorphenol, detomidine and midazolam, all administered i.m.1 There is one published case of tiletamine/zolazepam administered at 5.3 mg/kg to immobilize a babirusa for tusk extraction7 but the quality of anesthesia was not described. In this report we describe an immobilization protocol using premedication with i.m. xylazine (Gemini E, Vetus Animal Health, Burns Veterinary Supply, Rockville Centre, New York 11570 USA) and induction with i.m. tiletamine/zolazepam (TZ) (Telazol®, Fort Dodge, Fort Dodge, Iowa 50501 USA). Anesthesia was reversed with yohimbine (Antagonil, Wildlife Laboratories, Fort Collins, Colorado 80524 USA) and, in most cases, flumazenil (Mazicon, Hoffman-LaRoche Inc., Roche Animal Nutrition and Health, 45 Eisenhower Dr., Paramus, New Jersey 07652 USA) both administered either i.v. or i.m.

Fourteen babirusa (five females and nine males, ranging in age from 2-13 yr) were immobilized 24 times during a 3-yr interval. Xylazine ($\bar{x} = 1.25 \pm 0.33$ mg/kg; range: 0.82-2.07 mg/kg) was administered i.m. as a premedication. Initial sedative effects generally occurred within 10 min and consisted of ataxia although some animals were not sedate at 20 min. Degree of sedation varied with some animals becoming recumbent but the majority remaining standing. TZ was given i.m. ($\bar{x} = 1.82 \pm 0.63$ mg/kg; range: 0.86-3.59 mg/kg) 20 min after xylazine and animals became laterally recumbent within 23 ± 9 min (range: 5-41 min) of the xylazine dose. They could be handled within 10 min of the TZ injection.
Babiruus heart rates during anesthesia ranged from 35-88 beats/min (x̄ = 60 beats/min), respiratory rates ranged from 12-42 breaths/min (x̄ = 24 breaths/min), and temperatures ranged from 34.2-40.3° C. Variations in the heart and respiratory rates were most likely related to the plane of anesthesia.

Anesthesia was reversed with yohimbine (x̄ = 0.15 ± 0.04 mg/kg; range: 0.09-0.24 mg/kg) and, in most cases, flumazenil (1 mg/20 mg zolazepam), both given i.m. or i.v. After yohimbine and flumazenil were given i.m. animals were standing within 25 ± 22 min (range: 2-55 min) compared to 50 ± 31 min (range: 20-122 min) after i.v. administration. The longer time to effect when the drugs were administered i.v. versus i.m. was not expected. The route of administration was dependent upon ease of venipuncture and in some cases limb movement precluded venous access. Thus animals that received the i.m. doses may have been at a lighter anesthetic plane resulting in shorter recovery times. Alternatively, this may indicate that these drugs are absorbed equally well from an i.m. versus i.v. administration. Yohimbine, however, given i.v. to rocky mountain elk (Cervus elaphus nelsoni) resulted in a recovery time approximately 100 times faster then when the same dose was given i.m. In white-tailed deer (Odocoileus virginianus) reversal times were approximately the same when the i.m. dose of yohimbine is double that of the i.v. dose. Due to the lack of comparative studies on the efficacy of yohimbine given i.v. versus i.m. in domestic swine, and the different depths of anesthesia when these babiruus were reversed, no conclusions can be drawn regarding the sensitivity of babiruus to this drug, or the difference in absorption between i.v. and i.m. injections.

This protocol is safe, reversible, inexpensive, requires only small dart volumes and results in sedation and muscle relaxation sufficient for minor diagnostic and therapeutic procedures.

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LITERATURE CITED


A ONE STEP PROCEDURE TO REPAIR A CLASS III FRACTURE (EXPOSED PULPAL TISSUE) OF ANY CONTINUOUSLY GROWING TOOTH OR TUSK

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Abstract

Many exotic and wildlife collections include species that have continuously growing dentition (e.g., elephants, rodents, tusked pigs, rabbits, etc.). The traumatic fracture of dentition should be an anticipated clinical eventuality for the well prepared clinician. The treatment of the traumatically fractured continuously growing tooth presents the treating clinician with a number of difficult endodontic and restorative problems, decisions and treatment alternatives.

The repair of a broken mandibular right tusk of an African babirusa (Babyrousa babyrussa) provides a practical, single procedure, five-part solution to this clinical problem with a high probability of success, and with a minimum of post operative complications. A step-by-step hand out with a complete instrument, materials and equipment list with source suppliers will be included/available.
CLINICAL MANAGEMENT OF SEVERE NECROTIC LAMINAR DISEASE IN AN EASTERN BLACK RHINOCEROS (Diceros bicornis michaeli) ASSOCIATED WITH AN UNDETERMINED ETIOLOGY

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Abstract

Recently, a syndrome seen in the black rhinoceros of Zimbabwean origin only, (at the time of this writing) has been suggested as resembling purpura hemorrhagica in the domestic horse. Whether or not the case presented in this paper is a definitive representative of this syndrome has yet to be determined. One aspect of the purpura hemorrhagica like syndrome includes lesions of the feet similar to laminitis. All four feet were affected in this rhinoceros. The lesions appeared to develop initially as horizontal, linear directed, weeping ulcers along the coronary band lesions of varying feet. The length and depth of the ulcer, which foot was affected, as well as the location along the coronary band area also varied over the years. However, in the spring of 1996, they were noted to have been more severe in appearance. It is speculated that the lesions indicated a primary site which then lead to ischemic necrosis of much of the distal aspects of the feet. Most of the soles were sloughed leaving the corium exposed as the weight bearing surface. Both horns were also lost during the initial phases of the disease syndrome. An immune mediated response as seen in Type III hypersensitivity leading to antigen:antibody complexes with resulting vasculitis was suspected. Since the time of the initial tentative diagnosis listed, the purpura hemorrhagica like syndrome has been suggested. Frequent foot trimmings were required beginning in July 1996 for successful therapy. In addition to the surgical management of severe necrotic laminitis, pharmaceutical prescriptions included phenylbutazone (usually 6 g p.o., s.i.d. × 5 days post-immobilizations), trimethoprim-sulfa (#16 960 mg tablets p.o., b.i.d. × 13 wk), and Metronidazole (#50 500 mg tablets p.o., b.i.d. × 7 wk). Minor trimmings are still necessary in the spring of 1998 due to what would appear to be pockets being formed secondary to the more rapid wall growth over the slower growing underlying corium. To date, what was once considered potentially hopeless or at best chronic now appears to carry a prognosis of returning to a fully normal foot without the obvious waving of the nail wall as seen in the domestic horse with chronic laminitis.

Introduction

Purpura hemorrhagica has been associated most often with an earlier respiratory infection caused by Streptococcus equi in the domestic horse although an association has been noted with equine influenza as well. There are a few other less common associations. Although the actual cause of the syndrome is not known for sure, it has been strongly suggested that a hypersensitivity to streptococcal antigens leading to antigen:antibody complexes which would then produce the primary histologic lesion of aseptic vasculitis associated with hemorrhage and edema, the classic signs of
purpura in horses.\textsuperscript{2,6} This edema is most apparent in the distal extremities of all four limbs, the head, and the ventral midline. Leakage of serum may occur. When purpura occurs in the horse it is usually 2-4 wk post respiratory infection. Affected horses tend to retain their appetites. Treatment involves ridding any underlying bacterial infections, reducing the edema, and inhibiting the immune response. Penicillin and corticosteroids are the pharmaceuticals of choice.

Laminitis in the domestic horse is most commonly associated with a carbohydrate overload (i.e., grain overload). It is suggested that lactase-producing bacteria proliferate in the cecum releasing large volumes of lactic acid. This sudden drop in cecal pH kills the enterobacteria, releasing endotoxins. The combination of lactic acidosis and endotoxemia leads to the inflammation of the pedal laminae. Although blood flow actually increases in the feet, blood is shunted by arteriovenous shunts and therefore no blood reaches the laminae. Thus ischemic necrosis of the laminae occur. There are other conditions which have been noted to lead to the development of laminitis in the horse such as retained placentas, excessive water consumption following exercise, too much exercise, excessive trimmings, pastures with lush grass, and probably others. Treatment includes phenylbutazone for pain and improved blood flow into the corium, cold water soaks to reduce oxygen demand in the laminae and for pain, and corrective shoeing and trimming in the horse. Corticosteroids are contraindicated due to the inhibition or at least the delay of keratin synthesis. Antibiotics prevent secondary infections which could lead to abscesses. Exercise is to be limited to short walks or limited to small pens which encourages blood flow to the laminae while at the same time limiting excessive pressure on the same structures.\textsuperscript{1,3,5} It is further suggested that if aggressive daily foot care is unavailable then euthanasia should be considered.\textsuperscript{5}

**Case Report**

A 7.5-yr-old male eastern black rhinoceros (*Diceros bicornis michaeli*) was immobilized for multiple foot trimming to remove necrotic material involving the nail, sole, and corium of all four feet. It had been noted that the animal had experienced coronary band lesions off and on for several years, especially in the spring and summer months. These typically were weeping, crusty, linear directed ulcerated areas extending for various lengths along the coronary bands of varying feet every year. In the early summer of 1996 these lesions were noted to be more severe than noted during fly seasons from earlier years. Topical administration of a chlorhexadine acetate ointment (Nolvasan, Fort Dodge) was chosen to avoid topical corticosteroids in a black rhinoceros even though the minute amount present topically may have rendered this concern as unwarranted. In late July 1996 the first of a series of immobilizations were initiated to first evaluate the reason for recent lameness, why tarsal joints bilaterally and acutely felt hot to the touch the day before, and to determine the extent of coronary band involvement. Radiographs were unremarkable. It was during this initial immobilization that we discovered that a finger could be inserted deep into the foot through one of the ulcers. A Group C *Streptococcus* was grown from this wound but was dismissed as a contaminant. Subsequent immobilizations were conducted to begin the process of removing necrotic material from all four feet. Although, initially we attempted to leave the sole intact to provide a surface to walk on, much of it was necessarily removed from each foot due to the amount of necrotic corium that had to be removed underneath. This meant that the animal was forced to walk on
exposed laminar tissue which allowed for the introduction of foreign material such as pebbles, sand, urine, hay and other feed materials, etc. The decision to avoid bandaging was made due to the cost and difficulty of changing four bandages at least daily with the added risks of even more immobilizations. Feet trimmings were accomplished using X-Acto blades (Bob Corey Associates, PO Box 73, Merrick, NY 11566) which proved to be ideal for rapid, aggressive removal of dead tissue. We use these for routine elephant foot trimmings and consider them indispensable. The blades are available in a multitude of shapes and sizes which allow for precise excision. Corium, sole, and nail tissue were removed to the point of bleeding. Tincture of iodine was applied to cauterize and harden the exposed tissues. Multiple immobilizations allowed for staged excisions as the need arose as well as for the visualization of progress. Spooning of the exposed softer corium tissues was at times so pronounced that one could only imagine an end result of a spooned foot similar in appearance to a Shetland pony with chronic laminitis.

Further, early on in the disease process the animal had developed a number of severe decubital ulcers, especially over the bony prominences. The animal also had developed a severe ventral midline moist, necrotic dermatitis. One may now look back and wonder if ventral edema was first present, although none was noticed, or simply had developed secondary to staying moist and contaminated from sternal positioning and decreased activity. This ventral midline dermatitis responded well to firm scrubblings with iodine, scraping away loose necrotic tissue, and antibiotic coverage (trimethoprim-sulfa (#16 960 mg tablets p.o., b.i.d. for 13 wk), and metronidazole (#50 500 mg tablets p.o., b.i.d. for 7 wk). Nolvasan ointment was applied topically later when the wound was no longer moist. Management of the skin lesion complications progressed from the use of wood chips for cushioning (was beneficial, but contaminated the wounds) to making huge wood chip pillows out of sheets (impossible to move out when it was wet and acted as a constant source of contaminated moisture). We found a satisfactory solution in the use of public donated water bed bladders. These allowed pressure on the feet and skin lesions to be minimized but would pool surface fluids at times. The animal was visibly much more comfortable on these and even appeared to be euphoric at times.

We began supplementing the animal’s diet with gelatin to encourage nail growth. This is made into “brownies.” Later we added an essential fatty acid supplement to the “brownie,” which is flax seed based (Missing Link, Designing Health, Inc. 28310 Avenue Crocker, Unit G, Valencia, CA 91355 USA). It was hoped that adding these micronutrients might help in healing the skin lesions as well. I feel that browsers fed pelleted diets and processed hays would benefit from this addition.

Immobilizations were accomplished using carfentanil (1.2 mg i.m.) or etorphine (3 mg i.m.). Reversal was utilized using naltrexone (100 mg i.v. or 300 mg i.v., respectively). Hand injections in the exhibit with the animal were possible initially. Hiding behind poles with a Telinject dart system while someone distracted the animal was necessary as the animal felt better later on. Weight was estimated at 1000 kg. The animal was typically placed on phenylbutazone (usually 6 g p.o., s.i.d.) for 5 days following immobilizations to control pain. Further, as a nonsteroidal antiinflammatory blood flow to the corium is improved with phenylbutazone due its ability to decrease platelet aggregation. However, concern for gastrointestinal ulceration and the worry of
1000 kg being too comfortable on exposed corium clouded the decision to utilize phenylbutazone more fully.

Discussion

The purpura hemorrhagica like syndrome in black rhinos, which has recently been brought to our attention, does have characteristics that are similar to purpura in horses. Feet lesions that appear similar to laminitis have been noted to occur early in the disease but it has been suggested that these similarities are probably related to pressure associated with the edema. However, histopathology performed on affected areas in the rhino reveal a lymphocytic perivascularitis unlike the purpura in horses, which includes a vasculitis. Further, this syndrome in the black rhinoceros has been associated with a very low PCV (13) and low serum phosphorus. The rhino in this case maintained a PCV of 53 early, slowly dipped to 39 around 6 mo later, and steadily rose back to 53, 1 yr later. Serum phosphorus wavered from 3.2 and 4.8 mg/dl, normal values, throughout the same period. During the initial workup an IgG level was requested but no anti-rhino IgG was available then. A request for IgT was also requested but no reagent for rhino serum was available. Serology was negative for bovine viral diarrhea, equine herpes I, equine viral arteritis, eastern and western equine encephalitis, equine infectious anemia, and bluetongue were all negative. Leptospira serology indicated the following titers approximately 6 yr post single leptospira vaccination: bratislava 1:25, canicola 1:25, ictero 1:200, pomona 1:25, tarassovi 1:25. One year later the animal demonstrated an elevated gamma globulin consistent with chronic inflammation and a decrease in alpha 1 proteins indicating immunosuppression or immunodeficiency in this specific group of acute phase proteins (Lisa Michelle Tatum, DVM).

Conclusion

Although it is yet undetermined as to the definitive cause for the necrotic laminitis in this rhinoceros, clinical management proved successful when proper surgical management was combined with appropriate pharmaceutical coverage and an environment which promoted good nursing care and animal comfort. Concerns at this point include recurrence of the disease syndrome: a) the purpura hemorrhagica like syndrome, which has already occurred in at least one institution, resulting in death, and b) the laminitis, which commonly reoccur in horses due to now being predisposed. Unforeseen complications during regrowth such as foreign bodies within the previously exposed laminar tissue or open spaces deep to the wall due to different regrowth rates of the various tissues of the foot are also expected. Finally, one should take and store plenty of serum for future reference should this disease syndrome begin to demand serologic evidence not anticipated earlier.

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timely: “I’ve never seen that before,” “hang in there,” and my favorite: “I’m glad it’s not me!” provided moral support (?) and helped carry me through all this! A special thanks goes to Jeff Bullock, Animal Curator, for his permission and active participation in the ground breaking foot care of an endangered black rhinoceros. As no man is an island, I must place most of the credit for Ahoudi’s recovery with those who actually provided a healing environment and daily nursing care—his keepers: Amy Jirsa, Tonya Kuker and Janet Porter. Thank you all!

LITERATURE CITED

TREATMENT OF A NASAL ULCER IN A BLACK RHINOCEROS (*Diceros bicornis*) USING CRYOSURGERY

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Abstract

Case Report

A 22-yr-old female eastern black rhinoceros (*Diceros bicornis michaeli*) was presented for a hemorrhagic ulcer on the rostral-medial edge of its left nostril. The rhinoceros’ past medical history included previous episodes of mucocutaneous ulcers, heavy accumulations of tartar that resulted in the removal of several molars and premolars, chronic diarrhea (after negative evaluations for common causes of diarrhea, presumably due in part to malnutrition secondary to the oral lesions), a digital abscess, and a cranial horn avulsion. The animal had been vaccinated annually for leptospirosis (Leptoferm-5, SmithKline Beecham, West Chester, PA 19380 USA). A previous, mild nasal hemorrhage that presented 5 yr before this episode, had spontaneously resolved.

Seventy-two hours after presentation, the rhinoceros was anesthetized with 4 mg etorphine (Etorphine, Wildlife Laboratories, Fort Collins, CO 80524 USA) and 100 mg xylazine (Xyla-ject, Phoenix Pharmaceuticals, St. Joseph, MO 66504 USA). The animal was recumbent in 15 min, and blood was collected from the medial carpal vein for a CBC, serum chemistry profile, and for various research protocols. Examination revealed a 3 × 8 cm ulcer with a raised border and raw, bleeding surface in the left nares and a 5 cm by 10 cm ulcer inside the upper right lip margin. Bronchoscopy 30 cm into the nasal passage revealed blood draining from the ulcer, but no additional ulcers or masses. Punch and wedge biopsies were collected from the margins of both the nasal and oral ulcer. The hematocrit was 43.8% and there was a mild increase in the white blood cell count (17,800/ml) with a mature neutrophilia. Histologic findings were compatible with the superficial necrotic dermatopathy syndrome (formerly called mucocutaneous ulcerative syndrome by some) of captive black rhinoceroses.1,2

Over the subsequent 3 wk, the ulcer continued to hemorrhage. The rhinoceros was again anesthetized, and although repeat nasal endoscopy revealed no additional lesions, the ulcer now extended 13-14 cm up the nasal passageway. The rostral portion of the ulcer was cauterized with a carbon dioxide laser, and the caudal-most aspects of the lesion that could not be reached by the laser were treated with electrocautery. The hematocrit on blood collected from the medial carpal vein was 38.7%.
Four days later, significant hemorrhage was noted. Blood collected from an ear vein, revealed a hematocrit of 24%. In an attempt to control the hemorrhage, 1:25,000 epinephrine solution was sprayed onto the ulcerated area. However, marked hemorrhage continued overnight. The rhinoceros was anesthetized the following morning. The hematocrit (from the medial carpal vein) was 32.4%, and the WBC was again moderately elevated. A clotting profile was as follows: PT = 14.1 sec, PTT = 23.9 sec, and 288,000 platelets/ml. The nasal ulcer was more proliferative and there was a notable deficit (“cratered area”) in the caudal aspect of the lesion. Liquid nitrogen, applied with sterile gauze sponges, was used to freeze as much of the ulcer as possible. At the end of the procedure there was only mild hemorrhage and it was controlled with topical 1:10,000 epinephrine. Upon reversal, the animal sneezed violently and rubbed its nose on the floor, re-initiating the hemorrhage. However, it quickly subsided when again treated with topical 1:25,000 epinephrine. Due to decreased hemorrhage, the epinephrine was discontinued the following day.

In the subsequent 5 days, only occasional drops of blood were noted and the ulcer became notably smaller and drier in appearance. Serial hematocrits drawn from the ear vein ranged from 25.7-27%. On day 5, the cranial portion of the lesion was dry in appearance, and the internal affected mucous membranes were white, dry and delineated by a sharp margin. In the subsequent 4 wk the lesion completely resolved and only a small white scar remains. During this period, the hematocrit (from the ear vein) increased to 31.4%.

Discussion

The syndrome of superficial necrolytic dermatopathy has been reported as the most common disease of captive black rhinoceroses in North America. As in this case, ulcerative lesions, and sometimes proliferative lesions can be found on the skin, often starting over points of wear, and the mucous membranes of the oral and nasal cavities. Frequently the lesions recur.

Although most cases, particularly the milder forms, appear to spontaneously resolve, some do not. Various empirical treatments have been suggested (including topical and systemic antibiotics for secondary infections), however, no single treatment has consistently caused a reversal of the lesions. Biopsies are important in order to determine that the lesions are indeed representative of the superficial necrolytic dermatopathy, and not other ulcerative skin lesions of black rhinoceroses. Additionally, biopsies should be performed so that neoplastic disease such as fibrosarcoma or squamous carcinoma can be ruled out.

A remarkable feature of the ulcer in this case was the significant blood loss apparent both in the frank hemorrhage and in the hematocrit values. A notable difference between the hematocrit of ear vein samples from awake rhinoceroses and that of samples collected from the medial carpal vein of anesthetized animals was also noted and is similar to the findings of the others (M. Miller, personal communication).

In this rhinoceros, attempts to cauterize the lesion with laser therapy appeared to exacerbate the hemorrhage. Cryosurgical techniques produced a quick, effective resolution of the ulcerative
lesions. In general, the use of cryosurgery is limited in part by the ability to control its application (i.e., not freezing vital underlying anatomic structures). In this case, it was fortunate that the ulcerative lesion was along the nasal septum, and not adjacent to important nerves, vessels or tendons. On the basis of this single treatment, laser therapy should not be ruled out for future cases. However, given the results from this case, cryosurgery appears to be a potentially valuable therapy for the treatment of future ulcerative lesions that significantly compromise the overall health of a rhinoceros.

ACKNOWLEDGMENTS

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LITERATURE CITED

UMBILICAL HERNIORRHAPHY IN A JUVENILE ASIAN ELEPHANT (*Elephas maximus*)

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Abstract

A 2-wk-old female captive-bred 130 kg Asian elephant, born and housed at the Burnet Park Zoo in Syracuse, NY, was diagnosed with an umbilical hernia. The cord was sealed, but a 5 cm defect in the abdominal wall allowed tissues to protrude into the subcutaneous space. The length of the cone-shaped protruding tissues was about 5 cm. The hernia was located very caudally along the midline. Two weeks following the initial examination, characterization of the hernia was attempted with ultrasonography. Since no loops of bowel could be detected at this point, a conservative approach was elected. The animal was monitored for the following 3 wk. A small reduction in the diameter of the hernia was detected at 2 mo of age. At this time, the animal weighed 187 kg. Surgical correction was discussed with the zoo’s staff and elected because the defect had the potential to entrap a loop of bowel or interfere with reproductive potential in future years. An immediate surgical correction was elected to decrease the risks for anesthetic complications that would develop with increasing size. The trainers were able to achieve conditioning of the animals to reduce the stress of handling on the baby and facilitate its separation from the dam. It was elected to perform the surgery at the zoo utilizing a team from Cornell University.

The dam, weighing about 2850 kg, received 230 mg (0.08 mg/kg) of xylazine hydrochloride i.m. (Miles, Inc., Agriculture Division, Animal Health Products, Shawnee Mission, Kansas USA). The animal became sedated within 10 min, and its calf was moved into an induction area in front of its stall in the elephant house. The calf received 20 mg of xylazine HCl (0.1 mg/kg) and 300 mg of ketamine hydrochloride (1.6 mg/kg) i.m. (Phoenix Pharmaceutical, Inc., St Joseph, Missouri USA). Within 20 min, the calf was ataxic and head pressing but did not become recumbent. An intravenous catheter was placed in an ear vein and an additional 25 mg of ketamine HCl (0.13 mg/kg) combined with 2.5 mg of diazepam (0.013 mg/kg) (Schein Pharmaceutical, Inc., Florham Park, New Jersey USA) were administered i.v. The animal then became recumbent and was transported to the operating room. Two supplemental doses of 25 mg ketamine HCl were given i.v.: one during the transfer to the operating room, and the second 3 min after intubation until the isoflurane took effect.

Intubation was accomplished with auffed 14 mm internal diameter, endotracheal tube. A semi-closed rebreathing system was connected to a large animal anesthesia machine. Ventilation was spontaneous and isoflurane (Abbott Laboratories, North Chicago, Illinois USA) was delivered in oxygen. Monitoring included heart rate, respiratory rate, electrocardiogram, direct arterial blood pressure (catheter in ear artery), temperature, arterial blood gases, and pulse oxymetry. Hypotension
was corrected by adding an intravenous infusion of dobutamine hydrochloride at a rate of 10 mg over 50 min (Ben Venue Laboratories, Inc. Bedford, Ohio USA).

The animal was placed in dorsal recumbency and the area prepped for surgery. A 12 cm fusiform incision was made around the umbilical hernia. Following sharp dissection the hernial ring was identified. The size of the hernial wall defect was 7 cm long; the depth of the hernial sac was 10 cm. Within the hernial sac a 3-cm fibrous mass and another softer structure could be palpated. It appeared that the intestine could be herniated and adhered to the sac; thus it was decided to open the sac so that its content could be examined. Upon opening the sac, small intestine was present within the hernia. The palpably thick structure was identified as firm fibrous connective tissue, and the palpably softer structure was fat and small intestine. These were fully reducible due to lack of adhesions. The hernial sac was resected, and at that time some small intestine was seen prolapsing through the body wall defect. This defect was reapposed using #3 Vicryl® (Ethicon, Inc., Sommerville, New Jersey USA) in a simple interrupted pattern, which required 10-12 sutures. The loose fascial tissue immediately adjacent to the body wall was reapposed with 0 Maxon® (Davis & Geck Monofil, Inc., Manati, PR) in a simple continuous pattern. The subcutaneous tissue was also reapposed using the same suture material and pattern. A subcuticular suture was placed using 0 Monocryl® (Ethicon, Inc., Sommerville, New Jersey USA). Tissue glue was applied to the skin.

Ceftiofur (The Upjohn Co., Animal Health Division, Kalamazoo, Michigan USA) was administered at 430 mg i.m. (2.3 mg/kg). The entire procedure from the sterile preparation to the end of the surgery lasted 1 hr. Within a few minutes after terminating the isoflurane, the calf began to move its legs and was transported back to the induction area in the elephant house. The animal was extubated 10 min after the isoflurane was discontinued. Supplemental oxygen was administered via a nasal tube inserted in the trunk, alternating nostrils every 5-7 min. Fifteen minutes later, 2.5 mg of yohimbine HCl (Lloyd Laboratories, Shenandoah, Iowa USA) was given i.v. and 2.5 mg was given i.m. Five minutes and 15 min later, two additional doses of 2.5 mg of yohimbine HCl were administered i.v. The total yohimbine HCl dose was 0.05 mg/kg. Increased muscle tone and activity were then noted. Forty five minutes after extubation the animal picked its head up for the first time and 1.5 hr after extubation it became sternal and stood up. The animal was physically supported for 15 min until strong enough to ambulate without falling. The cow received 100 mg of yohimbine HCl i.m. (0.035 mg/kg) while the calf was being reintroduced.

Post operative care was greatly facilitated by the previous training of the elephants. It included: (1) a 5-day 24-hr watch of the baby and its mother by the trainers; (2) prevention of self-induced or mother-induced trauma to the surgical site; (3) taking daily rectal temperature (calf); (4) assessment of the wound; (5) antibiotics (Ceftiofur 1.1 mg/kg i.m. for five doses); (6) butorphanol tartrate (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa USA) 5 mg i.m. (0.027 mg/kg) every 6 hr for 48 hr post-surgery. Exercise was restricted to the stall for 14 days. Short daily outdoor sessions were then authorized under supervision. The calf had a tendency to roll and drag herself in the sand. The wound appeared to be healing well for 3 wk. A delay in healing was then noted and further examination revealed a purulent exudate around the scabs. A swab was submitted for aerobic bacterial culture and sensitivity. The wound was subsequently cleaned daily with a diluted Nolvasan® solution (Chlorhexidine diacetate, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa
USA). Thirty days post operatively, a strand of exposed sutures was removed from the subcuticular layer. This was thought to be at the source of the tissue reaction. The wound closed and healed without further complication. Thirty-three days post operatively the calf and the dam were allowed to go outside on exhibit with the other elephants. Daily trainer checks and biweekly assessments by staff veterinarians continued to confirm the resolution of the inflammatory process and the final stages of healing.
INTESTINAL VOLVULUS AND STRicture ASSOCIATED WITH A LEIOMYOMA IN A GREEN SEA TURTLE (*Chelonia mydas*)

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Abstract

A 30-kg female green sea turtle (*Chelonia mydas*) was presented to the Veterinary Medical Teaching Hospital, University of Florida, with a 2-mo history of anorexia, intermittent regurgitation, decreased fecal production, and positive buoyancy of the right side. Found stranded near Atlantic shore, Hutchinson Island, Florida, the turtle had been diagnosed as having a bowel obstruction and treated medically at a rehabilitation center prior to referral, with no response to therapy. Upon initial presentation, no abnormalities were detected on physical examination, and hematology and clinical chemistry results were within limits established for this species. Radiographs confirmed gaseous distension of bowel loops, suggestive of intestinal obstruction and the patient was placed on ceftazidime (20 mg/kg i.m. q 72 hr). Exploratory celiotomy was performed in order to identify and treat the cause of the obstruction. The turtle was induced with 1.15 mg of medetomidine (40 μg/kg) and 115 mg of ketamine (4 mg/kg) administered intravenously in the dorsal coccygeal vein and maintained on isoflurane and oxygen. The intestinal obstruction was initially palpated through a soft tissue approach into the right hindlimb fossa, but it could not be exteriorized. Through plastron osteotomy performed through the right abdominal scutes, a 540 degree torsion of the small intestine was identified. Following reduction of this torsion, an intestinal stricture was identified as well as a thickened bowel wall containing intramural nodules. Approximately 30 cm of abnormal bowel, including the stricture, were resected and submitted for histopathology. An end-to-end anastomosis was performed using techniques to accommodate the discrepancy in size between the sections of bowel orad and aborad to the stricture. The plastron osteotomy was stabilized using self-tapping screws and figure-eight wire. Fiberglass cloth and fast-drying epoxy were used to seal the defect. Atipamezole (4 mg) was administered intravenously in the dorsal coccygeal vein for reversal of the medetomidine and the patient recovered uneventfully.

Histopathology of the resected bowel segment identified a leiomyoma associated with the focal stricture. The margins of the resected segment were free of tumor. Markedly ectactic subserosal lymphatic and blood vessels occurred secondary to the torsion and accounted for the nodules noted during exploratory celiotomy. In addition, there was a moderate diffuse lymphoplasmacytic enteritis with widely scattered small granulomas centered around eggs consistent with the spirorchid trematode, *Laeredius laeredii*. 
The turtle was returned to the rehabilitation center 6 wk following surgery. At 4 mo post-surgery the turtle remained positively buoyant but able to rest on the tank bottom for extended periods of time. The fiberglass cloth and fast-drying epoxy patch had begun to separate from the plastron and the underlying tissues treated using antibiotic-impregnated ointment and a water-resistant adhesive dressing. Appetite and defecation remained steady and no further clinical signs of gastrointestinal obstruction were observed.
DERMATITIS CAUSED BY GROUP G BETA-HEMOLYTIC *Streptococcus* IN NILE HIPPOS (*Hippopotamus amphibius*)

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Abstract

Case Report

Dermatitis was noted in a group of three captive Nile hippos at the Milwaukee County Zoo. The group consisted of a 28-yr-old male, a 27-yr-old female, and their 21-yr-old female offspring. The hippos were separated by gender, but the two groups utilized several of the same areas at different times of the day. The housing for these animals consisted of an indoor pool, two indoor concrete floored holding areas, and an outdoor yard which has a traffic bond substrate and a concrete pool. Both pools are cleaned by hosing daily after draining, with a subsequent refill using city tap water. The indoor holding areas are hosed daily, and are cleaned with a quaternary ammonium disinfectant. The hippos are fed high fiber herbivore cubes and timothy/grass hay. No change in housing, feeding, or cleaning programs occurred prior to the development of dermatitis.

In February of 1997, the older female hippo developed numerous linear cracks in its skin, especially over the bridge of the nose and bilaterally along the shoulders and flanks. A 2-cm circular wound was present ventrally at the angle of the right mandible. Initial treatment included topical application of dilute chlorhexidine solution by sprayer to the entire body once daily which later changed to 0.1% gentamicin in glycerin. Over the next month, the animal was lethargic intermittently, would dog-sit, and appeared to be losing weight. Actual weights could not be obtained. The hippo was treated empirically with trimethoprim-sulfamethoxazole 45 g p.o., s.i.d. for 2 wk. Attempts to obtain skin samples via biopsy darts were unsuccessful. After oral treatment, the animal was less lethargic, but continued to lose weight.

Milder lesions, consisting of skin cracks over the face, limbs, and trunk, were present in the other two hippos. Over the next 6 wk, all hippos developed bilateral draining wounds under the mandible. A quick scalpel biopsy of the margin of a submandibular lesion in the older female was obtained through a partially opened stall door. The 1 × 2 cm flap of skin was submitted for histopathology, which revealed suppurative, multifocal epidermitis teeming with numerous dense colonies of gram-positive cocci, as well as severe hyperkeratosis and multifocal parakeratosis. Cultures of two different skin wounds were obtained in each of the two older animals. All four wounds grew a heavy growth of Group G beta-hemolytic *Streptococcus*. Moderate numbers of *Morganella morganii* and *Citrobacter freundii* also grew in one of the two cultures from each animal. The
isolated *Streptococcus* was susceptible to all drugs tested including trimethoprim-sulfa combinations, but had only intermediate susceptibility to gentamicin.

In April of 1997, the male was treated with oral trimethoprim-sulfamethoxazole 60 g p.o., s.i.d. for 2 wk. The mild lesions in the male hippo and the younger female began to resolve. Weight loss became marked in the older female hippo, and serosanguinous fluid began to ooze from the skin lesions. The older female was retreated with a higher dose of trimethoprim-sulfamethoxazole, 75 g p.o., s.i.d. for 2 wk, without any signs of improvement. Weight loss continued, and a large draining tract formed on the lateral aspect of the right carpal region. Feces of all hippos were negative for parasites and enteric pathogens including *Salmonella* spp., *Campylobacter* spp., and *Mycobacterium paratuberculosis*. The hippos were switched to a Mazuri herbivore diet fortified with natural vitamin E, beta-carotene, essential fatty acids, and organic zinc.

Immobilization of the older female for blood work and biopsies was planned. A medical infectious disease specialist recommended treatment with an oral penicillin, as streptococcal infections can show *in vivo* resistance to trimethoprim-sulfa drugs even when *in vitro* susceptibility is reported (G. Dorff, personal communication). Due to the severity of the illness in this animal, treatment with amoxicillin 30 g p.o., s.i.d. for 6 wk began in mid-May, ten days prior to the immobilization and workup. Attempts at intramuscular treatment by pole syringe were completely unsuccessful due to the thickness of the hippo skin and its aggressive reaction to the attempted treatment.

The older female hippo was moved into an elephant chute for containment immediately prior to immobilization. At its smallest setting, the chute measured 2 x 4.5 m, which still allowed the hippo to move forward and backward, as well as turn around inside the chute. While the dimensions of the chute were too large to safely manipulate an awake hippo, the containment it afforded proved very helpful during the immobilization. Ultrapotent narcotics were not used due to concern regarding collapse and death during anesthesia in this debilitated animal. The hippo was estimated to weigh 950 kg and was immobilized with 40 mg of detomidine and 60 mg of butorphanol delivered by a Cap-chur dart into the muscles of the upper neck region. The animal became recumbent at 7 min, but maintained limited movement of its head and limbs throughout the procedure. Respirations varied between 1-4 breaths/min. Measurements of oxygen saturation could not be obtained. Supplemental oxygen was supplied intranasally. Heart rate was 36 beats/min. Multiple skin biopsies were taken from the flank region and submitted for histopathologic examination and culture. The hippo was skin tested for tuberculosis using intradermal injections of mammalian isolates and PPD-bovis in the thinner skin adjacent to the ears. Blood was drawn from the ventral tail vein using an 18-ga needle attached to an extension set. The animal stood with little warning at 65 min after the initial darting, prior to the administration of 250 mg of yohimbine and 500 mg of naltrexone, which were subsequently given intramuscularly.

The skin test was negative for reaction to tuberculin at 72 hr by manual palpation. Evaluation of the hippo’s blood work proved problematic as normal values could not be obtained. The following values were possibly elevated: blood urea nitrogen 48 mg/dl, creatinine 1.8 mg/dl, globulin 4.0 g/dl, lactate dehydrogenase 1529 U/L, aspartate transaminase 251 U/L, and alanine transaminase 68 U/L.
Cultures of the biopsy sites revealed numerous organisms, but no *Streptococcus* spp. *Serratia liquifaciens*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Escherichia coli*, and moderate diphtheroids cultured from various sites were considered to be contaminants. Blood cultures were also contaminated, growing numerous gram-negative organisms including *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris* and *Enterococcus faecalis*. Fungal cultures of the skin were negative for growth. No serum antibodies to *Blastomyces* sp. were detected by radial immunodiffusion.

Histopathology of the skin taken ten days after beginning amoxicillin therapy revealed mild to moderate epidermitis with moderate numbers of unidentified fungal hyphae and spores in the keratin layers, along with occasional scattered cocci. Hyperkeratosis was present, but was much less severe than in the previous biopsy.

The skin of the older female hippo began healing within 1 wk after beginning amoxicillin therapy, and visible weight gain was noted by 3 wk. Scars remain present on this animal’s skin, but no new lesions have been observed over the last year.

**Discussion**

Group G streptococci are normal flora of the skin and mucosa of animals. However, these organisms can act as rapid opportunistic pathogens. Group G streptococcal infections tend to be severe and prolonged, and resemble those caused by Groups A and B. Skin infections and cellulitis in humans caused by Group G streptococci may be associated with abnormal lymphatic drainage or underlying malignancies. Bacteremia, endocarditis, and glomerulonephritis are possible, although uncommon, sequelae to infection with Group G streptococci. Suggested antibiotic therapies for humans with streptococcal skin infections include penicillin or a first-generation cephalosporin, with surgical debridement if indicated. Poor clinical response is often seen with erythromycin or chloramphenicol. Group G beta-hemolytic *Streptococcus* spp. was cultured at necropsy from an infected metacarpophangeal joint in an adult male hippo with a chronic draining toe lesion (R. Junge and R.E. Miller, unpublished data).

The dermatitis in these cases is likely the result of opportunistic infection of skin lesions by normal skin flora. The inciting factor for the development of skin infections in these hippos remains unclear. Topical or intramuscular treatment is recommended in zoo medicine texts, but topical treatment was ineffective in these animals. Intramuscular treatment was spectacularly unsuccessful. The mobility of the animal in the chute made neck injections unsafe, and penetration of the truncal skin could not be achieved with a pole syringe. Repeated darting was not used for antibiotic administration due to the need for multiple large darts, and concern that the hippo would become reluctant to enter the chute, making follow-up observations and manipulations more difficult. Hippos have a complex stomach with four sections, and although they are not true ruminants, efficacy of oral treatment with antibiotics was questioned. However, dramatic improvements in skin condition was noted immediately after starting oral amoxicillin. The fungal infection noted in the...
second biopsy may represent a secondary invasion of the wounds, which resolved spontaneously upon treatment of the primary skin disease.

Death during immobilization of hippos with ultrapotent narcotics has been reported.\textsuperscript{4,5} Juvenile hippos have been immobilized at the San Diego Zoo with detomidine and butorphanol.\textsuperscript{3} The low dose detomidine and butorphanol administered to this adult animal provided good immobilization for the procedures performed. However, the amount of head and limb movement retained, along with a surprise spontaneous recovery, made the use of a chute or containment device important for personnel safety. Pulse oximetry and electrocardiogram could not be obtained on this animal with our current probes. Anesthetic monitoring was limited to visual assessment of respiration rate and palpation of heart rate over the thorax. Blood collection is reported to be difficult in hippos.\textsuperscript{4,5} The ease of collection of large amounts of blood from the ventral tail vein in this animal may have been due to a light level of anesthesia, as the blood was collected in the 10 min prior to spontaneous recovery. The lack of accurate weights and reference ranges for standard hematologic and serum chemistry values hampered assessment of the animal’s condition during the course of the disease.

Conclusions

Opportunistic infection with group G beta-hemolytic streptococci should be included in the differential diagnosis of skin disease in hippos. Treatment of infected hippos with amoxicillin at 15 mg/kg p.o., s.i.d. appears to be effective. Detomidine and butorphanol can be used for immobilization of adult hippos, although containment of the animal within a chute or cage is recommended for personnel safety. Blood should be collected from normal hippos when possible, to aid in the development of reference ranges for this species.

ACKNOWLEDGMENTS

The successful resolution of this case would not have been possible without the hard work, persistence, and ingenuity of the Pachyderm staff. The Milwaukee County Zoo is also indebted to Dr. Gerald Dorff, MD for his advice and assistance with this and numerous other cases. As always, veterinarians across the country helped with advice and comments. Special thanks to the veterinarians of the San Diego Zoo, the St. Louis Zoo, and the Metro Washington Park Zoo for sharing anesthetic regimes, previous cases, and comparison tissues. Drs. Howard Steinberg and Annette Gendron-Fitzpatrick reviewed the histology slides. Dr. Bill Sadler and Karen Wright from Purina Mills, Inc. assisted in formulation and production of the fortified diet used during the treatment and recovery from dermatitis in these hippos.

LITERATURE CITED

SURVEY FOR Salmonella spp. IN CAPTIVE U.S. RHINOCEROSSES

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Abstract

Seventy-two zoological institutions and private ranches in the United States were surveyed for Salmonella culture results. Fifty-nine responded (82% return rate) with seven positive culture results reported from 1990-1997. With prevalence defined as a positive culture result in this survey, a 12% prevalence for responders was established and a 10% prevalence if nonresponders are assumed negative. Seventeen Salmonella isolations were made from 16 different animals (ten black, four white, and two Indian rhinoceroses). Nine different serotypes were reported with an additional two identified to the group level. Five serotypes were isolated more than once and of these, four were isolated at the same institutions. Two institutions experienced epizootics in which multiple animals were affected clinically.

Presentations included: asymptomatic (6), diarrhea (8), reluctance to stand (4), septicemic (5), and death (6). Two of the animals were in quarantine and five recently moved prior to their illness. Disease duration ranged from 3 days-3.5 mo. Clinical pathology changes reported in domestic large animal salmonellosis include: elevated plasma fibrinogen and neutrophilia or neutropenia with left shift in severe cases. Blood was obtained from five of the rhinos with clinical salmonellosis and demonstrated neutrophilia (2), lymphopenia (3), and degenerative left shifts (2). Two of these blood samples were obtained from moribund animals that died the same day as sample collection; these samples indicated hemoconcentration and one had evidence of prerenal azotemia.

Antibiotic treatment was attempted in six animals. Five received trimethoprim sulfa compounds (TMS) containing either sulfadiazine or sulfamethoxazole orally at 30 mg/kg every 12 or 24 hr. Two animals, one of which was initially treated with TMS but became anorexic, were given ceftiofur i.m. (Naxcel, The Upjohn Co., Kalamazoo, MI 49001 USA).

Six of the rhinoceroses died with four deaths attributed directly to salmonellosis. The primary pathology observed was edema and hemorrhage of the affected bowel with overlying fibrinosuppuration and multifocal lymphoplasmacytic gastritis, enteritis, and colitis. Three of these salmonellosis deaths had signs and lesions compatible with septicemia, such as suppurrative pneumonia, hepatitis, epicarditis, and omphalitis.

LITERATURE CITED
BRUCELLOSIS VACCINATION IN BISON

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Abstract

A current problem confounding the national effort to eradicate brucellosis from the United States is a reservoir of Brucella abortus infected bison (Bison bison) in Yellowstone National Park. This herd is a potential threat to the beef agribusiness surrounding the park since bison to cattle transmission of brucellosis has been documented under both experimental and field conditions. A possible solution to reduce this concern is vaccination of bison with the new cattle brucellosis vaccine, B. abortus strain RB51 (SRB51). This study evaluated the colonization and virulence of SRB51 in a high risk bison herd of 29 American bison which originated from a brucellosis reactor herd in Strong City, Kansas.

The herd was housed at a bison containment center at Texas A&M University and contained ten adult males, seven calves, and twelve adult females. The adult males and calves were vaccinated with the standard calfhood cattle dose of 1.8 × 10¹⁰ colony forming unists (CFU) of SRB51 subcutaneously while the adult females received the standard adult cattle dose of 1 × 10⁹ CFU. The vaccination strain was expected to not colonize or cause fetal pathogenesis in bison.

Following vaccination, serologic response was evaluated by western blot analysis which did indicate SRB51 specific antibodies. Additionally, all adult males, all calves, and three non-pregnant adult females were euthanatized at either 13 or 16 wk post-vaccination. Tissue samples were obtained at necropsy for Brucella culture from the liver, spleen, lymph nodes, and reproductive tract. The remaining adult females were in early gestation and were monitored until parturition for signs of fetal pathogenesis. Of these cows, six delivered healthy calves, one delivered a stillborn calf that was Brucella negative, and two failed to produce calves although had been determined pregnant.

From these results, administration of SRB51 to a high-risk, previously exposed bison herd did not appear to cause either prolonged colonization or a high rate of fetal pathogenesis following vaccination in early gestation. This study provided the evidence that SRB51 should be further investigated as a possible Brucella abortus vaccine in American bison. To address this issue, a vaccine efficacy trial is currently under evaluation.
PREVALENCE PATTERNS OF PATENT Baylisascaris procyonis INFECTIONS IN RACCOONS (Procyon lotor) IN WEST-CENTRAL ILLINOIS†

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This study is pending publication in the Journal of Zoo and Wildlife Medicine.

Abstract

The raccoon (Procyon lotor) is an omnivorous mammal that is widely distributed throughout North America. The raccoon has adapted well to both urban and rural environments with populations increasing in both areas. This generates greater contact opportunity between people and raccoons and creates potential for human and animal exposure to parasites normally harbored by these animals. One of the intestinal parasites, Baylisascaris procyonis, has been associated with fatalities in both humans and animal species. This parasite has a predilection for the central nervous tissue in aberrant hosts and has been reported as the cause of ocular, cerebrospinal, and visceral larval migrans in humans, avian, and other mammalian species.

Introduction

In this study, prevalence patterns of patent Baylisascaris procyonis infections were examined in raccoons from a state park (Siloam Springs State Park, Adams County, Mt. Sterling, Illinois USA) and a surrounding rural area (Adams County) in west-central Illinois. Differences in fecal shedding among raccoons by age, sex, geographic, and seasonal profiles were examined to provide more information on the epidemiology of this disease.

Methods

The study was conducted from September 1989-October 1993 with trapping seasons divided into fall (August-October) and spring (March-June). Following live capture, raccoons were sedated with Telazol® (Tiletamine HCl and Zolazepam HCl; Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, 50501 USA) at 5 mg/kg i.m. and fecal samples were collected rectally. Fecal material was stored in a 2.5% potassium dichromate solution until submitted to the University of Illinois (College of Veterinary Medicine, Urbana, Illinois, 61801 USA) for parasitologic testing.

Age was estimated in the field by evaluation of facial and body features, tooth eruption, and body weight. Raccoons were categorized as juveniles, yearlings, or adults. Juveniles were classified as animals born in the spring or entering their first fall for the current trapping year while yearlings...
were those in the subsequent spring and fall seasons. Adults were classified as animals past the second spring following birth. A first premolar was extracted from yearling and adult animals to confirm the age by evaluating the cementum annuli.6

To evaluate for eggs, fecal material was centrifuged (1,500 g for 5 min) and the fecal pellet re-suspended in Sheather’s sugar solution. A coverslip was placed on the tube, and the sample was centrifuged (1,200g for 10 min) a second time. The coverslip was placed on a slide and reviewed under light microscopy at 100-450x. The mean dimensions of the B. procyonis egg was 68 × 55 μm. The eggs were golden-brown, ellipsoidal, and had a granular texture.

Risk factors were screened at the univariate level and then compared, simultaneously, using logistic regression (SAS). Analyses were conducted using SAS with $P < 0.05$ considered to be statistically different.19,20

Results

Of 392 animals tested, 149 (38%) were found to be actively shedding B. procyonis. Juvenile raccoons were more likely have a patent infection (70%) than yearlings (31%) or adults (28%). Male raccoons were more likely have a patent infection (45%) than were females (30%). Raccoons captured in the park were less likely to have a patent infection (26%) than those in the rural area (46%). Raccoons captured during the spring season were less likely to be shedding eggs (34%) than those captured in the fall (41%). However, when juveniles were excluded from the comparison, because there were no samples collected from this age group in the spring, shedding of B. procyonis was lower in the fall (23%) than the spring (34%).

Discussion

Baylisascaris procyonis is the common roundworm of raccoons.19 Previous studies have reported prevalence of B. procyonis in raccoons from 7.5-82.0%.2,4,11,13,16,18 These reports were of adult worms diagnosed at necropsy except two reports which were from fecal samples with prevalence of 20.3 and 73%.13,18 The prevalence of egg shedding in this study (38%) is within the range but probably underestimates the true infection rate. Negative fecal samples are often the result of occult infections, which are not considered detrimental to aberrant hosts. Although active parasite burdens may not always be represented by evaluating fecal samples, prevalence of egg shedding is a measure of transmission and zoonotic potential. Characterization of age, gender, site, and seasonal trends are important in determining when the risk of exposure is greatest.

In this study, egg shedding was significantly higher in juveniles, relative to yearlings and adults. Juveniles become infected after ingesting infective eggs, infective intermediate hosts, and possibly through direct infection from their dams through the placenta or mammary secretions.11,13,18 Adult raccoons become infected predominantly by ingestion of infective intermediate hosts.11
Egg shedding in males was significantly higher than in females. It has been speculated that prevalence was lower in females due to transplacental and transmammary migration of larvae from females to juveniles thereby decreasing the developing number of mature adults.

Egg shedding was more likely in the rural area than the park area. Raccoons often use a latrine for defecation.3,23 There was less topographic relief in the rural area than park area. It has been reported that higher numbers of B. procyonis infections occur in areas with little topographic relief.4,19 Differences in habitat and other environmental influences could affect the social behavior and defecation site selection of raccoons. Further research is needed to explore ecologic mechanisms responsible for this difference.

It was reported that 80% of the B. procyonis detected in raccoons from New York were collected in the fall with the overall prevalence (for the fall) at 42.4%; this was nearly identical to the current study findings (41%). 13 The higher fall prevalence in the present study was due to the number of juveniles captured. Among yearlings and adults, fewer were shedding B. procyonis in fall than spring. The primary diet of raccoons in the spring is from animal sources.8 The pre-patent period for B. procyonis is 30-35 days.11 This provides adequate time for raccoons to develop higher rates of infection during the spring sampling season. Yearling and adult raccoons are more likely to feed on plant material during the fall, which might also account for the lower prevalence identified in these animals. The lower prevalence of egg shedding in adults during the fall also supports previous reports of self-clearance in the fall and possible resistance to subsequent infections.13 The prevalence of egg shedding was lower in the fall than the spring in adult females, however the difference was insignificant. This lower prevalence in the fall does support a role for transmammary larval transmission as parturition often starts in April, however it is variable.14 Further research is needed to determine if transmammary transmission does occur or if other factors, such as immunologic or dietary habits, may also play a role.

The findings of this study indicate the importance of preventative measures for individuals working with and around raccoons. The high prevalence of B. procyonis in juvenile raccoons poses a special threat to those who work closely with them, such as wildlife rehabilitators or pet owners. Children playing in sandboxes, on fallen logs, or areas where raccoons have established latrines are also at risk of exposure to potentially infectious larvae. Persons utilizing recreational facilities should also practice strict hygiene around areas potentially contaminated by raccoons. Bleach can be used to treat areas used by raccoons, although only fire destroys B. procyonis.11 Feeding of raccoons, which can concentrate them in large numbers, should be discouraged in both public and private settings. Raccoons in rehabilitation facilities or zoological parks should be quarantined from other species to decrease the risk of contamination.

ACKNOWLEDGMENTS

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U.S. DEPARTMENT OF AGRICULTURE ANIMAL CARE: REGULATION AND POLICY UPDATE

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Abstract

Several changes have recently occurred in the U.S. Department of Agriculture animal care regulations and policies that might affect zoo animal medicine. More changes are under consideration and may be adopted in the near future to improve the effectiveness of animal care programs. Some areas affected by these changes include:

1. Elephant TB testing, treatment, and travel restrictions (effective July 1, 1998);
2. Perimeter fencing (final ruling expected summer 1998); and
3. Risk-based inspection system to focus U.S. Department of Agriculture resources on problem facilities (effective March 1998).

Changes under consideration include:

1. Training and handling requirements for dangerous animals,
2. Primate enrichment guidelines, and
3. Specific space requirements for wild/exotic mammals.
BATS–PUBLIC HEALTH REGULATIONS AND ISSUES

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Abstract

 Nearly 1000 species of bats occupy an astonishing variety of ecologic niches. They range from solitary to colonial in their habits, from frugivorous to carnivorous in their diets, and from grams to kilograms in adult body weight. These animals have recently emerged as popular exhibition species at zoos while native species are receiving increasing attention in naturalist and conservation programs.

The international transport of bats is regulated by the International Air Traffic Association (IATA) that specifies the shipping containers that must be used. Recently revised, these requirements have become more flexible, but are likely to have additional changes suggested by the Chiropteran TAG and other experts. CDC’s Division of Quarantine has further observations on designs and procedures which make the importation process smoother.

Public health concerns are present in the potential for transmission of rabies and a number of other diseases by bats. Of the non-imported cases of rabies in humans since 1980, all have been determined to be bat-associated strains, rather than strains associated with domestic animals or terrestrial wildlife. The Council of State and Territorial Epidemiologists (CSTE), an influential public health body, has adopted a position statement which discourages having bats in situations where public contact may occur, such as free-flight exhibits. The Advisory Committee on Immunization Practices of the Public Health Service advises that persons in contact with bats be pre-immunized against rabies, and has updated its recommendations on post-exposure prophylaxis to include bat-associated situations which previously were thought not to require intervention. In addition to preventing any direct contact of the general public with bats, consideration should be given to guidelines where personnel contact does occur, such as zoo staff (including volunteers), wildlife rehabilitators, and private veterinarians and their clinical staff.
TUBERCULOSIS IN WHITE-TAILED DEER IN MICHIGAN: A REVIEW OF THREE YEARS (1995-1997) SURVEILLANCE

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Abstract

Mycobacteriosis is an important re-emerging zoonotic disease. The first known occurrence of self-sustaining tuberculosis in free-ranging cervids in North America has been recognized in Michigan since the Fall of 1994. In order to eradicate this disease, a multi-agency task force consisting of personnel from the MI Department of Natural Resources, MI Department of Community Health, MI Department of Agriculture, U.S. Department of Agriculture, and the Animal Health Diagnostic Laboratory was formed. While this task force has a number of ongoing responsibilities, this paper will concentrate on the techniques of surveillance, the results of the wild deer surveillance program over the last 3 yr, and the management strategies employed to control this tuberculosis outbreak.

A single hunter-killed white-tailed deer (Odocoileus virginianus) was identified as having Mycobacterium bovis infection during the Fall hunt of 1994. During the 1995 hunting season, 354 hunter-killed deer were examined for tuberculosis. These examinations consisted of gross and histologic evaluations, as well as Mycobacterial isolation from cranial and thoracic lymph nodes. The estimated prevalence rate from the 1995 survey was 5.0%. Subsequently, a special deer management area was formed around the known positive deer–DMU 452–and the annual hunting season survey was expanded in 1996 to include four counties. In 1996, 3,765 deer were surveyed; with an overall four county prevalence rate of 1.24%, but a prevalence of 2.33% within DMU 452. In 1997, a fifth county, immediately North of the original four, was added into the survey. Approximately 3,700 deer were surveyed in 1997; the overall five county prevalence was 1.97%, a prevalence of 2.25% within the four county area, and a prevalence of 4.44% within DMU 452. To date three coyotes have been found positive for tuberculosis, surveillance of a nearby free-ranging elk herd has been negative, all cattle tested within the five county area have been negative, and one captive deer herd–out of approximately 30 in the five county area–has been positive for tuberculosis (Fig. 1).

Since the prevalence rate has been increasing in the wild deer, and one infected captive deer herd has been identified, the tuberculosis task force will be implementing additional management strategies in 1998. The ongoing surveillance programs will continue for wild deer, wild elk, wild carnivores, captive deer, and domestic cattle in the five county area. The entire five county area will become a single deer management unit; within this unit increased hunting pressure will be utilized to decrease the deer herd density. Additionally, a voluntary ban on supplemental feeding of deer
will be made mandatory in 1998. By decreasing deer numbers, and eliminating the abnormal concentrations of deer at feeding stations, it is believed that the rate of transmission of tuberculosis will dramatically decrease. Hunter education concerning the lesions of tuberculosis, as well as increased field examination of carcasses at highway deer check stations will also be continued.

LITERATURE CITED

AGENTS OF HUMAN EHRlichiosis in white-tailed deer populations

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Abstract

In the last decade, two human ehrlichiosis agents have emerged as public health concerns in the United States: Ehrlichia chaffeensis, which causes human monocytic ehrlichiosis (HME), and an organism closely related or identical to Ehrlichia equi which causes human granulocytic ehrlichiosis (HGE). Both organisms apparently are transmitted among wildlife reservoirs and to humans by ticks. Serologic, PCR-based, and culture-based surveys of white-tailed deer populations have revealed that deer in the southeastern United States commonly harbor E. chaffeensis. Results of PCR tests also show wild deer are infected with the HGE agent and another novel Ehrlichia-like organism which to date has not been reported as a cause of human disease. The presence of multiple Ehrlichia spp. in wildlife reservoirs or tick vectors can present challenges to developing reliable diagnostic tests for epidemiologic surveys. However, in areas where purported tick vectors of both E. chaffeensis and the HGE agent coexist, deer populations may be coinfected with multiple Ehrlichia spp. In a survey of a deer population in coastal Georgia, serology and PCR indicated multiple individual animals had each been exposed to at least three Ehrlichia spp. Such data underscore the importance of white-tailed deer in the natural history of human ehrlichiosis, and indicate deer could serve as a valuable sentinel species for studies on the geographic distribution of human ehrlichiosis agents in the United States.
EMERGENCE OF CHRONIC WASTING DISEASE AS A DISEASE OF CONCERN TO MANAGERS OF CAPTIVE AND FREE-RANGING CERVIDS

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Abstract

Chronic wasting disease (CWD) of cervids has been recognized as a clinical syndrome for approximately 30 yr and as a transmissible spongiform encephalopathy for 20 yr. During most of that time CWD was primarily of interest to local wildlife managers in Colorado and Wyoming and a handful of researchers studying the few animal and human spongiform encephalopathies then known. However, since the identification of bovine spongiform encephalopathy (BSE) in the United Kingdom and especially since BSE was linked to a new variant of human Creutzfeldt-Jacob disease in 1996, considerable attention has been focused on all of the transmissible spongiform encephalopathies, including CWD. These diseases are thought to be caused by abnormal isoforms of normal cellular proteins called “prions.”

Chronic wasting disease is a fatal chronic central nervous system disease of captive and free-ranging mule deer (Odocoileus hemionus), white-tailed deer (Odocoileus virginianus), and Rocky Mountain elk (Cervus elaphus nelsoni). Chronic wasting disease occurs in captive research cervids in southeastern Wyoming and northcentral Colorado, and in ranched elk in two South Dakota facilities and one Nebraska facility. A few cases were diagnosed in zoological gardens and a game farm in the past, but CWD apparently did not persist in these locations. In free-ranging cervids, CWD is only known from limited contiguous areas in eastern Wyoming and Colorado. The recent diagnosis of CWD in commercial elk in South Dakota and Nebraska underscores the importance that veterinarians and owners of facilities housing cervids should be aware of the clinical presentation of this disease, epizootiology, and methods of diagnosis.

Studies of the epizootiology of CWD indicate lateral and possibly maternal transmission; the possibility of environmental contamination by the agent is being studied. Affected animals are older than approximately 18 mo of age, with the majority of cases in the 3- to 6-yr-old age range; however the age of development of clinical disease probably depends on when the animal was exposed and animals 12 yr of age and older have developed CWD. A minimum of about 18 mo incubation appears to be required for development of clinical signs. Onset of signs is subtle, often only recognized initially by those familiar with the individual animal. Early clinical signs include behavioral changes and weight loss. As the disease progresses, animals may develop a variety of signs including polydipsia, polyuria, hypersalivation, difficulties in swallowing, head tremor, ataxia, and hyperexcitability. Not every animal will display all signs; the disease in some elk may be quite
subtle. Aspiration pneumonia often occurs as a terminal event, thus CWD should be considered a differential in cases of pneumonia in prime-aged cervids.

Currently, diagnosis of CWD requires examination of the brain, at a minimum the obex region of the medulla oblongata, by histopathology. Confirmatory tests include immunohistochemistry, Western blotting, and negative stain electron microscopy. Considerable research is currently underway to define the distribution, prevalence, host range, pathogenesis, epizootiology, and approaches to diagnosis of CWD.
MYCOPLASMAL CONJUNCTIVITIS OF WILD FINCHES

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Abstract

 Conjunctivitis in house finches (Carpodacus mexicanus) due to infection with Mycoplasma gallisepticum was first reported in suburban Washington, DC in 1994. Since the initial observations of affected house finches in the mid-Atlantic region, the disease has become widespread and currently has been reported throughout the eastern range of the house finch in the United States and Canada, and by late 1995, mycoplasmal conjunctivitis was confirmed in American goldfinches (Carduelis tristis). House finches and American goldfinches remain the only species in which mycoplasmal conjunctivitis has been confirmed.

In natural and experimental infections, mycoplasmal conjunctivitis is characterized by chronic inflammatory changes in the eye and adnexae as well as the upper respiratory system. Grossly, there may be unilateral or bilateral eyelid swelling with feather loss and serous exudate. Mucoid nasal exudate may accumulate on the bill and face and plug the nares. Microscopically, lymphoplasmacytic inflammation with epithelial and lymphoid hyperplasia is present in the conjunctivae, cornea, nasal turbinates, and rarely the trachea. Ultrastructurally, large numbers of mycoplasmas adhere to mucosal surfaces.

The MG strain associated with finch conjunctivitis grows very slowly in culture and has been difficult to isolate. Molecular characterization of the house finch MG isolates collected from 1994-1996 throughout the range of the disease as well as 2 MG isolates from goldfinches suggested that a single strain of MG appears responsible for the widespread epornitic in wild birds. Furthermore, the isolates appeared genetically dissimilar to MG strains commonly associated with vaccination or clinical disease of domestic poultry. The original source of the MG strain responsible for conjunctivitis in wild finches remains undetermined.

The effect of mycoplasmal conjunctivitis on house finch populations has not been determined. Natural MG infection in captive house finches was highly transmissible and nearly all finches caged with infected birds developed lesions and antibodies. The severity of disease was evident as 25% of the naturally infected birds died or were euthanatized because of debilitation. Field surveys for MG in house finches and other wild passerines in northeastern Georgia in winter 1997-1998 revealed that house finch populations appeared low when compared to 1994-1996. Similar anecdotal information has been received from other areas of the eastern United States. Based on U.S. Fish and Wildlife Service Breeding Bird Surveys from 1966-1996, a declining trend in house
finch populations in several mid-Atlantic states has been detected since mycoplasmal conjunctivitis was first documented in 1994.

In addition to fewer house finches in northeastern Georgia in winter 1997-1998, there was a marked reduction in the prevalence of clinical disease and antibodies in house finches in the region. Gross conjunctivitis was observed in only one of 54 captured birds and only five of 54 were seropositive. Isolates of MG were obtained from the house finch with conjunctivitis and one clinically normal house finch. This is in stark contrast to the previous two winters when the vast majority of house finches captured in northern Georgia were infected with MG.

LITERATURE CITED

UNEXPLAINED NEUROLOGIC DISEASE IN BALD EAGLES IN SOUTHWESTERN ARKANSAS AND AMERICAN COOTS IN ARKANSAS, NORTH CAROLINA, AND GEORGIA

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Abstract

Morbidity and mortality in free-ranging bald eagles (Haliaeetus leucocephalus) and American coots (Fulica americana) due to a neurologic disease of undetermined cause, has occurred on three lakes in southwestern Arkansas, one lake in North Carolina and one lake in Georgia in recent years. A total of 58 eagles in Arkansas and an unknown number of coots at sites in each of the three states have been affected. The only consistent finding in affected birds was a microscopic lesion in the brain and spinal cord described as a vacuolar myelinopathy.1 This disease had not previously been reported in free-ranging wildlife. Extensive diagnostic evaluations, research and field studies have so far been unsuccessful in identifying the cause of these events.

LITERATURE CITED

DO NOT FORGET TO EDUCATE

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Abstract

Veterinarians work towards conserving animal species and environments. However, all the new techniques, computer models, PCR technology, embryo transfer, disease detection, etc. will not result in ecosystem preservation if one basic practice is forgotten: *Educating people about why to conserve.*

Conservation will only work if people understand what it is, why it is important and whose interest it serves. The facts show that on a world level, conservation is losing, mostly due to public ignorance. Wildlife veterinarians must take the education of people: students, the public, and fellow professionals as an integral part of their job. Numerous avenues exist to spread information about, and teach conservation education.

Education becomes even more important in field projects. Projects often take place in areas with limited access to news sources and suffer from information deprivation. Information shared in such areas gets attention. This is where the environmental message is so important. In lesser developed countries societies have the chance to effect economic development while protecting their environment, something the developed world was unable to do. However, these countries and their professionals are in dire need of information to apply this concept.

**Education**

Conservation education can been accomplished in the following ways.

1. School groups: Encourage visits to conservation facilities and being available to give lectures to primary, secondary, high and preparatory schools. It will always be difficult to change ideas, however, it is far easier to guide the creation of ideas. Children have yet to become fixed in their ideas and can be taught the important elements of ecosystem conservation. To state the obvious, the practice of conservation will soon be in their hands.

2. Graduate students: For those who work in universities this is bread and butter, however those of us outside the teaching world can become involved. On the veterinary side we know about preceptors, interns and residents but beyond that, relationships can be sought with local universities to encourage programs of day visits, workshops, lectures and sponsored research by students in other disciplines. In particular, for students in lesser developed countries, chances
for field experiences are few and difficult. While on field projects always locate and encourage local students and professional staff to participate.

3. Professional and fellowship groups: Be available to give lectures and provide information to veterinary and other related field associations about ecosystem health. Professionals in other fields may not be as aware of the issues and your words may invoke forces well beyond your normal circles. Other professional groups can see they have a stake in ecosystem health. Fellowship groups are usually a mixed assortment of professions. Any individual who hears your words can become an ally and carry your message further.

4. Governmental and Non-Governmental organizations: These organizations always desire input from individuals. Providing information and offering guidance to these is education that will provide direction for our society. Every level society has its corresponding Governmental and Non-Governmental organizations, from town zoning committee and neighbor beautification club to institutions at the highest level in capital cities. At any step along the way input from a wildlife veterinarian can have a profound positive effect.

5. Publications: Theses include journals and newspapers, brochures, newsletters and professional journals. We strive to publish in professional journals, but perhaps we are missing some valuable education opportunities. Create your own literature in the style that suits you and find an outlet for it. There are numerous publications, print and electronic, interested in pieces from a high profile profession like wildlife veterinarians. Keep a notebook of interesting articles handy to show interested persons and/or stocks of appropriate brochures for distribution. Good sources of literature are conservation organizations such as World Wildlife Fund (WWF), International Union for Conservation of Nature (IUCN), American Zoo and Aquarium Association (AZA), etc.

6. Media: When being featured in either simple news stories or major documentaries be sure to present a conservation message. High visibility projects on charismatic mega vertebrates generate attention in the public and media. A wildlife veterinarian can use that attention as an alert for conservation of all aspects of an ecosystem.

7. Research: Research is part of all our lives and is a form of education. Defining points and aspects about the world around us increases our knowledge and is education for all.

8. Biologic sample banks: Each time we handle an animal we have the opportunity to collect information, sometimes very unique information. Besides publishing your data there may be other individual or institutions interested in materials. Examples of potential homes are pathology repositories, teaching collections, and museums. With some species, particularly those with Species Survival Plans (SSP), wish lists of samples are published and should be investigated. In other cases word of mouth should be sought. Resources may be required to store and find homes for samples but we need to learn as much from each animal contact as possible.
9. Library materials: To increase the availability of information a useful occupation is finding, recommending, and/or donating relevant materials to libraries. This can be pursued at many levels: public, school or university; print or electronic; nationally or internationally. In particular, international donations of appropriate and timely materials to libraries in lesser developed countries provide unique access of information to a multitude of individuals.

10. One on one: Education can be on a personal and every day level. By ensuring that all the contacts throughout your daily routine understand what you are doing and why you are doing it you will receive greater cooperation and action. It is not necessary to always be professorial, but taking a few moments to teach can yield fine rewards. This includes teaching to everyone, from the test tube cleaner to the director of the institute.

Education is the cornerstone of environmental salvation. This is a responsibility of all wildlife veterinarians. It will require resources and time, however the result will be assistance toward our goal of healthy ecosystems. People can see the beauty of animals in national parks and zoos and they see what is beautiful. However it is up to us, conservation and veterinary staff to help people acquire an understanding of why it is beautiful. Good ecosystem health will never be achieved without education.

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LITERATURE CITED

A NEW WILDLIFE HEALTH PROGRAM FOR NEW ZEALAND

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Abstract

Many species of New Zealand’s endemic wildlife are threatened with extinction. Captive management and intensive in-situ management are being used to help reverse the decline of ecosystems and the critically endangered species they contain. Disease could have a huge effect on these vulnerable populations. The New Zealand Department of Conservation (DOC) has taken a proactive approach on the issue of wildlife health. A new wildlife health program aimed at disease prevention in wild and captive native fauna is being developed. The program includes the development of a new wildlife pathology database, the production of disease investigation protocols and health guidelines for DOC staff, the provision of technical advice, the production of guidelines for wildlife health research and for the collection of biomedical data.

Introduction

There has been considerable discussion in recent years concerning the need to evaluate the effect of disease on the reintroduction, repatriation and translocation of species of high conservation value.1

There have been very few detailed reviews of the disease status in New Zealand’s wildlife2 and the collection of baseline biomedical data has been limited.3

There have however, been discussion papers on the effect of diseases on breeding and translocations4 and there have been general suggestions on disease avoidance in the conservation of threatened species in New Zealand.5 These considerations have also been discussed elsewhere.6,7

This paper discusses the development of a new wildlife health program for New Zealand.

Background

New Zealand has a unique flora and fauna. There are over 70 endemic species of birds, two species of bats, two species of pinnipeds, approximately 75 endemic species of reptiles and five endemic species of frogs. One thousand years of human settlement has had a severe impact on New Zealand’s biodiversity.8 More than 50 species have become extinct in this time and several hundred more are presently at risk from extinction. Included as causal factors are the introduction of mammalian predators including the feral cat, mustelids and three species of rat; habitat destruction,
degradation and fragmentation and competition from introduced herbivores such as the brush-tailed possum (*Trichosurus vulpecula*).

Listed amongst the endangered are several species whose total population number less than 200. These include the kakapo (*Strigops habroptilus*, a large, flightless, nocturnal parrot), the takahe (*Porphyria mantelli*, a large flightless gallinule), the Okarito brown kiwi (*Apteryx australis*) and the black stilt, (*Haemotopos novaeezelandiae*). Several other species are also critically endangered.

Conservation techniques used in New Zealand include the development of island reserves that are free from introduced species, mainland habitat restoration and captive management. The restoration of off-shore islands has meant that several species are now only found there.

Many threatened species are now managed through recovery programs that have been developed by conservation managers and other interested groups. These programs involve intensive management of the wild population. In addition, several of the programs require a captive management component to help restore populations.

Translocating animals from the wild to establish new populations and the reintroduction of animals from captivity into the wild are all very important aspects of the intensive conservation management.

There is a clear risk of disease becoming an important factor in the further decline of threatened species as they become more intensively managed. Potentially many pathogens can be transferred with the individuals thus introducing further challenges to possibly disease-naive populations. Increases in the population density can, in turn, add to environmental stresses that may increase susceptibility to disease.

There have been a few incidences within New Zealand where disease has played an impact on the captive and wild populations of endangered species. Avian pox and malaria were found in a captive population of the endangered New Zealand dotterel (*Chadrius obscurus*), which meant that they could not be released into the wild. Several critically endangered black robin (*Petroica traversi*) developed avian pox which contributed to the deaths of five to six fledglings in 1985. Erythroblastosis, the avian leucosis complex, was diagnosed in Antipodes Island parakeets (*Cyanoramphus unicolor*), which had come into direct contact with New Zealand parakeets.

### New Zealand’s Wildlife Health Program

The idea of a veterinarian to provide advice to the New Zealand statutory body responsible for conservation was first suggested in 1986. Recent wildlife health issues within the Department of Conservation (DOC), as it is now called, highlighted the need for a more proactive approach to health and disease management. Dr Milton Friend from the National Wildlife Health Research Center in Madison, Wisconsin USA, visited New Zealand in 1993 to review the threat of disease to threatened species management in New Zealand.
Following the visit, a working group comprising veterinarians and DOC staff defined the health management requirements. This resulted in a document: *Guidelines for the Health Management of Species under the Wildlife Act*. These guidelines are managed by a DOC scientist and a veterinarian employed on contract to the DOC and based at New Zealand’s veterinary school in Palmerston North. The author was employed to develop a health program for New Zealand’s native wildlife.

The new wildlife health program covers many areas. The tasks can be grouped under the following headings.

1. **Defining Wildlife Health Standards**
2. **Information Management**
3. **Technical Advice and Information Dissemination**
4. **Coordination of Resources**

**Defining Wildlife Health Standards**

As part of this new position, it has been most important to set and maintain standards relating to the health management of wildlife both in the wild and in captivity. The guidelines are used by DOC staff to improve and standardize the health management of threatened species.

Initially, generic health management guidelines were produced for captive facilities and island reserves which included health monitoring programs for wildlife, recommendations on the movement of animals and personnel and on quarantine and hygiene standards.

Health Management Plans have also been produced for particular species (e.g., kakapo and takahe). The plans include health monitoring, biomedical data collection, hygiene, translocation, necropsy and common diseases and treatments.

Standard protocols have also been produced for necropsy procedures, clinical pathology and baseline health data collection and health monitoring procedures for birds and reptiles.

Risk analysis procedures have been outlined for wildlife translocations and developed initially for the introduction of exotic disease into native psittacines.

**Information Management**

A new computerized database of wildlife health and disease has been developed in MS Access, which evaluates information on mortality, morbidity and biomedical health data. The New Zealand Wildlife Mortality database incorporates retrospective and prospective wildlife health and disease data from all diagnostic laboratories, conservancies and captive institutes. The information can be used to further enhance health management strategies and guidelines and act as a disease surveillance and monitoring tool. One aim was to develop a database structure that is compatible with other regional and global wildlife disease database systems and which allows the sharing of
relevant and useful information. The structure has been based on the Canadian Cooperative Wildlife Health Center database.

In order to gather useful information for data input it has been necessary to develop new submission forms and necropsy forms. These have been distributed to all potential submitters.

All tissues and wax blocks are also catalogued for retrospective analysis in combination with the New Zealand Registry for Animal Pathology.

A bibliography of native and exotic diseases has been catalogued for reference and future planning.

**Technical Advice and Information Dissemination**

A major part of the work involves giving technical advice to veterinarians, DOC conservation staff, the National Kakapo Team on managing individual cases, and disease outbreaks, nutrition, quarantine and translocation procedures. In some instances, there is a need to be involved directly in field investigation.

Further advice is given on native wildlife submissions to the Veterinary school, which includes the interpretation of results and recommendations for management. Offering advice and facilitating wildlife health research is another aspect to the position. DOC staff are also trained in wildlife health procedures including necropsy procedures and health data collection.

All information and data gathered from a variety of sources is disseminated to wildlife managers and veterinarians in order to enhance the management of the threatened species. This is achieved through scientific publication, other publications and direct contact.

**Coordination of Resources**

Criteria have also been developed for the best use of the National Wildlife Surveillance fund. This fund is offered to all holders of native wildlife and wildlife managers on a prioritized basis. The aim is to ensure that the costs involved in diagnosing the cause of mortality is not hampered by lack of funds when this information would be very useful.

In order to gather as much useful information for the Mortality database as possible, it has been necessary to coordinate information from all the diagnostic laboratories and all personnel holding wildlife.

The conservancies and recovery groups are given advice on how to budget for health management annually.

A national network and database of veterinarians has been produced to encourage participation in recovery groups, and assistance with ex situ and in situ wildlife conservation programs.
Liaising with outside agencies including the Ministry of Agriculture veterinarians, non government conservation organizations, zoos and other universities is required to facilitate the achievement of wildlife health tasks as outlined above.

**Future Developments**

As wildlife health management develops in New Zealand, there will be opportunities to employ further staff to expand on the developments and to undertake postgraduate health research. Training videos will be produced for necropsy procedures and a manual will be developed which will bring all existing guidelines and procedures together.

A New Zealand Wildlife Health Centre that will bring together rehabilitators, oil spill response personnel, clinicians, pathologists, ecologists, nutritionists and epidemiologists is under development.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**

POSTGRADUATE EDUCATION IN ZOO AND WILDLIFE VETERINARY SCIENCE THE MASTER OF SCIENCE COURSE IN WILD ANIMAL HEALTH AT LONDON

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Abstract

There has been a growth in the number of veterinarians involved in the veterinary care and welfare of both captive and free-living wild animals. The opportunities for postgraduate veterinary education in the science and medicine of wild animals is limited and there is a need to produce more veterinarians with this specialist knowledge. The Master of Science Course in Wild Animal Health jointly run by the Institute of Zoology (Zoological Society of London) and the Royal Veterinary College (University of London) was established in 1994 to meet this requirement. The participants are given tuition through lectures, tutorials and demonstrations, and gain practical experience in the field of wild animal medicine. Participants are assessed on examination papers, course work, a research project and an oral examination. Twenty-nine veterinarians from 18 countries in six continents have graduated from the course of which 18 have already found posts working with free-living or captive wildlife.

Introduction

There has, over the last 30 yr, been an enormous increase in our knowledge of wild animal disease 2 and a growth in the number of veterinarians involved in the veterinary care and welfare of both captive and free-living wild animals. During this period, interventions for reasons of health, welfare and the conservation of free-living wild animals have been undertaken with increasing frequency. 1,7,9,10 Such actions require specialist veterinary input, for example, in assessing and controlling the risk of accidental disease introduction to wildlife, domestic animal and human populations associated with wildlife translocations. In addition, a wide range of wild animals is now kept in captivity: in zoos for conservation and exhibition, in laboratories for research purposes and as pets.

Tuition in the veterinary care of wild animals receives relatively little attention in the already crowded undergraduate veterinary curriculum. Consequently, there is a growing need for veterinarians (and related scientists) with specialist knowledge of the management of wildlife and the control of diseases of both free-living and captive wild animals. 8 Hatt and others 3 have shown that opportunities for postgraduate education in wild animal health throughout the world are limited. Degree courses in wild animal health of which the authors are aware include a Masters Course in
Wildlife Diseases offered by the University of Pretoria, Republic of South Africa, Faculty of Veterinary Science; a Master of Science Course in Wildlife Medicine at the University of Sydney, Australia and a Master of Science Course in Wildlife Medicine at the University of Melbourne, Australia.

The Institute of Zoology, Zoological Society of London and the Royal Veterinary College, University of London collaborated for a number of years to provide undergraduate teaching in the field of wild animal health and since 1991 have run an elective course in zoo and wildlife medicine for final year BVetMed students. In addition, the Institute of Zoology provided a 6-mo postgraduate course, to which the Royal Veterinary College contributed, in wildlife husbandry and disease in 1990 and 1991.

The first Master’s Course in Wild Animal Health commenced in October 1994 with twelve participants from six different countries attending. In the first 3 yr of the course, 29 veterinarians representing 18 countries from six continents have graduated. A further fourteen participants are attending the 1997/98 course, of which six individuals are from previously unrepresented countries.

The Master of Science Course in Wild Animal Health

Entry requirements. The course is primarily for qualified veterinarians. However, provision is made to be able to accept biology graduates with appropriate experience and qualifications under some circumstances (but such students are unable to undertake certain veterinary procedures on the course). Proficiency in spoken and written English is essential.

Inquiries and applications for the course. From the first announcement of the course until October 1997, 572 inquiries from 59 countries have been received about the course. 136 applications have been received for the first three courses and a total of 100 offers made for places. The most common reason for non-attendance on the course for those offered a place has been insufficient funding.

Structure of the course. The course, which lasts 12 mo, comprises 1) a taught component, occupying three academic terms leading to examinations in June, and 2) an individual research project, carried out during July, August and September, leading to the final assessment. The taught component of the course is divided into six modules and consists of lectures, tutorials, demonstrations, practical work and site visits (October-May). Tuition is provided by speakers from the Institute of Zoology, the Royal Veterinary College and numerous other zoological and veterinary centres, drawing upon expertise from both within and outside Europe. Module A is the Foundation Course in Wild Animal Health and includes, population biology, conservation genetics, the impact of diseases on populations, diversity in anatomy and physiology, management of wild animals, artificial reproductive techniques, welfare of captive and free-living wildlife, organization of zoo programs for ex-situ conservation, nutrition, legal and ethical aspects and key issues in sustainable utilization. Module B includes teaching on epidemiology, statistics and information systems. Non-infectious diseases are the subject of Module C including nutritional diseases and toxicities. Module
D is titled Infectious Diseases and Disease Investigation and Module E contains tuition in Therapeutics, Preventive Medicine and Imaging. Restraint (including Anaesthesia) and Aspects of Reptilian and Avian Surgery are the subjects in Module F.

Participants are encouraged to make an assessment of the Course by scoring each lecture, tutorial, demonstration or practical for scientific content and presentation and space is available on the assessment form for additional comments where necessary.

**Practical experience.** Participants are expected to acquire practical experience in duty groups at the Zoological Society of London’s collections, Regents Park (London Zoo) and Whipsnade Wild Animal Park. On two days each week, the participants attend clinical and post-mortem cases with the Society’s veterinarians. At other times there are opportunities to keep up to date with clinical cases. To broaden the range of practical experience offered, visits are arranged to other institutions, for example, to the RSPCA wildlife hospital in Norfolk, for the handling of pinnipeds. The course also includes training in the use of firearms and remote injection techniques.

**In-course assessment.** Each participant undertakes four assignments during the taught component of the course in which a subject area related to the particular module in progress must be thoroughly researched. Examples of assignments that have been undertaken include: the population dynamics of the Sumatran tiger (*Panthera tigris sumatrae*) (Christopher Dutton 1994), mycotoxicosis in free-living wildlife (Karina Wrigley 1995) and chemical restraint and surgical anaesthesia of amphibians (Jean-Michel Hatt 1996). Each participant gives an oral presentation to the rest of the class and a Course Director, and provides a two page written summary.

The participants are required to submit a casebook of four clinical and/or pathologic cases with which they have personally being involved. Cases may involve aspects of free-living or captive wildlife and describe population, group or individual animal problems.

**Research projects.** Each student is required to undertake an individual research project, of a practical nature, on an approved aspect of wild animal health, and to submit a typewritten report not exceeding 10,000 words. Approximately 12 wk during the summer is allocated to project work but the majority are planned during the early months of the course. Examples of projects that have been undertaken include: “An Attempt to Characterize a Poxvirus from Nile Crocodiles (*Crocodylus niloticus*)” (Norman Mukarati 1995), “An Investigation of the Interspecies Variation in Pharmacokinetic Parameters of Enrofloxacin (Baytril) in Relation to Bodyweight” (Catherine Brown 1996), and “A Histopathological Study of the Possible Effects of Mercury Contamination in the Liver of Harbour Porpoises (*Phocoena phocoena*) from British Waters” (Julie Barnes 1995). Some projects have already been published4,5,6,11 and it is expected that further publications based on the MSc projects will appear in the near future.

**Examinations.** Participants are assessed on their performance in two 3-hr and one 2-hr written examination papers, course work, the project report and an oral examination.
Careers of Graduates following Completion of the Course

As expected, given the depth of the syllabus and the diverse job opportunities in the wild animal health field, the interests and careers of those who have graduated from the course have varied. Of the 29 veterinarians who have graduated from the course to date, seven have found work with free-living wild animals, seven have gained posts working with captive wild animals, four with both free-living and captive animals and a further three primarily with domestic animals but with some free-living or captive animal involvement. Of the 29 graduates, six are working in government posts, three in zoological collections, seven in university posts and two in rehabilitation centres.

Conclusions

Three Master of Science in Wild Animal Health courses have been completed and a further year is in progress. The level of interest in the course and the success that graduates have shown in finding posts in the veterinary care and medicine of captive and free-living wildlife suggests that the course has fulfilled a need for postgraduate education in wild animal health.

ACKNOWLEDGMENTS

We would like to thank the many people who have contributed to the course: members of staff at the Institute of Zoology and the Royal Veterinary College who have assisted with planning, organization and teaching; the many external lecturers for dedicated tuition; the external examiners J Baker, JE Cooper and IF Keymer, and the course participants.

LITERATURE CITED

A PSITTACOSIS OUTBREAK IN COSTA RICA ASSOCIATED WITH PET BIRDS IMPORTED FROM THE UNITED STATES

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Abstract

The importation of pet birds into the United States has been regulated to insure that exotic avian diseases do not find their way into the poultry industry or human populations. However, evidence exists suggesting that birds infected with chlamydia psittaci are being exported from the United States to Costa Rica. The association between imported birds, an outbreak of psittacosis specifically affecting a large collection of psittacines in Costa Rica, and the potential impact the importation of disease can have on endangered wild birds will be discussed.

Psittacosis (Chlamydia psittaci) has not been documented in Costa Rica’s wild nor captive native bird populations, nor in random testing of psittacines in Peru. The prevalence of this zoonotic disease among pet bird populations in the United States, as randomly detected using indirect fluorescent antibody serology (IFA) is 5-6% (C. Cray, personal communication), and has been reported to be as high as 70% in selected U.S. populations, utilizing other detection methods cited in this study. Because of the complex biology of these obligate intracellular bacteria, detecting exposure and defining infection can be challenging. As well as IFA, diagnostic methods used in this study include immunoassays (ELISA), direct compliment fixation (DCF), elementary body agglutination (EBA) and gene based diagnostics such as polymerase chain reaction (PCR) technology.

Pet bird ownership is common in Latin America. Although the traditional source is poaching, pet bird importation is not uncommon, and many non-native species are noted throughout Costa Rica. In a separate study of captive birds in Costa Rica, 78 of 261 birds examined (30%) were non-native species, and of those, 57 were old world species. In 1995 a flock of over 50 psittacines recently imported from the United States were found to be clinically ill. Subsequently five of 26 survivors tested positive for psittacosis (IFA and/or ELISA).

More recently, more birds imported from the United States have been implicated in an outbreak of psittacosis among a collection of 132 psittacines, including 94 macaws which are in a captive breeding-for-release program. As a result, 66 scarlet macaws (Ara macao) and 28 great green macaws (Ara ambigua) have been placed at risk. Both species are considered highly endangered (CITES I), and remaining wild populations are estimated at fewer than 400 scarlets and perhaps as few as 40 pairs of green macaws in all of Costa Rica. In November 1995, all birds in the collection had been examined and were seronegative for psittacosis (IFA). A protocol for new acquisitions was established, including quarantine and chlamydia testing. Unfortunately, in June of 1996 after only
a brief quarantine, a scarlet macaw was moved directly into the main breeding facility, which consists of two large open-air aviaries one containing 32 scarlets, the other 26 green macaws. Hatched in Costa Rica from native parents this scarlet originated from a facility which frequently imported psittacines from the United States. Many of the birds at that facility were free-flying, no quarantine protocol was followed; native birds were housed with imports. In August 1996 that facility provided a second scarlet with the same background. The bird entered quarantine, and was subsequently found to be seropositive (IFA) for psittacosis. Further testing revealed that the first acquisition was also positive (IFA), as now were its cage-mate and other birds in the aviary, some of which were clinically ill. The entire facility was quarantined and all birds were treated for psittacosis with a diet containing 1% chlortetracycline for 45 days. An extensive environmental cleanup operation was also carried out. Additional tests (EBA, DCF and IFA) indicated that further exposure had occurred.

IFA may detect humoral antibodies for up to 7 mo after an infected bird has been successfully treated for psittacosis (C. Cray, personal communication). Eleven months after the chlortetracycline treatment was completed, birds were re-tested. Evidence of exposure and/or infection had increased in the main breeding aviaries. Newly positive birds were identified throughout other areas of the project, young birds being particularly affected.

A second attempt to eradicate the disease was begun. Despite the expense and risks, a regime of doxycycline injections was selected. Infection does not provide effective immunity and failure to eliminate the organism from the environment is a common source of re-infection. Thus, another extensive environmental clean-up took place. It may be impossible to eradicate psittacosis from a flock of this size (A.M. Fudge, personal communication). C. psittaci in fecal material persists in the environment and is easily aerosolized. Birds sharing the same airspace as infected birds are considered exposed (A.M. Fudge, personal communication). The flock from which the first two seropositive scarlets originated is still free-flying in a remote area of Costa Rica near private reserves and a national park, possibly posing a direct threat to wild psittacines.

Largely because of economic risks to the poultry industry, the U.S. Department of Agriculture requires quarantine, exotic disease testing and prophylactic treatment with chlortetracycline of psittacine birds imported from Latin America into the United States. Interestingly however, none of these are required by agencies in either government to import birds into Costa Rica. Tourism is Costa Rica’s leading source of foreign currency and relies heavily on a reputation of abundant flora and fauna. The presence of free flying psittacines is one of the attractions most frequently cited by tourists visiting Costa Rica. Because both scarlet and green macaws are large, colorful, raucous birds they are particularly appealing to tourists. Considering tourist expenditures to see macaws, Vaughan estimated that a free flying macaw in Carara National park, Costa Rica, may earn as much as $20,000.00 per year as an attraction. Similarly, Munn concluded that a macaw in Peru can generate up to $4,700.00 each year. By either estimate, psittacine associated ecotourism is big business. As traditional habitat destruction and poaching continue to represent a significant threat to populations of wildlife, it is important to prevent the possibility of further extinction pressure in the form of an imported disease, such as psittacosis.
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LITERATURE CITED

6. Convention in International Trade in Endangered Species
CONTROL OF RABIES IN FRANCE: EVALUATION OF DIFFERENT PROGRAMS, THEIR COST AND EFFICIENCY

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Abstract

Fox rabies established itself in France in 1968. Since 1989, the incidence of the disease has dramatically declined. This decline is a direct result of fox vaccination campaigns using vaccine baits distributed by helicopter over the infected zones.

However following this campaigns we observed a significant increase in fox populations and there is a common opinion of game keepers and country inhabitants that fox density are higher than before the rabies outbreak. As a result, we observed a decrease in the number of foxes marked with tetracycline (a biologic marker incorporated in baits) and in the percentage of immunized foxes. Furthermore, we had to tackle several residual foci, mainly near the borders with Belgium and Germany.

Program Implementations

This new epidemiologic problem required us to improve the efficacy of vaccination campaigns while studying their cost-effectiveness.

The first steps were to improve the quality system of the whole process:

1. We carefully planned each helicopter flight by mapping the areas indicating precise densities of baits that had to be evenly distributed. After every flight, the flight parameters were checked and recorded.
2. We titrated vaccine baits before and after dropping. Previous studies had demonstrated the paramount importance of vaccine titre and its stability in the fields.
3. Field trials were organized over large areas for testing the effect of several parameters. We examined the increased density of baits in relation with fox density index. We examined the effectiveness of distribution campaigns at various seasons. Two distributions (one during spring, one during autumn); three distributions (spring-summer and autumn); two distributions (spring and autumn) with a double distribution during the same season as a short delayed booster. We examined distribution system by helicopter only, or combined with a hand distribution at the entrances of fox dens.
The results obtained on the percentage of foxes marked with tetracycline, and of immunized adult and cub foxes, were compared between large adjacent areas either treated according to the new, or the classic vaccination campaign protocols.

Cost effective protocols are proposed in the context of emergency or difficult situations, these being a new rabies outbreak occurring in an area densely populated with foxes, or remnant foci in endemic areas.
ISOLATION OF A NOVEL VIRUS DURING AN INVESTIGATION OF A MASS MORTALITY OF COARSE FISH IN THE ERNE RIVER SYSTEM, IRELAND

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Abstract

A novel virus-like particle was isolated from wild coarse fish during an investigation of mass mortalities of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) in the Erne river catchment, Ireland. The culture and electron microscopic characteristics of this isolate appear to be different from those of viruses previously isolated from fish in Ireland and also from those of viruses isolated elsewhere from perch or roach.

Introduction

About 75% of the earth’s surface is covered in water. Only 3% of this is fresh water, the remainder is in the marine environment. Three quarters of the fresh water is found in the frozen form at the earth’s poles and in glaciers. The remainder constitutes our rivers and lakes.

Although only a small part of the planet’s total water, the rivers and lakes are a vital constituent of the circulatory system in the purifying water cycle; from sea to cloud to land to sea. Fresh water rivers and lakes are an important natural resource and provide us with an essential fresh water supply network throughout major land masses. The vital position of rivers and lakes within a major natural cycle makes the health of these freshwater bodies of paramount importance. Knowledge about health factors in these ecosystems may also provide us with key indicators of general environmental health which stretch beyond the geographic boundaries of lake shores and river banks.

Anyone who has set out to investigate disease problems in rivers or lakes soon comes to realize the vastness of this environment, despite it representing such a small percentage of the earth’s waters. Epidemiologic disease information is generally more readily available on land dwelling animals and more basic work is required for many species of fish.

In recent years veterinary investigations of wild fish diseases have been carried out to enable the development of a National Fish Health Management Plan with a view to assisting the rational and sustainable use of Ireland’s natural fisheries resources. As with any endeavor where background information is scarce, the initial findings often raise more questions than they provide answers.

Ireland has approximately 140,000 hectares of fresh water lakes and 14,000 kilometers of main channel rivers. There are strong recreational fisheries interests in these fresh waters. From a wildlife vet’s point of view this proves a major benefit. Fish inhabit an environment where it is difficult to
oversee their well-being and they would often go unnoticed but for anglers. It is common for wildfish “disease” problems to be first noticed and brought to the forefront by anglers. Such problems include mass mortalities, decreased availability of fish, skin diseases noticed on caught fish, odd shaped fish or fish behaving strangely.

Report

Investigations of wildfish diseases, in common with any wildlife disease investigation, generally lead to the identification of a number of disease agents. Some of these may be intimately associated with the problem under investigation, some may be “normal background” and others may be unknown entities requiring further study. This communication reports preliminary findings of the isolation by culture and the detection by electron microscopy of a novel virus-like particle from wild coarse fish during an investigation of mass mortalities of perch and roach in the Erne river catchment, Ireland. The complete findings from this investigation are currently being analyzed.

The River Erne catchment covers approximately 4340 square kilometers. It is the fourth largest river in Ireland. The catchment covers areas in both the Republic of Ireland and Northern Ireland. The river rises in Beaghy Lough in County Cavan in the Republic of Ireland. It flows 64 miles through Loughs Gowna and Oughter and Upper and Lower Lough Erne before entering the sea at Ballyshannon, County Donegal. For 30 miles between Crossdoney in County Cavan to the town of Enniskillen, County Fermanagh it is difficult to distinguish the river as it winds its way through hundreds of interconnected loughs nestling among the drumlin hills of Counties Cavan and Fermanagh.

Many of the waters in the Erne catchment are used for sport fishing. Sport fishing is of economic importance to this predominantly rural area since it attracts tourists who use local facilities. Many tourists visit Ireland primarily to fish. Sport fishing consists of game or coarse fishing. The vast majority of fishing sites within the Erne catchment are for coarse fish (bream Abramis abrama, roach Rutilus rutilus, perch Perca fluviatilis, pike Esox lucius and tench Tinca tinca) but game fish (salmon Salmo salar and trout Salmo trutta) are also a relatively popular angler’s quarry on the river system.

A major incident of coarse fish mortalities had occurred in the lakes of the Erne. Initial reports were of tens of thousands of dead and dying fish fry in Loughs Gowna and Oughter in the summer months. Perch were the predominant species affected in Lough Gowna but the majority of later reports involved both perch and roach. Later again, smaller numbers of perch up to 5-yr-old were reported dead or dying. By mid-August, 14 other lakes within the Erne system were experiencing similar problems. By the end of August it was estimated that over one million fish had died (A. Ni Shuilleabhain, Northern Regional Fisheries Board, personal communication).

Samples of perch and roach fry from Loughs Gowna and Oughter and Annaghmakerrig Lake were submitted to virology testing procedures similar to those laid down under EC Decision 92/532/EEC.4

Inoculated cultures were incubated at 15°C and 22°C for seven days and inspected daily for the presence of cytopathogenic effect (CPE) at 40x magnification. Flasks and trays with no CPE after
1 wk were subcultivated using the following procedures. Aliquots of medium from all wells constituting the primary culture were pooled following one freeze-thaw cycle of the cultures and 0.1ml of this inoculated at 1:1 and 1:100 dilutions. These were then incubated and examined as for the primary cultures.

CPE consisting of a slow thinning of cell monolayers was noted initially from perch tissues on bluegill fry (BF-2) cell lines at 15°C after seven days. CPE also developed from perch tissues on the chinook salmon embryo (CHSE) and epithelioma papulosum cyprini (EPC) cell lines and from roach tissues on BF-2 cell lines after serial passage. The CPE resultant from inoculae of tissues from both perch and roach occurred earlier (within three days in some instances) after serial passage. Overall however, despite attempts to improve methodology, the extent of CPE in repeat wells and flasks and between species was somewhat variable suggesting culture conditions may not be ideal.

The CPE was not affected by neutralizing antibodies against IPN (specific divalent antiserum to reference strains Sp and Ab), infectious hematopoietic necrosis (IHN) (group specific antibody) or viral hemorrhagic septicaemia (VHS) (group specific antibody).

Suspensions for electron microscopic (EM) examinations were from cultures in which CPE had developed. These were put through a freeze-thaw cycle and centrifuged at 3,000 rpm for 20 min to sediment tissue culture cells and debris. The resultant supernatant was ultracentrifuged at 60,000 rpm for 2 hr. The sediment was stained with 1% phosphotungstic acid, ph 6.5 for 1 min and examined with a Philips Model EM 201 at 30,000x magnification.

Attempts to identify viral particles by EM examination proved negative initially suggesting that viral titres may be low, again possibly due to the lack of ideal culture conditions. It has not been possible to achieve sufficient titers from cultures of roach tissues to detect virus by EM. However, icosahedral particles approximately 70nm in diameter with an electron dense core and with an apparent less dense capsid were found in tissue cultures of samples of perch tissues.

Discussion

Although, worldwide, there are numerous diseases in fish that are associated with viruses, there are reports of only three viruses, capable of causing fish disease, which have been cultured in Ireland. These are infectious pancreatic necrosis (IPN) virus, pike fry rhabdovirus and salmon pancreas disease virus (SPDV).

The described culture and EM characteristics of this isolate appear to be different from those of viruses previously isolated from fish in Ireland and also from those of viruses isolated elsewhere from perch or roach. The relationship of this isolate to other known viruses deserves further study.

No viruses were isolated during investigations of diseases affecting coarse fish in England, Scotland and Ireland during the 1960’s and 1970’s, despite attempts by some investigators. More recently, the effects of pollution have been attributed as the principal cause of wild fish deaths in Ireland.
There is insufficient information at present to determine the role of this isolate in the fish deaths in the Erne and further work is necessary in regard to this area.

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LITERATURE CITED

COMPLIANCE WITH DRUG ENFORCEMENT ACT, FOOD AND DRUG ADMINISTRATION AND THE ANIMAL WELFARE ACT: A CHALLENGE FOR NATURAL RESOURCE AGENCY VETERINARIANS

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Abstract

Historically, veterinary involvement in wildlife conservation began out of concerns over animal welfare, human safety, and restricted access to immobilizing drugs. There are three specific federal laws in the United States that require veterinary interaction with or supervision of biologists engaged in conservation work. These are, the supply of scheduled substances for anesthesia under the Controlled Substances Act; enforced by the Drug Enforcement Administration (DEA); the “extra label” use of most prescription drugs in most wildlife species under the Animal Medical Drug Use and Control Act (AMDUCA); enforced by the Food and Drug Administration and the welfare and handling of some wildlife, which falls under the Animal Welfare Act (AWA); enforced by the U.S. Department of Agriculture. Fortunately or unfortunately, wildlife are of peripheral interest to each of these agencies or laws, and the responsibilities of the wildlife veterinarian are often unclear. In general, the agencies enforcing these laws are not extremely helpful or responsive to wildlife veterinarians asking for advice on how to comply with the law. Thus, these laws are a two edged sword, they encourage the involvement of wildlife veterinarians, but they leave those veterinarians without clear procedures to follow or direction as to how to help their agency comply with the law.

In general, the government wildlife veterinarian is seldom able to be present to directly supervise all wildlife immobilizations, drug administrations, and research projects involving live animals, but may be held personally and professionally responsible for accidents or violations of the law. Biologists and the administrators of wildlife agencies want the veterinarian to facilitate their work, but not to complicate their activities. Thus, there is a natural tendency for tension to develop between the veterinarian and the wildlife biologist/agency. Dealing appropriately with this tension is a major challenge for wildlife veterinarians, both in the United States and worldwide.

On the positive side, the lack of standard operating procedures for drug use and dispensation and animal welfare allows the wildlife veterinarian the opportunity to develop those policies and procedures. We have developed a set of draft policies to allow wildlife veterinarians employed by government agencies to meet the requirements of the law in these three areas, hopefully to both improve professionalism and facilitate the legitimate work of wildlife agencies, while minimizing the risk of accidents and/or personal or legal censure. In brief, drugs are obtained by the veterinarian for the wildlife agency and dispensed through an organized system to biologists. Biologists must
record each use of drugs, report regularly to the veterinarian, and all drugs must be accounted for. Wildlife projects that may fall under the AWA are reviewed by an agency institutional animal care and use committee, similar to those used by universities. Regular formal training in animal capture, legal considerations, pharmacology, emergency procedures, animal welfare and care are offered. Although the length and scope of these draft policies greatly exceed what can be presented in these proceedings, they are available for review and comment on an Internet web page at www.absc.usgs.gov/research/vet. Organized bodies of wildlife veterinarians should consider endorsing these draft policies in concept and/or developing their own draft policies. We must hang together or we may hang separately.
CUTANEOUS CHYTRIDIOMYCOSIS IN AMPHIBIANS: AN EMERGING DISEASE?

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Abstract

Fungi in the Phylum Chytridiomycota (chytrids) are ubiquitous microscopic organisms that reproduce asexually by means of motile, uniflagellate spores (zoospores). Many chytrids are saprobes in aquatic and terrestrial habitats. Other species are obligate or facultative parasites of other fungi, algae, vascular plants or invertebrate animals but none has previously been recognized as a parasite or a pathogen of vertebrate animals.1,2,6,7

From September 1996 through October 1997, the deaths of 24 juvenile blue poison arrow frogs (Dendrobates azureus), 4 juvenile green-and-black poison arrow frogs (Dendrobates auratus), 3 aged adult White’s tree frogs (Litoria caerulea), and an adult ornate horned frog (Ceratophrys ornata) at the National Zoological Park (NZP) were associated with cutaneous infections by chytrids. Affected frogs were housed in close proximity in NZP’s Reptile House. Anorexia and lethargy were noted in two blue poison arrow frogs 1 day before their deaths. Ventral erythema with multifocal patches of soft, brown shedding skin was seen in one White’s tree frog the day prior to its being found dead. However, most frogs died without prior clinical signs of disease. At gross necropsy, the skin along the ventral aspects of the body of many of the frogs was granular and discolored brown. Histologically, cutaneous lesions were most prominent over the ventral abdomen, pelvis, and hind legs and were characterized by epidermal hyperplasia and hyperkeratosis associated with numerous, 10-30 μm chytrid thalli within the superficial epidermis. Multifocal epidermal degeneration with minimal to mild inflammation also was often present. The cause of death in affected frogs was attributed to disruption of normal cutaneous function, which in amphibians may include water absorption, osmoregulation, respiration, and electrolyte maintenance.3

Three forms of the chytrid thalli were identified in the skin by light microscopy: uninucleated forms with homogeneous basophilic cytoplasm, multinucleated forms containing lacy to microvesiculated cytoplasm, and thick-walled sporangia containing multiple 2-3 μm round spores. Sporangia usually had single tubular extensions (discharge papillae) directed toward the skin surface. Histologic special stains revealed that all forms of the fungus were periodic acid-Schiff (PAS) positive and stained with Gomori’s methenamine silver (GMS). They did not stain with Gridley’s fungal stain and were not acid-fast. The spores were gram-positive and stained weakly with Giemsa. Transmission electron microscopy demonstrated that spores had flagella and that these zoospores had features characteristic of chytrids, including kinetosome props, a terminal plate in the axoneme core, and mitochondria with plate-like cristae. Other ultrastructural features, such as ribosomes grouped in a mass, placed the
organism within the order Chytridiales.\(^2\) Phase contrast microscopy revealed that living zoospores released from zoosporangia in fresh skin samples from infected frogs swam with a single, posteriorly-directed, whiplash flagellum. Pure cultures of the chytrid were established from the skin of a blue poison arrow frog and from a green-and-black poison arrow frog; the fungal isolates from the two species of frogs were morphologically indistinguishable and represent a new genus and species of chytrid fungi.

These findings indicate that chytrids can infect vertebrate tissue and act as significant pathogens in frogs. Although the infective agent has not previously been recognized as a member of the Chytridiomycota, a review of pathology records at NZP revealed other cases of cutaneous chytridiomycosis in a White’s tree in 1988 and an ornate horned frog and another White’s tree frog in 1990. The disease was previously described in captive arroyo toads (*Bufo microscaphus californicus*), amargosa toads (*Bufo nelsoni*) and a woodhouse toad (*Bufo w. woodhousei*) and the causative agents were characterized as “fungal-like protists.”\(^5\) A fatal dermatomycosis in captive dwarf African clawed frogs (*Hymenochirus curtipes*) was probably caused by chytrids instead of the zygomycete *Basidiobolus ranarum*, as previously reported.\(^4\) Cutaneous chytrid infections have been recently identified in captive amphibians at several U.S. zoos, affecting species such as false tomato frogs (*Dyscophagus guineti*), Cowan’s mantellas (*Mantella cowanii*), a Solomon’s Island eyelash frog (*Ceratobatrachus guentherii*), an European green toad (*Bufo viridis*) and a giant palm salamander (*Bolitoglossa dofleini*) (T. McNamara, M. Greenwell, K. Wright and M. Garner, personal communication). Chytrid fungi have been associated with significant population declines in several species of wild frogs from Australia (L. Berger, personal communication) and leopard frogs (*Rana yavapiensis* and *R. chiricahuensis*) in Arizona (G. Bradley, personal communication). In most instances, the causative organisms were initially thought to be protozoa because of the morphology of chytrid thalli and zoospores on light microscopy.

The apparently sudden emergence of cutaneous chytridiomycosis in captive and wild amphibians is noteworthy, particularly in view of increasing recognition of worldwide declines in populations of amphibians. Further studies to better characterize this disease and the possible co-factors that predispose amphibian skin to chytrid infection are warranted.

LITERATURE CITED

CARDIOPULMONARY AND ANESTHETIC EFFECTS OF PROPOFOL FOR INDUCTION AND MAINTENANCE OF ANESTHESIA IN GREEN IGUANAS (*Iguana iguana*)

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Abstract

To determine the cardiopulmonary effects and usefulness of intraosseous propofol as an anesthetic in green iguanas (*Iguana iguana*) following a single bolus and during constant rate infusion for 30 min.

Animals

Fourteen clinically healthy green iguanas of unknown sex and age on loan from a local zoological institution were used.

Methods

In the first trial, four iguanas were induced with propofol administered intraosseous (IO) at 10 mg/kg. Immediately following induction of anesthesia iguanas were intubated and end tidal CO₂ (EtCO₂) concentrations were monitored continuously. Heart rate, respiratory rate, and rectal body temperature were recorded 1 min prior to propofol administration, every minute for the first 5 min, then every 5 min until recovered from the anesthetic event. Functional hemoglobin oxygen saturation (SpO₂) was recorded every minute following induction for the first 5 min and every 5 min until recovery.

In a second trial ten green iguanas were administered intraosseous propofol at 5 mg/kg for induction of anesthesia. Anesthesia was maintained for 30 min by constant rate intraosseous infusion of propofol at 0.5 mg/kg/min. Iguanas were not intubated and EtCO₂ was not monitored. Heart rate, respiratory rate, rectal body temperature, and SpO₂ were recorded as in the first trial. In addition, venous blood gas tensions were determined 1 min prior to propofol administration and at 1, 5, and 30 min post induction.

Results
In the first trial, induction time was 1.2 ± 0.6 min. Baseline heart rate was 74 ± 8 beats/min. A significant but transient decrease in heart rate was seen 1 min after propofol administration (60 ± 5 beats/min). All iguanas were apneic following induction and spontaneous ventilation resumed within 5 min. A significant decrease in respiratory rate from 26 ± 8 breaths/min to 2 ± 3 breaths/min was observed 1 min following propofol administration. This decrease persisted until 25 min where it was no longer different from Time 0 (T₀). End-tidal CO₂ concentrations decreased from 46 ± 7 mm Hg at 4 min of immobilization to 32 ± 6 mm Hg at 30 min of immobilization.

In the second trial, induction time was 3 ± 2 min following administration of 5 mg/kg propofol. No significant changes in heart rate were seen throughout the infusion period; however at 35 min a significant decrease in heart rate was noted. The baseline heart rate was 59 ± 17 beats/min. Heart rate after 30 min of immobilization was 51 ± 12 beats/min. A significant decrease in heart rate occurred at 35 min and persisted until 120 min after the initiation of propofol administration. Apnea was observed in six iguanas between 2 and 15 min after the onset of propofol administration. Apnea persisted to 120 min in two of the six iguanas. A significant decrease in respiratory rate was observed after 2 min of immobilization (12 ± 7 breaths/min) when compared to T₀ (27 ± 13 breaths/min). Decreased respiratory rates persisted to 105 min of immobilization. SpO₂ values decreased from 79 ± 10% at 5 min of immobilization to 64 ± 16% at 30 min. The venous pH decreased from 7.4 ± 0.2 at 1 min to 7.2 ± 0.2 at 30 min. Recovery time was 57 ± 27 min. Recoveries were smooth and excitement-free.

Conclusions

Propofol is an effective agent for induction and maintenance of anesthesia in green iguanas. Propofol administration was associated with clinically substantial changes in cardiopulmonary function. It is recommended to intubate, provide oxygen supplementation, and assist ventilation following administration of propofol to prevent hypoxemia and hypercapnia. Further studies are needed to see if a dose less than that used in this study might shorten the recovery time as well as decrease the cardiopulmonary depressant effects.

LITERATURE CITED

Abstract

An epizootic characterized by progressive lethargy, anorexia, ocular discharge, edema, weakness, and paraparesis attributed to a new *Mycoplasma* organism, tentatively named *Mycoplasma lacerti*, has been documented in a captive herd of male American alligators (*Alligator mississippiensis*).\(^1,2\) Minimum inhibitory concentrations (MIC) for nine antibacterial agents were determined for isolates obtained from symptomatic alligators.\(^3\) Based upon these results, enrofloxacin and oxytetracycline were chosen for pharmacokinetic evaluation. Preliminary kinetic parameters of both drugs administered intravenously in American alligators have been described.\(^3\) Further characterization of enrofloxacin and oxytetracycline disposition in the American alligator was performed.

The ten alligators used in this study were captive-reared, unknown gender, clinically healthy, and ranged in weight from 2.1-9.7 kg. Alligators were acclimated to 27°C for five days prior to the study and maintained at this temperature during the period of sample collection. Prior to drug administration, a time zero blood sample was obtained for baseline analysis and generation of a standard curve. All alligators were manually restrained and blood samples collected from the supravertebral vein and placed into lithium heparinized tubes. Five alligators received oxytetracycline (Liquamycin LA-200, 200 mg/ml, Pfizer, New York, New York 10017 USA) at 10 mg/kg as a single bolus administered in the left biceps brachii muscle. Blood samples for analysis were collected at 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, and 192 hr following drug injection. Five alligators received enrofloxacin (Baytril, 22.7 mg/ml, Bayer Co., Shawnee Mission, Kansas 66201 USA) at 5 mg/kg as a single oral bolus, administered through a pre-measured polyurethane tube passed through the mouth into the stomach. Blood samples for analysis were collected at 1, 4, 8, 12, 24, 36, 48, 60, 72, and 96 hr following drug injection. All alligators were fasted prior to and during the study period. Plasma was separated by centrifugation, aliquoted into 1 ml plastic cryotubes and frozen at -10°C.

Sample analysis for both drugs was performed using high-performance liquid chromatography (North Carolina State University, Raleigh, North Carolina 27606 USA). Ciprofloxacin, a known metabolite of enrofloxacin, which exhibits a similar spectrum of activity, was also assayed. The limit of
quantitation (LOQ) for enrofloxacin was 0.05 µg/ml and the limit of detection (LOD) 0.02 µg/ml. For oxytetracycline, the LOQ was 0.25 µg/ml and the LOD approximated 0.20 µg/ml. For each animal, a plasma drug concentration-versus-time curve was generated. Compartmental modeling was performed using computer software for polyexponential curve stripping, fitting and least squares parameter estimation of the data (RSTRIP II version 1.0, MicroMath, Salt Lake City, Utah 84110 USA).

A two-compartment model best described both enrofloxacin and oxytetracycline. Oxytetracycline exceeded the established MIC for *Mycoplasma lacerti* of 1.0 µg/ml, with a mean peak plasma concentration of 6.85 µg/ml occurring at 1 hr post-injection. Average plasma concentrations of oxytetracycline at 192 hr post-injection were four times the minimum inhibitory concentration. Plasma enrofloxacin concentrations remained below the target MIC of 1.0 µg/ml throughout the sampling period, with a mean peak plasma concentration of 0.45 µg/ml occurring at 24-hr post-administration. Mean plasma ciprofloxacin levels did not rise much above the LOD (0.02 µg/ml), with average concentrations ranging from 0.06-0.12 µg/ml throughout the sampling period.

Based upon the data evaluated here, enrofloxacin administered orally to fasted American alligators at 5 mg/kg is not expected to achieve minimum inhibitory concentrations established for *Mycoplasma lacerti*. Although present, the small concentration of ciprofloxacin detected should not significantly contribute to the in vivo efficacy of enrofloxacin. Long-acting oxytetracycline, administered intramuscularly to American alligators at 10 mg/kg, is expected to exceed minimum inhibitory concentrations established for *Mycoplasma lacerti*, with average plasma drug levels maintained above the target MIC at seven days post-administration.

**LITERATURE CITED**


SEDATIVE AND CARDIOPULMONARY EFFECTS OF MEDETOMIDINE AND ATIPAMEZOLE IN AMERICAN ALLIGATORS (Alligator mississippiensis)

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Abstract

The American alligator (Alligator mississippiensis) is an important species in both captive and wild environments where it may become necessary to capture and restrain for physical examination, medical treatment, ecologic research or translocation. Most alligator handling requires some degree of manual restraint with or without sedation, which may result in dangerous contact for both the handler and alligator.

Historically, anesthetic agents in alligators have produced little or no effect, unpredictable recoveries or death.1 Increased contact with these animals has created a need for a sedative agent that is safe, easy to administer, provides adequate analgesia, and produces a rapid recovery. Alpha_2 agonists provide sedation, analgesia and muscle relaxation. Antagonists are available to reverse these effects. The alpha_2-agonist, medetomidine, has recently been approved for use in the United States, along with its specific reversal agent, atipamezole. This project was designed to evaluate the sedative and cardiopulmonary effects of both of these agents in an effort to identify a safe and effective sedative for alligators that can be reversed at the end of the procedure.

Fourteen farm-raised American alligators, averaging 114 cm (snout-vent length), were used in this study. An initial physical examination was performed under manual restraint and baseline data collected, including heart rate (HR), respiratory rate (RR), and cloacal temperature (T). Environmental temperatures were recorded throughout the day. Following collection of baseline data, each alligator received 0.15 mg/kg medetomidine (Dormitor®, 1 mg/ml, Pfizer Animal Health, New York, NY 10117 USA) i.m. in the left anconeus muscle, followed in 30 min by atipamezole (in a volume equal to the medetomidine; Antisedan®, 5 mg/ml, Pfizer Animal Health, New York, NY 10017 USA) in the right anconeus muscle using a 22-ga, 2.5 cm needle. Time to relaxation, resistance to handling, and loss of righting reflex were recorded as indicators of sedation. Ocular reflexes were recorded to assess depth of sedation and analgesia was assessed by absence of withdrawal reflex. Heart and respiratory rates were recorded at 5, 10, 15 and 30 min after medetomidine and at 5 and 15 min after atipamezole. Heart rate was determined by doppler ultrasound with the probe placed on the ventral midline just caudal to the pectoral girdle. Respiratory rate was determined by observation of thoracoabdominal excursions.
Three blood samples (2 ml each) were collected from the ventral tail vein of each alligator. A baseline sample, prior to any drug administration, and samples 15 min after medetomidine and atipamizole administration were collected. Packed cell volume (PCV), total protein (TP), partial pressures of CO$_2$ (PCO$_2$), and O$_2$ (PO$_2$), bicarbonate (HCO$_3$), oxygen saturation and blood pH were evaluated.

Heart rate, respiratory rate, packed cell volume, total protein, PCO$_2$, PO$_2$, HCO$_3$, oxygen saturation and blood pH were evaluated over time using Friedman’s nonparametric analysis of repeated data. A significant difference was determined at $P < 0.05$. Where differences were apparent, Rhyne and Steels method for comparison of related samples to a control (time 0) was used with an experimental-wise error of alpha = 0.05.

There was a significant difference in heart rate ($P = 0.001$) and respiratory rate ($P = 0.001$) over time. Comparisons between time periods and baseline showed a significant decrease in heart and respiratory rates at times 5 and 30 min after medetomidine and a return of heart rate to baseline at 5 and 15 min after atipamezole administration. HCO$_3$ also showed a significant decrease after administration of medetomidine (15 min) with a return to baseline after atipamezole (15 min). Other blood parameters, including PCV ($P = 0.02$), PCO$_2$ ($P = 0.027$), oxygen saturation ($P = 0.006$) and blood pH ($P = 0.001$), showed a significant decrease from baseline after medetomidine but did not return to baseline after atipamezole administration. Although these parameters were statistically different, there appeared to be no clinical significance.

Sedation was recorded in all 14 alligators. The loss of the righting reflex was also recorded in all test subjects, although the time to righting loss was variable. As for safety and reversibility, all 14 animals recovered rapidly and uneventfully. Further evaluation of these drugs in different size alligators and other crocodilians needs to be undertaken to evaluate their overall effectiveness.

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LITERATURE CITED

A 58 kg 7- to 10-yr-old Loggerhead sea turtle (Caretta caretta) was injured in an oilrig explosion off the coast of Louisiana on November 20, 1997. The turtle was flown by helicopter to the Sea Turtle Research and Rehabilitation Facility at the National Marine Fisheries Service in Galveston, Texas. Initial examination revealed a full-thickness, midline cranial to caudal carapacial fracture. Serosanguinous fluid was present at the fracture site. Blood and mucus were orally expelled during respiratory excursions. The turtle demonstrated rear limb paresis. These clinical signs were compatible with pulmonary trauma and possible spinal cord injury secondary to shell trauma. The referring veterinarian performed radiographs. Enrofloxacin (Baytril, Bayer Corp., Shawnee Mission, Kansas 66201 USA) 5 mg/kg i.m., s.i.d., e.o.d. and parenteral penicillin at 10,000 IU/kg s.i.d., e.o.d. were prescribed due to possible wound contamination.

The turtle was transported to the Houston Zoological Gardens’ veterinary hospital the following day exhibiting no change in physical condition. The animal was not severely debilitated, yet was considered a significant anesthetic risk. Additional radiographs were obtained but were not contributory to determining the extent of spinal cord injury or the severity of pulmonary trauma. Additional visceral trauma was not evident. The diagnosis, based on history and clinical signs, was severe carapacial fracture secondary to trauma, with presumed pulmonary and equivocal spinal cord trauma.

The carapace was lavaged and scrubbed with povidine-iodine scrub and braced with orthopedic plates, screws, and wires on either side of the fracture. The turtle was tolerant of all procedures, and returned to the rehabilitation facility immediately following repair.

Rehabilitation included initial placement in shallow water to minimize movement, with gradual increase in water depth over a period of approximately 1-2 wk. The turtle was slowly introduced to food. Parenteral penicillin and enrofloxacin were continued for 2 and 4 wk respectively. The turtle’s progress included absence of oral blood and mucus after 2 days in rehabilitation, learning to swim in shallow water without the use of the rear legs, and resumption of eating after 3 wk in rehabilitation. The turtle regained use of the rear limbs in early January 1998. It currently exhibits normal behavior and is scheduled for release from Galveston Island in late spring of 1998.
DERMATOPHILUS INFECTIONS IN A TORTOISE COLLECTION

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Abstract

Dermatophilus chelonae was isolated from one Egyptian tortoise (Testudo kleinmanni) and four bowsprit tortoises (Chersina angulata) at the Knoxville Zoo. The tortoises were housed indoors in adjacent sections of an open tripartite, wooden box.

The Egyptian tortoise died without premonitory signs. Dermatophilus chelonae was cultured from a large intra-coelomic granuloma.

The infections in the bowsprit tortoises were manifested as dermatitis in all four tortoises and as bilateral, septic arthritis in two of the four animals. The cutaneous lesions were raised, yellow-white nodules, 0.2-0.5 cm in diameter, covered with dry flaking skin. Each tortoise had multiple skin lesions (>10), most frequently on the neck and in deep recesses around the neck and legs. Two tortoises also had bilateral septic arthritis of the stifle joints. The severity of the lesions required euthanasia of one tortoise. Another tortoise died following 11 mo of antibiotic treatment.

Treatment consisted of debriding skin lesions with a cotton-tipped applicator dipped in a dilute povidone-iodine solution (Burns Veterinary Supply, Rockville Centre, New York 11570 USA). Infected stifle joints were debrided and flushed with a dilute povidone-iodine solution. Affected tortoises were treated with parenteral ampicillin and amikacin. Intramuscular enrofloxacin, amoxicillin and oral metronidazole were also administered to each tortoise. Lesions usually regressed within 3-6 mo following treatment but recurred in each tortoise within 12 mo. Prolonged amoxicillin therapy (SmithKline Beecham, Westchester, Pennsylvania 19389 USA; 10 mg/kg i.m. q 48 hr for 30-45 days) and keeping the animals in a drier environment appears to be successful in resolving the infections.

Our experience suggests that the unique microscopic morphology of Dermatophilus sp. may cause it to be easily missed in lesions and cultures from reptiles. The organisms were seen as branching, gram-positive filaments and coccoid forms without longitudinal planes of divisions by light microscopy. Considering the size and superficial location of lesions and the chelonid’s environment, contamination is sometimes hard to avoid.

Dermatophilosis is a widely recognized skin disease of mammals caused by the actinomycete, Dermatophilus congolensis. This organism occasionally causes extra-epidermal infections in
mammals. In addition, dermatophilosis has been observed in several lizard species, a boa constrictor (Constrictor constrictor) and an American alligator (Alligator mississippiensis).

LITERATURE CITED

ISOLATION OF VIRUSES FROM LAND TORTOISES IN SWITZERLAND

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Abstract

A number of viral infections have been reported in tortoises. These include reports of herpesviruses associated with necrotizing stomatitis, which appears to be responsible for significant morbidity and mortality among captive land tortoises in Europe and has also been reported in the United States.2,5,7,9,11-15,17 Iridoviruses have been identified by electron microscopy in lesions of tortoises suffering from hepatitis and respiratory disease.8,16,23 Togaviruses have been isolated from the blood of Texas tortoises, but have not been reported in association with any disease.4,21 Reoviruses have not been reported in chelonians, but have been identified and isolated in other reptiles.1,3,10,19,22 The only viruses isolated from and associated with a distinct disease process in land tortoises are herpesviruses.2,14 Koch’s postulates have not been fulfilled for any viral infections of tortoises. This study reports the isolation of herpes-like, irido-like, and reo-like viruses, as well as an unidentified, nonenveloped virus from organ samples of land tortoises.

In 1996, 315 organ samples from 27 land tortoises necropsied at the Department of Zoo Animal Pathology, Institute of Animal Pathology, Bern were tested in cell culture for the presence of cytopathic agents. The tortoises tested consisted of nine Hermann’s tortoises (Testudo hermanni), seven spur-thighed tortoises (T. graeca), four red-foot tortoises (Geochelone carbonaria), four leopard tortoises (G. pardalis), one yellow-foot tortoise (G. denticulata), one radiated tortoise (G. radiata) and one parrot-beaked tortoise (Homopus aereolatus). The animals tested were chosen on the basis of pathologic and histopathologic findings indicative of a virus infection, including inclusion bodies and unclear diagnoses.

Organ samples were collected in cell culture medium (basal medium Eagle) (BME) with Earle’s salt solution (Seromed, Biochrom KG, Berlin, Germany) and frozen until testing. Before testing, the samples were thawed at room temperature, sonified and centrifuged at low speed (1000xg, 5 min) for the removal of cell debris. Supernatant was inoculated onto Terrapene heart cells (TH-1, CCL (50), ATCC, Rockville, Maryland USA) and chicken embryo fibroblasts (CEF) prepared according to Schat and Purchase.20 All cultures were incubated at 28 °C and observed daily for cytopathic effects (CPE). If no CPE was visible after 14 days, a second passage was performed. Cytopathic agents were tested for their sensitivity to chloroform, inhibition of replication in cell culture by 5-iodo-2’-deoxyuridine (IUDR) and cell culture supernates were examined by electron microscopy.
Herpes-like viruses were isolated in TH-1 cell cultures from the esophagus of two, and the small intestine of one, spur-thighed tortoises and the tongue, trachea and brain of one yellow-foot tortoise. The isolates caused round cell formation in TH-1 cells typical of tortoise herpesviruses\textsuperscript{2,14} and no CPE in CEF. They were further identified as herpesviruses on the basis of their sensitivity to chloroform, inhibition of replication by IUDR and by electron microscopy. Histopathologic examination also revealed eosinophilic intranuclear inclusion bodies in cells of affected organs.

Irido-like viruses were isolated from the tongue, trachea, lung, liver, esophagus, small and large intestine and cloaca of one and the tongue of another Hermann’s tortoise. These isolates caused a round cell CPE and vacuolization in both TH-1 cells and CEF. They were also found to be sensitive to chloroform and IUDR, and electron microscopy revealed viral particles morphologically consistent with iridoviruses. Histopathologic examination of tissues of these animals revealed basophilic intracytoplasmatic inclusion bodies in several organs.

A reo-like virus was isolated from the tongue, esophagus, lung, liver and kidney of one spur-thighed tortoise. This isolate caused syncytium formation in CEF and no CPE in TH-1 cells. The isolate was not sensitive to chloroform or IUDR, showing it to be non-enveloped and possibly an RNA-virus. Electron microscopic examination revealed reo-like virus particles in the cell culture supernatant.

An agent that caused lysis in TH-1 cells, but no CPE in CEF was isolated from a number of organs from two leopard tortoises, three Hermann’s tortoises, and four spur-thighed tortoises (Table 1). This agent was not sensitive to chloroform or IUDR, could be serially passaged in TH-1 cell culture and was able to pass through a 100 nm filter without a reduction in titer. Despite repeated efforts, we have not yet been able to visualize it in an electron microscope, despite the fact that it reaches titers of 7-8 log\textsubscript{10} TCID\textsubscript{50}/ml (mean tissue culture infectious dose) in TH-1 cell cultures. Herpesviruses were also isolated from three of the spur-thighed tortoises. All of the animals from which these unidentified viruses were isolated suffered from disease symptoms typically described for herpesvirus stomatitis. Histologic examination demonstrated intranuclear inclusion bodies in various tissues in all but one of the Hermann’s tortoises. The significance of these viruses in the disease process is unclear. Since they replicate faster in cell culture than herpesviruses, it is possible that all of the animals from which these viruses were isolated were also infected with herpesviruses which could not be isolated because the cell monolayer was destroyed by this agent first.

Koch’s postulates have not yet been fulfilled for any viral infections of tortoises. Although histologic findings have so far strongly suggested that herpesviruses are responsible for viral stomatitis in tortoises,\textsuperscript{18} the fact that another virus was isolated from so many animals also diagnosed with herpesvirus infection suggests that it could also be a factor in this disease. It is also possible that this virus is a secondary infection in animals whose immune systems have been weakened by other disease processes. The irido-like virus infection in the two Hermann’s tortoises was also clinically similar to herpesvirus stomatitis. These data indicate that a number of viruses are potentially virulent for tortoises and are therefore of concern for owners, breeders, zoos, the pet trade and conservation programs. It is important for veterinarians to consider various viral diseases as differential diagnoses in tortoises. Studies to identify and characterize the isolated viruses are ongoing.
ACKNOWLEDGMENTS

The authors would like to thank Dr. Werner Herbst, Institut für Hygiene und Infektionskrankheiten der Tiere, Justus Liebig University, Giessen, Germany and Dr. Peter Wild, Institute of Veterinary Anatomy, Laboratory for Electron Microscopy, University of Zurich, Switzerland for electron microscopy.

LITERATURE CITED


Table 1. Animals and organs from which an unidentified, nonenveloped virus was isolated.

<table>
<thead>
<tr>
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<tr>
<td>Leopard tortoise</td>
<td>1</td>
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<td>Tongue, brain</td>
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<td></td>
<td>3</td>
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MYCOPLASMA SURVEY OF CAPTIVE AND FREE-RANGING EASTERN BOX TURTLES (Terrapene carolina carolina) IN NEW YORK

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Abstract

Infectious diseases are a significant factor in chelonian conservation programs, especially for release and translocation projects.3,5 Mycoplasma agassizii upper respiratory tract infection has caused widespread morbidity in captive and free-ranging desert (Gopherus agassizii) and gopher (G. polyphemus) tortoises and has been observed in captive spur-thighed (Testudo graeca) and star (Geochelone elegans) tortoises.1,5,6,8,9

A mycoplasma survey of 70 (35 male, 34 female, and 1 not sexed) free-ranging and 34 (14 male, 18 female, and 2 not sexed) captive adult eastern box turtles (Terrapene carolina carolina) was conducted between June 1995 and October 1997. Free-ranging turtles were collected from six locations in Westchester, Nassau, and Suffolk counties New York. The majority (53) were from one study site.3 The free-ranging turtles were clinically healthy except for six which had aural abscesses. They were collected by hand, held for less than 2 days before sampling, and then released at the original collection site. Captive turtles included a colony of nine turtles (C1) and 25 that were presented to wildlife rehabilitators or the Department of Herpetology (C2). The second group included long term pets, animals donated by members of the public due to illness (upper respiratory tract disease, aural abscesses, overgrown beak, subcutaneous abscesses, and general unthriftiness with weight loss), and turtles of unknown background. These may have been recovered from the wild, but collection location and duration in captivity were unknown.

Heparinized blood samples were obtained from all turtles by jugular, or in a few cases by occipital sinus, venipuncture. Plasma was separated by centrifugation and frozen at -30 °C until assayed by a plasma enzyme-linked immunosorbent assay (ELISA) validated for the detection of antibodies to M. agassizii in desert and gopher tortoises.8,9 This assay is cross reactive with antibodies of a range of chelonian species although antibodies of the genus Testudo are poorly cross reactive (D. Brown, unpublished data). An ELISA ratio of sample to negative control was calculated for each sample. Samples with a ratio of ≤ 2 were interpreted as negative, 2-3 as suspect, and > 3 as positive. Those cutoffs were conservatively based on the variance of ELISA ratios of healthy and experimentally infected tortoises, with the objective of minimizing the risk of false negatives.
Free-ranging turtles were frequently seropositive (31.4%, with 18.6% serologically suspect and 50% seronegative). The single large free-ranging population had more seropositive turtles (39.6%, with 22.6% serologically suspect and 37.7% seronegative) than the pooled data from all other collection locations (5.9% seropositive, 5.9% serologically suspect, and 88.2% seronegative).

Significantly fewer captive turtles (C1 and C2) were seropositive (14.7%, with 5.9% serologically suspect and 79.4% seronegative) than were free-ranging turtles (Chi Square Test; \( P < 0.05 \)). The captive colony (C1) had similar numbers of seropositive turtles (22.2% with 77.8% seronegative) as did those presented to wildlife rehabilitators (C2) (12% seropositive, 8% serologically suspect, and 80% seronegative).

Seropositive turtles were not associated with aural abscesses, upper respiratory tract disease, or other illnesses. Two free ranging turtles and 13 captive turtles were sampled multiple times (two to three times during an 8- to 14-mo interval). Serologic status remained the same for 10 turtles (nine negative and one positive) and converted from one serologic status to another (negative to positive one time, positive to negative three times, and suspect to negative one time) in five turtles.

Nasal washes were performed with sterile 0.9% saline and frozen at -30 °C until analyzed. Samples from seropositive turtles were cultured in SP4 mycoplasma broth and aliquots tested by polymerase chain reaction (PCR) testing utilizing *Mycoplasma* genus specific reaction conditions. No *Mycoplasma* spp. were isolated in culture or identified by PCR testing of nasal wash samples.

These serologic results suggest that captive and free-ranging eastern box turtles in New York have been exposed to a *Mycoplasma* spp. Until an organism is isolated it is impossible to determine if it is the same species as that infecting the desert and gopher tortoise. *M. agassizii* has, however, recently been isolated from a free-ranging Florida box turtle (*Terrapene carolina bauri*) with upper respiratory tract disease (D. Brown, unpublished data). Although captive turtles are potential candidates for repatriation, novel pathogens may have devastating consequences when introduced into naive populations. Until more is known, seropositive box turtles should not be released or translocated into populations which are seronegative or of unknown serologic status and contact between seropositive turtles and other chelonians should be avoided.

**ACKNOWLEDGMENTS**

This project was funded, in part, by a grant from the Wildlife Conservation Society’s Freed Foundation Species Survival Fund. The authors thank Andy Sabin for his support of this project and his contributions to the Reptile Conservation Station. The authors appreciate the efforts of volunteers who assisted with box turtle collections and technicians who assisted with sample processing.

**LITERATURE CITED**

THE CONSERVATION, BIOLOGY, AND COMMON HEALTH PROBLEMS OF KIWI

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Abstract

Kiwi (Apteryx sp.) numbers in the wild are declining. Their unique biology makes them susceptible to introduced predators. Incubating eggs removed from the wild, raising the chicks, and then releasing them back into the wild are being used on an experimental basis to overcome the effects of high neonatal mortality. The conservation status, biology, and the veterinary care of kiwi are discussed in this paper.

Introduction

There are at least four species of kiwi: the brown kiwi (Apteryx mantelli) in the north island and at Okarito in the south island, the tokoeka (Apteryx australis) in the southern south island and on Stewart island, the great spotted kiwi (Apteryx haastii) in the northern South island, and the little spotted kiwi (Apteryx owenii) now restricted to Kapiti Island and several offshore islands.1

Due to the declining wild population, The Kiwi Recovery Program was launched in 1991 with the aim of “maintaining and, where possible, enhancing the current abundance, distribution and genetic diversity of Kiwi.”2 Kiwi have been held in captivity since 1932 and the first captive breeding occurred in 1945.3 As of January 1998, the international studbook shows there are 80 kiwi held in captivity in 15 institutes in New Zealand and 34 held in 13 institutes overseas.4

Conservation Status

Reasons for Decline

The range and numbers of kiwi have been reduced since humans arrived in New Zealand, but particularly over the last 100 yr. The reasons for the decline include forest clearance, which has reduced available habitat and produced fragmented populations. However, the most important factor is the introduction of predators. Possums damage eggs, mustelids and feral cats kill young chicks, and dogs and ferrets can kill adults. Up to 95% of all chicks are being killed within the first 2 mo of life in some areas.5

Operation Nest-Egg

This has been established as an experiment to allow the hatchlings to grow to a weight where they can defend themselves in the wild. Eggs are removed from wild kiwi nests and incubated in artificial incubators. Ten institutes have been involved with varying success.3 Chicks are given earthworms...
within the first week and then are encouraged to eat the artificial diet. Birds are held in captivity for
up to 5 mo or until 1000g, and then released. None of the released birds over 1000g have succumbed
to cats or stoats, but some of the birds have died due to other predators (e.g., dogs and ferrets). The
plan is to change some of the release procedures and sites to improve success.

**Biology**

Flightless and with only vestigial wings remaining, kiwi occupy a secretive, nocturnal niche feeding
on leaf litter and soil invertebrates, and fallen fruits. Adults will live on average 20 yr in the wild, but
may live up to 40 yr.

They have several unusual physical features. They have small eyes, but still have good night vision.
They hear well and have an excellent sense of smell, the nostrils being uniquely placed at the tip of
the long bill. The feathers are very simple, with a single rachis and unlinked barbs, and hang like
hairs. Wings are vestigial and they have no tail. Legs are short and very powerful, and the feet have
four toes with claws, which are used for fighting and digging. They also have paired functional
ovaries, a remnant of a diaphragm, and a body temperature and metabolic rate which is lower than
most birds.

Female are larger and heavier than males. The female lays one to two eggs, 2-4 wk apart, which are
very large and comprise up to 15-20% of the body weight of the female (approximately 375-450 g).
They eggs have a very long incubation period (80-85 days). Each chick hatches fully feathered and
first leaves the nest after 1 wk, when it will feed unaccompanied. In the first seven days, a steady
weight loss is normal. The chick will remain in the area until the next breeding season when it will
disperse.

**Common Health Problems**

**Trauma**

Aggressive exchanges between birds have often resulted in the death of one of the birds. Trauma to
the bill tip has been seen in captive kiwi. Repair is very difficult, due to the narrow fragile nature of
the bill. Despite attempts at repair, the bill tip often becomes ischemic. Some birds have been known
to adapt. Birds that have been caught in traps are sometimes brought to a captive facility. Often the
leg is irreparable and amputation is indicated. Birds will often adapt, but amputees only survive for
short periods.

**Embyronic mortalities**

Embyronic mortalities are commonly seen in kiwi eggs that have been incubated artificially. The
standard procedure for incubation is not fully understood. Bacterial contamination is seen and
abnormalities in yolk sac internalization are commonly seen in eggs subjected to high incubation
temperatures. Incubation length should be 80 days plus. Chick and embryo deformities have been
seen and these include crossed or bent bills, curled toes, and anophthalmia.6
Ventricular Foreign Bodies
Ingestion of foreign bodies has been a common cause of death in adult kiwi. Small nails, pieces of metal, and soft materials can be found in the substrate and are readily ingested, leading to perforation of the stomach wall and leakage of ventricular fluids into the coelomic cavity. This can often lead to peritonitis and death. Surgical removal by way of a proventriculotomy and treatment with broad-spectrum antibiotics has been successful. It is important when changing the substrate to check for foreign bodies. A metal detector can be helpful for identifying metal in the substrate.

Egg related peritonitis
Egg related peritonitis is a common cause of mortality in breeding females. Death has occurred within several days of laying in some birds. Considering the enormous size of the egg, it is not surprising egg peritonitis does occur commonly. Treatment consists of long term antibiotics, supportive care, and possibly peritoneal lavage. It is important at all times to gently examine breeding females, especially immediately prior to egg laying, and to ensure the birds are not overweight.

Yolk Sac Retention and Infection
One of the common problems encountered in the first 3 wk of life is retention of the yolk sac, with or without infection. Normally, the yolk should be absorbed within the first 2 wk in most birds, but in certain instances yolk digestion and absorption slows or ceases. Clinical signs include continued weight loss beyond normal weight loss in the first 10 days, failure to eat, weakness, depression, dyspnea, abdominal distention, and often the inability to stand correctly. The retained yolk sac is often 20-40% of the body weight of the chick. Diagnosis is based on the symptoms and a doughy mass on abdominal palpation. Radiography reveals an enlarged mass. The etiology is not clearly understood, but includes sub optimal incubation conditions, excessive handling, systemic disease, or infection of the sac. Infection has been associated with *E. coli*, *Proteus* sp., and *Streptococcus* sp. Treatment consists of surgical removal, with enteral nutritional supportive care and broad-spectrum antibiotics.

Cryptococcosis
This disease has been seen only once in the veterinary field in New Zealand. It was seen in a 22-yr-old female North Island brown kiwi, *Apteryx australis mantelli*. No gross abnormalities were seen on necropsy. Several of the organs showed a multifocal to diffuse inflammatory reaction associated with cryptococcal organisms. The organism cultured from liver samples was *Cryptococcus neoformans var gattii*. It is thought that birds are not very susceptible to cryptococcus species because of their high body temperatures. It does not grow readily above 40°C. However, kiwi have a much lower body temperature (around 37.5°C). Cryptococcus will grow readily under these conditions. This organism is generally associated with two species of Australian gum trees, *Eucalyptus camaldulensis* and *E. tereticornis*. Neither of these two species was found in the kiwi enclosure substrate. It is believed that this species of cryptococcus can be associated with other species of gum.

Aspergillosis
Aspergillosis has been the cause of death several times in adult kiwi. Birds usually show weight loss, whilst still maintaining a reasonable appetite. Terminally they show dyspnea and marked depression. Characteristic fungal plaques are seen on air sacs and granulomata are seen in the lungs and throughout the coelomic cavity.

Coccidiosis
Coccidiosis has been seen in many kiwi. The disease is seen in the first 2 yr of life, but most commonly in the first 6 mo. The disease is associated with diarrhea, severe dysentery and melena, inappetence, dehydration, and weakness. Birds soon die if not treated. On histologic examination, a massive colonization of the intestinal mucosa associated with coccidial life-forms is seen. Evidence of various life-forms has also been seen in the renal pelvis in a 1-mo-old kiwi and in the liver parenchyma, in the bile ductules, and in the pancreas of other kiwi. An inflammatory reaction was seen at each site. The etiology is presumed, at this stage, to be an *Eimeria* sp. Diagnosis is based on clinical signs and the presence of large numbers of oocysts on fecal flotation. The condition has been successfully treated with toltrazuril (Baycox, Bayer Ltd., New Zealand) at 7-10 mg/kg for 2 days. Supportive care is also required. The area where kiwis live should be 'spelled' wherever possible. Removal of all of the substrate to a depth of 30 cm and replacement with fresh leaf litter should be considered at least yearly. Regular fecal flotation should be performed on juveniles (e.g., every 3-4 wk until 18 mo of age). If oocysts are detected, they should then be treated and the substrate changed, or the bird removed from that area. Much is still to be discovered about the epidemiology, pathogenesis, and etiology of this condition and its presence in the wild is being evaluated.

Visceral Gout
Visceral gout has been seen on several occasions in chicks within 4 mo of hatching. There have been no reports of this condition in adults. Chicks are usually found dead. Possible causes of gout include excessive protein in the diet coupled with high levels of calcium, vitamin D₃, and dehydration. Visceral gout was associated with congenital ureteral obstruction in one neonate.

Suspect Biotin/Pantothenic Acid Deficiency
Within 3 wk of developing generalized skin lesions, several adult kiwis had died despite treatment. The lesions resembled seborrheic dermatitis. Exudative encrustations were seen on the head, around the mouth and ears and, later, on the feet. Histologically there was a marked hyperkeratosis with exfoliating sheets and inflammatory crusts associated with mixed bacteria and micro abscesses. The history indicated that the vitamin supplementation had been absent from the diet for only 3 wk. Treatment with B vitamins quickly and successfully reversed the signs. Other birds died before a diagnosis was made. Similar conditions have been reported in other captive institutes. The condition most closely resembles biotin or pantothenic acid deficiency, which is seen in domestic chickens.

Miscellaneous health problems
Generalized steatitis has been seen adults kiwi possibly associated with vitamin E deficiency. Adult birds have been found dead with significant hemorrhage in the coelomic cavity associated with hepatic lipidosis. Pneumoconiosis is seen commonly as an incidental finding in kiwi. It is thought to be associated with the inhalation of fine dust particles through the distally placed nostrils.
Coagulative necrosis of the lung, liver, and thymus, associated with tachyzoites, were seen in a juvenile little spotted kiwi. This was thought to be due to toxoplasmosis, but this has not yet been confirmed. Generalized avian tuberculosis has been seen in an adult kiwi. Miliary white nodules were seen throughout the liver, on the serosal surface of the spleen, gizzard, and the intestinal tract. Tetramisole toxicity and death has occurred when birds had been wormed with the anthelmintic, Aviverm.™. The dose rate was not known. It is therefore not known whether it is a species related or a dose related sensitivity to the drug. Fenbendazole and ivermectin at standard dose rates are safe to use. The nematodes *Cyrnea aptericis* are found in the gizzard and *Heterakis gracilicauda* in the large intestine. Normally these nematodes cause little pathology, except when found in high numbers.

**LITERATURE CITED**

VETERINARY INVESTIGATION OF THE YELLOW-FOOTED ROCK-WALLABY
(Petrogale xanthopus xanthopus) FOR REINTRODUCTION

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Abstract

In September 1996, ten captive yellow-footed rock-wallabies (YFRW) were reintroduced to Aroona Sanctuary in the Northern Flinders Ranges, South Australia. The reintroduction of the YFRW was to trial the effectiveness of captive propagation and release as a recovery tool for this species. This program, facilitated by the Royal Zoological Society of South Australia (RZSSA), illustrated the importance of cooperation, preparation, commitment of time and money, and veterinary involvement in a reintroduction program. Veterinary involvement included disease surveillance, selection of the fittest individuals for release, and acclimatization, monitoring, and pathology after release. Comparisons of microflora and parasite loads between captive and wild YFRW showed little variation. Consequently no pharmacologic intervention was deemed necessary prior to reintroduction, apart from vitamin E/selenium supplementation. Short-term success can be credited, with an 80% survival rate and first generation juveniles surviving past weaning.

Introduction

The YFRW exists in isolated colonies throughout its former range in South Australia (SA), New South Wales and Queensland. The SA subspecies, Petrogale xanthopus xanthopus, suffered a large decline since European settlement, due to habitat destruction, hunting, and the introduction of eutherian predators. Although hunting was outlawed in 1912, the pressures of carnivores, introduced competing herbivores, and pastoralism have created isolated pockets of animals with all its attendant problems. Although environmental management will be the key to the recovery of the YFRW, a trial reintroduction of captive-bred animals was sanctioned at a meeting convened for Petrogale spp. in 1994. Adelaide Zoological Gardens (AZG) has managed a captive colony of YFRW for 108 yr. Capture of wild males and their incorporation into the studbook has seen this healthy captive population grow to 136 animals in eleven Australian institutions.2 Seventy of these are kept in 6000 m², natural mallee paddocks at the Society’s open range zoo, Monarto Zoological Park (MZP). The zoo joined with the Department of Environment Heritage and Aboriginal Affairs (DEHAA) and Optima Energy (formerly the Electricity Trust of SA) to conduct the reintroduction trial. Support was sought and gained from the Zoological Boards of Victoria and New South Wales, the local community and surrounding pastoralists of Leigh Creek.

The site chosen for reintroduction was Aroona Sanctuary, the property of Optima Energy, and associated with the township of Leigh Creek. The sanctuary supported a YFRW colony, until they became locally extinct in 1982. The threatening processes at this site are not known, but assumed to
be a combination of high resident fox numbers, goat and sheep migration to the central Aroona Dam, and shooting. Apart from being within the historic range of YFRW, this site was chosen due to its association with Optima, its remoteness from wild YFRW colonies, and suitable habitat. Optima had initiated feral cat and herbivore control programs in association with revegetation at Aroona Dam. An intensive fox baiting and shooting program was added with further involvement from Optima and a commitment from RZSSA. The surrounding pastoralists embarked on a project of creating a 10-kilometre radius of 1080 baiting around the Sanctuary in January 1996. This “Buffer Zone” has proven very effective with spotlighting assessments showing a dramatic decrease of foxes in the area. (J. Crutchett, personal communication)

Disease Surveillance

Epidemics associated with high mortality rates in free-ranging macropods are rare in Australia. Diseases, such as toxoplasmosis, coccidiosis, necrobacillosis, and anemias caused by erythrozoites and globocephaloïdes, have been isolated from macropod epidemics, however most of these disease outbreaks are associated with environmental stressors. Factors, such as drought, flood, nutrition, and overcrowding, have a large role to play in macropod dieoffs. Recently the epidemiology of macropod herpesviruses and epidemics in *Macropus* sp. caused by Wallal/Warrego viruses have been investigated as pathogens for this family of marsupials. Wallal virus was described in 1995 in response to an epidemic of blindness in wild kangaroos and euros across NSW, Victoria, and SA in 1993, 1994, and 1995. The Wallal and Warrego viruses are insect-borne, orbivirus-related organisms. Macropod herpesvirus (MHV) is widespread in Australian macropods and has caused fatalities in captive macropods, but no clinical cases have been reported in the wild. Once again clinical expression of this disease is likely to be a multifactorial problem resulting in immunosuppression.

Serologic testing
Serologic testing for MHV, Wallal virus and toxoplasma resulted in no sero-conversion in 38 captive MZP YFRW (54 animals tested for Toxoplasma) or 3 wild YFRW from the Northern Flinders Ranges. From wild euros (*Macropus robustus erubescens*) inhabiting Aroona Sanctuary in 1996, there was 100% sero-conversion to MHV, 66% conversion to Wallal virus and no sero-conversion to toxoplasma. Four of five cats sampled from Aroona Sanctuary did show antibodies to *Toxoplasma gondii*.

Gastrointestinal bacteriology and parasitology
An extensive variety of organisms were isolated from feces (Table 1). Significant organisms in captive macropods could be *Eimeria* spp. and *Salmonella typhimurium*, however they are not considered to be pathogenic in wild macropods, unless other factors causing microbiologic imbalance are experienced. Most were readily cultured from a high proportion of the captive and wild macropods with no clinical evidence of disease or poor condition.

Skin scraping
Skin scrapings and hair samples revealed Aspergillus versicolor and Heterodoxus ampullatus present at low levels in the captive population. Fungi have been isolated from hair samples from wild wallabies and the Heterodoxus louse is common in this species. There were no clinical signs or histories of disease associated with either of these ubiquitous organisms.

**Selection of Individuals for Release**

Wallabies were caught in large hanging nets set up in corral-style cul-de-sacs along fences. All animals between the ages of 2 and 5 yr were anaesthetized with isoflurane via a face mask. Comparative weights, condition scores, pelage, and ectoparasite burdens were used as indicators of general health. Thorough clinical examinations were performed on each animal, paying particular attention to tooth/gum margins because of the common incidence of necrobacillosis (lumpy jaw) in captive macropods.

Over seventy complete blood profiles and biochemistries have been collated from 1995-1996 to create the “normal” blood profile for this species in captivity (values will be available in the next ISIS Reference Values, MedARKS). This profile helped to assess the health status of captive and wild YFRW. No inflammatory or organ problems were detected. Limited sampling ($n = 4$) from wild macropods demonstrated a mean of 9.27 mg/L alpha-tocopherol. Sampling of captive YFRW ($n = 69$) showed a mean of 2.06 mg/L. While this may not indicate a deficiency in these captive wallabies, Selenium/vitamin E was injected intramuscularly at 0.02-0.025 ml/kg (Vitamin-E-Selen, 150 IU vitamin E and 0.5 mg Selenium /ml).

**Social and Environmental Acclimatization**

The release group comprised two males and eight females. Six months prior to reintroduction, two groups of potential release animals, each with two males and seven females, were placed under minimal surveillance with no supplementary feeding and natural exposure to wedge-tailed eagles, a very common predator at Aroona Sanctuary. Species of browse and grasses similar to those at Aroona Dam could be found in their enclosures. Fecal analysis of scats from wild wallabies, euros, and the captive group were conducted to determine food preferences. It took a few months after release for the captive adults to change their food preference to predominantly browse, similar to YFRW in the wild. After a second veterinary examination, radio-collars were fitted to ten wallabies to test individual responses. The collars had been tried for 6 mo on female wallabies at AZG, without event. One female panicked in response to the collar, which was immediately removed and the wallaby was replaced with another female. One month prior to release, in-pouch joeys were removed from these females for hand-rearing. This procedure would avoid peak lactation at the time of release, and synchronize a birth at the point of release, thereby minimizing the time before the first generation of wild YFRW emerged from the pouch.

**Monitoring and Deaths**
Monitoring of the wallabies after release was accomplished by radiotelemetry. Radio-collars of individual frequency were fitted with mortality switches. A remote system of triangulation for locating each wallaby was used at least twice a day. Tracking teams provided continual assessment for the first 40 days, then once per month for the next 8 mo and once every 3 mo thereafter. This remote tracking system ensured minimal human interference to the wallabies after release, allowing rapid establishment of home territories. The wallabies have never ventured further than 1 km from the release site. Females have formed small home ranges with the male’s territory overlapping most of these.

Two wallabies have died since release. The first death was that of a male, 1 mo after release. Post-mortem examination identified accelerated autolysis in the thoracic cavity, but no diagnosis. The second wallaby, a female, died 6 mo after release. Post-mortem revealed severe purulent pleuropneumonia. *Actinobacillus* sp. has been cultured. No evidence of physical stress or injury was found to explain the origin of this pneumonia. The female had a 140 day old healthy, female joey in the pouch. There was no indication of predation, starvation or parasitic diarrhea in either autopsy. A trapping program in April 1998 allowed six of the eight remaining radio-collars to be replaced and samples to be taken for future veterinary and biologic research.

**Discussion**

Veterinary investigation prior to release showed a high degree of correlation between organisms inhabiting the captive and wild YFRW. It was decided not to try to eradicate any of the organisms in the captive stock because of unknown consequences to the immunity and microbiologic balance. Vaccination against Wallal virus, MHV and *Toxoplasma gondii* is not possible and the risk of cross infection from the wild euros and feral cats at Aroona Sanctuary remains. A case of poxvirus causing clinical dermatitis was confirmed in a euro at Aroona and this disease may also be able to infect the released YFRW. Collection of blood after a period of pursuit and handling did not appear to produce a stress leukogram in the majority of YFRW, as indicated by the high lymphocyte/neutrophil ratio. Individuals occasionally show marked elevations in creatinine phosphokinase, but no overt cases of myopathy resulted. As the majority of animals examined at MZP satisfied selection criteria, reproductive histories formed the basis for final selection.

**Conclusion**

The success and motivation toward local conservation inspired by this reintroduction, are the result of a well planned release protocol and several years of communication, dedication and commitment by the RZSSA. The positive results of this project extend beyond the survivability of the YFRW. In 1997, a large Land Care grant was awarded for expanding and improving the feral animal eradication program into the whole of the Aroona catchment basin. The local area school has received a grant to establish an automated tracking system. Optima Energy has funded a biologic survey of the region and the release group and their progeny also have the potential to provide ecotourism and research.
Australian animals and their organisms are unique, and epidemiology of their diseases is poorly understood. Veterinary involvement in reintroductions in Australia is important in assessing risk. The surveillance of disease, selection of individuals for release, adequate acclimatization and monitoring ensures maximum survivability of release animals and minimal impact on endemic populations. This is critical when dealing with the remaining fragmented populations of our many threatened species and to date the success of mainland Australian reintroductions has been poor.1

ACKNOWLEDGMENTS

The author acknowledges the “team” for funding and faith! The RZSSA, especially Ed McAlister, Suzy Barlow and John Crutchett, Optima Energy, especially Beat Odermatt, DEHA, Zoo Boards of Victoria and NSW, Leigh Creek Area School, especially Colin Murdoch, the pastoralists, Allan Salisbury (Transceiver Services, SA) and Steve Lapidge. Vetlab, South Australia, the Australian Animal Health Laboratory, Victoria and the Queensland Agricultural Biotechnology Centre performed the clinical pathology and serology.

LITERATURE CITED

Table 1. Selection of microorganisms and results of the survey.

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<td>100 (10)</td>
<td>100 (5)</td>
</tr>
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<td>73 (41)</td>
<td>30 (10)</td>
<td>60 (5)</td>
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<td>100 (6)</td>
<td>100 (3)</td>
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<tr>
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<td><em>Geotrichum</em> sp.</td>
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<td>Macropod herpesvirus</td>
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<td><em>Wallal virus</em></td>
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<td><em>Toxoplasma gondii</em></td>
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PARASITIC DISEASE IN THE LITTLE PENGUIN (Eudyptula minor) WITH PARTICULAR REFERENCE TO Contracaecum eudyptulae (NEMATODA: ASCARIDOIDEA)

Richard J. de B. Norman, BVSc, MVS, MACVSc

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Abstract

Little penguins (Eudyptula minor) dying on Phillip Island, near Melbourne, Australia, between 1992 and 1995 were necropsied to examine the relationship between the nematode parasite Contracaecum eudyptulae, Johnston and Mawson, 1942, and this host. 64% (227 of 353) of penguins examined were killed by foxes or road traffic. Factors, including starvation, fungal pneumonia, parasitic cholangiohepatitis, coccidial nephritis, enteritis, septicemia, neoplasia, and possibly hyperthermia contributed to the deaths of the remaining 36% (126 of 353). Ages of penguins examined ranged from a few days post-hatching to mature adults, with 118 unfledged chicks (i.e., chicks that had not yet gone to sea) and 228 adults and post-fledging juveniles. Archived fixed stomachs from adult penguins collected in 1979 and 1980 were also examined for lesions.

Unfledged chicks were initially infected with C. eudyptulae when fed by regurgitation by parents. Lesions associated with C. eudyptulae infection varied in number, size, and severity. The smallest lesions were superficial foci of epithelial erosion or coagulative necrosis associated with a single nematode attached by its mouthparts to the gastric mucosa, or occasionally the esophageal mucosa. Nematodes penetrated the compound tubular glands of the proventriculus and initiated proventricular gland abscesses. Proventricular or ventricular ulceration resulted from nematodes invading the lamina propria and submucosa. Chronic infection resulted in obliteration of compound tubular proventricular glands, with fibrosis and granulomas at the sites where nematodes were embedded in the tissue. Rarely, nematodes reached the muscularis or serosa. Healed lesions with scarring and distortion of the mucosa were sometimes observed in mature penguins. Nematodes could be expelled by regurgitation, and gastric lesions were sometimes found without any worms being present, particularly in fledged birds.

Prevalence of infection with C. eudyptulae was 68% (mean intensity: 46; range: 1-374; S.D. 63.5; n = 228) and the prevalence of lesions was 66%.

The prevalence of infection amongst penguins which died due to disease or starvation was 76% (mean intensity 79.1; range: 1-374; S.D. 83.0; n = 89) and the prevalence of lesions was 78%. The prevalence of infection amongst penguins which were killed by foxes or road traffic was 64% (mean intensity 24.7; range: 1-186; S.D. 32.7; n = 139) and the prevalence of lesions in this group was 60%.
The prevalence of infection amongst unfledged penguins was 98% (mean intensity 56.5; range: 1-244; S.D. 55.1; n = 112) and the prevalence of lesions was 77%. The prevalence of infection amongst fledged penguins was 52% (mean intensity 35.8; range: 1-374; S.D. 69.9; n = 114) and the prevalence of lesions was 61%. Fledged penguins which were killed by foxes or road traffic had a prevalence of infection of 49% (mean intensity 10.5; range: 1-68; S.D. 13.9; n = 77) and the prevalence of lesions was 56%. Adult penguins killed in 1979 and 1980 had a prevalence of gastric lesions of 68% (72 of 106).

Other pathogenic helminths found included the tapeworm, *Tetrabothrius lutzi*, the liver fluke, *Mawsonotrema eudyptulae*, and a new schistosome.
COCKATOO CONSERVATION IN WESTERN AUSTRALIA: EFFORTS FOR CARNABY’S COCKATOO (Calyptorhynchus latirostris)

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Abstract

Introduction

The arrival of Europeans in Western Australia in 1827 was the beginning of rapid changes to the landscape. The area most affected was the major agricultural region in the southwest of the state, extending from the Murchison River, 400 kilometers north of Perth, inland to Norseman, and south to the coast at Esperance. Five

Eight species of cockatoo occur in this region and have been impacted upon by environmental changes that followed European settlement and agriculture practices.

The short-billed or Carnaby’s white-tailed black cockatoo (Calyptorhynchus latirostris) are endemic to this area. Although population estimates are high, there is much concern for the long-term well being of this species due to reduced breeding activities in many areas.

In 1995, collaborative conservation efforts between the Department of Conservation and Land Management, Perth Zoo, private aviculturists, landowners, and local shires began to halt the decline in suitable habitat and increase the breeding success of both captive and free ranging cockatoos.

Ecology and Breeding Biology

The Carnaby’s cockatoo migrates between the inland eucalypt woodland breeding grounds and the more coastal areas in the non-breeding season. The breeding season is July to December with one to two eggs laid in a deep nest hollow, 2 m above the ground. Very large old growth eucalypts of the species E. salmonophloia and E. wandoo are preferred. The chick fledges at 3 mo of age and is fed for a further 4 mo by the parents. After this, the birds form flocks and migrate to the coastal areas for the rest of the dry summer months, returning to woodlands after April or May, when the winter rains begin.

Around the Perth metropolitan area, the cockatoos move into pine plantations and stands of Pinus pinaster to feed on the cones and new twig growth. Two
The main food item for the Carnaby’s cockatoo in the wheatbelt region are the seeds and flowers of native plants such as banksia, dryandra, grevillea, and hakea. Erodium, an introduced weed, is also fed upon during the limited time its seeds are available.3

Carnaby’s cockatoos become sexually mature at 4 yr of age and may live up to 60 yr. They form very strong pair bonds and return to the same area to breed year after year, and in some cases, use the same hollow.6

**Threats to Survival**

The enormous amount of land that has been cleared for agricultural use in the cockatoos’ range means that the native vegetation needed for food and nest sites is scarce and scattered. Breeding success of this species is dependent on these two resources.4

Carnaby’s cockatoos must compete with other deep hollow nesters such as galahs and corellas for nest sites. Galah numbers have increased with the introduction of European agrarian practices. Additionally this species nest year round and may already occupy favored sites when breeding season begins for the white-tailed black cockatoo.

Poaching of young chicks and eggs from nests not only has the immediate effect of decreasing the fledglings for the current breeding season, but may impact further breeding success as nest hollows may be damaged or destroyed by the poachers. Although illegal, some white-tailed black cockatoos are still shot by farmers.

Some of the last remaining significant stands of native vegetation occurs along road verges and railway easements. Birds may be hit by cars when they are on the ground feeding near the roads. Many injured cockatoos are collected by CALM officers each year.

**Conservation Efforts: Ex Situ**

The Western Australian Department of Conservation and Land Management allows zoos, wildlife carers, and private aviculturists to hold white-tailed cockatoos classified as ‘derelicts’. This includes birds that are injured from gunshot and road accidents, and younger birds that are too imprinted for successful release to the wild. A licensing system is used to record these birds and they remain the property of the government. Since 1994, a program to confirm the species and sex of these birds, and pair them up for captive breeding has begun. People holding the birds are asked to voluntarily participate in the program which in some cases involved the relocation of the birds to another site. All birds are brought to Perth Zoo for an examination where the birds have a blood sample collected for DNA research, a microchip is placed, and surgical sexing is done, if the birds are immature.1

The goal of this program is to increase the number of provenanced black cockatoos in aviculture.
In 1996, CALM began another captive breeding program for the Carnaby’s cockatoo. This program involved taking 60 birds from the wild as eggs and chicks. These birds were divided among five private aviculturists for raising. The first year 56 birds were fledged. At 12 mo of age, the birds had blood samples taken and were microchipped by zoo vets. CALM will receive 20% of the birds with the rest retained by aviculturists. CALM will sell its birds by public auction, with the money put into a trust to fund this and future captive breeding programs for other WA threatened bird species.

In 1997, 40 birds as chicks only were collected for this captive breeding program. As the goal of 100 captive provenanced Carnaby’s has been reached, no more collection from the wild is planned. There have been very few captive breedings of this species and until the birds sexually mature in 4 yr and captive breeding occurs, the success of this program is unknown.

Other ex situ conservation efforts have focused on increasing public awareness of the plight of Carnaby’s cockatoos in the wild. A cockatoo information center has been created in the vicinity of Perth Zoo cockatoo aviaries. Also near the aviary, native plants and graphics have been used to highlight the important habitat contained in road verges.

**Conservation Efforts: In Situ**

Education staff at Perth Zoo have organized a joint venture involving Agriculture WA, CALM, primary schools, and farmers. Farmers near the town of Coorow, 250 kilometers north of Perth, were contacted and agreed to participate. Volunteers collected seeds from native plants on these farms identified as cockatoo food species. A Perth primary school accepted the job of extracting the seeds, then sowing the seeds collected, and caring for the seedlings in their school nursery. A primary school in the rural area was also contacted. Their role was to monitor where and on what the Carnaby’s were feeding, to monitor nests, and to monitor flight paths to identify remaining patches of native vegetation that the birds were using for food.

Recently the Perth school has potted the native plants and the students will plant the seedlings at the farmer’s property and meet with their ‘sister’ school in the project.

CALM wildlife officers are also working to educate local governments responsible for road verges about the importance of this remaining native habitat for nesting sites. In some areas, the only remaining native gums of suitable size for nesting are next to roadways. This location makes the vegetation vulnerable to future roadwork and the nestlings easy prey for poachers. Only through education and recognition by the shire governments about the importance of these verge areas can they be preserved.

The conservation efforts aimed at Carnaby’s cockatoos have brought together a wide range of parties. The program can be used as a model not only for other cockatoo species, but also for other Australian native species whose numbers are declining for many of the same reasons. Co-operation and education are the key ingredients that can make any conservation effort a success.
ACKNOWLEDGMENTS

The author wishes to thank the staff of CALM and Perth Zoo who have assisted with this project and this manuscript.

LITERATURE CITED

MEDETOMIDINE-KETAMINE IMMOBILIZATION AND ATIPAMEZOLE REVERSAL OF EASTERN GREY KANGAROOS (*Macropus giganteus*)

**Geoffrey W. Pye, BVSc, MSc¹** and **Rosemary J. Booth, BVSc, MVS²**

¹Currumbin Sanctuary, Currumbin, Queensland 4223, Australia. Present addresses: Exotic Animal, Wildlife, and Zoo Animal Medicine Service, College of Veterinary Medicine, Kansas State University Manhattan, KS 66506 USA; ²Healesville Sanctuary, Healesville, Victoria 3777, Australia

**Abstract**

In 30 immobilizations, eastern grey kangaroos (*Macropus giganteus*) were induced with an i.m. mixture of medetomidine (40.2 ± 4.7 μg/kg) and ketamine (4.0 ± 0.5 mg/kg). Induction was smooth, time to lateral recumbency was 6.1 ± 2.0 min, with the depth of anesthesia being heavy sedation in six cases and light anesthesia in 24 cases. In 24 cases, immobilization was reversed with atipamezole (0.20 ± 0.02 mg/kg i.m.). Recovery to standing occurred in 30 ± 11 min and full recovery occurred within 79 ± 17 min. In six cases, immobilization was reversed with atipamezole (0.22 ± 0.04 mg/kg i.v.). Recovery to standing occurred in 29 ± 20 min. No fatalities and no anesthetic complications occurred in this study. It is concluded that immobilization with medetomidine (40 μg/kg i.m.) and ketamine (4 mg/kg i.m.) and reversal with atipamezole (0.2 mg/kg i.m.) is a safe and reliable method for the chemical restraint of eastern grey kangaroos. To ensure smooth recovery, atipamezole should be administered i.m. at least 30 min after the immobilization dose.

**Introduction**

Eastern grey kangaroos (*Macropus giganteus*) are common in the wild in Australia and in captive situations throughout the world. Chemical restraint is a necessary tool in the successful veterinary management of both captive and wild populations of macropods. Rapid and smooth induction and recovery are required to avoid trauma-related injuries and capture myopathy in macropods. A variety of drugs and mixtures of drugs have been used to chemically immobilize macropods, including thiopenitone, pentobarbitone, phencyclidine, phencyclidine-acepromazine, etorphine, etorphine-acepromazine, etorphine-methotrimeprazine, etorphine-ketamine, droperidol, droperidol-fentanyl, azaperone, alphaxalone-alphadolone, alpha-chloralose, xylazine, ketamine, ketamine-xylazine, and tiletamine-zolazepam. ²⁻¹¹,¹³,¹⁶⁻²⁵ Macropods are prone to self injury during prolonged recovery periods following anesthesia. Recovery periods of between 2-4 hr have been observed following immobilization of macropods using ketamine-xylazine and tiletamine-zolazepam. The use of medetomidine or ketamine alone can be unreliable and immobilized animals can be unpredictable for handling. The purpose of this study was to develop a safe, reliable, and reversible immobilization protocol for eastern grey kangaroos using medetomidine-ketamine and atipamezole.

Medetomidine-ketamine and atipamezole have been used in a variety of nondomestic species at dose ranges of 13-140 μg/kg of medetomidine, 0.8-8.0 mg/kg of ketamine and atipamezole at an
atipamezole: medetomidine ratio of approximately 2-5:1 (μg:μg). \(^{1,12,15,26}\) When doses of ketamine at the higher end of the range are used, it is recommended that atipamezole doses at the lower range be used to avoid the problems of “residual ketamine effect.”\(^{12}\) Medetomidine-ketamine has been administered i.m. in the red-necked wallaby (*Macropus rufogriseus*) at 100 μg/kg of medetomidine and 5 mg/kg of ketamine.\(^{12}\) In general, a combination of medetomidine at 50-100 μg/kg and ketamine at 2-3 mg/kg i.m. has been recommended for use in Australian native fauna.\(^{4}\)

**Methods**

From July 1996-May 1997 at Currumbin Sanctuary, Australia, medetomidine-ketamine was used to immobilize healthy eastern grey kangaroos during an anti-GnRH vaccine trial to facilitate translocation, clinical assessment, and pouch examination in 30 cases. Twenty-nine females were immobilized by hand injection and one male was immobilized via blow dart. Body weight ranged from 12-35 kg (average 21 ± 5 kg) and age ranged from 18-46 mo (average 30 ± 8 mo). Equal volumes of 1 mg/ml medetomidine (Domitor®, Novartis Animal Health, Wentworthville, NSW 2145, Australia) and 100 mg/ml ketamine (Ketamil®, Ilium Veterinary Products, Smithfield, NSW 2164, Australia) were given i.m. Doses were calculated on body weight estimates or previous weights using a dosage rate of 0.04 ml/kg (1 ml/25 kg). Atipamezole 5 mg/ml (Antisedan®, Novartis Animal Health, Wentworthville, NSW 2145, Australia) was administered i.v. in six cases and i.m in 24 cases at 0.2 mg/kg, a volume equal to the volume of medetomidine given. Time to lateral recumbency during induction and time to standing and full recovery following reversal were recorded. Animals in lateral recumbency, but aware of sound, movement and painful stimuli were considered under heavy sedation, while animals in lateral recumbency and unaware of all but deep pain were considered under light anesthesia. In eight cases, body temperature, heart rate and respiratory rate were recorded. Anesthetic records were collated on computer using MedARKS (ISIS, Apple Valley, MN 55124 USA).

**Results**

Dosage rates of 0.04 ml/kg on estimated or previous weights resulted in dose rates calculated on actual weights of 40.2 ± 4.7 μg/kg of medetomidine (range: 31.8-50.0 μg/kg) and 4.1 ± 0.5 mg/kg of ketamine (range: 3.2-5.0 mg/kg). Mean time to lateral recumbency was 6.1 ± 2.0 min (range: 3-12 min). Induction was smooth and the depth of anesthesia produced was light anesthesia in 24 cases and heavy sedation in six cases.

Physiologic data were collected in eight cases, 20 min postimmobilization: body temperature was 36.0 ± 1.6 °C (range: 33.9-38.0° C), heart rate was 44 ± 18 beats per min (range: 22-68), and respiratory rate was 34 ± 20 respirations per min (range: 8-60). No fatalities and no anesthetic complications occurred during this study.

In 24 cases, immobilization was reversed with atipamezole at a dose of 0.20 ± 0.02 mg/kg i.m. (range: 0.17-0.22 mg/kg). Atipamezole was administered 40 ± 13 min after the immobilization drugs were given (range: 22-60 min). Times to standing were 30 ± 11 min (range: 16-60 min) and times to
full recovery were within 79 ± 17 min (range: 57-98 min). All recoveries were smooth in cases where the antagonist was given > 24 min postadministration of medetomidine-ketamine. In the three cases in which the antagonist was given ≤ 24 min of the immobilization drugs, recovery was characterized by hypersalivation, anxiety, and ataxia. It is believed that these reactions resulted from “residual ketamine effects.”

In six cases, recovery was effected with atipamezole at a dose of 0.22 ± 0.04 mg/kg i.v. (range: 0.16-0.25 mg/kg). Atipamezole was administered 60 min following administration of the immobilizing drugs in all six cases and times to standing were 29 ± 20 min (range: 2-48 min). Intravenous use of atipamezole appeared unreliable in duration of recovery period. Two recoveries were considered rough with excessive ataxia following very rapid recoveries (range: 2-5 min). It is unlikely that this was due to “residual ketamine effect.” Sudden awareness following immobilization may explain these reactions. Recovery in the other four cases was smooth (range: 37-48 min).

Discussion

Based on the results of 30 immobilizations, medetomidine at a dose of 40 μg/kg mixed with ketamine at a dose of 4 mg/kg administered i.m. produces a safe and reliable method of chemical restraint of eastern grey kangaroos. Atipamezole at a dose of 0.2 mg/kg produces reliable reversal of the medetomidine but should be administered i.m. at least 30 min postimmobilization to avoid “residual ketamine effect.”

ACKNOWLEDGMENTS

Thanks to Wes Caton, Dr. Katie Reid, Heidi Hellingman, Nadia Valzacchi, and Sue Whyte for their assistance in this study.

LITERATURE CITED

TAIL AMPUTATION IN A RED KANGAROO (Macropus rufus): CLINICAL MANAGEMENT AND LONG TERM MAINTENANCE

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Abstract

It is well known that macropods utilize the tail as an aid in balancing.¹² The evolution of penta-pedal gait allows the kangaroo to move more economically than similar sized running bipeds or quadrupeds.¹ These facts cause obvious concerns when considering the ramifications of tail amputation.

A 5-yr-old intact female red kangaroo (Macropus rufus) was presented with an abnormal positioning of the tail and weight loss of several days’ duration. Physical examination revealed cellulitis and devitalization of the distal ½ of the tail. Radiographs revealed osteomyelitis of several coccygeal vertebrae. Amputation of an estimated 85% of the tail was performed under general anesthesia. The animal recovered uneventfully and adapted well to the loss of the tail.

Case Report

A 5-yr-old intact female red kangaroo (Macropus rufus), weighing 17 kg, was presented with an abnormal positioning of the tail and weight loss of several days’ duration. The animal was housed with 28 additional conspecifics of various ages and genders in a five-acre naturalistic exhibit during the day. During the evening, the animals were housed in an outdoor gravel and sand yard measuring 25 × 20 m, with access to indoor wooden stalls with cement floors. The kangaroos were maintained on a pelleted alfalfa based diet (Mazuri ADF, Purina Mills, Inc., St. Louis, Missouri 63166 USA), with alfalfa hay and a variety of natural enrichment “treats” consisting of whole wheat bread, bamboo, vegetables, and fruits.

The animal was immobilized with 275 mg ketamine hydrochloride (Ketaset, Fort Dodge Laboratories, Fort Dodge, Iowa 50501 USA) and 75 mg xylazine hydrochloride (Rompun, Bayer Corp. Shawnee Mission, Kansas 66201 USA), and maintained on 2.5% isoflurane (Aerrane, Ohmeda Pharmaceutical Products Division, Inc. Liberty Corner, New Jersey 07938 USA) via facemask. Intravenous buffered lactated fluids (LRS, Abbott Laboratories, Chicago, Illinois 60064 USA) were administered at 20 ml/kg/hr. Physical examination revealed devitalized “dry gangrene” tissue of the distal half of the tail with a 8 × 5 cm necrotic area over the ventral aspects of coccygeal or caudal (Cd) vertebrae 9-11. Survey radiographs revealed osteomyelitis of Cd 5-6 and Cd 11-12. Blood for a complete blood count (CBC) and selected serum chemistries were unremarkable.
The combination of weight loss, necrosis, devitalization, and ascending osteomyelitis precluded medical therapy, and the tail was amputated. Standard technique for tail amputation as for dogs was performed. After sterile preparation, the skin was incised in a double, dorso-ventral “V” over Cd 3-4, with the dorsal aspect extending beyond the ventral aspect. The median caudal artery and the paired lateral caudal arteries and veins were identified and ligated with 3-0 polyglactin (Vicryl, Ethicon, Inc. Somerville, New Jersey 08876 USA). Blunt dissection of the sacrocaudal musculature continued to the coccygeal vertebrae. The coccygeal vertebrae and tail were removed after separation of Cd 3 and 4. Approximately 80 cm of the tail was removed. The remaining tissue was irrigated with 100 mg of gentamicin sulfate (Gentocin, Schering-Plough Animal Health Corp., Omaha, Nebraska 68103 USA) in 10 ml of a 0.9% saline solution (0.9% NaCl, Abbott Laboratories). Fascia muscle sheaths were sutured together in a linear pattern with 2-0 polyglactin simple interrupted sutures. The skin was closed with 1-0 nylon non-absorbable sutures (Ethilon, Ethicon, Inc.) in a simple interrupted pattern.

The remaining 8-10 cm of tail was bandaged and changed every 6-9 days during immobilization with 2 mg/kg tiletamine/zolazepam (Telazol, Fort Dodge Laboratories). A total of six bandage changes were performed. Aerobic cultures revealed scant growth of beta-hemolytic *Streptococcus*, heavy growth of *Klebsiella pneumoniae*, and heavy growth of *Enterococcus faecalis*. Anaerobic cultures were negative. Based upon aerobic sensitivities, antibiotic therapy was initiated with 28 mg/kg sulfadimethoxine/trimethoprim (Tribrissen 48%, Mortar and Pestle Pharmaceuticals, Des Moines, Iowa 50310 USA) intramuscularly once daily for 14 days. Sutures were removed 5 wk after the initial surgery.

Only minor difficulty was noted when the animal started to recover, but adaptation to the tail loss was rapid. The tail “stub” does not touch the ground, yet the animal is able to stand erect when startled. When walking, the kangaroo places noticeably more direct weight upon the tarsal joints. This may predispose to future arthritic problems but has not been noted to date. The muscles of the hind limb have hypertrophied, especially what appear to be the gluteal and thoracolumbar muscles, and the animal has minimal balancing problems. On very rare occasions, the kangaroo cannot right itself from a laterally recumbent position. In these instances, keepers have assisted in righting the animal.

Tail amputation in this kangaroo was considered a complete success, and would be contemplated in any severely traumatized, neoplastic or infectious process involving the tail of macropods housed at the Kansas City Zoological Gardens.

ACKNOWLEDGMENTS

The author greatly appreciates the efforts of the animal health and zookeeper staff for post-operative care of this kangaroo.

LITERATURE CITED

THE CREATION OF GUIDELINES FOR THE USE AND HANDLING OF CANADIAN WILDLIFE - HOW DID WE GET HERE AND WHERE ARE WE GOING?

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Abstract

The Canadian Association of Zoo and Wildlife Veterinarians (CAZWV) was formed in 1989 to help establish a network of interested veterinarians and as a professional resource on issues concerning the health management of captive and free ranging wildlife. To this end the mission statement of the association is: “to promote and contribute to the health management of free ranging and captive wild animals and in so doing enhance the well being of these animals.”

Two major projects have been undertaken by the CAZWV to support the mission statement. Firstly, the association has developed a training course and accompanying manual “The Chemical Immobilization of Wildlife” that is used to train non-veterinary wildlife health professionals in the handling of wildlife in Canada.

Secondly, the association initiated a process to help establish national guidelines for the care and handling of wildlife in Canada. In doing so we sought the assistance of the Canadian Council on Animal Care (CCAC). The CCAC is a non government agency which has previously published Guide to the Care and Use of Experimental Animals (Volumes 1 and 2),1,2 as well as supporting Codes of Practice for most farm animals, including most recently, a code of practice for the Care and Handling of Farmed Deer (Cervidae).

The CCAC has now taken on the primary responsibility of developing Guidelines of the Use and Handling of Canadian Wildlife with the support of the CAZWV and with funding provided by the Max Bell Foundation. Under the direction of a Subcommittee of the CCAC Guidelines Committee, a process has been set in motion to collect information from a broad variety of experts. These experts have experience with various genera of animals, management issues, nutrition, and specific handling and immobilization procedures. The information will be peer reviewed, edited, and collated into a formal publication. It is anticipated that the first draft will be completed by the fall of 1998.

LITERATURE CITED

COMPARATIVE CARDIOPULMONARY EFFECTS OF MEDETOMIDINE-ZOLAZEPAM-TILETAMINE AND TELAZOL® IN POLAR BEARS (Ursus maritimus)

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Abstract

Six captive polar bears (Ursus maritimus) were immobilized to compare the effects of Telazol® with a medetomidine-Telazol® combination (MZT). Telazol® was administered at an estimated dose of 6.5 mg/kg. Medetomidine was administered at an estimated dose of 52 g/kg combined with tiletamine at an estimated dose of 0.86 mg/kg, and zolazepam at an estimated dose of 0.86 mg/kg. Animals immobilized with MZT appeared to be in a “deeper” plane of anesthesia than those anesthetized with Telazol® alone. Heart rate, respiratory rate, PaO₂, and BE were significantly lower with MZT than with Telazol® alone. Hypoxemia was present with MZT and was most severe at the 15 min reading. Systolic, mean, and diastolic arterial pressure were significantly higher with MZT. Both combinations can be used to produce safe, effective immobilization, for at least 1 hr in polar bears.

Introduction

Telazol® (zolazepam + tiletamine) is the drug of choice for immobilization of polar bears (Ursus maritimus). Reasons for the popularity of this drug combination include: relatively small drug volume, rapid induction and safe reliable immobilization.6,9 The major disadvantage of Telazol® is lack of reversibility and prolonged recovery. Medetomidine is a potent alpha-2 adrenoceptor agonist that has been used in combination with ketamine, to produce immobilization in a variety of domestic and non-domestic animals.3,4,5,7,8,10 The sedative effects of medetomidine are readily reversed with atipamezole, a potent alpha-2 adrenoceptor antagonist. A “reversible” combination, such as this, is desirable in some situations.11 Sudden recoveries from medetomidine-ketamine immobilization have been reported in brown bears.8 Following similar incidents of sudden recovery in polar bears immobilized with medetomidine-ketamine we developed a medetomidine-Telazol® combination (MZT), in an attempt to decrease the risk of sudden recoveries.1 A pilot study of the combination in black bears was performed.2 The major side effects were hypertension and hypoxemia.2 The following study details the cardiopulmonary effects of (MZT) and Telazol® in captive polar bears.

Materials and Methods

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The bears immobilized in this study were six captive polar bears that were captured in Churchill, Canada, during October 1996, as part of the Manitoba Department of Natural Resources’ polar bear control program. These animals were captured at least 7 days prior to our experiments and were maintained individually in cages with access to fresh water only. Polar bears naturally fast at this time of the year, and in this area, had been without food since the ice melted in Hudson Bay, during early July.

Six bears were immobilized with both combinations. Initial choice of immobilizing combination was random. Treatments were administered at least 5 days apart. Immobilizing drugs were administered, with a pole syringe into the muscles of the shoulder or neck. Weight was estimated initially, and following immobilization the bears were weighed to determine actual dose received. Medetomidine was administered at an estimated dose of 52 μg/kg. This was combined with tiletamine at an estimated dose of 0.86 mg/kg, and zolazepam at an estimated dose of 0.86 mg/kg. These dosages were based on a pilot study in black bears and on field studies of the combination. The medetomidine was reversed with atipamezole, which was administered at four times the medetomidine dose. Half of the atipamezole was administered i.v. and half was administered i.m. Telazol was administered at an estimated dose of 6.5 mg/kg. This dose was based on doses reported in the literature, and on a field study of this combination.

Once the bear was immobilized it was removed from the cage and a 20-ga, 5-cm catheter was placed in the femoral artery. The catheter was connected to a Baxter transducer, which was, in turn, connected to a Propaq 400 monitor. The arterial line was used to measure heart rate, direct arterial pressure, and to remove arterial blood samples for blood gas analysis. A 14-ga 6-cm catheter was placed in the jugular vein, and blood was removed for CBC and hemoglobin determination. A lead II ECG was constantly monitored to characterize arrhythmias. A Nellcor Durasensor 100A oximeter probe was placed on the tongue for constant monitoring of hemoglobin saturation. Respiratory rate was determined by observation of chest excursions. Rectal temperature was determined with a digital thermometer. One hour following administration of the immobilizing drugs the monitors were disconnected and the bear was returned to its cage. Immobilization produced by MZT was reversed with atipamezole, and bears receiving Telazol were maintained in sternal recumbency and recovered spontaneously. Repeated measures ANOVA was used to compare between treatments. One way ANOVA was used to compare differences over time within treatment groups. Comparison among means at specific time points was performed with a Bonferroni test. A significance level of \( P < 0.05 \) was used in the analysis of the results.

**Results**

Calculation of the actual drug doses received by these bears (all reported values are mean ± SD) revealed that they received a dose of 74.8 ± 11.8 μg/kg of medetomidine plus 2.2 ± 0.3 mg/kg of Telazol. Reversal was achieved with a 261 ± 105 μg/kg of atipamezole. The time from drug administration to immobilization was 3.7 ± 2.7 min following administration of MZT. The actual dose of Telazol administered was 8.2 ± 2 mg/kg. The time from drug administration to immobilization was 3.7 ± 1 min. Animals immobilized with MZT appeared to be in a “deeper” plane.
of anesthesia than those anesthetized with Telazol® alone. A pronounced spike in blood pressure and heart rate could be elicited when the nail bed was compressed with a hemostat during immobilization with Telazol®. No change in heart rate or blood pressure occurred with the same procedure during immobilization with MZT. No arrhythmias were noted with either of the treatments. Heart rate, respiratory rate, PaO₂, and BE were significantly lower with MZT. Systolic, mean, and diastolic arterial pressure were significantly higher with MZT. Physiologic data are listed in Tables 1 and 2.

Discussion

Hypertension and bradycardia are common findings in animals anesthetized with medetomidine-based protocols. Hypertension results from peripheral activation of alpha-2 receptors. Bradycardia is likely due to reflex increase in vagal tone. Bradycardia can result in decreased cardiac output and oxygen delivery.4,10 Similar cardiovascular changes were noted during medetomidine-ketamine immobilization of polar bears.3 PaO₂ was significantly lower with MZT. Animals were hypoxemic (PaO₂ < 60mm Hg) at the 15-min sampling time. PaO₂ was increased to above 60 mmHg by the 30-min blood gas sample. PaO₂ continued to increase over time with MZT. Oxygenation was similar in black bears immobilized with this combination.2 Hypoxemia is common in ruminants immobilized with medetomidine-ketamine.4,5,7 Ventilation-perfusion mismatch with increased venous admixture is the most likely cause of hypoxemia.3 Respiratory depression was minimal. Normal PaCO₂ for most species ranges between 35-45 mmHg. The highest PaCO₂ with Telazol® was 48±3 mm Hg at the 15-min reading. The highest PaCO₂ encountered with MZT was 47±4 mmHg at the 30-min reading. Application of a hemostat to the nail bed resulted in a significant spike in heart rate and blood pressure during immobilization with Telazol®. Heart rate and blood pressure did not change significantly during application of the hemostat in animals immobilized with medetomidine-ketamine. This is a very crude test of analgesia, but it does suggest that analgesia is better with MZT.

Conclusion

Both of these combinations can be used to produce safe, effective immobilization, for at least 1 hr in polar bears. Hypoxemia was present with MZT and was most severe at the 15-min reading. These animals were ventilating adequately (PaCO₂ 44 ± 9 mmHg at 15 min) and hypoxemia should respond to supplemental oxygen administration. Oxygenation was excellent with Telazol®, it would still be good clinical practice to monitor saturation with a pulse oximeter and administer supplemental oxygen if hypoxemia is encountered.

ACKNOWLEDGMENTS

The authors would like to thank C. Elliot, M. Ramsay, M. Swain, the Manitoba Department of Natural Resources, the Churchill Northern Studies Center and the Churchill Health Center for their assistance with this study. The authors also thank Farmos Pharmaceuticals and Pfizer Animal Health for providing the medetomidine and atipamezole used in this study.
study. M. Cattet gratefully acknowledges the financial support of the Medical Research Council of Canada and the United States National Science Foundation.

LITERATURE CITED

Table 1. Physiologic data following the administration of medetomidine + tiletamine + zolazepam (Mean ± SD).

<table>
<thead>
<tr>
<th>Time from immobilization</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate(^a)</td>
<td>54 ± 14</td>
<td>46 ± 12</td>
<td>41 ± 10</td>
<td>42 ± 8</td>
</tr>
<tr>
<td>Resp. rate(^b)</td>
<td>5 ± 3</td>
<td>6 ± 2</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Mean art. press.(^b)</td>
<td>237 ± 32</td>
<td>228 ± 24</td>
<td>209 ± 14</td>
<td>203 ± 17</td>
</tr>
<tr>
<td>Temperature °C</td>
<td>36.7 ± 0.5</td>
<td>37.2 ± 0.5</td>
<td>37.4 ± 0.6</td>
<td>37.6 ± 0.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.29 ± 0.05</td>
<td>7.27 ± 0.02</td>
<td>7.28 ± 0.01</td>
<td>7.29 ± 0.02</td>
</tr>
<tr>
<td>BE(^b)</td>
<td>-5.6 ± 1.3</td>
<td>-5.1 ± 0.8</td>
<td>-5.0 ± 1</td>
<td>-5.0 ± 1</td>
</tr>
<tr>
<td>PaO(_2) mm Hg</td>
<td>53 ± 9</td>
<td>62 ± 9</td>
<td>68 ± 11</td>
<td>78 ± 9(^w)</td>
</tr>
<tr>
<td>PaCO(_2) mm Hg</td>
<td>44 ± 9</td>
<td>47 ± 4</td>
<td>45 ± 3</td>
<td>43 ± 5</td>
</tr>
</tbody>
</table>

\(^w\)Significant difference within treatment (difference between 15 and 60 min measurement).

\(^b\)Significant difference between treatments.
Table 2. Physiologic data following the administration of Telazol (Mean ± SD).

<table>
<thead>
<tr>
<th>Time from immobilization</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82 ± 9</td>
<td>71 ± 21</td>
<td>68 ± 16</td>
<td>69 ± 17</td>
</tr>
<tr>
<td>Resp. rate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 ± 2</td>
<td>7 ± 2</td>
<td>6 ± 3</td>
<td>9 ± 4</td>
</tr>
<tr>
<td>Mean art. press.&lt;sup&gt;b&lt;/sup&gt;</td>
<td>147 ± 26</td>
<td>146 ± 18</td>
<td>161 ± 22</td>
<td>164 ± 11</td>
</tr>
<tr>
<td>Temperature °C</td>
<td>37.2 ± 0.2</td>
<td>37 ± 0.2</td>
<td>36.8 ± 1.3</td>
<td>36.6 ± 10.14&lt;sup&gt;W&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>7.26 ± 0.01</td>
<td>7.28 ± 0.01</td>
<td>7.29 ± 0.01</td>
<td>7.30 ± 0.02&lt;sup&gt;W&lt;/sup&gt;</td>
</tr>
<tr>
<td>BE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-4.4 ± 0.5</td>
<td>-4.0 ± 0.7</td>
<td>-3.6 ± 0.7</td>
<td>-3.5 ± 0.8</td>
</tr>
<tr>
<td>PaO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; mm Hg</td>
<td>80 ± 15</td>
<td>104 ± 20</td>
<td>98 ± 13</td>
<td>108 ± 15&lt;sup&gt;W&lt;/sup&gt;</td>
</tr>
<tr>
<td>PaCO&lt;sub&gt;2&lt;/sub&gt; mm Hg</td>
<td>48.3 ± 3</td>
<td>46 ± 3</td>
<td>45 ± 2</td>
<td>44 ± 2</td>
</tr>
</tbody>
</table>

<sup>W</sup>Significant difference within treatment (difference between 15 and 60 min measurement).

<sup>b</sup>Significant difference between treatments.
CARDIOPULMONARY RESPONSE OF ANESTHETIZED POLAR BEARS (Ursus maritimus) TO RERAINT AND SUSPENSION BY NET

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Abstract

Since 1995, at least three anesthetized polar bears have died while suspended in nets during research and management programs. A study was undertaken to determine the effect of suspension by net on the cardiopulmonary function of anesthetized polar bears. Eight captive polar bears were anesthetized with Telazol® (mean = 9.5 mg/kg, SD = 2.29 mg/kg). Arterial blood pressures of anesthetized polar bears increased significantly, and failed to return to baseline values, when the bears were suspended in the net. Bears are at risk of developing clinically significant acute hypertension following net restraint. It is recommended that alternative methods of suspending anesthetized bears above the ground for purposes of research and management be considered.

Introduction

In the management, conservation, and research of captive and free-ranging wild species, anesthetized animals are often restrained and positioned in different postures, some of which may markedly compromise cardiopulmonary function. However, the cardiopulmonary effects of restraint and posture have received little attention in wildlife anesthesia, although they are well-documented in human and domestic animal anesthesia.⁸⁻¹¹ In our ongoing investigation of the physiology of polar bears (Ursus maritimus), we often restrain and suspend anesthetized polar bears by net for up to 5 min to enable us to determine their body mass. Similarly, during wildlife management operations, anesthetized polar bears are translocated while suspended in a cargo net beneath a helicopter for flights lasting between 20-30 min. Since 1995, at least three anesthetized polar bears have died while suspended in nets during research and management programs but, in all cases, the cause of death remains unknown.²³ In an effort to improve methods of anesthetizing and handling polar bears, and to potentially reduce anesthesia-related morbidity and mortality in these animals, we designed and conducted a study to determine the effect of restraint and suspension by net on the cardiopulmonary function of anesthetized polar bears. Eight captive polar bears were anesthetized with Telazol® (mean = 9.5 mg/kg, SD = 2.29 mg/kg).

Materials and Methods
In Hudson Bay, most polar bears naturally fast on land between July and November. During this time, the bay is free of ice and polar bears are no longer able to capture their principal prey, ringed seals (*Phoca hispida*). However, as the ice begins to re-form in late October, polar bears become increasingly active and begin to concentrate along the Hudson Bay coast, and in the vicinity of Churchill. Those bears posing a threat to the inhabitants of Churchill are captured by the Manitoba Department of Natural Resources and moved to a restricted-access facility within which they are maintained in individual pens. Once the ice re-forms over Hudson Bay, captive bears are anesthetized and translocated from Churchill to the coastal sea-ice by helicopter.

Eight captive polar bears were anesthetized with Telazol® (mean = 9.5 mg/kg, SD = 2.29 mg/kg) by pole syringe during November 1997, at Churchill, Manitoba. Anesthetized polar bears were prepared for cardiopulmonary measurements by aseptically cannulating their femoral artery with an 18 ga × 10 cm arterial catheter. The catheter was subsequently secured with ligatures and connected to a pressure transducer, via non-compliant plastic tubing filled with heparinized saline. The transducer was calibrated and connected, in turn, to a physiologic monitor used to measure direct systolic, mean, and diastolic arterial pressure, and heart rate. Percent saturation of hemoglobin with oxygen (SaO₂) was monitored using a reflectance probe inserted in the rectum, and connected to a pulse oximeter.

Physiologic parameters measured were arterial pressures, heart rate, respiratory rate, SaO₂, and rectal temperature. We collected arterial blood samples at pre-determined times for hematology and blood gas analyses. Blood samples were chilled in ice water immediately following collection. Within 3 hr of collection, hematologic and blood gas analyses were completed using an automated blood counter and blood gas analyzer. All blood gas analyses were corrected for body temperature and hemoglobin concentration.

Complete physiologic measurements were recorded every 5 min during three experimental phases. Phases 1 and 3 each lasted 10 min, whereas phase 2 lasted 15 min. Within each phase, arterial blood samples were collected only during the first and last sets of measurements. During phases 1 and 3, anesthetized polar bears were unrestrained and positioned on the ground in dorsal recumbency. During phase 2, polar bears were suspended in a net from a 3-m high tripod constructed of telescoping aluminum poles. While in the net, bears maintained a semi-seated or reclined position with their head unsupported and either laying forward on their chest or laterally against their shoulder, similar to the posture assumed by bears during helicopter translocations. After measurements were completed, monitoring equipment was disconnected and the arterial catheter was removed from the polar bear. To statistically compare cardiopulmonary responses among the three phases, we used one-way ANOVA for repeated measures, and the Bonferroni-multiple comparison test. Significance was determined for statistical test values where the probability of a Type I error was less than 5 percent.

**Results**

Although no bear appeared at risk of cardiopulmonary failure while suspended in a net, many indicators of their cardiopulmonary function changed significantly among phases. In particular,
arterial pressures were significantly greater during phase 2, than during phases 1 and 3 (Fig. 1). Anesthetized bears also consistently showed signs of increased arousal (e.g., head, tongue, and limb movement) while suspended in the net. Most blood gas parameters also changed significantly among phases (Fig. 1). Arterial blood pH, PaO₂, and SaO₂ were significantly increased, and PaCO₂ was significantly decreased, during phase 3 when compared to values observed during the previous two phases. Arterial base excess and HCO₃⁻ concentration did not change significantly among phases.

Discussion

Arterial pressures typically remain stable during postural changes in awake animals, and stabilize to normal values slowly following postural changes in anesthetized animals. The arterial blood pressures of anesthetized polar bears in this study increased significantly, and failed to return to baseline values, when the bears were suspended in the net (e.g., end-expiration mean arterial pressure increased 17-49% among individual bears). We suggest that the restraint and concurrent body compression imposed during suspension was sufficient to squeeze blood from skeletal muscle and venous reservoirs of the abdomen toward the heart, thereby increasing venous return. In effect, and possibly mechanism, restraint by net was similar to military anti-shock trousers, an inflatable garment used to combat shock and increase peripheral vascular resistance. Whatever the mechanism, the acute pressure changes we observed suggest anesthetized polar bears may be at risk of developing clinically significant hypertension when suspended in a net. Arterial pressure did not decrease towards baseline values during the period of suspension, suggesting that additional or alternative mechanisms may have come into effect. One possible explanation is that suspension by net incited a stress response. The resulting sympathetic activity then caused blood pressure to rise by direct cardiovascular stimulation, and by indirect stimulation through the release of epinephrine and norepinephrine into the blood. Evidence for a stress response is circumstantial, as we did not measure plasma catecholamine levels. However, we did observe increased arousal of bears suspended in the net, consistent with increased sympathetic stimulation.

Changes in the blood gas parameters of anesthetized polar bears were largely caused by anesthesia with Telazol, and less so by restraint and suspension by net. The pattern of change in pH, PaO₂, PaCO₂, and arterial base excess in this study is similar to that observed in previous studies. Commonly, pH and PaO₂ values progressively increase, and PaCO₂ values progressively decrease, following anesthesia with Telazol (e.g., ≥ 15 min post-administration). However, arterial base excess remains relatively static throughout anesthesia such that the combined blood gas results indicate an initial, transient, mild respiratory acidosis. Less apparent, but also common between this study and previous studies, is large variation in blood gas values between individual bears. For example in this study, when comparing PaO₂ values of the arterial blood samples collected from each bear while suspended in the net, the values ranged from 55-137 mm Hg (7.3-18.3 kPa). When examined individually rather than as the mean PaO₂ value for eight bears, the blood gas responses of some bears indicated they were at risk of developing clinically significant hypoxemia following restraint and suspension by net (e.g., PaO₂ ≤ 60 mm Hg [8.0 kPa] for 3 out of 16 arterial samples collected).
In conclusion, our results indicate that most bears will be at risk of developing clinically significant acute hypertension following restraint and suspension by net as imposed in this study, whereas a smaller number of bears may be at risk of developing serious hypoxemia. We, therefore, recommend developing alternative methods of suspending anesthetized bears above the ground for purposes of research and management. It appears methods using minimal restraint may ultimately prove safer for bears, but this will not be established until newer methods are rigorously compared against existing methods. Finally, we suggest similar studies of cardiopulmonary response to restraint and postural change, in other captive and free-ranging wild species, may contribute to reducing anesthesia-related morbidity and mortality in wildlife research, management, and conservation.

ACKNOWLEDGMENTS

We gratefully acknowledge N. Campbell, C. Cassidy, M. Dyck, C. Hutchins, and C. Morran for their assistance in collecting data and analyzing blood samples. We thank the Churchill employees of the Manitoba Department of Natural Resources, particularly Wade Roberts and Jack Batstone, for their cooperation and assistance. This research was funded, in part, by operating grants from the Wildlife Health Fund of the Western College of Veterinary Medicine, the Manitoba Department of Natural Resources Research Fund, the U.S. National Science Foundation, and the National Science and Engineering Research Council of Canada. Further financial and logistic support was received from the Churchill Northern Studies Centre (1997 Northern Research Fund), the Churchill Regional Health Authority, the Medical Research Council of Canada, and the Keewatin Region Health Centre Laboratory.

LITERATURE CITED

Figure 1. Effect of restraint and suspension by net on arterial pressure and blood gases of eight anesthetized polar bears. Mean and standard error represented at each measurement time.
EFFECTS OF BACK-MOUNTED RADIO TRANSMITTERS WITH A SUBCUTANEOUS ANCHOR ON DAY-OLD MALLARD DUCKLINGS: PRELIMINARY RESULTS

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Abstract

Transmitters may have deleterious effects on wild ducklings which may make them more susceptible to mortality from starvation, chilling, exhaustion, predation and parasites. Captive studies report nil to little effect of transmitters but ducklings in those studies were maintained in ideal conditions. In contrast to other captive studies, this research suggests back-mounted radio transmitters with a subcutaneous anchor have a negative effect on duckling growth, behavior and survival.

Introduction

Waterfowl broods are difficult to monitor during their first 2 wk of life because of high mobility and low visibility. To overcome these obstacles, researchers rely on radiotelemetry as a tool in brood ecology. Most radiotelemetry studies on brood survival and duckling survival have employed radio-marked adult females with broods. In wild ducklings, brood cohesion can make it extremely difficult to distinguish duckling and brood mortality from brood abandonment. Therefore, more accurate measurements of duckling fate can be achieved by radio marking individual ducklings within the brood.

Transmitters may have deleterious effects on wild ducklings which may make them more susceptible to mortality from starvation, chilling, exhaustion, predation and parasites. Ducklings raised by wild mallards (Anas platyrhynchos) are often moved over land considerable distances within the first few days of life, they must forage for food and rely on the female for warmth. Studies of wild duckling survival have conflicting conclusions on the impact of back mounted radio transmitters. Some studies report little impact from the transmitters, while other studies report very high mortality. Sub-lethal effects of the transmitters may include sub-optimal growth which may reduce long-term survival of juveniles. If radio transmitters have an impact on duckling growth and survival, results of studies done on wild ducklings may be less meaningful. Conservation efforts will be more likely to succeed if they are based on more reliable knowledge.

Captive studies report nil to little effect of transmitters but ducklings in these studies were maintained in ideal conditions. Ducklings were housed without the female, were provided with ad libitum food and had access to an artificial heat source. Therefore, the purpose of this study was to determine the effects of transmitters applied to day-old ducklings by evaluating behavior and weight gain post-operatively in a semi-natural environment.
Materials and Methods

Five broods (36 ducklings) were hatched by adult female mallard ducks housed in outdoor pens at St. Denis Wildlife Refuge, Saskatchewan (52°13'N, 106°04'E). Females were housed individually in pens measuring approximately 3 × 10 m comprising of 70% brome grass and 30% artificial pond. Water was pumped from a nearby pond as needed to maintain water levels. Day-old ducklings were divided randomly into two matched groups where half the ducklings in each brood received back-mounted transmitters (1.8-2.0 g) with a subcutaneous anchor\(^{11}\) (transmitter group), and half the ducklings did not receive a transmitter but were handled for the same amount of time (control group). Anchors were placed subcutaneously through a 5-mm incision after injection of lidocaine at the surgical site. The incision was closed and the anchor was sutured in place with 4.0 silk. All ducklings had web tags placed after topical administration of lidocaine for individual identification.

Ducklings were weighed prior to transmitter attachment, daily for the first week and every 2 days thereafter for 30 days. To mimic natural environmental conditions where ducklings are moved over land by the female, food was restricted to 75% of energy requirements for duckling growth for the first 2 days and 110% for the remainder of the experiment. Access to an artificial pond containing invertebrates was not limited and ducklings were exposed to normal daily environmental conditions. All ducklings with radio transmitters and one randomly chosen control duckling from each brood were euthanatized humanely at end of experiment. Euthanatized ducklings and ducklings found dead had complete necropsies and histology. Results from necropsies will not be discussed.

Ducklings were observed or video recorded for a minimum of 30 min daily for the first week. Only observations done for the first week will be discussed. Behavior of each treatment group was recorded every minute as locomotion (walking or swimming), foraging, comfort behavior (preening, stretching), resting (sleeping, sitting or loafing) or out of sight (not visible to the observer). The behavior of the majority of ducklings for each treatment group was considered the behavior of that group. Each behavior was calculated as percent time spent performing the above behaviors as matched groups in each brood. In addition, every preening event was recorded and each event was separated by a different behavior. Preening was calculated as preening event per duckling/min.

Survival data were analyzed with a Fisher’s exact test. Duckling behavior (transmitter groups verses control groups) were analyzed using Wilcoxon matched-pairs signed ranks tests since distributions were non-normal. Duckling weights were analyzed using Student’s t test. A difference was considered significant at \( P \leq 0.05 \).

Results

Five ducklings (four females and one male) that received transmitters died and one female duckling in the control group died (Table 1). The mean time to death was 3.5 days. Deaths of ducklings in the
transmitter group was significantly greater than ducklings in the control group (Fisher’s exact test, 1-tailed, \( P = 0.0403 \)). Six ducklings from three broods escaped from the pens and were excluded from the study after escape. All ducklings lost weight following hatch. Female ducklings in both the transmitter and control groups took 6 days to gain weight to match or better hatch weight. At day 6, female ducklings in the transmitter group were 2 g lighter than ducklings in the control group. Male ducklings in the control group took 5 days to match or better hatch weight while ducklings in the transmitter group took 6 days. Female ducklings in the transmitter group weighed less than ducklings in the control group from day 1-30. There was no significant difference between hatch weight in females (Student’s t test, \( P = 0.2 \)). Male ducklings with transmitters were significantly heavier at hatch (Student’s t test, \( P = 0.05 \)) but were lighter than ducklings in the control group from day 9-30.

Ducklings with transmitters preened the surgical area more often (0.08 preening events/duckling/min) than ducklings without a transmitter (0.01 preening events/duckling/min Wilcoxon test, 1-tailed, \( P = 0.04 \)). Ducklings with transmitters were also observed shivering, and pulling on the transmitter body and antenna. The hen was observed stepping on the antenna, preventing ducklings with transmitters from moving on several occasions. Although no statistical difference was found in other aspects of behavior, some trends observed may become significant when sample size is increased. Ducklings with transmitters spent more time out of sight (12 %) and less time foraging (14 %) compared to ducklings in the control group (5 % and 17 %, respectively).

Discussion

In contrast to other captive studies, this research suggests back-mounted radio transmitters with a subcutaneous anchor has a negative effect on duckling growth, behavior and survival. Ducklings with transmitters had higher mortality, decreased weight gain, and preened more frequently than ducklings without transmitters. In addition, ducklings with transmitters spent more time out of sight and spent less time foraging. Ducklings with external transmitters show areas of increased surface temperature in thermographic images suggesting that heat loss may be increased in ducklings with transmitters.\(^2\) Thermal effects may be significant for ducklings because of small body size, limited thermogenic capacity, and relatively ineffective insulation.\(^2,9\) Transmitters may also disrupt the plumage\(^12\) thereby increasing thermoregulatory costs in cold, windy weather. Increased heat loss, demonstrated by shivering, may have resulted in hypothermia and death of the five ducklings that died with transmitters. In addition, ducklings with increased heat loss may not gain weight effectively. Ducklings may seek more sheltered areas of the pen which may have been demonstrated in more time spent out of sight. Energy requirements of maintaining body temperature may result in decreased foraging activity and thus lower body weights.

Although results from this study are preliminary, there appears to be some important survival growth, and behavioral changes of ducklings with radio transmitters compared with ducklings without transmitters. Further research is required to increase sample size. This study will be completed during the 1998 field season.

ACKNOWLEDGMENTS
This research was supported by the Canadian Wildlife Service, Delta Waterfowl Foundation, and the Wildlife Health Fund, University of Saskatchewan. I would also like to thank my supervisor Dr. Alex Livingston and Dr. Robert Brua for their assistance in writing this abstract. Special thanks to Lise Tellier for technical support during this study.

LITERATURE CITED

Table 1. Survival of mallard ducklings after placement of a radio transmitter with a subcutaneous anchor.

<table>
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<th>Treatment</th>
<th>Died</th>
<th>Lived</th>
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<td>12</td>
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</tr>
<tr>
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MALIGNANT FIBROUS HISTIOCYTOMA IN A SWIFT FOX (Vulpes velox)

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Abstract

An 8-yr-old, 3-kg male swift fox (Vulpes velox) housed at the Saskatoon Zoo was presented for examination of an ulcerated mass on the right hind leg. The fox was in good body condition and showed no lameness of the affected leg. The mass was on the medial aspect of the limb in the dewclaw area, and was firm and well circumscribed. Blood and urine samples, radiographs of both hind limbs and of the thorax, fine needle aspirates, impression smears and two wedge biopsies of the mass were taken under isoflurane (Isoflurane USP, Abbott Laboratories Limited, Montreal, Quebec, Canada) anesthesia. The mass was identified as a fibrosarcoma based on the biopsy results. Bloodwork showed mild abnormalities attributable to stress and chronic inflammation. No evidence of metastases was found radiographically.

Considering the location and behavior of this type of tumor (high rate of local recurrence after excisional biopsy), it was elected to amputate the affected limb. Following this, further histopathology of the tumor led to a revised diagnosis of malignant fibrous histiocytoma, giant cell variant, which was supported by immunohistochemistry. Electron microscopy results are pending.

A literature search found no reports of neoplasia in swift foxes, and only one report of malignant fibrous histiocytoma in a fox (genus was not specified). Canine cases have been reported, but this type of tumor appears to have a fairly low incidence in the pet dog population when compared to other soft tissue sarcomas. The diagnosis of malignant fibrous histiocytoma is particularly challenging and cannot be made by light microscopy alone, as much histologic overlap exists between different sarcomas and other neoplasms. Accurate diagnosis requires the use of more specialized techniques such as immunohistochemistry and electron microscopy. Malignant fibrous histiocytomas are locally invasive tumors with poor metastatic potential and a high rate of local recurrence after excision in domestic canids. Radical excision with wide margins of healthy tissue, cutting through bone if necessary, is recommended. Adjuvant radiation therapy or a second surgery should be considered following incomplete resection.

LITERATURE CITED

RECOVERY PLAN FOR THE VANCOUVER ISLAND MARMOT (*Marmota vancouverensis*)

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Abstract

The Vancouver Island marmot (*Marmota vancouverensis*) is one of the rarest mammals in the world and the only indigenous Canadian mammal to be designated as endangered. The total population is 150 or less and is limited to two small areas of Vancouver Island in British Columbia (B.C.). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) Recovery Plan for the Vancouver Island Marmot provides an overview of activities recommended by the national Vancouver Island Marmot Recovery Team to achieve down-listing of *M. vancouverensis* from its federal and provincial endangered status.

Factors contributing to the decline of marmot numbers appear to include weather, predation, disease, human activities, and reduced landscape connectivity associated with dispersal into harvested forest sites. The Recovery Team considers that down-listing of the species from endangered to threatened should not occur until a total population of 300-400 animals is established in at least two metapopulations. Captive breeding, combined with reintroductions into suitable habitat presents the best only option to increase marmot numbers to achieve this goal. Experience with captive breeding of other marmot species from western Europe and Asia provides evidence that this can be accomplished with limited technology. A Captive Breeding Plan has been developed and is an integral component of the Recovery Plan. The primary goals of the captive breeding program are to produce marmots for reintroduction into historic habitats, to maintain captive animals for genetic preservation, non-invasive research and for educational and promotional display. The program will receive direction from the conservation program under the guidance of the Recovery Plan and Recovery Team.
ARTIFICIAL BREEDING AND THE TIMING OF STANDING ESTRUS OF ARTIFICIALLY SYNCHRONIZED WAPITI (Cervus elaphus) ON FARMS

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Abstract

With a view to establishing the time of standing estrus in a commercial herd of wapiti (Cervus elaphus), estrus was synchronized a total of 47 times among 23 multiparous hinds over a 3-yr period. All hinds were multiparous females selected for entry into an artificial insemination (AI) program. Progesterone containing (9%) intravaginal devices (CIDR) were inserted on day 0 and the hinds were placed in a paddock with a vasectomized experienced stag. On day 14 the CIDRs were removed between 2000 h & 2100 h, and each hind was treated with PMSG. The stag was marked with raddle paint. The hinds were checked at dawn, mid-day and dusk for paint marking over the flanks or rump. Paint marking was taken as evidence of standing estrus. Insemination was carried out during a 60- to 63-hr period after CIDR removal. The overall conception rate as judged by ultrasonography was 74% but the calving rate was only 64%. Of the six twin conceptions only one came to term as twins, another as a single calf. The other four animals that had had twin conceptions did not carry to term and one of these has remained barren.

Introduction

Artificial breeding of wapiti (Cervus elaphus) in North America is now a commercial success. Several hundred inseminations have been carried out in the last 5 yr (Bringans, personal communication). Artificial insemination is used primarily in order to try and achieve larger sets of antlers in male progeny. For convenience and because estrus detection without teaser stags is difficult hinds are synchronized and time inseminated.1

There are no objective indicators of genetic quality in wapiti in North America, although sire referencing schemes have been developed in New Zealand in the conspecific, but much smaller, red deer.1 North American wapiti sires are selected solely on phenotypic characteristics. The main criteria in the market place are either the velvet antler weight and character, or the hard antler score. Both are judged in one of several continent-wide competitions sponsored by regional or provincial breeders associations and all coordinated under the umbrella of the North American Elk Breeders Association.

Materials and Methods
The criteria for selection into the program included a multiparous history, with no record of calving trouble, a quiet nature and ease of handling, and the desired genetic background. Hinds were also selected only if their body condition score was between 3 and 4 (scale 1-5; 1 being very thin, 5 being overfat). Each hind is reported as a different case for each year unless otherwise indicated.

To establish the time of standing estrus after synchronization in a commercial herd of wapiti, estrus was synchronized with a regime that involved the use of a device containing 9% progesterone into the vagina (CIDR, CHH Plastic Products, Hamilton, New Zealand) and the administration of PMSG at the time of CIDR removal. CIDRs were inserted on day 0 and the hinds were placed in a paddock with a vasectomized experienced stag. On day 14 the CIDRs were removed between 2000 h and 2100 h, and each hind was treated with PMSG (210 I.U. in year 1, 200 I.U. in year 2, and 190 I.U. in year 3).

Estrus was synchronized a total of 47 times among 23 multiparous hinds, 12 hinds in year 1, 16 hinds in year 2, and 19 hinds in year 3. All hinds were multiparous females selected for entry into an artificial insemination (AI) program.

As soon as the hinds had been treated and released back to their paddock, the stag had a mixture of automotive grease, engine oil and blue paint smeared over its ventral thorax, medial forelegs and anterior hind legs above the stifles. The animal was then returned to join the hinds. The hinds were checked at dawn, mid-day and dusk for paint marking over the flanks or rump. Paint marking was taken as evidence of standing estrus.

Insemination was carried out during the standard 60-63-hr period after CIDR removal. The dose of semen used was estimated to be about 20 million live sperm.

After insemination the hinds were held for 10 days in a paddock without a stag. They were then allowed to join a natural breeding group.

Conception was detected by ultrasonography in the period 45-64 days post insemination. However, the identity of the sire of each calf born to any hind that was inseminated was confirmed by DNA parentage testing.

Results

One hind was raddled within 15 hr of CIDR removal in 2 consecutive years. In year 1, it conceived to AI at 61 hr post CIDR removal despite having no detectable mucus in the vagina. In ten cases (21%) hinds were raddled between 39 and 46 hr after CIDR removal. In 30 cases (64%) hinds were raddled between 46 and 58 hr of CIDR removal. A total of five (11%) different hinds (one in year 1, two in each other year) showed no evidence of raddling.
When examined four of the hinds that lacked raddle marks showed no evidence of ovulation. The fifth one had good uterine tone and moderate amounts of cervical mucus. The animal was inseminated and conceived.

Overall conception, as judged by ultrasonogram data was 74%. In year 1, nine of 11 bred hinds conceived to AI, three of the conceptions being twin pregnancies. In year 2, 11 of 14 bred hinds conceived, three of these being twin conceptions. In year 3, 11 of 17 hinds conceived, one being a twin. Of the seven double conceptions, only two came to term as twins, another as a single calf. Of these two twin sets, one involved live female calves of 17 kg and 11.5 kg. The other twin pair of opposite sexes were born dead after they became trapped in the vagina together and were only discovered at 4:30 a.m. Delivery was assisted. The other four animals that had had twin conceptions did not carry to term.

Directly related to the pre-parturient losses seen in twin conceptions, the calving rate fell to 64%. DNA parentage testing revealed a mean gestation length of 247 ± 5 days.

Discussion

Not only is the detection of estrus in wapiti difficult and time consuming, but the use of raddle harnesses and other paint marking devices as used in the sheep and cattle industries has proved to be unsatisfactory. This may be due to the near vertical position taken by the stag at time of copulation and hence its failure to exert any downward pressure on the hind. However, during pre-copulatory low mounts, which are not always seen, there is considerable pressure exerted. The failure of conventional heat detecting devices may also be due to the fact that the heavy winter coat of wapiti, which is fully grown in by the advent of the breeding season, does not take raddle paints or chalk markers very well.

The conception rates achieved are a reflection of the average results obtained across North America (Bringans, personal communication). Best results have been as high as 100% conceptions in groups of up to 20 hinds.

It has been shown that during a period from 40-60 days after breeding measurement of fetal size and other features of the pregnant uterus can be used to accurately predict conception date within less than 2 days. The 10 day window therefore offers reasonable certainty of expected calving dates.

DNA data banks of specific named sires now exist in several laboratories in North America and New Zealand so that parentage confirmation is becoming more widely used in sales, both for buyer satisfaction and in some cases jurisdictional confirmation of purity.

The dose of PMSG was reduced in each of the three successive years of the program in order to try and counteract the unwanted incidence of twinning. Twinning is rare in wapiti and generally considered to be <0.5%. However there are instances of exceptionally high incidence of natural twinning on some wapiti farms (Bergen, personal communication). There is a potential for
freemartinism in different sex twins, but the risk does not appear to be great (Bergen, personal communication).

In the one pair of live-born twins seen in this study one animal was markedly lighter than the other. Normal birth weights for single wapiti calves are about 18 kg. Calves born under 11.4 kg in weight have a less than 50% chance of survival, and calf weight at birth has a direct relationship with weight at 4 wk and at weaning. The smaller twin was successfully artificially reared.

The fact that four animals did not carry to term can be considered a distinct disadvantage from a commercial standpoint. One of these hinds was culled after remaining barren for 2 yr.

LITERATURE CITED

A PROTECTED CONTACT APPROACH TO ANESTHESIA AND MEDICAL MANAGEMENT OF AN ASIAN ELEPHANT (Elephas maximus)

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Abstract

Introduction

Invasive medical and surgical treatment of elephants managed by protected contact is a difficult and complex undertaking. Protected contact (PC) implies that the animal and handlers remain safe during routine husbandry procedures and access to the animal is restricted. Approximately half of the North American captive elephants are in a PC or voluntary contact program. When intensive medical or surgical procedures are required on PC animals, a successful outcome depends largely on the consistency of cooperation and access.

Foot abscesses are common in captive elephants: in one study 50% of captive elephants had a foot related medical problem. Gage4 outlined a protracted course of treatment followed by surgery and then post operative care required by an elephant with phalangeal osteomyelitis. However, the animals that have been treated by these intensive methods are tractable, free contact elephants1,2. This paper describes the management of pododermatitis comprising of 3 yr of medical treatment, including two general anesthetics in a PC elephant.

Case Study

Ganesha, a 16-yr-old, 5,000 kg male Asian elephant (Elephas maximus) at the Calgary Zoo, developed a fistulous draining tract associated with the left fore second digit. This toe had been treated 5 yr previously at another institution before the animal was translocated to Calgary. Purulent discharge was evident from a solar defect with associated onychitis and paronychitis above the affected toe. Almost 1.5 yr of conservative treatments followed. Access to the affected foot was through a metal mesh welded wall with a “foot door” (8 cm × 92 cm, 79 cm from the floor).

Periodic survey radiographs of the left fore were obtained with a Mini X Ray 300 (MinXray Inc., Evanston, Illinois USA) set at 60 Kvp & 1.0 sec for A/P views and 80 Kvp & 1.0 sec for lateral views. A contrast agent (Omnipaque300, Sanofi/Winthrop, Markham, Ontario, Canada L3R 6H3) was used to help define the extent of the tract and monitor bone involvement. The right fore foot was also radiographed as a comparative normal. An attempt at sonography was unsuccessful in further defining the infection.
The toe continued to deteriorate clinically so an intensive flushing regime was started. The tract was irrigated and flushed (s.i.d. - t.i.d.) with antibiotics based on cultures and sensitivities. Treatment sessions lasted 10 to ≥ 70 min and occurred from one to three times a day prior to the first surgery. Despite this intensive medical treatment, serial lateral and anterior/posterior radiographs indicated a progression of the osteolytic infection involving the second phalange.

At this point, a decision was made to attempt surgical curettage. Due to the animal’s size, the expected duration of surgery and our inability to ensure that the elephant was properly positioned during anesthesia, a system of air cushions (Jumbo Lift International Air Cushion Systems) was set up to support the animal. An immobilizing dose of 10 mg carfentanil (Wildnil, Wildlife Pharmaceuticals Canada, Inc., RR1, Callander, Ontario, Canada P0H 1H0) was given intramuscularly by restraining the elephant in a hydraulic elephant hugger.

To debride the draining tract, a solar surgical approach was performed. Post surgical management required the animal to wear a protective boot which was designed by the elephant keepers and constructed at a local tent and awning manufacturer. Once or twice daily bandage changes, as well as periodic radiographs and microbiologic monitoring lasted 14 mo before a second surgery was performed.

The second surgical approach was via an incision above the nail to allow an enbloc resection of the distal third of the second phalanx. Intra operative hemorrhage was controlled by elevating the limb with the aid of an overhead hoist. Post operative infections delayed healing in both cases.

Routine complete blood counts and full biochemical profiles were collected on a regular schedule throughout this period. The elephant presented its ear through an “ear door” (25 cm × 92 cm, 135cm from the floor) in the mesh wall. Ear access also facilitated i.v. injections for antibiotics or sedatives. Occasionally, usually during musth, sedatives were used to perform very painful or invasive treatments such as removing excess granulation tissue. Standing sedation with intravenous xylazine (Rompun Bayer Inc., Agriculture Division, Animal Health, Etobicoke, Ontario, Canada M9W 1G8) was required three times to facilitate debridement and flushing of the tract. Careful titration of the xylazine dose (33-72 µg/kg) was required so that it would leave its foot on the foot plate and not become so sedate as to be unresponsive to verbal commands. Partial reversal with atipamezole (Antisedan, Orion Corporation, Orion-Farmos, Espoo, Finland) at 8-14 µg/kg made the animal more responsive in cases of heavy sedation.

Discussion

The elephant was target trained by bridging and rewarding desirable behaviors and ignoring unwanted behaviors. “Time outs” were effective when the animal stopped responding to commands in a positive manner. The animal learned to approach the open door, turn and put whichever foot was requested through the foot door opening onto a flat metal stand for treatment. Various types of stands were used to facilitate different approaches to the foot. Although obviously painful, most treatments
were accomplished simply by positively reinforcing the desired behavior: leaving its foot out on the
treatment stand.

This conditioning process was initiated to ensure the animal’s cooperation in or out of musth,
throughout painful and sometimes lengthy treatments. The animal tolerated flushing, debriding,
packing, antibiotic infusion and soaking of this foot. From time to time, the elephant did kick at staff
and swipes with its trunk through the foot door did occur.

Radiographs were accomplished by conditioning the animal to place its foot on a piece of plywood
the size of our radiograph plates. When the animal could be trusted to place its foot down gently, not
crushing the plywood in the process, we attempted both lateral and A/P views. Introduction of the
radiograph machine did not require any special conditioning. We had our portable machine mounted
on a trolley for easy maneuvering. When radiograph sessions became routine, ultrasound examination
was added.

A decision to open the foot surgically to drain and debride the abscess meant we had to ensure we
could protect the foot postoperatively from environmental contamination. A sterile bandage covered
by a protective boot kept the foot clean and dry. The elephant learned to accept the boot by carefully
conditioning the animal to increasing amounts foreign material attached to its foot. Initially, a cloth
anklet was rubbed behind its foot, then attached to its ankle until finally the full slip-on boot fastened
with three seat belts behind the carpus was accepted. During the conditioning process, the keepers
made a point of prodding the foot with an ankus so that the animal would become tolerant of pain
associated with this foot. This conditioning was accomplished just prior to the first surgery.

To ensure proper support of as much of the elephant’s dependent side as possible during general
anesthesia, preplacement of the air bags was required. This was accomplished after induction and just
prior to sternal recumbency. Once laterally recumbent, the bags were inflated and surgery started.
Upon recovery the air bags and support belts had to be removed once the animal had moved off of
them without leaving them long enough for to be destroyed. Ideally, we should have conditioned
the elephant to walk into the hugger over the top of these deflated bags to ensure full coverage of the
animal’s down side. No attempt was made to accomplish this, although it is probably possible.

Intensive treatment, surgery and painful postoperative care in elephants are possible in a protected
contact management system. Several factors contributed to our success: attention to positive
reinforcement of desired behaviors, a slow increase of noxious stimuli and dedicated keeper staff.
Although “surgery is difficult and the aftercare horrendous”3 it is not necessary to omit any treatment
modality from an ideal regime. Successful management of PC animals ensures that all captive
elephants have access to the same quality of medical care.

LITERATURE CITED


ANESTHESIA OF WOOD BISON (Bison bison athabascae) WITH MEDETOMIDINE-TELAZOL AND XYL AZINE-TELAZOL COMBINATIONS

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Abstract

The study was designed to determine the efficacy and physiologic effects of two potentially useful combinations for the immobilization of bison. Medetomidine-Telazol® (MZT) is a potent, low volume solution that may be a good choice for immobilization of free ranging bison. Xylazine-Telazol® (XZT) is a higher volume solution that may be more effectively used on captive or game-farmed bison. Seven male wood bison (Bison bison athabascae) received 1.5 mg/kg of xylazine + 3 mg/kg of Telazol®, and on another occasion 60 µg/kg of medetomidine + 1.2 mg/kg of Telazol®. Both combinations produced effective immobilization. Induction was significantly faster with XZT; recovery was significantly faster with MZT. Quality of immobilization was good with both treatments. Blood pressure was significantly higher with MZT. The major complication was hypoxemia which occurred with both combinations.

Introduction

Bison are becoming increasingly popular as a game farmed species. Bison must be immobilized for a variety of reasons, and can be a difficult animal to work with. Historically muscle relaxant drugs, such as succinylcholine were used to produce immobilization. Humane aspects and low therapeutic index limit the acceptability of this drug. High doses of xylazine have been advocated, this often fails to immobilize the animal and can result in bloat, regurgitation, and other complications Telazol® has been used, but it can result in rough inductions and recoveries. Medetomidine-ketamine immobilization has not been reported in North American bison but has been reported in Wisent (Bison bonasus).6 Potent narcotics, such as carfentanil, will produce effective immobilization of free ranging bison.4,7 Many wildlife managers and veterinarians are unwilling to use this drug due to safety concerns.

The following paper details results of a study designed to determine the efficacy and physiologic effects of two potentially useful combinations. Medetomidine-Telazol® (MZT) is a potent, low volume solution that may be a good choice for immobilization of free ranging bison. Xylazine-Telazol® (XZT) is a higher volume solution that may be more effectively used on captive or game-farmed bison, where small, accurate darting systems are less critical.
Materials and Methods

This study was performed during February of 1998, in Elk Island National Park, Alberta, Canada. Seven male wood bison were used in the study (five 3-yr-old, one 4-yr-old, and one 5-yr-old). The average weight of the bison was 413 ± 35 kg (386-462 kg). Bison were fasted for at least 24 hr prior to drug injection. Each bison received both combinations in random order. The treatments were administered 1 wk apart to allow for drug metabolism and excretion. The drug solutions were prepared as follows. For XZT 2.5 ml of xylazine was added to a vial of Telazol®, the resulting solution had a volume of 2.8 ml and contained approximately 89 mg/ml of xylazine and 178 mg/ml of Telazol®. For MZT 2.5 ml of 10 mg/ml medetomidine solution was added to a vial of Telazol®, the resulting solution contained approximately 8.9 mg/ml of medetomidine and 178 mg/ml of Telazol®.

On the day of the study the bison was moved through a handling facility into a hydraulic chute. The bison was weighed, and received either 1.5 mg/kg of xylazine + 3 mg/kg of Telazol®, or 60 µg/kg of medetomidine + 1.2 mg/kg of Telazol®. The drugs were administered by hand injection into the gluteal muscle mass. Following injection the bison were released from the chute and moved into a holding pen. The time from injection to sternal recumbency and head down were recorded. Once it was safe to move into the pen the bison was restrained in lateral recumbency. A 20-ga, 5-cm catheter was placed in the saphenous artery for pressure measurement and arterial blood sampling. The catheter was connected, with non-compliant tubing, to a Baxter® pressure transducer. The transducer was connected to a Propaq 104 EL® physiologic monitor. ECG leads were placed in a three lead axis, and a lead II ECG was constantly monitored. Body temperature was measured rectally with a digital thermometer. Heart rate (HR), respiratory rate (RR), body temperature (temp) and mean arterial pressure (MAP) were recorded every 5 min. PaCO2, PaO2, pH, and base excess (BE) were measured with a blood gas analyzer at 15, 30, 45, and 60 min post injection. Blood gas samples were stored on ice, and analyzed within 3 hr from collection. Samples were corrected for hemoglobin concentration and body temperature.

Physical qualities of immobilization, incidence of ruminal tympany and regurgitation were also noted. At 60 min post injection equipment was removed from the animal and alpha-2 antagonist agents were administered. Atipamezole was administered, at a dose of 90 µg/kg i.v. and 90 µg/kg i.m., to antagonize medetomidine. Tolazine was administered, at a dose of 1.5 mg/kg i.v. and 1.5 mg/kg i.m., to antagonize xylazine. The time from antagonist administration to sternal recumbency and standing was recorded.

Results

A comparison of HR, RR, MAP and temp can be found in Figure 1. A comparison of pH, PaO2, PaCO2 and BE can be found in Figure 2. Hyperpnea was present with both combinations, RR was
significantly lower with XZT. RR did not change significantly over time. HR was not significantly different between treatments and did not change significantly over time. MAP was significantly lower with XZT, and did not change over time with either treatment. Temp was not significantly different between treatments and did not change over time. Arterial pH was not significantly different between treatments. Arterial pH increased significantly over time with both treatments. Both treatments produced some hypoventilation, evidenced by increased PaCO₂. PaCO₂ was significantly higher with XZT. PaCO₂ increased significantly over time during immobilization with MZT. Both combinations produced hypoxemia (PaO₂ < 60 mm Hg) throughout the immobilization. PaO₂ decreased over time with both treatments. The lowest mean PaO₂ produced by MZT was 46.9±7.6 mmHg at 45 min post injection. The lowest mean PaO₂ produced by XZT was 44.4±5.3 mmHg at 30 min post injection. BE was not significantly different between treatments, and increased significantly over time with both treatments.

Immobilization data can be found in Table 1. Induction was significantly faster with XZT, recovery was significantly faster with MZT. Quality of immobilization was good with both treatments. XZT appeared to be slightly shorter acting, with some animals demonstrating ear and limb movement at 55 min post injection. Mild ruminal tympany was present with both treatments. One animal regurgitated 16 min post reversal with tolazoline. The average drug volume used was 7 ml with XZT and 2.78 ml with MZT.

Discussion

Both of these combinations should prove to be useful for immobilization of bison. The major complication of immobilization was hypoxemia. Hypoxemia is common in ruminants anesthetized with medetomidine based protocols. Hypoxemia has also been noted during xylazine sedation or anesthesia in ruminants. Two other factors probably contributed to hypoxemia. The animals were restrained in lateral recumbency, this was done to facilitate instrumentation of the animal. Sternal recumbency is a preferable position, and should result in less hypoxemia. Ruminal tympany probably contributed to hypoxemia. Hypoventilation was present with both combinations, but was not severe (PaCO₂ was always < 60mm Hg). Hypoventilation tended to increase over time with MZT, perhaps as a result of ruminal tympany. Since hypoventilation was not severe the major cause of hypoxemia was probably ventilation-perfusion mismatch. Increased ventilation-perfusion mismatch has been noted in sheep anesthetized with medetomidine-ketamine. Hypoxemia should respond to supplemental inspired oxygen, and supplemental oxygen is recommended with both of these treatments. When possible, the animal should be maintained in sternal recumbency, to decrease ventilation-perfusion mismatch and facilitate oxygenation.

Blood pressure was significantly higher with MZT. Medetomidine is more potent and selective for the alpha-2 receptor than xylazine. The increased blood pressure is most likely the result of peripheral alpha-2 receptor activation, produced by medetomidine. BE and pH were both low in the early stages of immobilization. These values probably reflect increased muscle activity in the bison prior to immobilization. The animals were agitated at being handled and restrained in the chute. The decreased pH and BE may also have been the result of poor tissue perfusion early in the
immobilization period. BE and pH improved over time as a result of decreased muscle activity, or increased perfusion. There was no respiratory compensation over time, in fact, the animals were experiencing a respiratory acidosis from the increase in PaCO₂.

Body temperature did not change over time. The mean temperature over time was 40.2°C with MZT and 40.3°C with XZT. This probably is a slight increase in temperature for this species and may reflect increased activity and stress during handling.

Ruminal tympany was never severe, nor was regurgitation a problem. In an unfasted animal both of these complications have the potential to be more severe. Physical characteristics of immobilization suggest that XZT may be more useful for immobilization of game farmed or captive animals. Currently XZT would be the most economic combination, using our formulation it needs to be delivered at a relatively high volume, necessitating the use of larger, less accurate darts. Recovery is also more prolonged with XZT, this would not be a serious problem in a confined animal, but free ranging animals may be at increased risk of predation. MZT can be delivered in a low volume, induction time is significantly lower than with XZT, but should still be suitable for most situations, recovery is significantly quicker than with XZT. Small volume and rapid recovery makes this combination more attractive for free ranging animals. The animals used in this study were free ranging animals that were acutely confined for the purpose of this study, and were unaccustomed to handling. Induction time may be shorter in game farmed animals that are more accustomed to human manipulation. Antagonists were administered half i.v. and half i.m. Several animals experienced full recoveries in less than 1 min. Both atipamezole and tolazine are active i.m., and i.v. administration is probably not advisable unless a safe location can be reached in less than 1 min.

Conclusions

Both of these combinations will produce effective immobilization of bison. The major complication is hypoxemia. Supplemental oxygen is recommended, and maintenance in sternal recumbency should also be of some benefit, when possible. Recovery can be very rapid if the antagonists are administered i.v., particularly following atipamezole administration. Intravenous administration of the antagonist should only be considered if a safe location can be reached quickly.

ACKNOWLEDGMENTS

We thank Saskatchewan Agricultural Development Fund for funding this study. We also thank Rob Kaye, and Ron Larson (Park Wardens), the maintenance staff of Elk Island National Park, Dr. M.J. Limojes, and M. Read for assistance with this study. We thank the staff of the Fort Saskatchewan Hospital for assistance with the laboratory work, and we thank Orion Pharmaceuticals for the medetomidine and atipamezole used in this study.

LITERATURE CITED
Table 1. Comparative immobilization features of medetomidine-Telazol (MZT) anesthesia with atipamezole reversal, and xylazine-Telazol (XZT) anesthesia with tolazoline reversal, in wood bison.

<table>
<thead>
<tr>
<th>Feature (min)</th>
<th>MZT+Atipamezole</th>
<th>XZT + Tolazoline</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>Induction time - sternal</td>
<td>7.5</td>
<td>2.11</td>
<td>4.1</td>
</tr>
<tr>
<td>Induction time - head down</td>
<td>8.8</td>
<td>2.10</td>
<td>5.5</td>
</tr>
<tr>
<td>Recovery time - sternal</td>
<td>1.6</td>
<td>0.94</td>
<td>5.6</td>
</tr>
<tr>
<td>Recovery time - standing</td>
<td>1.7</td>
<td>0.82</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Paired t-test used to compare immobilization data of seven wood bison. Statistical significance at $P < 0.05$ indicated by asterisk.
Figure 1. Comparison of physiologic responses of seven subadult wood bison during anesthesia with MZT, and during anesthesia with XZT. Means and standard deviation bars are presented for wood bison anesthetized with MZT ("), and with XZT (!). Significant differences ($P < 0.05$) between drug treatments at specific time points are indicated by an asterisk (*).
Figure 2. Comparison of blood gas responses of seven subadult wood bison during anesthesia with MZT and during anesthesia with XZT. Means and standard deviation bars are presented for wood bison anesthetized with MZT (*) and with XZT with (!). Significant differences (P < 0.05) between drug treatments at specific time points are indicated by an asterisk (*).

REPEATED IMMOBILIZATION OF A GREVY’S ZEBRA (Equus grevyi)
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Abstract

A 4-yr-old, female, Grevy’s zebra (Equus grevyi), weighing an estimated 350 kg was immobilized five times in a 6-wk period to manage a traumatic carpal joint injury. In all situations the zebra was fasted 12-24 hr and was active and alert with good body condition. Immobilization took place in a 6 x 10 m wooden stall. Ambient temperatures ranged from 15-21°C, while humidity ranged from 50-90%. The animal was premedicated with detomidine HCl (Dormosedan, Pfizer Inc., West Chester, Pennsylvania 19380, USA) administered i.m. via blowdart (Telinject, Saugus, California 91350, USA), followed by induction with carfentanil citrate (Wildnil, Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado 80524, USA) administered i.m. also by blowdart. Naltrexone (Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado 80524, USA) was given divided i.v. and i.m. by hand for reversal.

The average detomidine dose used was 13 mg. Carfentanil was given an average of 21 min after detomidine at a dose of 12 mg in all instances. The average time to recumbency following carfentanil administration was 13 min. The naltrexone dose used was 600 mg i.v. plus an average of 510 mg i.m.. The average time to standing after naltrexone administration was 4 min. The included table details the results of the repeated immobilizations.

There are several reports in the literature involving immobilization of Grevy’s zebra with variable results.1-4 This report describes an immobilization protocol that was used repeatedly over a short period of time with excellent results.

LITERATURE CITED

Table 1. Summary of repeated immobilization of a Grevy’s zebra.

<table>
<thead>
<tr>
<th>Date</th>
<th>Detom. Dose (mg)</th>
<th>Carf. Dose (mg)</th>
<th>Interval between D &amp; C (min)</th>
<th>Time to Recumbency</th>
<th>Naltrex. Dose (mg)</th>
<th>Time to Standing (min)</th>
<th>Duration of Recumbency (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 Feb</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>9</td>
<td>600 i.v. 600 i.m.</td>
<td>5</td>
<td>104</td>
</tr>
<tr>
<td>22 Feb</td>
<td>10</td>
<td>12</td>
<td>33</td>
<td>14</td>
<td>600 i.v. 600 i.m.</td>
<td>3</td>
<td>66</td>
</tr>
<tr>
<td>1 Mar</td>
<td>15</td>
<td>12</td>
<td>17</td>
<td>14</td>
<td>600 i.v. 450 i.m.</td>
<td>5</td>
<td>65</td>
</tr>
<tr>
<td>15 Mar</td>
<td>15</td>
<td>12</td>
<td>23</td>
<td>11</td>
<td>600 i.v. 450 i.m.</td>
<td>4</td>
<td>63</td>
</tr>
<tr>
<td>30 Mar</td>
<td>15</td>
<td>12</td>
<td>20</td>
<td>19</td>
<td>600 i.v. 450 i.m.</td>
<td>4</td>
<td>69</td>
</tr>
<tr>
<td>Average</td>
<td>13</td>
<td>12</td>
<td>21</td>
<td>13</td>
<td>600 i.v. 510 i.m.</td>
<td>4</td>
<td>67</td>
</tr>
</tbody>
</table>
CHRONIC LYMPHOCYTIC ENTERITIS ASSOCIATED WITH WASTING AND COPPER DEFICIENCY IN INTENSIVELY MANAGED MUSKOXEN (Ovibos moschatus)

John E. Blake, DVM, MVSc, Janice E. Rowell, BS, MS, PhD, and Lans Teal BS

1Institute of Arctic Biology, PO Box 757000, University of Alaska Fairbanks, Fairbanks, AK 99775 USA; 2Musk Ox Development Corporation, PO Box 587, Palmer, AK 99645 USA

Abstract

From 1990 through 1994, a captive herd of musk ox (Ovibos moschatus) experienced substantial losses in all age and sex groups. Adults showed two primary patterns of mortality: sudden death without warning or within 24-72 hr of recumbency, and a chronic wasting syndrome. The principal necropsy findings in the acute cases were an enlarged, pale liver and a chronic lymphocytic enteritis with villous atrophy. Livers were small and dark in the chronic form. Liver copper levels were between 1 and 8 ppm. Dietary changes and discontinuation of an in-feed anticoccidial medication resulted in an improvement in condition of the herd and a substantial reduction in mortality.

Case Report

From 1990 through 1994, the Musk Ox Farm in Palmer, Alaska experienced substantial losses in all age and sex groups. Adults showed two primary patterns of mortality: 1) A sudden death syndrome which frequently presented as unexpected death with no prior clinical signs, or sudden-onset recumbency followed by death within 24-72 hr. These animals were generally in good body condition with abundant internal body fat and an enlarged, pale liver. There was a distinctive clustering of these cases in the late summer and early fall. 2) A chronic wasting syndrome characterized by progressive weight loss for 6-18 mo, sometimes with a brief recovery period. These animals usually showed facial hair loss, dry brittle discolored hair coats, and periodic drooling. Dead animals had abundant body fat but showed marked muscle wasting. The liver in these animals was dark and slightly smaller than normal.

Both types of mortality had the following common pathologic features: a chronic lymphocytic enteritis with villous atrophy, atrophy of the exocrine pancreas, splenic hemosiderosis, abundant internal body fat, loss of alveolar bone with tooth root abscesses, and extremely low liver copper levels (1-8 ppm). An aggressive plan of diagnostics was implemented with a careful analysis of herd records. Bacteriologic, virologic, and parasitologic investigations were unrevealing. Affected animals showed mild anemia and haptoglobin was not detected in serum of affected animals. Serum copper levels were not indicative of tissue levels. Ceruloplasmin levels were not evaluated. By fall 1993 over 95% of the herd showed symptoms of the chronic wasting syndrome. Although, animals dying suddenly in early fall were considered at necropsy to be in good body condition, herd records indicated that poor weight gains and slight declines occurred in all but one animal during the summer,
a time of maximal weight gain in muskoxen. In addition to adult losses the herd was experiencing high losses due to abortion, stillbirth and calf mortalities.

**Discussion**

We believe that the different clinical presentations in the adults represent various nutritional deficiencies that were secondary to the chronic enteritis. Copper deficiency and a protein losing enteropathy were identified in these animals but multiple vitamin deficiencies may also have been present. Although a definitive etiologic agent was never identified we propose that the intestinal lesion was the result of a chronic irritant.

Management changes included: 1) an overall reduction in quantity of pelleted feed; 2) pelleted feed offered three times weekly instead of everyday; 3) a larger amount of browse was offered starting in summer 1994; 4) decoquinate (Decoxx R, Rhone-Poulenc, Atlanta, Georgia USA), an anticoccidial drug incorporated into this herd’s pelleted ration (0.02%) in 1990, was removed on November 1, 1993.

The overall herd condition improved over the summer of 1994 and winter 1995. Starting in 1996, the annual adult mortality rate decreased to less than 1%. Unfortunately, too many management changes were implemented to determine which was most effective. However, based on experience with muskoxen at the Institute of Arctic Biology, we suspect that the reduction in quantity and frequency of feeding pellets coupled with the increase in browse was insufficient to produce such a dramatic change in herd health. The chronic feeding of decoquinate is more likely to have induced the changes seen. Long-term feeding is not recommended by the manufacturer.

A similar problem to the sudden death pattern described above occurred in spring 1991 at the Large Animal Research Station, Institute of Arctic Biology. Two male muskoxen died unexpectedly following a 2-day course of illness. These animals had mild-moderate muscle wasting, abundant internal body fat, and a markedly enlarged, pale liver. There was marked lipid accumulation within hepatocytes and bile stasis. Liver copper levels were 1 and 2 ppm. Unlike in the Farm herd, there was no enteritis in either of these animals. Pasture and hay trace mineral analyses were not available for that time period. However, subsequent analyses in 1994 indicated that the pasture housing these two animals becomes severely deficient in copper and cobalt by mid-August and the hay obtained from the Fairbanks/Delta Junction areas was equally deficient. For these two animals we suspect that a primary nutritional deficiency occurred with resultant lesions closely resembling ovine white-liver disease. Unfortunately elemental cobalt levels are of little value and samples were not available for vitamin B12 or metabolite analyses.

In conclusion, these disease investigations emphasize the importance of careful dietary management in captive muskoxen. Additionally, this work suggests that both primary and secondary copper deficiency can occur in captive muskoxen. Although requirements are unknown, both muskox facilities currently feed 35-45 ppm of copper in their pelleted rations. Specific dietary manipulations are underway to better evaluate copper requirements in this species.
GROWTH IN TWO EARLY-WEANED BLACK AND WHITE RUFFED LEMURS (Varecia variegata variegata)†

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†Information presented and published in the 1998 Proceedings of the Comparative Nutrition Society, No. 2, August 1998. Copyright 1998 Comparative Nutrition Society. All rights reserved.

Abstract

Growth rates of two infant hand-reared black and white ruffed lemurs (Varecia variegata variegata) were measured from 32 days until 5 mo of age. The diet consisted of pureed pumpkin, banana, grape juice and high-fiber primate biscuits. Body weights steadily increased throughout the whole feeding period. The overall average daily gain for each lemur, from day 32 to day 122, was 23.9 g and 21.3 g respectively. The weight gains obtained were comparable to those previously published for this species.

Introduction

The propagation of the black and white ruffed lemur (Varecia variegata variegata) in captivity has been very successful. Between July and December 1997, 70 black and white ruffed lemurs were born in a managed captive breeding program. The current population of ruffed lemurs exceeds 500 animals in more than 120 institutions worldwide. A majority of the infant black and white ruffed lemurs born into captivity are parent-reared. This paper describes a management situation that involves the hand-rearing of two 32-day-old female orphaned black and white ruffed lemurs. Originally triplets were born, one of which died shortly after birth. Documentation will provide the ages and weights of the surviving two infants as they are introduced to a soft-food diet at an age of 32 days, and then later switched to an adult diet that consists of primate biscuits and pieces of different fruit.

Methods

The 32-day-old female orphaned ruffed lemurs were housed together in one outdoor enclosure (wire-mesh and plywood shelter) at St. Catherines Island, Midway, GA from May-September 1997. The total enclosure measured 7.01 m long, 2.31 m high and 1.22 m wide, and provided the animals with both an indoor (length: 2.44 m; height: 2.31 m; width: 1.22 m) and an outdoor environment. The infants were fed a diet consisting of pureed pumpkin, banana, grape juice and high-fiber primate biscuits (Table 1). The four ingredients were mixed in a food processor and the resulting paste was rolled into balls with a diameter of 2 cm. Dry primate biscuits were also provided with the daily
ration. The diet was offered ad libitum, and fed for a total of 142 days, after which time the lemurs were switched to a primarily primate biscuit diet. The ration was divided into 2.5 servings for the first 2 mo. Two servings were offered, one in the morning (0900 hr) and another in the afternoon (1500 hr), and half a serving was provided at noon (1200 hr). Body weights were collected prior to being placed on the soft-food diet. Because animals were consuming the diet, and by visual observation appeared to be healthy and alert, they were not handled (weighed) until 30 days after the initial body weight collection. Thereafter, they were regularly weighed every 2-5 days. The soft-food diet was gradually discontinued when the lemurs were 142 days old, and from this point on they were consuming a solid-food diet or adult diet.

**Results**

The initial body weights taken at day 32 were considered normal for this species, 361 g (ID# 398) and 301 g (ID# 323). After 34 days consuming this diet, the animals weighed 700 g and 640 g, respectively (Fig. 1). The daily average weight gain during this period was 10 g for each lemur. Thereafter, daily weight gain ranged from a maximum of 80 g to no weight gain at all (Fig. 2). Their body weights steadily increased throughout the whole feeding period (Fig. 1). The overall average daily gain from day 32 to day 122 was 23.9 g (ID# 398) and 21.3 g (ID# 323- Fig. 2). The body weights at age 122 days were 1.9 kg and 1.7 kg respectively. One of these females (ID# 323) died of causes unrelated to nutrition after the data collection period. The other one is still alive at St. Catherines Island, Midway, GA. Although no feed intake was measured, palatability and acceptance did not seem to be a problem.

**Discussion**

Comparative data for hand-reared ruffed lemurs are available, but it appears that most of these data were obtained when ruffed lemurs were fed a milk substitute diet. The objective of these studies was the comparison of mother-raised vs. hand-raised and this may explain the focus on use of milk substitutes. The weight gains obtained at St. Catherines Island Wildlife Survival Center were comparable to those previously published for this species. Meier and Willis established a 15 g/day gain between 1-4 mo of age, when hand-reared ruffed lemurs were fed a formula consisting of whole cow milk and human infant rice cereal. The average daily weight gain for this study, during that same period, was higher (22.6 g/day).

These data will contribute to the knowledge on hand-rearing lemurs, and may also present an alternative feeding option for young black and white ruffed lemurs.

**LITERATURE CITED**

Table 1. Ingredients in soft-food diet (by weight) fed to orphaned black and white ruffed lemurs (Varecia variegata variegata) from 1-5 mo of age.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent of diet (%) by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-fiber primate biscuit¹</td>
<td>55</td>
</tr>
<tr>
<td>Red grape juice - whole</td>
<td>13</td>
</tr>
<tr>
<td>Banana, peeled</td>
<td>15</td>
</tr>
<tr>
<td>Pureed pumpkin</td>
<td>17</td>
</tr>
</tbody>
</table>

¹HMS high-fiber primate biscuit; Bluffton, Indiana USA.

Table 2. Calculated nutrient composition of soft-food diet, fed to early-weaned black and white ruffed lemurs (Varecia variegata variegata).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>43.4</td>
</tr>
<tr>
<td>Crude Protein (DM basis)</td>
<td>23.7</td>
</tr>
<tr>
<td>Fat (DM basis)</td>
<td>4.6</td>
</tr>
<tr>
<td>Soluble Carbohydrates (DM basis)</td>
<td>46.9</td>
</tr>
<tr>
<td>Calcium (DM basis)</td>
<td>.88</td>
</tr>
<tr>
<td>Phosphorus (DM basis)</td>
<td>.62</td>
</tr>
<tr>
<td>Ash</td>
<td>6.3</td>
</tr>
<tr>
<td>Acid-detergent Fiber</td>
<td>18.5</td>
</tr>
</tbody>
</table>
Figure 1. Body weights (g) of two orphaned ruffed lemurs (*Varecia variegata variegata*) fed the previously described diet from 32-122 days of age.

Figure 2. Daily weight gain (g) of two orphaned black and white ruffed lemurs (*Varecia variegata variegata*) fed the previously described diet at an early age.
PREVALENCE OF INTESTINAL PARASITES AND HEMOPARASITES IN VARIOUS Amazona spp. DESTINED FOR RELOCATION IN GUATEMALA

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Abstract

Introduction

An integral component of contemporary wildlife conservation is the relocation of threatened or endangered species. There are several important considerations for a successful reintroduction program. One component is that relocated populations be free of pathogens. A relocated population may introduce pathogenic infectious agents that could threaten the health of the wild population of the same or a different species.1,5,6,8,9,17 As the result of introduction of novel or emerging diseases, epizootics can cause complete or severe decimation of a population.1,5 Diseases can also have subcatastrophic effects such as reduced survival and reproduction as well as increased susceptibility to predators or environmental stress.1 The threat of epizootics caused by relocations is ever present but economic resources often limit preventative measures. A concerted effort must be made to evaluate the health status of the relocated populations prior to release.

Methods

Approximately 300 Amazon parrots (Amazona autumnalis autumnalis, Amazona ferinosa guatemala, Amazona albifronsus albifronsus, and Amazona xantholora) were confiscated by government officials from poachers, and assigned for rehabilitation by a non-governmental organization, Associacion de Rescate y Conservacion de Animales Silvestre (ARCAS). The birds were to be relocated to Peten province in northeastern Guatemala. Prior to release they were evaluated for the presence of intestinal and blood parasites.

A total of 95 blood and 75 fecal samples from birds were examined. Blood smears were air-dried, fixed with 100% methanol, and stained with Diff-Quick (Mercedes Medical, 1435 Tallevast Rd, Sarasota, FL 34243). Fecal samples were collected and placed in 2% formalin. Standard sedimentation and floatation techniques were used.

Results
No hemoparasites were observed in any of the smears. The prevalence of intestinal parasites was 3/75 (4%) with all of the parasites being *Isospora* spp.

**Discussion**

The low prevalence of intestinal parasites could be attributed in part to the small sample volume of feces samples and the fact that only one sample was taken from each bird. The lack of intestinal parasites in psittacines could be due to the lack of a complete parasitic life cycle in these arboreal species that eat predominantly fruits and nuts. The low prevalence of hemoparasites in this study agrees with results of other evaluation of neotropical birds. This is in contrast to the remainder of the world where hemoparasite prevalence is much higher. The low prevalence of intestinal parasite found in this study coincides quite well with other reports involving wild psittacines.

Despite the apparent freedom of these birds from intestinal and blood parasites, medical evaluation of wildlife, such as these parrots, destined for reintroduction or translocation is essential.

**LITERATURE CITED**

ANTIBIOTIC USE IN ORNAMENTAL FISH†

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†Article has been modified from a chapter to be published in: Current Veterinary Therapy XIII, (Kirk and Bonagura eds.), W.B. Saunders Co., Philadelphia, Pennsylvania, 1998.

Abstract

Introduction

Certainly one of the most commonly asked questions concerning the practice of ornamental fish veterinary medicine is, “what antibiotic should I use and what’s the dose?”—straight forward and uncomplicated question with a complex and frequently ambiguous answer. Of course the problem lies in the lack of sound pharmacokinetic data available and the overwhelming number of species involved. When environmental differences such as temperature, pH, and water hardness are tossed into the equation, selecting a drug and dosing regimen becomes even less objective.

Historically, most treatment of ornamental fish diseases has been performed by non-veterinarians using empirical dosing regimens of a variety of chemotherapeutics. The Food and Drug Administration is currently examining the wide availability of prescription drugs, especially antibiotics, and may possibly enforce the restrictions on their availability to the lay public within the next few years. Such measures will necessitate sound pharmacokinetics studies to support clinical use of antimicrobials in ornamental fishes by licensed veterinarians. Very little research related to pharmacology has been reported in ornamental fishes. What little information exists is based on clinical efficacy and in-vitro trials using a number of different antimicrobials.

The purpose of this review article is to familiarize the reader with the most current pet fish pharmacokinetic information, provide a list of frequently used antibiotics, and discuss the variables that influence antibiotic dosing regimens in ornamental fish. Several general texts discuss antibiotics and their use on ornamental fishes in some detail.3,5,7

Dosing Routes

Due to their aquatic nature, generally small size, and frequently large numbers, a variety of atypical methods are utilized to deliver antibiotics to the ornamental fish. Standard parenteral methods can and commonly are used to dose aquarium fish with antibiotics, but the clinician must also be familiar with the terminology applied to water borne treatments. Table 1 contains these important definitions.
**Drugs and Dosages**

The majority of the current information on antibiotics used in aquarium fish has been extrapolated from the finfish aquaculture literature. There are a number of reasons for this, most of which revolve around funding for sound pharmacokinetic research. A recently published review article summarizes the entire body of literature on this subject. There are currently only two antibiotics approved for use in fish intended for human consumption (Romet-30® and Terramycin for Fish®). Most of the literature dealing with antibiotic usage in aquarium fish is empirical and anecdotal. One recent study reports on the pharmacokinetics of enrofloxacin in the red pacu, a tropical aquarium fish closely related to such important species as the tetras, hatchetfishes, silver dollars, and headstanders. Fortunately, the veterinarian treating aquarium fish can apply current extralabel drug use regulations when selecting and initiating antibiotic therapy. Whenever possible, appropriate microbiologic culture and sensitivity results should be applied when selecting an antibiotic course of treatment. The majority of ornamental fish pathogens are gram-negative bacteria that are susceptible to a number of antibiotics. Efforts to follow a complete treatment course are encouraged to help reduce the potential for producing antibiotic resistant organisms. Indiscriminate use of antibiotics in the aquarium fish industry both in the United States and abroad has made antibiotic resistance a significant problem. At times, appropriate microbiologic diagnostics are financially or logistically unavailable. In such cases, selecting an antibiotic that has good activity against gram-negative organisms may be the clinician’s only recourse.

Table 2 lists a number of antibiotics that are available to the zoo veterinarian and private practitioner which are clinically effective for treating bacterial disease in aquarium fish including pond koi. This table must be treated with respect and the clinician must understand that the routes and dosages are only guidelines and that a biotest is in order when dealing with an unfamiliar drug, fish species, particular aquatic environment, or all of the above.

**LITERATURE CITED**


**Table 1.** Routes of antibiotic administration for ornamental fish.
**Bath**- Usually refers to a treatment in which the drug is dissolved in the water in which the fish are swimming. The treatment usually lasts at least 15 min and less than 24 hr. Dosage is usually based on volume of water and not on fish biomass.

**Dip**- Refers to a treatment in which the fish is submerged in a particular solution for between 1 sec and 15 min. Water volumes are usually smaller than those of bath treatments and drug concentrations are frequently higher.

**Flush or Flow Through**- Requires constant water flow. Most frequently used in raceways or narrow vats. Medicant is added to inflow area and fish are exposed to drug as it passes over them with the water current. Similar to dip procedure except fish may not have to be removed from their normal holding area.

**Indefinite Bath**- Medication is added to aquarium or pond and usually there is no water change or immediate retreatment.

**Injection**- The antibiotic is given by injection with the aid of a hypodermic needle and syringe. Routes may be subcutaneous, intradermal, intramuscular, intravenous, and intraperitoneal.

**Oral**- Medication is mixed with the food in order to treat the fish. Usually done by incorporating drug into a gelatinized food mixture. For larger fish patients, medication can be placed in a chunk of food and then fed or force fed to the patient.

**Topical**- The antibiotic is applied directly to the lesion.

* Discontinue chemical (e.g., carbon) filtration during immersion treatments as this will inactivate the medicant. Adequate aeration is also important during any water treatment.

**When antibiotics are used as bath treatments they should be used daily for 5-7 days. Water changes (at least 50%) should take place between treatments.

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**Table 2.** Antibiotics for ornamental fish.
Aztreonam: 100 mg/kg i.m. or i.p. q 48 hr for 15 days. Used primarily by koi hobbyists. Effective against *Aeromonas salmonicida*. Dosage information courtesy of Spangenberg & Hedrick, UC Davis.

Chloramphenicol: 20-40 mg/kg i.m. or i.p. q 48 hr for 15 days. Human health issues, wear gloves when handling.

Enrofloxacin: 5-10 mg/kg given i.m. or i.p. q 48 hr for 15 days; injectable or oral form 5-10 mg/kg p.o. for 10-14 days or 0.1% (10 mg/10 g) can be used in food and feed fish for 10-14 days; 2.5-5 mg/L as a 5-hr bath, repeated q 24 hr for 5-7 days, 50-75% water change between treatments.

Erythromycin: 50-100 mg/kg p.o. q 24 hr for 10 days. Bath formulations also available.

Kanamycin sulfate: 50-100 mg/L as a 5-hr bath, repeated q 72 hr for three treatments, 50-75% water change between treatments. Available through pet products suppliers.

Metronidazole: 400 mg/L as a 5-12-hr bath, repeated q 24 hr for 3 consecutive days; 0.2% (20 mg/10g) in food and feed for 10 days. Good for anaerobes and some flagellates.

Nitrofurazone: 20 mg/L as a 5-hr bath, repeated q 24 hr for 5-7 days, 50-75% water change between treatments. Water soluble formulations work best.

Oxytetracycline: 20-50 mg/L as a 5-24-hr bath, repeated q 24 hr for 5-7 days, 50-75% water change between treatments; 25 mg/kg given i.m. or i.p. q 24 hr for 5-7 days; 50 mg/kg p.o. q 24 hr for 10 days. Many resistant bacteria.

Silver sulfadiazine cream: Apply cream directly to wound q 12 hr. Allow affected area to remain out or water for 30-60 sec while medication is absorbed.

Triple antibiotic ointment: Apply ointment directly to wound q 12 hr. Allow affected area to remain out or water for 30-60 sec while medication is absorbed.

Trimethoprim/Sulfamethoxazole: 30 mg/kg p.o. q 24 hr for 10-14 days; 0.2% (20 mg/10g) in food and feed to fish for 10-14 days; 20 mg/L as a 5-12-hr bath, repeated q 24 hr for 5-7 days, 50-75% water change between treatments. Very effective as a bath treatment.
MANUALLY INDUCED PARTURITION OF TWO YELLOW SPOTTED STINGRAYS (Urolophus jamaicensis) WITH DYSTOCIAS

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Abstract

Yellow spotted stingrays (Urolophus jamaicensis) or yellow stingrays are popular display aquarium elasmobranchs which are viviparous. Their gestation period is not well documented but other species of the same genus have terms between 3 and 4 mo (T. Tricas, Florida Institute of Technology, personal communication). Yellow spotted stingrays commonly conceive in captivity but some fail to deliver offspring. The underlying etiology is uncertain, but improper environmental cues such as water temperature and salinity changes, photoperiod, nutrition, social structure, bottom substrate or any combination of the above could contribute to the inability for gravid rays to deliver. Manually induced parturition is a viable option to resolve yellow spotted stingray dystocias.

Cases

Two adult pregnant yellow spotted stingrays were approximately 2 mo past their expected parturition dates. Rays were kept in artificial sea water (Forty Fathoms Crystal Sea®, Marine Enterprises International, Inc., Baltimore, MD 21221) with a crushed coral substrate. Salinity (31 ± 1 ppt), temperature (23 °C ± 1 °C), and pH 8.3 ± 1 were stable and ammonia, nitrite, and nitrate were within acceptable parameters. The rays were fed a mixture of squid, shrimp, clams, and gel food at approximately 1-3% of their body weight daily. The rays were housed with clear-nosed skates (Raja eglanteria), cownose rays (Rhinoptera bonasus), Atlantic stingrays (Dasyatis sabina), southern stingrays (Dasyatis americana), blunt-nosed stingrays (Dasyatis sayi), and bonnethead sharks (Sphyrna tiburo).

Visual examination and palpation of the distended coelomic wall indicated undetermined number of viable pup(s) which were observed swimming inside the uterus with a characteristic undulating motion of their pectoral fins. Both rays were induced through digital manipulation.

Throughout the dystocia the females continued to eat and behave normally. Females were anesthetized with 300-400 ppm tricaine methanesulfonate (Finquel®, Argent Chemical Labs, Redmond WA, 98052 USA) and the cloaca canulated with a beveled 6-ml syringe case to examine the functional “cervix.” A Kelly forceps was introduced into the cervix, which was dilated by rotating the instrument in a slowly expanding circular motion. Clear uterine fluid evacuated once the cervix was expanded and a gloved finger was introduced to continue dilating the cervix. The lumen was gently expanded to approximately 2 cm where two fingers could be placed into the uterus to locate one of the pups’ tails. Remember: most ray pups resemble miniature adults and have venomous
barbs which could cause the surgeon serious injury and pain. Once the tail was pulled through the cervix, gentle but steady pressure was placed on the coelomic cavity to expel the fetuses from the uterus. Pups that could not be extracted in this manner were carefully removed by gentle traction on the barb using padded hemostats, thus pulling the fetus out by its tail.

Six pups were delivered alive, four from one ray and two from the other. Ray pups started respiring 30-130 sec after delivery. It is uncertain whether this was a physiologic response or a result of the tricaine anesthesia. Four months after manual induction the adult females were once again gravid.

Pharmacologic methods to induce parturition are currently being investigated as well as natural history data, such as gestational times and parturition cues to determine when intervention is necessary.

ACKNOWLEDGMENTS

We thank the staff of the North Carolina Aquarium, Fort Fisher.
HUMORAL IMMUNE RESPONSE TO DNA-MEDIATED VACCINATION IN SELECTED AQUATIC SPECIES

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Abstract

Immunization using DNA, or nucleic acid vaccination, represents a potentially powerful, low-risk method for protection of valuable aquatic species from common diseases associated with high mortality or morbidity. Studies investigating immune responses to DNA-based vaccines in a variety of animals, including mice, fish, pigs, chickens, cattle, and non-human primates, show great promise for this technology as a new weapon for the prevention of infectious disease. Animals kept in aquaria and zoos may be exposed to a variety of pathogens that can ultimately result in fatalities and endemic infections, sometimes with severe economic implications. Factors contributing to successful plasmid vaccination of certain aquatic species, specifically elasmobranchs, penguins, pinnipeds, and cetaceans, are not well-defined to date. This study investigates humoral responses of African black-footed penguins (Spheniscus demersus) and chain dogfish (Scyliorhinus retifer) inoculated with a plasmid encoding β-galactosidase.

Humoral response to plasmid vaccination was investigated in twelve adult penguins (six males and six females). On Day 0, the diluent control group (n = 6) received sterile phosphate-buffered saline (PBS) by i.m. injection, the test plasmid group (n = 3) received 50 µg of the test plasmid pCMV-β (containing the gene for β-galactosidase) in PBS, and the control plasmid group (n = 3) received 50 µg of pCI (lacking the gene for β-galactosidase) in PBS. Serum was harvested weekly for antibody screening. After 3 wk, the six birds that received saline were divided equally, assigned to the other two groups, and given the appropriate injection of test or control plasmid. This approach was used to achieve ultimate group sizes of six birds for each of the three conditions, while minimizing the total number of animals (12) devoted to the study. Blood samples were obtained weekly for 6 wk post-inoculation with plasmid from all birds. On Day 21, a booster vaccine (identical to the first vaccine) was given to the twelve plasmid-vaccinated birds.

Penguin sera were screened in duplicate for antibodies against β-galactosidase using ELISA. Anti-chicken IgG, shown to have an acceptable binding efficacy to penguin serum immunoglobulins, was employed as secondary antibody. The geometric mean of ELISA titers for all six penguins of each group (saline, pCI, or pCMV-β) was calculated for each day serum was collected. Results show that penguins inoculated with the pCMV-β construct produced measurable anti-β-galactosidase antibodies when compared to control animals. On Days 21, 28, and 42, there was a statistically significant difference (using single factor ANOVA) between the mean of log-transformed titers of control birds.
(saline- or pCI-inoculated) versus birds vaccinated with the pCMV-β reporter plasmid. These results demonstrate that African black-footed penguins, directly transfected with the gene encoding β-galactosidase, are capable of mounting a humoral response against the in vivo expressed protein.

The portion of the study examining antibody responses of adult chain dogfish to i.m. plasmid vaccination is in progress. On Day 0 and Day 21, two groups of dogfish received either 50 µg of the control plasmid pCI (n = 3), or 50 µg of the test plasmid pCMV-β (n = 4), respectively. Serum was harvested every 7-21 days for 18 wk post-inoculation. ELISA is being used to screen the dogfish sera for anti-β-galactosidase antibodies, using anti-sandbar shark IgM as secondary antibody. Initial results indicate that β-galactosidase as an antigen may not elicit strong immune responses in chain dogfish. Although DNA vaccination has been investigated in several teleost species, plasmid vaccination in an elasmobranch species has not been reported; thus, data from this trial represent an exciting preliminary look at the humoral immune response of elasmobranchs to nucleic acid vaccines.

This study has demonstrated that African black-footed penguins are capable of immunologically responding to foreign proteins that are transferred in the form of plasmid DNA. Analysis of immune response to DNA-mediated vaccination in chain dogfish is still underway. As an extension of the study reported here, humoral responses to DNA vaccination in pinnipeds (Mirounga angustirostris and Phoca vitulina) and cetaceans (Tursiops truncatus) are currently being investigated at the Marine Mammal Center (Sausalito, CA) and the U.S. Navy Marine Mammal facility (San Diego, CA), respectively. Research at these separate facilities using protocols similar to those reported here should yield intriguing discoveries in the fields of aquatic animal medicine and vaccinology.

Once proven to induce an immune response to well-characterized protein antigens, plasmid vaccination can be used as a tool for screening genes encoding potentially protective antigens from various bacterial, viral, or parasitic pathogens. Knowledge gained from this work will be integral in the future development of nucleic acid vaccines against infectious diseases of aquatic species in both captive and wild environments.

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This project was supported in part by the Sea Research Foundation in Mystic, Connecticut. The authors wish to thank the members of the Mystic Aquarium husbandry staff whose time and efforts made this study possible, as well as Vickie Burnley and Chanagun Chitmanat of the University of Georgia for excellent technical assistance. We are also grateful to Dr. John Marchalonis at the University of Arizona for his time and the donation of anti-sandbar shark IgM.
IMMOBILIZATION OF CALIFORNIA SEA LIONS (Zalophus californianus) USING MEDETOMIDINE AND KETAMINE AND REVERSAL WITH ATIPAMEZOLE

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Abstract

Safe and efficacious immobilization of marine mammals continues to be an area of intensive investigation. Particularly challenging are large, free-ranging otariids that are not easily manually restrained and which do not have readily accessible blood vessels that allow intravenous administration of short-acting agents that can be safely titrated to effect. For these animals, administration of anesthetic agents continues to be best accomplished by the intramuscular (i.m.) route. Many currently used i.m. agents are associated with prolonged recovery times that can create problems in field conditions. The recent introduction of the α2-agonist medetomidine to North America may provide a distinct advantage over previously used agents because of its reversibility by the α2-antagonist atipamezole.

From May 1997-February 1998, 16 male and 20 female California sea lions (Zalophus californianus), varying in weight from 18-145 kg, were immobilized for a variety of medical procedures at a rehabilitation center in Northern California using a combination of medetomidine and ketamine. Atropine (0.02 mg/kg) was given i.m. to each animal at least 10 min prior to administration of the medetomidine/ketamine combination. Each animal was given 140 μg/kg medetomidine and 2.5 mg/kg of ketamine i.m. by either hand injection (n = 32) or blowdart (n = 4). Both drugs were administered together in the same syringe. Sites of injection included muscle immediately surrounding the pelvis, femur and tibia and muscle overlying the scapula.

Time to immobilization (mean ± SD) for hand injection was 8.8 ± 5.4 min and was significantly (P < 0.01) lower than for those animals immobilized by blowdart (16.8 ± 5.9 min). An adequate plane of anesthesia was not achieved in seven animals, two of which had been blowdarted, and additional ketamine (1/2 of the original dose) was given to four animals. The remaining three animals were physically restrained to complete the desired procedures. Nine animals were intubated during the procedure. Five of these were intubated after being given medetomidine/ketamine only while the remaining four were masked down with isoflurane to allow intubation.

Total immobilization times varied from 17-57 min with a mean of 31.3 ± 10.1 min for animals that were given only the initial dose of medetomidine/ketamine and were not placed on gas anesthesia. Recovery times for these animals were 9.9 ± 6.1 min after being given 200 μg/kg atipamezole i.m. No animals died during the study.
Disadvantages of medetomidine/ketamine use in sea lions include a moderate variation in induction time and plane of anesthesia. Since there was a significant difference in these parameters between animals anesthetized by hand injection and by blowdart administration, where penetration into muscle may not have been reliably achieved, it is thought that some of the variation may be due to injection into poorly vascularized sites such as blubber. In addition, the commercially available 1.0 mg/ml solution of medetomidine requires that very large volumes be used at the recommended dose for sea lions. Lyophilization followed by reconstitution to a concentration of 10 mg/ml produces a much more manageable injection volume.

The advantages of medetomidine/ketamine include safe and reversible immobilization that can be easily administered by the i.m. route and that produces a plane of anesthesia that is sufficient to carry out most routine diagnostic procedures.
PRELIMINARY PHARMACOKINETICS OF SINGLE-DOSE INTRAMUSCULAR BUTORPHANOL IN ELEPHANT SEALS (*Mirounga angustirostris*)

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Abstract

The Marine Mammal Center (TMMC) in Sausalito, CA, is a rehabilitation facility for marine mammals which strand along the central and northern California coast. Animals presented to TMMC have a wide variety of injuries and diseases,1 many of which require pain management. Butorphanol tartrate (Torbugesic®, Ft. Dodge Animal Health, Overland Park, KS 66225 USA), a synthetic opioid analgesic with mixed agonist-antagonist activity, is approximately five times more potent than morphine and until recently was not listed as a controlled drug.2 While it is commonly used as an analgesic at TMMC, pharmacokinetic values in marine mammals have not been established. Extrapolation of doses used in domestic animals may not be appropriate in marine mammals, where regional differences in blood flow at common injection sites might significantly affect drug absorption.3 This pilot study was conducted to determine the pharmacokinetics of single-dose intramuscularly administered butorphanol in elephant seals, one of the major species handled at TMMC.1 Four weaned elephant seal pups (mean weight = 66 ± 28 kg) held at TMMC were included in this study. To facilitate repeated blood collection and minimize the stress associated with multiple restraints, a 19-ga, 36 inch epidural catheter (Encapsulon, Teleflex Inc. USA, Duluth, GA 30136 USA) was placed in the dorsal intravertebral vein of each subject. Seals received 0.055 mg/kg of butorphanol by intramuscular injection in the right shoulder. Blood samples were collected at 0, 5, 15, 30 and 45 min, and 1, 1.25, 1.5, 2, 3, 4, 5, and 6 hr post-injection of the drug. Blood plasma butorphanol concentrations were analyzed by high-performance liquid chromatography using a reverse-phase C-18 column (Supelco C-18, model 088498AD, Supelco, Bellefonte, PA USA) and electrochemical detection at 900mV (LC-4B, Bioanalytical Systems, West Lafayette, IN 47906 USA). Plasma butorphanol concentrations were quantitated using nalorphine (Alltech Applied Sciences Laboratories, State College, PA 16801 USA) as an internal standard. Butorphanol was absorbed rapidly with mean peak plasma concentrations of 241 ng/ml (range: 87-635 ng/ml) occurring at the first time point, 5 min post-injection. Plasma butorphanol concentrations could still be detected up to 5 hr post-injection. No adverse reactions were observed in any seals, however they were subjectively sedated for up to 3 hr post-injection.

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LITERATURE CITED

BIOMEDICAL MANAGEMENT OF THE MOST ENDANGERED PINNIPED IN U.S. WATERS: THE HAWAIIAN MONK SEAL (Monachus schauinslandi)

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Abstract

The Hawaiian monk seal (Monachus schauinslandi) is the only marine mammal located entirely within U.S. waters where it is the most endangered pinniped. Beach counts have declined sharply since 1985 and current abundance is approximately 1,300-1,400 seals. Population trends most likely will continue to decline due to high juvenile mortality and low reproductive recruitment at French Frigate Shoals, Northwestern Hawaiian Islands (NWHI), where the largest of the six main reproductive populations can be found. Conceivably, this species could be on the verge of extinction in 20 yr. Since 1981 captive care and release programs have been an integral part of management efforts to conserve the species. Three methods have been used including headstart; direct translocation from one site to another; and transport to Oahu for rehabilitation, followed by release into a depleted wild monk seal subpopulation. Headstart was a technique that first was used in 1981 at Kure consisting of on-site protection of weaned pups from shark predation and adult males. Since 1984, female pups from French Frigate Shoals were rehabilitated in Oahu and then released into the NWHI after 8-10 mo. To date, rehabilitation efforts have been halted by the development of an ocular condition of unknown etiology affecting 12 female pups captured during the summer of 1995. The seals have not been released because of the risk of spreading the disease to the wild population. Attempts to document health and disease in the species began following an epizootic of unknown etiology (although ciguatera poisoning has been suggested) that killed ~50 seals in Laysan, NWHI in 1979. Causes of mortality in the population have been identified based on field necropsies of dead individuals and in some cases, on histopathologic studies. The primary causes of monk seal mortality from 1981-1995, based on gross and histopathologic examination of 65 monk seals were identified as: emaciation in 36 seals (35%), trauma in 19 seals (29%), infectious disease in 10 seals (15%), and undetermined mortality in 8 seals (12%). Gastrointestinal parasite burdens were abnormally high in 45 (69%) seals. Their significance also remains unknown. The Hawaiian Monk Seal Epidemiology Task has been recently developed to take a proactive approach in identifying health and disease parameters in the population. Its primary objectives are to centralize and maintain a serum/specimen bank, construct a long-term plan for epidemiologic research and implement an action plan for unusual mortality events.
USE OF DEPOT LEUPROLIDE AND CYPROTERONE TO CONTROL AGGRESSION IN AN ALL MALE CALIFORNIA SEA OTTER (Enhydra lutris nereis) COLONY

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Abstract

Male marine mammals in zoos and aquariums are sometimes maintained alone or in same sex groups for behavioral demonstrations, to prevent breeding, control aggressive behavior, or retain the genetic representation of animals for future reproduction. These management schemes may be complicated by undesirable behaviors (aggression, anorexia, and loss of operant training). While castration effectively minimizes these problems, anesthesia and surgery are required and it eliminates future reproduction.

Leuprolide acetate is a long acting, but reversible, gonadotropin-releasing hormone (GnRH) agonist. Administration initially results in an increase in follicle stimulating hormone (FSH) and luteinizing hormone (LH) causing transiently elevated testosterone. A progressive suppression then occurs reducing circulating testosterone levels to those of a castrate. Leuprolide acetate has been previously demonstrated to suppress testicular function in Atlantic bottlenose dolphins (Tursiops truncatus). 2,3 Cyproterone is an orally administered testosterone receptor blocking agent. In addition to blocking androgen receptors, it exerts a negative feedback on the hypothalamic pituitary axis inhibiting LH secretion and consequently suppresses testicular testosterone production.

Four male California sea otters (Enhydra lutris nereis) were exhibited together at the Aquarium for Wildlife Conservation. As they matured, intraspecific aggression developed until eventually they could no longer be maintained as a compatible colony. During this study (July 1996-December 1997) parenteral leuprolide acetate in either a 1-mo (phase 1) 1,4 or 4-mo (phase 2) depot suspension, or oral cyproterone acetate (phase 3), were administered in succession to control aggression.

Four otters were included in phase 1.1,4 One otter died of causes unrelated to the study and the remaining three otters were included in phases 2 and 3. Ages and weights of otters ranged from 3.5-6 yr, and 20-34 kg, during the course of the study. Otters were manually confined in an otter restraint device for venipuncture and all injections.5

During phase 1 otters received 3.75 mg (0.11-0.19 mg/kg) of a 1-mo depot formulation of leuprolide acetate i.m. at monthly intervals. Initiation of drug administration in each otter was staggered so animals received three to seven injections in a 6-mo interval. In phase 2 otters were given 30 mg (0.9-1.1 mg/kg) of a 4-mo depot formulation of leuprolide acetate administered either i.m. or s.c. twice...
at intervals of 3.5-4 mo. Leuprolide was administered in the dorsal surface of a pelvic limb. In phase 3, otters were given 50 or 75 mg (1.5-2.3 mg/kg) cyproterone acetate p.o., s.i.d. for 84 days.

Testicular lengths of two otters were measured before the initial treatment and at the end of each phase. Marked testicular atrophy occurred and was maintained in all phases. Pretreatment testicular length (mean ± SD)(53 ± 3.5 mm) was significantly greater (paired t-test; \( P < 0.05 \)) than at the end of each phase (35 ± 2.5 mm, 27.5 ± 2.5 mm, and 26.8 ± 2.6 mm for phases 1, 2, and 3; respectively). Mean testicular length in phase 1 was significantly greater (Tukey Honest Test, \( P < 0.05 \)) than in phases 2 and 3; there was no significant difference in testicular size between phases 2 and 3.

Testosterone levels were determined by radioimmunoassay before treatment and then monthly in phase 1; three to five times at 4- to 6-wk intervals in phase 2; and two to three times 4-12 wk after initiation of treatment in phase 3. Testosterone levels of the youngest otter were below the assay detection limit (< 0.05 ng/ml) on all sampling dates. Pretreatment values for the two others ranged from 0.31-2.28 ng/ml. Testosterone levels in phase 1 decreased after 1 mo of treatment (< 0.05-0.19 ng/ml) and after 2 mo of treatment all were undetectable. In phase 2, all testosterone levels except one (0.7 ng/ml) were below the assay detection limit. In phase 3, testosterone was below the assay detection limit on all sampling dates.

Despite the effectiveness of leuprolide at inducing testicular atrophy and testosterone suppression, with consequent reduction in intraspecific aggression, treatment was discontinued due to adverse injection site reactions. These consisted of anorexia and depression with moderate to marked injection site lameness, swelling, or sterile abscesses. Preventing or treating these reactions with either diphenhydramine (1.5 mg/kg p.o., b.i.d. for 7 days) or flunixin meglumine (0.9 mg/kg p.o., s.i.d. for 5 days) was not successful while carprofen (1.5-2 mg/kg p.o., b.i.d. for 5-10 days) was a more effective treatment. Some otters were also treated with trimethoprim sulfa (33.6 mg/kg p.o., s.i.d. for 10 days). No adverse effects were noted in phase 3.

Administration of depot leuprolide acetate or cyproterone acetate was successful in suppressing testosterone and controlling aggression in an all male sea otter colony. This enabled otters to be maintained in a more compatible social group. Leuprolide was more effective than cyproterone in controlling aggression. Compared to castration, advantages of these medications include no requirement for anesthesia or surgery and reversibility. Disadvantages of leuprolide are the drug cost, injection site reactions, and the necessity for frequent animal handling. Cyproterone acetate is currently not commercially available in the United States but offers the advantage of oral administration with no animal handling required. These treatments have potential application for the control of male-associated undesirable behaviors in sea otters in zoos and aquariums. A trial with a long lasting GnRH agonist implant (deslorelin) is currently underway.

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LITERATURE CITED


PRELIMINARY FINDINGS OF COLITIS CONDITIONS IN CETACEANS: RECOMMENDATIONS FOR DIAGNOSIS AND CLASSIFICATION

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Abstract

Diseases of the intestinal system of most species are usually the most difficult to understand, diagnose and properly treat. This is also true in cetacean species where gastroscopy is often limited to the first of multiple chambers, where fecal material is already liquid and where sampling is complicated by acquisition and difficulties in handling. Clinical disease of the small and large bowel is poorly understood. The small numbers of cetaceans maintained in controlled environments affects the progress, understanding and classification of small and large bowel disease conditions.

Antibiotic use has resulted in better survival rates in cetaceans but it is often empirical and is affected by experience, comfort and prejudice. As we incorporate more antibiotics into general use and see more clinical manifestations of intestinal disease we will all benefit from developing diagnostic protocols which will help to facilitate our understanding of these processes.

Human classifications of bowel diseases include inflammatory bowel disease (Crohn’s disease, ulcerative colitis, lymphocytic colitis, collagenous colitis), irritable bowel disease (etiology unknown), and infectious bowel disease (travelers diarrhea-bacterial, viral, parasitic). Another common bacterial infection, Clostridium difficile, is often linked to antibiotic use and their secondary effects on the intestinal flora.

Cetaceans that are showing signs of intestinal illness will often lose their appetite as well as show possible signs of depression, lethargy, isolation and decreased cooperation. The first diagnostic tests done are usually a complete blood count and serum chemistries. Bloodwork findings may include an elevated white count, decreased red cell indices, elevated fibrinogen, elevated sedimentation rate, and a decreased serum iron. Often on the basis of an increased white count antibiotics are begun. If all returns to normal the diagnostic workup usually stops so there is little data regarding the initial involvement of intestinal disease. If the abnormalities persists then additional procedures are pursued.

Typical diagnostic techniques for bowel disease in cetaceans should include aerobic and anaerobic cultures, cytology of fecal material, and direct parasite exam. To aid in bacterial flora interpretation it is recommended that fecal cultures be done on animals while they are clinically normal. Anaerobic cultures are useful in determining the presence of possible pathogens and in correlating this with cytology results. A research project done at SeaWorld on clostridial organisms showed that the vast majority of isolates were Clostridium perfringens type A and that about 70% were toxin producers.
The diagnosis of diarrhea is related to the frequency of defecation rather than the consistency of the stool observed. Feces for cytology are collected with a flexible open ended tube with sterile technique. Cytology results in colitis cases may vary in findings and severity. Inflammatory cells may be classified based on morphology and numbers. The bacterial flora is evaluated for composition and morphology. Special note is taken when there is a predominance of one morphologic type such as bacilli with the presence of spores that may indicate clostridial involvement.

One technique that has not been adequately utilized is colonoscopy. It is often assumed that the size of the rectum and the liquid fecal material will make this a low yield procedure. This deserves further evaluation for possible inclusion as a more common technique, although typical scope diameters may be a limiting factor for some clinicians. With an increased number of interested clinicians we may then approach a classification system similar to the human field.
RECOGNIZING AND BALANCING THE BENEFITS AND RISKS OF ENVIRONMENTAL ENRICHMENT

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Abstract

As veterinarians, we each are in a position to impact the success and scope of enrichment activities taking place at our zoo. Our most basic involvement with enrichment often is to decide which enrichment techniques can be used safely for animals in our collection. There are basically two issues to consider. First, does this item pose a risk to the animal and, second, will this item provide any benefit to the animal? One of the veterinary medical credos is “above all, do no harm,” so it’s logical for veterinarians to want to err on the side of caution. As we become more adept at providing for the physical and medical needs of the animals under our care, we need to turn our attention toward their psychologic needs. Providing environments that stimulate a greater range of natural behaviors, decrease stress and increase activity is the next step towards optimizing health.

The scientist in me likes to see proof that enrichment works; I want to know that enrichment efforts will pay off and provide real benefits to animals. The more familiar I have become with enrichment literature, the more convinced I have become of the benefits to animal health.

With research, it has been demonstrated that enrichment can provide solutions to some of the behavioral and veterinary problems we encounter in captive animals and can improve overall well-being. One problem that can be addressed is autoaggression or self-directed behavior. Hair plucking and self-biting in primates, feather plucking in birds and tail sucking/chewing in cats are all examples of self-aggressive behaviors seen in zoos. These behaviors have been alleviated or reduced by providing enrichment activities designed to provide more opportunities for natural behaviors such as foraging or food manipulation.\(^2,11\) Aggression, stereotypic locomotion and low activity level can also be reduced with certain enrichment strategies.\(^9,10,12\) Regurgitation and reingestion (r/r) is an abnormal behavior seen in great apes that has been shown to be reduced in animals offered browse, straw and other forage items.\(^1,3\) Dental health can be improved with the provision of bones and rawhide chew toys.\(^5,6\)

Veterinary cases are also made manageable through operant conditioning, often considered a form of enrichment. At the Detroit Zoo, we have been able to train 2/3 of our 15 chimps to accept hand injections of anesthetic drugs. This has made annual physical exams much less stressful for both the animals and the personnel involved. With similar techniques other zoos have been able to manage a diabetic drill baboon with regular blood collection and insulin injections, use a blood pressure cuff to monitor woolly monkeys with hypertension and train a macaque to present its neonate for supplemental bottle feedings.\(^7,8\) Hoofstock have been able to be conditioned for venipuncture and
other veterinary procedures. In the hospital, we use enrichment for patients that need to be pulled from social groups during a time of healing. There are numerous examples of animals that couldn’t have been treated successfully without these techniques.

The risks associated with enrichment can be thought of as either physical or behavioral. When we think about physical risks, we think about animals becoming caught in tires, impacting on burlap bags, or becoming sick after eating non-processed food items like live fish. There is almost nothing in the literature about enrichment hazards, so we find it necessary to anticipate potential problems. It’s sometimes hard to separate problems associated with enrichment from problems associated with captive living in general. It is my impression that the incidence of enrichment related injury and death is very low. Many accidents occur because of poor coordination of enrichment efforts or lack of maintenance of enrichment items and exhibit features. If we compare an enriched exhibit to a barren one, it’s obvious an enriched environment is more complex and will present more dangers if not properly maintained. Perhaps the larger risk is spending valuable time on enrichment techniques that are not effective or do not provide the greatest potential benefit. Without assessing our efforts we risk cluttering animal enclosures with objects that are of little interest to animals and have no positive impact on behavior.

Both the physical risks and behavioral risks can be diminished by setting up a program to coordinate enrichment efforts. Program paperwork doesn’t have to be complex or time-consuming, but should provide basic information to allow enrichment ideas to be evaluated and improve in efficacy over time. Individual enrichment ideas can be thought of as falling somewhere on a continuum of low risk to high risk and a continuum of low benefit to high benefit. The goal should always be to maximize benefit with the lowest risk. Keepers should be encouraged to try new and creative ideas that meet this goal while learning to fine tune techniques and make simple ideas more effective.

Enrichment is an effective tool for solving many problems found in captive animals. The potential benefits of a well-executed enrichment program are numerous. By understanding and being familiar with research, we can identify trends and define enrichment strategies most likely to provide widespread benefit in our collections. We can start to design environments that not only address specific problems, but meet the physical and psychologic needs of animals.

LITERATURE CITED

THE USE OF POSITIVE REINFORCEMENT TECHNIQUES IN THE MEDICAL MANAGEMENT OF CAPTIVE ANIMALS

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Abstract

Positive reinforcement training is gaining acceptance as a valuable animal care and management tool to aid in husbandry activities, veterinary procedures, and research protocols. The benefits of such work include less stress on the animal, greater flexibility and reliability in data collection, and a reduced use of anesthesia. This paper presents examples of the use of training techniques to address various medical situations in a number of species in the zoological setting.

Introduction

The comprehensive use of positive reinforcement training has revolutionized the way we care for captive animals.4,6 By using recognized techniques, many tangible results and benefits can be achieved. Animals are desensitized to frightening or painful events, like getting an injection, so the stress associated with these events is significantly reduced.5,7 Animals gain the opportunity to voluntarily cooperate in these procedures, rather than being forced to comply. With a greater accessibility to more cooperative animals comes the opportunity to initiate preventative medicine practices and to explore techniques previously seen as less practical on a routine basis such as ultrasound or tube insertions for artificial insemination.3 With this cooperation comes a reduction in the use of restraint and anesthesia.1 Many husbandry and veterinary procedures can be implemented with less disruption to all animals, by reducing the need to separate animals from their social groups for many procedures. Finally, experience has shown that trained animals maintain a high degree of reliability in participating in these procedures and are less stressed while doing so.8

Methods

The training referred to throughout this paper, and recommended as the approach of choice, is positive reinforcement training. Animals are reinforced with pleasurable rewards for the desired behavioral response. Operationally, it means that the positive alternatives are exhausted before any kind of negative reinforcement is used. On the rare occasions when an escape-avoidance technique is necessary, it is kept to a minimum and balanced by positive reinforcement the vast majority of the time. Punishment, which by definition is used to eliminate a behavior, is only appropriate in a life threatening situation for person or animal. To dispel a common misperception, positive reinforcement training does not require any food deprivation. Animals are fed their daily allotment of food, and rewards for training use that diet, or consist of extra treats. Finally, this training relies on voluntary cooperation by the animal to be successful.
Through a process termed desensitization, animals learn to tolerate presumably scary or uncomfortable stimuli. In basic terms, desensitization is a process designed to “train out,” or overcome, fear. By pairing positive rewards with any action or object that elicits fear, that fearful entity slowly becomes less negative, less scary, and presumably less stressful. Using this technique, animals have been desensitized to husbandry and veterinary procedures, new enclosures, unfamiliar people, negatively-perceived people like the veterinarian, novel objects, strange noises, and other possible aversive stimuli. In fact, the authors have previously reported that animals being desensitized to specific stimuli can, over time, become generally desensitized to anything novel or unexpected.²

**Voluntary Acceptance of Injections**

One of the most useful applications of husbandry training is the conditioning of animals to voluntarily accept injections. When training an animal to accept an injection, the feeling of a needle piercing the skin is a potentially frightening and painful experience. Effective desensitization requires pairing many positive rewards directly with that experience, or with a similar experience. Training may include pairing positive rewards with the experience of being touched with a progression of items, starting with the trainer’s finger, then a capped syringe, and then to a needle with the end cut off so it is blunted, and finally the real needle. The animal must experience this over and over again, with the touch slowly moving from very light to the final experience of actually piercing the skin. If desensitization is done well, the animal will voluntarily accept the injection and recognizable signs of stress and fear will be diminished or absent. To date, injection training has been successfully implemented with many different species and it continues to be a priority behavior for many zoos.

**Husbandry Training of Elephants in Protected Contact**

Protected contact, as a system for managing elephants, is based on the use of positive reinforcement techniques. All elephants in protected contact should be trained on a wide range of husbandry behaviors, including: skin care, body exam, foot care, tusk trim, blood sampling, vaginal exam, and rectal palpation. Until very recently, many in the zoo community were skeptical of the ability to provide comprehensive medical care for animals functioning in a positive reinforcement based system where compliance in behaviors is voluntary. That skepticism is eroding away as more examples of successful medical treatment under these conditions are being produced. The following examples illustrate the advances being made in the management of elephants in protected contact.

The Houston Zoo manages two male and four female Asian elephants in a protected contact system. Thailand, the 33-yr-old bull, has had chronic nail cracks and abscesses in its front feet for over 10 yr. Prior to protected contact, this elephant was maintained in a no contact system, which meant no routine foot care was being performed. Even as nail condition worsened, only minimal foot work was possible. With the introduction of protected contact and positive reinforcement techniques, the animal was easily trained to present its feet through an opening in the training wall or the barn door for foot work.
Over the past 5 yr, the elephant has tolerated routine trimming as well as deep trimming into the abscessed areas. The animal has also complied in daily treatment of the abscesses and regular foot soaks in epsom salts or Nolvasan (Chlorhexidine diacetate, Fort Dodge, Overland Park, KS 66210 USA) and warm water once or twice daily for 10 min. With the expanded access to the elephant, cooperation with diagnostic techniques was now possible. Radiographs were taken to determine the depth of the infected tissues and to see if there was any bony involvement. Radio-opaque dye was injected into the hole in the foot so that the tract could be identified. The elephant was trained for the procedure by first teaching the animal to extend a front leg through the foot hole and place its foot on a custom built foot rest. Next an old radiograph cassette was used to train the animal to hold steady with the plate in a variety of positions under and around its foot. The final step was to move the large machine in position for the procedure while the animal placed and held its foot in the proper positions.

Currently, the animal’s feet are greatly improved. Granulation beds have formed where the abscesses were and only small holes are visible on each foot. Routine foot care continues. The feet of this elephant will always be a concern, but through training the keepers and veterinarians maintain the ability to monitor and treat the animal’s condition as necessary.

In another case, Kiba, a young bull, was born at the Zoo in 1987 with an umbilical stump that was excessively long and soon became infected. Although it was treated daily with Betadine (Purdue Frederick, Norwalk, CT 06850 USA) the infection persisted and a cantaloupe sized bulge remained present on its abdomen. In February 1992, the elephant was sedated and an ultrasound exam on the herniated area was done, specifically, to check the integrity of the abdominal wall and the potential for entrapment of intestinal loops. The ultrasound showed the area to have healed well. In November 1995, the animal’s umbilical area appeared very swollen. The immediate concern was that a loop of bowel had become trapped in a previously undetected defect. An ultrasound was again needed, but this time, the elephant staff had the opportunity to train the elephant for this procedure. The animal was taught to present its body parallel to the training wall. The animal was then desensitized to the ultrasound exam including palpation of the area, the close proximity of the equipment, the feeling of the contact gel and pressure of the transducer.

This elephant is an extremely responsive animal and was ready for the ultrasound within days. Fortunately, the ultrasound showed no loops of bowel or defects; the swelling was likely due to mild trauma. The swelling decreased within 2 wk and has not reoccurred.

Medical Training and the Human/Animal Bond

In November 1994, trainers noticed that lower teeth appeared loose and ulcerations were present on the mandibular tissue in a California sea lion, Gertie. In December, the animal was immobilized for a biopsy of the ulcerations and to radiograph the mandible. The lower left incisors had severe bone resorption around the roots; the adjacent teeth (right central incisor and left canine) were loose and
easily removed. The animal’s condition significantly worsened when a pronounced swelling at the medial aspect of the lower left premolar appeared. Due to the severe deterioration of the mandible, a mandibulectomy was scheduled. The oral lesions appeared aggressive and necrotic, predominantly involving the proximal left mandible, part of the right, and the surrounding tissue. Diagnosis showed widespread squamous cell carcinoma throughout the lower mandible.

The prognosis for such a radical surgical procedure was not favorable, but the sea lion came through the procedure better than expected. Several days following the surgery, the animal was completely lethargic, not eating, and in an overall depressed condition. The animal was injected with analgesic antibiotics but little to no response was the result. The decision to euthanatize the animal was made as her condition worsened and recovery seemed unlikely. A trainer with a 7-yr history training this animal in a variety of husbandry and show behaviors was brought in as a last resort. Immediately, the sea lion responded to the trainer and was eating fish within days. A diverse behavioral repertoire offered many options to monitor the healing progress as well as help the animal re-learn to eat. The animal was trained for a full mouth exam, allowing all teeth and areas of the mouth to be touched and manipulated. This behavior proved instrumental in the recovery. As the area began to heal, suture material had to be trimmed as it worked out of the tissue. This involved the animal holding its mouth open and allowing scissors in and around the surgery site. Topical treatment and cleaning of the site was required and the animal tolerated this remarkably well.

The sea lion’s recovery took over 3 mo, but was fully successful. The animal was reintroduced to the group and the exhibit pool. Full recovery was largely due to the training that had occurred in the years before the surgery and the trust between animal and trainer that is an inherent and powerful part of positive reinforcement training.

The Versatility of Husbandry Training

As husbandry training grows in the zoological community, many applications and benefits not initially perceived continue to emerge. Some examples of novel application of husbandry training include:

- getting saliva samples on cotton balls from gorillas
- training free-ranging hoofstock to accept yearly vaccinations
- milking a female rhinoceros for supplementation of hand raised offspring
- performing a vaginal swab on a female warthog
- training female drill baboons on tube insertion for artificial insemination
- blood collection on rhinos, tapir, and adult chimpanzees
- getting weights on rhino, pygmy hippos, giant anteater, and tapirs
- holding giant anteaters on target while body measurements are taken

Conclusions
In conclusion, positive reinforcement training is gaining stature among animal managers and veterinarians as a useful tool for enhancing animal health and husbandry. The applied use of positive reinforcement techniques provides the means to pro-actively address a wide range of medical conditions and to develop and implement an effective program of preventative medicine. These benefits make training a valuable part of any animal management program and have significant implications for overall animal care and welfare.

LITERATURE CITED

VETERINARY PROCEDURES FACILITATED BY BEHAVIORAL CONDITIONING AND DESENSITIZATION IN RETICULATED GIRAFFE (Giraffa camelopardalis) AND NILE HIPPOPOTAMUS (Hippopotamus amphibius)

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Abstract

Introduction

Medical and diagnostic procedures can be challenging with exotic species. The large species are especially challenging due to the infeasibility of physical restraint for most contact procedures.

Historically, procedures on large or dangerous exotic species necessitated immobilization or general anesthesia. This produces inherent risks with altered physiology, recumbency and trauma during induction and recovery. The use of narcotics is common in these instances. These agents pose additional risks to the user of these drugs. Renarcotization and even death can be subsequent results of large animal chemical immobilizations.

Animal behavioral conditioning and desensitization techniques have been employed for years on domestic animals. More recently these techniques have been incorporated into several animal programs in zoos worldwide. These techniques have been used with a wide variety of species from birds to elephants.1,4,7 The most well-known methods used involve marine mammals (T. Lacinek, personal communication).3

At Busch Gardens in Tampa, Florida many areas of the zoo department are employing techniques of conditioning and desensitization to decrease the need for immobilizations especially in high risk animals.2 These include giraffe, black rhinoceros (Diceros bicornis)6 and Nile hippopotamus.5,8 The focus of this presentation will be on giraffe and hippopotamus.

Giraffe

A corral and chute system was constructed to facilitate working giraffe as a herd for elective procedures. The procedures include vaccinations, injectable deworming, tuberculosis testing, and phlebotomy. Body weights are also recorded with the use of a platform scale built into the last section of the four section chute.

The corral and chute system is designed in three parts (Fig. 1). The first is a corral that is used to hold the group of animals to be worked through the chute. When possible the animals are held in this area overnight to let them acclimate and calm down prior to moving them individually through the.
chute. This helps greatly to minimize excitement and potential trauma during the chuting episode. This also helps cut down on time. A group of 12 animals can be worked in under 2 hr once they are familiar with the system and it becomes a regular routine.

The second area is a smaller semi-enclosed barn adjacent to the chute. This area has a series of large gates that can be closed behind the animal as it advances into the next part of this area. This serves to confine the giraffe into a smaller and smaller area until it can eventually be encouraged through the entrance to the chute.

The animal then must enter the chute (Fig. 2) in order to advance out of the small area where it is eventually confined. It advances to the front of the first of three sections where a split swing gate is closed and secured behind the animal to prevent it from backing up. The animal is worked in this area. Once it is calm, the door in front of it is opened and it advances into the second area. The giraffe is allowed to calm down to prevent bolting through the last door. Then it is allowed to advance into the third compartment where it is weighed on an electronic scale. Once a weight is recorded and the animal is fairly calm, it is released into an adjacent coral that is opened to the exhibit enclosure.

Conditioning these animals to a chute has also facilitated anesthetic procedures. The chute is confining enough to allow for more control during the induction and recovery phases of the procedure.

**Hippopotamus**

Two of the five (2.3) Nile hippopotamus currently residing at the park have been maintained in an exclusively outdoor sand and water enclosure (Fig. 3) for over 34 yr. For many of those years these two female Nile hippopotamus have been conditioned to approach a fence when they saw a keeper with a white bucket approach. A white, 5-gallon bucket was used to carry their grain and fruit at meal times. Even when the times varied, these animals remembered the significance of the white bucket.

We decided to make use of this simple conditioned behavior to allow medical and diagnostic procedures to be performed on these high anesthetic risk animals. The hippos were initially taught to approach a specific place along the fence to receive hand feeding of grain and fruit from the bucket. Mouth opening and closing was taught to occur on cue and reinforced with food.

The next step was to build a makeshift stanchion along the fence line. The animal was worked and conditioned in this area while being fed. In this stanchion we were able to perform minor diagnostic procedures such as ultrasonography, punch biopsies of a perineal mass and bacterial culture.

When this conditioning was consistent, a temporary stanchion was constructed along the fence with a head and tail gait (Figs. 4.1 and 4.2). The hippos were conditioned to stand in the structure for longer periods of time each day. Occasionally manipulation of the animals would occur to
desensitize them to various veterinary procedures. In this system we were able to perform a more extensive biopsy of a perineal mass and explore the region with the use of topical and local anesthesia.

In the future a more permanent structure is to be constructed in the animals’ enclosure. Work in this structure is to be a routine for both hippos. Desensitization of various body areas is to be incorporated into this routine to facilitate medical or diagnostic procedures while avoiding the high risk of general anesthesia or immobilization in this species.

A recently constructed house was constructed for 2.1 unrelated hippopotamus. A chute has been built into the aisle way that provides access to and from the exhibit. The animals must routinely pass through this chute at least twice daily. Soon they will be conditioned to stay in the chute for a period of time with the gates closed. Gradually they will receive similar conditioning as the older females in the pond exhibit. This will facilitate achieving the goal of having all hippos in the park conditioned to be handled for routine medical and diagnostic procedures without anesthesia or sedation.

Summary

Through the use of conditioning and desensitization two high anesthetic risk species have been able to be handled for routine veterinary care on a regular basis. This facilitates safe handling of valuable animals as well as provides more opportunities to add to normal data on these species in zoos worldwide. Conditioning in this way has also allowed close examination of animals that develop health problems without the need for anesthetic agents. As the use of these techniques becomes more widespread, the availability of more biologic and anatomic parameters in a greater variety of species will facilitate the management and preservation of endangered and threatened animals in captivity.

LITERATURE CITED

BEHAVIORAL AND MEDICAL THERAPY FOR SELF-MUTILATION AND GENERALIZED ANXIETY IN A BONOBO (Pan paniscus)

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Abstract

Case Report

Treatment of abnormal or undesirable behaviors with behavior modification and medical therapy is relatively new to the field of veterinary medicine. Few published reports on the use of anti-depressants and anti-psychotic drugs in exotic animals exist. This report describes the successful treatment of a nonhuman primate with a combination of behavior and medical therapy based on extrapolation of similar treatments used in human psychiatric medicine.

On July 8, 1997, the Milwaukee County Zoo (MCZ) received “Brian” an 8.5-yr-old, 35-kg male bonobo (Pan paniscus) from another institution. The animal was born at that institution and had been housed with its father and other troop members until 7.5 yr of age. Reports from the staff indicated that the bonobo was regularly severely intimidated by its father. During this time, the bonobo developed the persistent self-mutilating behavior of inserting its fingers and/or entire hand into its rectum (termed “fisting”).

Believing that this behavior may have been caused by chronic stress and mental trauma, the animal was removed from the group in October 1996, and housed in isolation for 8 mo prior to its arrival at the MCZ. The “fisting” behavior continued while in isolation, occasionally with enough intensity to cause rectal bleeding. Treatment with acepromazine 12.5 mg orally every 8 hr was begun in November 1996. Effects were minimal and treatment was discontinued shortly thereafter. Magnetic resonance imaging was performed in December 1996 to determine if an underlying physical problem was causing the behavior. The rectum and lower colon were palpably thickened, which was attributed to chronic trauma, however, no other abnormalities were noted. Treatment with fluoxetine (Prozac, Eli Lilly & Co., Indianapolis, Indiana USA) 16 mg orally once a day was initiated in December 1996, and 14 days later it was noted that there was improvement in both the severity and frequency of the “fisting” behavior. Therapy was continued until just prior to shipment to the MCZ. To try to provide the animal with a better social environment, it was sent to the MCZ for integration into a large group of bonobos. The bonobo troop consisted of one juvenile and four adult females, and two juvenile and two adult males. In addition, the MCZ has an active operant conditioning and medical behavioral training program, and it was hoped that behavioral therapy would reduce the self-mutilation behavior.
When the bonobo arrived at the MCZ, it seemed both angry and frightened. The keeper staff noted several behavioral abnormalities which included: inserting its fist into its rectum, inducing vomition, pacing, constant hand-clapping, rubbing genitalia on sharp objects, self-mutilation by ripping at fingernails and toenails, inability to sleep or rest during the day, spitting and generalized aggression toward the keepers. Volunteer observers were recruited to monitor the type and frequency of these behaviors.

Behavior modification was used in an attempt to alleviate the problems; medical therapy was not immediately instituted. While isolated during the quarantine period, short training sessions praising desired behaviors, and ignoring undesirable behaviors were begun. Frequent small feedings were offered to keep the animal active and occupied. It was observed that induced vomition increased after fruit was eaten, therefore, fruit was removed from the diet, and the frequency of vomition decreased. Training and enrichment were difficult because the animal was extremely fearful of all new objects, including toys and food items used for behavioral enrichment. Nonetheless, some improvement in behavior were obtained, but after several weeks improvement reached a plateau.

One and one half months after arrival, the animal was cleared from quarantine and introduced to the other troop members. The animal was fearful of adult male bonobos, it had problems eating in a group, and it had poor play and reconciliation behaviors. Solutions to help the animal adapt included placing it in small social groupings with calm, gentle animals and keeping life routine and predictable. This strategy appeared to work for a couple months, when improvement stopped and behavior regressed.

A decision was made to seek consultation with a psychiatrist. The consultation included a “case conference” with zoo staff where the psychiatrist reviewed the animal’s developmental history and the dynamics of its seeming self-mutilating and obsessional “fisting.” There was agreement that the behavior increased when the bonobo was anxious or under stress and seemed to have both regressive and auto-erotic components to it. A plan was devised to use medications to deal with the obsessional anxiety, and behavioral efforts to introduce the animal to females and the usual matriarchal society of bonobos with the goal of promoting a more normal mature sexual outlet. The behavioral changes were staged to occur as the bonobo bonded with its keeper, allowed itself to be pampered by two female (older) companions and gradually resocialized with other bonobos. The process was accompanied by regular discussions with the consultant and modifications in the staging of socializing events according to the animal’s progress and improvement. All keeper-animal interactions were kept calm and positive. Many (≥ 5/day) short, positive training sessions were performed to integrate the animal into the medical behavior training program, and to keep it occupied in an attempt to decrease the undesirable behaviors.

Paroxetine (Paxil, SmithKline Beecham Pharmaceuticals, Philadelphia, PA 19101) 10 mg was administered orally once a day initially, but after five days administration increased to twice a day. Within 1 wk, the animal appeared calmer. Induced vomiting stopped after 2 wk. Changes noted by the observers included slower eating habits, ability to rest/sleep during the day, cessation of pacing, decreased aggression toward the keepers, and an increased attention span during training sessions.
with the ability to focus on tasks and learn new behaviors. The “fisting” behavior was reduced, but still occurred at perceived high anxiety times, such as immediately before meals. Diazepam 2.5 mg once daily in the morning was initiated to curb anxiety levels, with moderate success. Paroxetine is an antidepressant which acts as a potent and highly selective inhibitor of neuronal serotonin reuptake. In addition to its antidepressive effects, it is also highly effective in treating obsessive-compulsive disorders, and panic disorders. Reported side effects in humans include nausea, somnolence, insomnia, dizziness, asthenia and ejaculatory disorders. Its use is contraindicated in patients taking monoamine oxidase inhibitors. Paroxetine was chosen over other selective serotonin reuptake inhibitors (SSRIs) because of its antiobsessional effects, and because of its more immediate onset and the hope for quick reduction of anxiety.

The bonobo has now been introduced into the large bonobo group and is learning the social skills necessary to interact successfully with peers. The animal is successfully participating in the MCZ medical behavior training program. Although self-mutilation behaviors still occur occasionally, the animal continues to improve and adapt. Routine treatment with diazepam has been discontinued because of poor acceptance, without ill effects. It is hoped that with continued therapy, with adjustments as necessary, quality of life will remain high throughout the animal’s life span.

This case illustrates that behavioral and medical therapy, carefully chosen and implemented, can work effectively to treat undesirable and self-destructive traits exhibited by animals.

LITERATURE CITED
OPERANT CONDITIONING OF DIABETIC PRIMATES TO ACCEPT INSULIN INJECTIONS

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Abstract

Introduction

Diabetes can affect a wide variety of primates. At the Los Angeles Zoo, we currently have two cases of diabetic primates that have been or are in the process of being trained, using operant conditioning methods, to accept insulin injections without any type of manual restraint.

Case Reports

“David,” a red-tailed moustached guenon (Cercopithecus cephus cephus), was found to be diabetic in May of 1997 after a routine preventive medicine exam. This animal was wild-caught and estimated to be 24 yr of age. The animal lived with two other animals, and when its diet was evaluated, it was determined that this guenon was eating primarily the grapes and bananas. The diet was altered, however the keeper had difficulty getting the animal to eat other items in the exhibit environment. After 3 mo of no improvement on an oral hypoglycemic agent, the guenon was brought to the health center to live in a large squeeze cage and undergo intensive training. According to its keepers, the guenon was very aggressive to people and other animals and “didn’t like anybody.” This monkey was high-strung, suspicious, cautious, and reacted quickly and instinctually. Operant conditioning was started and the animal worked with one person for two sessions a day. A clicker and colored target was used with food rewards being given for the proper response. Diet was drastically altered to increase fiber and eliminate sugar and was accepted readily. This diet was used during training, then the remainder given after the training session. This initial training allowed the animal to become comfortable with the trainer and learn what was expected and how to successfully respond. Within 2 mo it was expert at stationing and putting its arm through the bars to touch the target. The animal never became comfortable having its arm held or manipulated, and would retreat when its arm was handled. However, when it would approach in a less formal manner, it became apparent that the animal liked to present by laying down with its back facing its trainer in a submissive manner. The animal would allow its back and other parts of its body to be scratched. Training was then altered to have the animal present in this manner. The animal was moved to a double dog-run-type chain link outdoor enclosure. Training rapidly progressed within another 2 mo, from scratching its back, to pinching the skin, to poking with a needle, to administering a small amount of cold saline s.c., to administering insulin. The animal is currently in the process of being regulated and since the animal does not allow blood collection, it is monitored via urine analysis for
glucose. The next step will be to return the animal to the exhibit and work with it in the back holding area there.

“Tule,” an 18-yr-old DeBrazza guenon, was being treated with prednisone for inflammatory bowel disease. Glucose and ketones were present in the animal’s urine; it was taken off the prednisone and then re-evaluated in November of 1997. The animal was severely compromised and diagnosed as a diabetic that needed immediate insulin therapy. (The animal was later diagnosed with Cushing’s disease as well.) The guenon was brought to the health center, housed in a large squeeze cage and given twice daily insulin injections in the tail or rump by veterinary personnel with the use of the squeeze. Training was instituted in a similar manner as outlined above with the red-tailed moustached guenon however, the initial goal was solely to get the animal to relax and provide it a positive experience with a person. The training was done at a separate time and by a different person so as not to associate the negative experience of being squeezed and getting injections with training. Initially, this animal was very ill and stressed but as it began to relax and its health improved, the training progressed to involve more touching and handling. The next step was to incorporate the injections. Progress was made very rapidly at this point; to date the animal allows injections with minimal squeezing and much less stress. This was an especially challenging case due to the animal’s medical condition necessitating treatment with restraint while simultaneously undergoing training.

**Conclusions**

These cases demonstrate that diabetic primates can be managed successfully and without stress with injectable insulin therapy long-term. The training required differs depending on the species and temperament of the animal. The animal gives the trainer cues as to what is the most comfortable and least threatening way to give the injections, and the manner of treatment and monitoring can then be tailor fit for the animal and its living environment.

**ACKNOWLEDGMENTS**

The author would like to thank and commend the animal care and veterinary staff involved for their intense efforts, patience and perseverance that resulted in such positive outcomes for these animals.

**LITERATURE CITED**

COMPULSIVE BEHAVIOR: RECOGNITION AND TREATMENT

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Abstract

Introduction

It has long been recognized that otherwise healthy animals, when kept in captivity, may develop strange behaviors. These behaviors appear abnormal because they are performed out of context, are often exaggerated, directed towards unnatural stimuli or objects, and are often performed repetitively in a constant manner. Well-known examples include pacing in polar bears and other carnivores, tongue playing in giraffes and okapis, feather plucking in parrots, repetitive regurgitation and reingestion in primates, and weaving in elephants. Compulsive behavior is very common in domestic livestock. In these species such behavior, particularly when stereotypic, has always been considered to be confinement-induced conflict behavior, and has been linked to specific husbandry practices. Compulsive behavior is also common in pets. Stereotyped compulsive behavior is one of the most studied abnormal behaviors in domestic animals, and much interest has been devoted to the topic in laboratory animals as well. Although early progress has been made to validate the diagnosis of Compulsive Disorder, further work is needed. The following working definition of Compulsive Disorder (CD) has been proposed: “behaviors that are usually brought on by conflict, but that are subsequently shown outside of the original context. The behaviors might share a similar pathophysiology (e.g., changes in serotonin, dopamine and beta-endorphin systems). Compulsive behaviors seem abnormal because they are displayed out of context and are often repetitive, exaggerated or sustained.”

Causes of Compulsive Disorder

Compulsive behaviors are considered to be an expression of stress, frustration, and/or conflict. Various forms of conflict behavior are caused by frustration or conflict and have been studied in a variety of species. Prolonged and/or repetitive frustration and conflict may result in the conflict behaviors becoming generalized to other contexts (i.e., they emancipate from their original cause). They are also believed to evolve gradually from more variable behavior to short sequences of a few simple behavioral elements. Furthermore it appears that the level of arousal necessary to trigger the performance of these behaviors diminishes as the behavior develops into a compulsive disorder.

While the above outlined development of compulsive behavior may be typical for locomotory behavior, case histories of affected dogs indicate that self-directed oral behavior is displayed from the start in various contexts, and does not increase in frequency. Also, these behaviors are typically
shown in situations with little outside stimulation, but presumably a high level of internal arousal. There is some evidence that locomotory and oral compulsive behaviors differ neurophysiologically as well.²

From clinical cases in dogs it is obvious there may be genetic factors controlling the development of CD: some breeds may be particularly susceptible to developing a CD and others may develop a particular compulsive behavior if the environment is conducive to the development of CD. A genetic predisposition for certain compulsive behaviors has also been demonstrated in lines of Thoroughbred horses,³³ and breed has been shown to affect the likelihood of various compulsive behaviors being performed by horses.²²

Any stressor, be it social, climatic, nutritional, or disease-related, is likely to contribute to the performance of compulsive behavior. These factors may either be causal, or they may simply increase arousal levels and thus the likelihood that an already established compulsive behavior is performed (i.e., act as modulating factors).²⁵

**Pathophysiology of Compulsive Disorder**

The pathophysiology of CD is not well understood. Most evidence stems from drug effects on the performance of compulsive behavior. Large doses of dopaminergic drugs such as amphetamine and apomorphine are effective in inducing stereotyped behavior in animals³ or exaggerating spontaneous compulsive behavior,² while the dopamine antagonist haloperidol results in suppression of spontaneously occurring stereotyped behavior.¹⁶

Beta-endorphins have been implicated in stereotypy performance because beta-endorphin receptor blockers can be effective in reducing stereotypies.⁵,⁶ However, the concept that performance of stereotypies is rewarded by endorphin release is no longer supported: cribbing in horses did not result in an increase in blood endorphin levels, and their pain sensitivity was actually increased during cribbing compared to when they were not cribbing.¹⁹ Furthermore it has been suggested that beta-endorphins may play a significant role only early on in the development of stereotyped behavior.¹⁶

Because of similarities of animal CD and human obsessive compulsive disorder, drugs inhibiting serotonin re-uptake have been used to treat dogs with CD.⁹ The effectiveness of such drugs implies that serotonin is involved in animal CD. Direct evidence of serotonin involvement has also been presented.³² However, the role of serotonin in CD is not well understood.¹⁵

**Treatment**

Treatment consists of environmental modification and, where necessary, pharmacologic intervention. In the following, treatment is listed in order of implementation.
1. If possible, the cause of the problem should be identified and addressed. The environment should be changed to accommodate the most important species-typical behaviors. Environmental enrichment is not useful unless it specifically targets the behavior that is frustrated, and/or the behaviors most commonly performed by the species in question.

2. Stressors may be additive, and once a compulsive behavior is established, environmental stress may serve to perpetuate it. This includes situations where important releasing stimuli for species typical behavior are lacking, and situations in which an aversive stimulus such as inappropriate climatic conditions, an aggressive group member, or proximity of visitors cannot be avoided. It is therefore indicated to try to reduce environmental stress as much as possible.

3. In most cases, particularly in those that have been going on for a long time, drug therapy may prove necessary. Beta-endorphin antagonists such as naloxone, nalmefene and naltrexone have been suggested to be used for treatment. Beta-endorphin antagonists have high first pass metabolism and a short half-life, and most are only effective as injectables. Only naltrexone is available as an oral formulation, because in humans its first metabolite, 6-beta naltrexol, is an active beta-endorphin antagonist. However, this metabolite is not formed in some species such as dogs, and clinical suppression of compulsive behavior in dogs is short-lasting. In spite of a report supporting its effectiveness at 2.2 mg/kg p.o., s.i.d.-b.i.d. in dogs, its use for the treatment of CD, at least in dogs, must be questioned.

Haloperidol has been used experimentally to reduce compulsive behavior in many species. It proved effective in suppressing stereotyped jumping in bank voles. Haloperidol decanoate was used to reduce bar biting in sows at 250 mg i.m. per sow. Haloperidol suppressed tongue playing in cattle. A dose for haloperidol has not been established for companion animals. Landsberg et al. list 1-4 mg per dog b.i.d., p.o. This author has used it only in a few cases of dogs with compulsive disorder at 1-2 mg per dog, invariably without success. The use of haloperidol for treating feather picking in birds was reported. In one case two African grey parrots were treated successfully with haloperidol at 0.4 mg/kg/day for 7 mo.

As is the case with human obsessive compulsive disorder, pharmacologic intervention is most likely achieved with serotonin re-uptake inhibitors. A clinical trial involving 51 dogs with a variety of compulsive behaviors has been performed for the tricyclic antidepressant, clomipramine. Some success was reported for treatment of feather picking in psittacine birds with clomipramine at 1 mg/kg s.i.d. or divided b.i.d., p.o. Clinical trials on cases of canine acral lick dermatitis have been performed for clomipramine, fluoxetine and sertraline. Paroxetine has also been used clinically, but its effect has not been evaluated. Fluoxetine has been used successfully to suppress pacing in a polar bear at the Calgary Zoo. Fluoxetine was given at 0.62 mg/kg s.i.d. for 77 days, then the dose was increased to 1 mg/kg s.i.d. In companion animals we usually give a drug for 3 wk after it appears to have an effect, then wean off gradually over 3 wk to avoid a rebound effect.
4. Instead of modulating the brain serotonin system by inhibiting the re-uptake and metabolism of serotonin in the pre-synaptic neuron, a tryptophan supplement can be fed. Tryptophan is a precursor of serotonin. Some success in the treatment of compulsive behavior in horses has been reported at a dose of 2g/horse b.i.d., or approximately 5 mg/kg b.i.d.23

5. In persistent cases a program of counter-conditioning (more correctly termed response-substitution) might be considered. If this option is chosen, treatment has to be implemented with great consistency in order to be effective. It is very important that the animal be distracted every time it is about to perform the compulsive behavior, and an alternative behavior be solicited.

LITERATURE CITED

Hook Lake Wood Bison Recovery Project: An Attempt to Eradicate Bovine Tuberculosis and Brucellosis from a Wood Bison Herd in Northern Canada

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Abstract

In 1990, the federally appointed Northern Diseased Bison Environmental Assessment Panel recommended the depopulation of tuberculosis and brucellosis infected free-ranging bison (Bison bison athabascae) herds in and around Wood Buffalo National Park and their replacement with disease free wood bison. An alternative approach to depopulation was conceived jointly by the Deninu Kue First Nation and the Government of the Northwest Territories and is being implemented as a pilot project for one infected bison herd. Over three successive calving seasons, 60 newborn calves were captured from the wild and placed into an isolation facility at Fort Resolution, Northwest Territories. The neonatal calves were treated with prophylactic antibiotics to prevent infection with Mycobacterium bovis and Brucella abortus microorganisms potentially present perinatally. Calves were housed in isolated pairs until they reached 12 mo of age. Multiple testing with the intradermal tuberculin test for tuberculosis and serology for Brucella antibodies revealed one caudal fold test positive calf. The reactor calf was slaughtered with its pen mate, but bacterial culture was unable to isolate any Mycobacterium species. The remaining 58 calves have remained test-negative for brucellosis and tuberculosis.

Introduction

Approximately 2,800 free-ranging bison (Bison bison athabascae) in northern Canada are found in populations known to be infected with Mycobacterium bovis and Brucella abortus. Two thousand and one hundred of these are located in Wood Buffalo National Park. The remainder range in areas adjacent to the park in northeastern Alberta and the Northwest Territories. The ongoing presence of both diseases in the region pose a threat to the conservation and recovery of healthy free-ranging bison populations, the ecologic, cultural and economic value of bison to local communities and the country, and the disease-free status and international reputation of the commercial bison and cattle industries in Canada.

In the absence of management support, infected herds have declined by 80% since 1970. In 1990, the federally appointed Northern Diseased Bison Environmental Assessment Panel6 recommended depopulation of tuberculosis and brucellosis infected free-ranging bison herds in and around Wood
Buffalo National Park and their replacement with disease free wood bison. The recommendation was strongly protested by many sectors of the Canadian public. The major reasons for opposition were impairment of ecosystem integrity and loss of genetic diversity represented in the infected herds.

Recognizing that the establishment of healthy bison populations is a desirable alternative to the status quo, the Deninu Kúe First Nation (Fort Resolution, NWT), supported by the Government of the Northwest Territories, initiated a long term recovery program for the Hook Lake herd located in the Slave River Lowlands. Elements of the program include habitat renewal using prescribed burning, establishment of a disease-free captive breeding herd based on the capture and prophylactic antibiotic treatment of neonatal calves, the gradual elimination of the infected parent herd largely through aboriginal subsistence hunting, isolation of the depopulated bison range, and reintroduction of disease-free bison.

**Methods**

Newborn calves were captured by helicopter-based net guns during May 1996, 1997 and 1998. In 1997 and 1998, calves were tested for antibodies against *Brucella abortus* using the Brewer’s Card Test (BCT) immediately after capture at a field staging site. Test positive calves were returned to their individual site of capture as determined by global positioning system and released. Test negative calves were transported to an isolation unit in Fort Resolution, NWT. They were housed in isolated pairs in 1.3 × 2.5 m boxes for 2 wk during which they were treated with intramuscular antibiotics. Calves were ear tagged with a numbered plastic disk at the time of capture, and with a tamper-proof metal tag provided by the Canadian Food Inspection Agency (CFIA) at the first disease test.

Calves were fed reconstituted milk replacer (22% fat and 24% protein by weight) (Brown’s Feeds, Airdrie, Alberta). High quality legume hay was offered ad libitum. Calf starter was provided from 2 wk after capture until the end of August. After that time, rolled barley and alfalfa pellets were provided at a rate of 1 kg and 0.5 kg/hd/day respectively. Upon completion of injectable antibiotic therapy, calf pairs were released into isolated 13 × 23 m paddocks.

In the absence of antibiotic sensitivity testing of the two microorganisms, the antibiotic protocol was selected based on information available from the veterinary and medical literature. Oxytetracycline (Liquamycin LA-200®; Rogar/STB Inc., London, Ontario) and dihydrostreptomycin (Ethamycin®; Rogar/STB Inc., London, Ontario) were administered by intramuscular injection at 10 mg/kg every other day for 14 days. Isoniazid (pms-INH7; Pharmascience, Montreal, Quebec) was administered orally in the daily milk feedings for 5 mo at 10 mg/kg UID. Isoniazid therapy was terminated 1 mo before the first intradermal TB test was carried out on each calf cohort. Enrofloxacin (Baytril®; Haver, Etobicoke, Ontario) and rifampin (Rofact®; ICN Canada Ltd., Montreal, Quebec) were also administered orally in the milk ration at 10 mg/kg and 15 mg/kg UID respectively.

To date, calves captured in 1996 have been tested five times for brucellosis and tuberculosis between November 1996 and February 1998. The intradermal caudal fold test using *M. bovis* PPD tuberculin was run in November 1996, February, April and November1997, and February 1998. The 1997
calves have been tested two times during the last two test periods. The Blood Tuberculosis Test (BTB) was run on whole blood and sera collected in March 1997 from 20 calves. The assay series was performed at the BTB Diagnostic Laboratory at Texas A&M University. Serum from each of the five test periods was tested for \textit{B. abortus} antibodies at the isolation unit using the Brewer’s Card Test. Additional serum was sent to the CFIA’s Animal Disease Research Institute in Lethbridge, Alberta, where it was tested using two or three of the following assays: Buffered Plate Antigen Test, Standard Tube Agglutination Test, and Compliment Fixation Test.

\textbf{Results}

Fourteen female and six male newborn calves were captured in 1996. In 1997, 26 calves were captured. Five calves tested positive on field screening for \textit{B. abortus} antibodies and were released at their respective capture sites. Sixteen female and four male test-negative calves were transported to the captive breeding facility at Fort Resolution.

All tests conducted in November 1996 were negative. In February 1997, one female calf tested positive on the caudal fold test for tuberculosis. A swelling >10 mm was observed on visual inspection and palpation. Due to logistic constraints a comparative cervical skin test could not be conducted within the required 10 day interval following tuberculin injection. The test positive calf and its pen mate were slaughtered on March 5, 1997. No lesions were grossly visible on post mortem examination. Histopathology revealed acid fast bacilli in a minute mineralized granulomatous lesion in a mediastinal lymph node. Bacterial culture failed to isolate any \textit{Mycobacterium} spp. All BTB tests performed on blood collected on March 4 were negative, including samples from the two slaughtered calves.

\textbf{Discussion}

Part of the protocol employed during this study (orphaning neonatal calves and elimination of test reactor pairs) was adapted from a successful approach used previously to eradicate tuberculosis and brucellosis from captive wood bison\textsuperscript{2}. In addition to orphaning and depopulation of isolated pairs, the Hook Lake project employed the prophylactic use of antibiotics. Anti-tuberculosis drugs have been used in deer, kudus, oryxes, camels, monkeys, and great apes in captivity.\textsuperscript{8,12} The antibiotic sensitivity of the strains of \textit{B. abortus} and \textit{M. bovis} found in bison in northern Canada has not been evaluated, but is the subject of a current study. Therefore, antibiotics were selected based on published findings from previous studies in which antibiotics were used to combat infections with \textit{M. bovis} or \textit{B. abortus} in other captive ungulate species. In the absence of controls, the efficacy of individual components of the protocol used in our study can not be fully assessed. Success will be determined by the ability of the protocol to establish a disease-free herd.

It was unfortunate that the single positive reactor to the caudal fold tuberculin test was not subsequently tested with the comparative cervical test to distinguish a possible infection by the most likely atypical mycobacterium, \textit{M. avium}, before slaughtering the calf and its pen mate. Potential avian hosts of \textit{M. avium} were abundant around the bison pens, including ravens (\textit{Corpus corax}),
common grackles (*Quiscalus quiscula*), and snow buntings (*Plectrophenax nivalis*). The incidence of atypical mycobacterial infections in people in the Northwest Territories (53 cases since 1990) provides an indication of the ubiquitousness of these organisms in the environment. Although there is a possibility that the calf was infected with an atypical mycobacterium, the location of the single detected lesion in a mediastinal lymph node is consistent with the pathogenesis of *M. bovis*. Identification of the acid fast bacilli detected in tissues from the test positive calf was unsuccessful, as bacterial culture did not isolate any *Mycobacterium* species.

We suggest that the risk associated with latent infections with either organism is minimized by the protocol applied in this study which includes field screening of neonatal calves for *Brucella* antibodies, administration of appropriate antibiotics, and regular disease testing. Multiple testing of the captive population will continue at a frequency of at least twice per year over the long term. One critical period remains for expression of latent *B. abortus* infections. Bovine calves infected by *B. abortus* in utero or after ingestion of infected milk may maintain a latent infection with seroconversion following their first calving or abortion in the case of females, or later in life in the case of males. Hence, it will be important to physically isolate primiparous cows during pregnancy beginning at 5 mo of gestation prior to the third trimester when brucellosis induced abortions may occur, and to monitor for anti-brucella antibodies for several weeks following parturition.

The approach being attempted in this study to eradicate *M. bovis* and *B. abortus* through salvage and creation of a healthy captive breeding herd may offer a possible alternative to the mass slaughter of wild bison populations and the attendant loss of genetic diversity that was proposed previously. However, latent undetected infections may yet compromise the project. Even if successful, establishing a clean captive herd represents only one step towards reestablishing a disease-free wild population. Additional measures, including a range of options identified in the planning of the program, will be required to ensure complete eradication of reservoirs of the microorganisms and protection of healthy bison from reinfection once they are restored to the Hook Lake bison range.

ACKNOWLEDGMENTS

Planning a project such as this involved the support of many organizations and individuals. The patience and indulgence of many Aboriginal Elders in Fort Resolution in accepting unfamiliar technology is recognized. The Deninu Kúe First Nation and Aboriginal Wildlife Harvesters Committee were strongly supportive of the project. Financial support for the operation was provided by the Government of the Northwest Territories Department of Resources, Wildlife and Economic Development.

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USE OF AZAPERONE WITH ZUCLOPENTHIXOL ACETATE FOR TRANQUILIZATION OF FREE RANGING WOOD BISON AND IMMOBILIZATION WITH CARFENTANIL AND XYLAZINE

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Abstract

Two hundred and seventy six Wood bison (Bison bison athabascae) were captured in March of 1997 and 1998 to determine tuberculosis (Mycobacterium bovis) and brucellosis (Brucella abortus) status in Wood Buffalo National Park, Alberta, Canada. Adult males were immobilized by helicopter darting with 7.2 ± 2.6 μg/kg carfentanil (Wildnil, Wildlife Pharmaceuticals, Fort Collins, Colorado, 3 mg/ml) and 87.6 ± 30.1 μg/kg of xylazine hydrochloride (Rompun, Bayer Inc., Etobicoke, Ontario, Canada). Mean induction time was 8.7 ± 6.1 min and mean reversal time (from anaesthetized to standing) was 6.3 ± 2.3 min using a single i.m. injection of naltrexone hydrochloride (Wildlife Pharmaceuticals, Fort Collins, Colorado USA) at a ratio of 132 ± 23 mg naltrexone per mg carfentanil. Induction time was strongly related to darting site, with most rapid inductions occurring with darts into large muscle masses. No cases of renarcotization were observed and all bison appeared clinically normal three days after the first capture.

Adult female and juvenile bison were captured by helicopter net-gunning and held in 3 × 3 m steel pens covered with polyurethane tarpaulins for 72 hr. Short-term tranquilization was achieved with 0.053 ± 0.015 mg/kg intravenous azaperone hydrochloride (Stresnil, Jannsen Pharmaceutica Inc., Mississauga, Ontario, Canada) and medium term tranquilization with 0.61 ± 0.20 mg/kg i.m. zuclopenthixol acetate (Clopixol-Accuphase, Lundbeck Inc., Montreal, Quebec, Canada). A significantly higher ($P < 0.05$) dose of 0.74 ± 0.21 mg/kg was used initially in the first field season, but a lower dose of 0.50 ± 0.099 was tried in 1998 due to the sedative effects observed after release from the pens. Eleven animals were intentionally not given the LAN in 1997 and a marked increase in excitability in the pens was observed compared with treated animals.

Three days after initial capture and processing, penned animals were immobilized with 4.4 ± 1.8 μg/kg carfentanil and 58.5 ± 28.4 μg/kg xylazine fired from a dart pistol. Mean induction time was 4.9 ± 3.6 min and mean reversal time was 7.5 ± 3.3 min with a dose of 155 ± 23.9 mg naltrexone per mg carfentanil. Xylazine reversal was included in 1998 using tolazoline hydrochloride (Tolazine, Lloyd Laboratories, Canada) intravenously at 1.7 ± 0.26 mg/kg. No significant difference ($P < 0.05$) in reversal time was observed between animals receiving tolazoline plus naltrexone versus those receiving naltrexone alone.

Three capture related mortalities were observed in 1997 and six in 1998. The lower dose of zuclopenthixol in the 1998 season along with deeper snow and warmer ambient temperatures may
have contributed to the additional capture related mortality observed in the 1998 season. carfentanil and xylazine proved to be a very effective and safe immobilization regime for both free ranging and penned bison at the doses used, resulting in very good muscle relaxation and minimal side effects. The most common side effects observed were regurgitation and vocalization. Tolazoline commonly produced muscle fasciculations and increased gastrointestinal motility. Zuclopenthixol in conjunction with azaperone seems to be a very effective calming regime for wood bison used at 0.7 mg/kg, with duration of tranquilization varying between two and four days.
CURRENT CAPTURE TECHNIQUE AND DRUG DOSAGE REGIME FOR THE IMMobilIZATION AND TRANQUILIZATION OF FREE-RANGING BLACK RHINOCEROS (Diceros bicornis bicornis) IN NAMIBIA

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Abstract

A dosage of 3.5-4.5 mg of etorphine combined with 120-250 mg azaperone or 80-100 mg xylazine was used to immobilize 172 free-ranging black rhinoceros (Diceros bicornis bicornis) from 1990-1997 in Namibia. With good dart placement induction time was 5-7 min. Rare adverse respiratory and cardiovascular depression has been reversed by i.v. administration of respiratory stimulant, (200-300 mg doxapram) and a partial narcotic antidote (10 mg nalorphine). Neuroleptanalgesia is routinely reversed with diprenorphine. Injury during loading, especially in rough terrain has been greatly reduced by “walking” the rhino after narcotic effect is partially antagonized with a low dose of nalorphine. Aggression and stress of rhino confined in bomas is reduced when administering a combination of long-acting tranquilizers: 100-200 mg Clopixol-Acuphase and 100-200 mg Trilafon. Black rhino immobilization and translocation using present capture and handling techniques has been highly successful. No rhino have died as result of the drug combination used, and translocation related losses have been low. The capture and translocation success has been superb with the present dosage regime and capture technique.

Introduction

Black rhinoceros have been immobilized regularly in Namibia since the 1960’s for certain management reasons including translocation. Over the years the combination of immobilizing drugs used and capture procedure has been altered to reach a safe effective drug combination and optimal handling technique for black rhinoceros. Since 1990, the drug combination administered to immobilize black rhinoceros and the handling technique used by the Game Capture Unit of the Ministry of Environment and Tourism in Namibia have been fairly consistent. During the period from 1990-1997, a total of 73 black rhinoceros have been immobilized for translocation. Almost all of these rhinoceros were immobilized for initial capture, placed in a boma to settle down and were subsequently immobilized at least once more for further procedures and loading for onward transport. Additionally, 94 free-ranging black rhinoceros have been immobilized for other procedures. Neuroleptanalgesia in these was reversed on site, once the procedures had been completed. Five free ranging black rhinoceros were immobilized for medical examination and treatment. Some of these were in very poor state and condition prior to immobilization.

Methods
Standard capture technique:2 The Ministry of Environment and Tourism in Namibia make use of trackers on the ground as well as aircraft (Cessna 172/182) to locate black rhinoceros. A helicopter is called in once a suitable animal has been spotted. The helicopter is only used for darting and lands near the rhino as soon as it becomes immobile. The plane circles above the rhino and guides the ground team into position. This routine greatly reduces helicopter flying time and thus the cost of the capture operation. Once all staff is in position, the plane resumes the search for other rhino.

It takes 5-10 min for the rhino to become immobilized with the drug combination used. The required drug doses are administered by injection. The animal is doused with water to prevent its body temperature rising above 39° C. Even though immobilized black rhinoceros are usually placed in sternal recumbency, it helps to let a rhino lie on its side, with the axilla and groin turned into the wind, to assist in cooling the animal down. The horn tip is cut off to prevent it being broken during confinement. Blood and tissue samples are taken, and microchips implanted subcutaneously as well as into both horns. Dental casts are taken for age determination.

A crate is then placed in front of the rhino. A rope fitted around the head of the rhino is pulled through the crate and secured to a vehicle. A team of laborers holds this rope and, once the rhino stands up following the administration of the anaesthetic antidote, pulls the rhino into the crate. In addition, the rhino is helped to its feet and directed into the crate by prodding from behind. Black rhinoceros do not like to walk backwards out of crates, therefore the crates are loaded into the truck with the rhino facing backwards. Once loaded into the crate, the rhino is transported immediately to a holding pen and off-loaded, or loaded over onto a mass crate. If rhino are transported directly after capture, they should reach their destination within 20 hr, where they are boma-trained for 4-8 wk, prior to release. If this is not feasible due to the traveling distance involved, the rhinoceros are boma-trained near the capture site for at least 4 wk, and are then transported to their new environment, where they are held in bomas for an additional two wk.

Rhino capture in Namibia takes place during the cooler hours of the day to reduce the chance of heat stress. In addition, all rhino translocations are normally carried out before the end of May. This ensures that all boma confinement and long distance transport are finished before the cold winter months and that there is still enough good quality browse available once the rhino are re-released. Depending on the boma size six to eight black rhinoceros are caught at a time.

Drug combination administered: Black rhinoceros are preferably darted from helicopter as described above. A Cap-Chur 3cc syringe with NCL-3 needle and standard tailpiece is delivered using a Palmer extra long-range projector. Considerable power is needed to penetrate the 3.5 cm thick skin. The dart is usually placed in gluteal muscles of the rump. When darting rhinoceros on foot, a Cap-Chur 2cc syringe with NCL-3 needle and plastic tailpiece is delivered using a Palmer extra long range projector or a Pneudart powder projector. In this case any suitable large muscle mass is used (often shoulder muscles or neck muscles).
A dosage of 3.5-4.5 mg of etorphine HCl (M99, C-Vet, South Africa) is used for adult rhino.¹ The exact dosage rate depends on the size and condition of a rhino. This is combined with 120-250 mg azaperone tartrate (Azaperone, Kyron Laboratories, Pty. Ltd., South Africa) for the tranquilizing and muscle relaxant effect. Alternatively 80-100 mg xylazine (Bayer, Leverkusen, South Africa) can be used. The induction time is considerable reduced if the tranquilizer or muscle relaxant is added. Additionally, with such a combination the trembling effect due to the M99 in black rhinoceros is greatly reduced. When hyalase is available, 4500 IU of this drug is added. The use of hyalase does increase the absorption of the drugs significantly, if darts are well placed and thus shortens induction time by more or less 1 min. Often a slightly higher drug dose is used when darting from helicopter in bad terrain, to get rhino down quickly. Lower doses are used for immobilizing rhino in bomas, where they are confined and a slower downtime is acceptable. Dosage rates, especially the tranquilizer dose, have to be reduced for animals in poor condition.

Most black rhinoceros will show effects of the drugs within 3 min. They may slow down, ears turn back and they exhibit a typical high-stepping gait. It becomes almost impossible to steer rhino away from dangerous terrain once the drugs have taken effect. Therefore darting is done on suitable terrain and the darted rhino is pushed to the most open, level area slowly, until they become affected. Usually the rhino will be immobilized 5-10 min after darting (preferably within 5-7 min). The induction time is directly related to the drug dosage used and closely correlated to good dart placement, which enables deep intra-muscular injection of the entire dart volume. If no effect is seen after 5 min, usually another full drug dose is given. If drug effect is seen, but a rhino is not down within 10 min a further partial or full dose is given, depending on terrain, drug effect, etc.) It is always preferred to dart rhino from helicopter. This enables easier and better dart placement. The rhino can be directed away from steep slopes or gullies. It is possible to re-dart from the helicopter, if the drugs do not effect the rhino sufficiently. The veterinarian reaches the rhino immediately once it is immobile.

Once the rhino is reached an intravenous catheter is placed immediately in an ear-vein to keep the venous system open for drug administration. The respiration is checked. A respiratory stimulant (e.g., 200-300 mg of doxapram; Dopram, Continental Ethicals, Pty., Ltd., South Africa) and a partial narcotic antidote (e.g., 10 mg nalorphine) are kept handy. This can be administered routinely, but may cause the rhino to get up with external stimuli. If the breathing rate is reduced to less than 6/min, the doxapram and/or nalorphine are administered intravenously. Additionally, the colour of the mucous membranes is monitored. The blood vessels on the back of the ears help to indicate blood pressure. If the mucous membranes are bluish/grey or the blood vessels are not prominent 10-20 mg of nalorphine and 10-15 ml of doxapram is administered. If breathing rate remains down/irregular, mucous membranes remain bluish and blood pressure does not increase a repeat dose of doxapram and/or nalorphine or complete reversal is indicated. This happens rarely in black rhinoceros. The heart rate is usually in the range of 60-90 beats/min.

Black rhinoceros respond very well to the antagonistic effect of diprenorphine HCl (M50-50, C-Vet, South Africa). A dosage of 2.4 mg diprenorphine/mg etorphine is routinely administered intravenously to reverse the anaesthetic. The rhino will respond by twitching the ears and becoming
alert within 45-90 sec of intravenous injection. Usually a rhino is back on its feet within 60-120 sec of reversal agent administration. If a rhino goes down in terrain unsuited for loading into a crate, or for complete reversal, the rhino is blindfolded and a person is positioned on either side of the animal. A rope is placed behind the posterior horn around the head and another rope on one of the hind-feet, to act as a brake. Staff keep the ropes under some tension, after the intravenous administration of 20-40 mg nalorphine. The rhino is prodded after about 3-5 min. Failing to get up, additional doses of 10-20 mg of Nalorphine are used at 5-min intervals. Once the rhino is up on its feet, it can be led to suitable terrain be exerting tension on the ropes and pressure on the side of the neck and head, while prodding from behind. The rhino can be walked straight into the crate or a complete antidote is given for release. This method of “walking a rhinoceros” after partial narcotic reversal is now used routinely to load rhino into the crate. This reduces the chance of injury to the rhino or staff during this procedure. The use of pure antagonists (e.g., naltrexone or nalaxone) to reverse the narcosis in black rhinoceros has not been necessary, but these drugs could be used, should an animal not respond well to other antidotes.

The use of muscle relaxant or tranquilizer not only improves the level of neuroleptanalgesia, but also is ideal for reducing the stress of rhino confined in crates after capture. During long transport, if a rhino become restless, it is sometimes necessary to top up the tranquilizer. azaperone is usually administered intramuscularly in increments of 100 mg.

Black rhinoceros, which are confined in bomas during translocation are injected intramuscularly with 100-200 mg zuclopenthixol acetate (Clopixol-Acuphase, Lundbeck, South Africa). This long-acting tranquilizer has an excellent sedative effect, which lasts for 3 days in black rhinoceros. This is ideal to reduce initial aggressiveness of rhino confined in bomas and minimizes injury to the rhino and damage to the boma. Clopixol-Acuphase is used in combination with 100-200 mg trilafon enanthate (Trilafon, Scherag, South Africa) injected intramuscularly. The effect of Trilafon is not as noticeable as that of Clopixol-Acuphase, but is important in minimizing aggression and stress experienced in confined rhino for around 14 days. After 2 wk rhinos are typically used to the boma routine and seem to tolerate the confinement without further sedation. For long distance transport 100-200 mg Clopixol-Acuphase is used successfully. Usually no top-up dosage is required.

Rhino are routinely treated with topical (in dart wounds) as well as systemic broad-spectrum antibiotics, B vitamins, selenium and vitamin E, endo- and ecto-parasiticides.

Results

During the period from 1990-1997, a total of 73 black rhinoceros were immobilized for translocation. Only one of these rhino died during the capture. This rhino bull was initially captured and placed in the boma successfully, but managed to break open the boma gate and escaped. The rhino ran almost without a break and without drinking for three days, through communal farmlands and even through a small village and had to be recaptured. While being loaded this rhino seized and died. Over-exhaustion and hypo-glycemia caused this death.
A group of six black rhinoceros kept in a boma during the winter of 1995 lost condition due to the poor quality of browse available. A sudden, severe cold spell resulted in the death of three of these rhino. One rhino broke off its horn in the boma and was released. The carcass of this bull was found 3 mo later. This mortality could have resulted from the trauma sustained in the boma.

In the period from 1990-1997, 69 black rhinoceros were released in a new environment. All releases were very closely monitored. Only two of these rhino died in the post-release period. One rhino failed to find water and disappeared into mountainous terrain, where it died. A young heifer, which had lost condition post release and was finally severely injured by an adult black rhinoceros bull also died 2 mo after release from the boma.

All 94 free-ranging black rhinoceros captured for a combination of procedures from 1990-1997 survived the capture and procedures.3

Five black rhino presented with varying degrees of debilitation or injury during the study period. One of these had sustained severe fighting wounds, another had severe burn wounds sustained during an extensive veld-fire and a third rhino had been stuck in the mud of a waterhole. These three rhino died in spite of attempted treatment, but had all survived the immobilization and transport to a boma. Another rhino with fighting wounds and one with fractured metatarsal bones II, III & IV, were treated and confined in bomas and healed, so that they could be re-released.

Discussion

During the years 1990-1997, a total of 172 free-ranging black rhinoceros have been immobilized for capture and translocation, or for a procedure to be carried out on site. Of these only one bull, who was severely stressed, died on re-capture. Not one of the many rhino immobilized, died while confined in a boma. Thus the success rate of immobilization using the combination of 3.5-4.5 mg M99 with either 120-250 mg Azaperone or 100 mg Xylazine, must be regarded as a very safe dosage regime. The induction time of 5-7 min with good dart placement using this dose is acceptable. Very few respiratory or cardio-vascular problems have occurred. When there have been respiratory problems, these are well-controlled using respiratory stimulant and partial narcotic anti-dote. Injury during loading, especially in rough terrain has been greatly reduced by “walking” the rhino after narcotic effect is partially antagonized with a low dose of Nalorphine. Aggression and stress of rhino confined in bomas is reduced when administrating a combination of long-acting tranquilizers: Clopixol-Acuphase and Trilafon. The entire translocation process using present capture and handling techniques has been highly successful.

Off the 9.6 % (7/73) that died during or after translocation, nearly half died due to adverse climatic conditions. The low post release losses (2/69) have been achieved by minimizing stress during the entire translocation. The adequate use of long acting tranquillizers and good handling facilities and techniques play an important role in achieving this level of success.

ACKNOWLEDGMENTS
The success of black rhino translocations in Namibia during 1990-1997 could only be achieved with the help of the dedicated staff of the Game Capture Unit, Ministry of Environment and Tourism in Namibia.

LITERATURE CITED

THE HEALTH OF RED SQUIRRELS (Sciurus vulgaris) IN TRANSLOCATION STUDIES

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Abstract

The feasibility of the translocation and introduction of red squirrels (Sciurus vulgaris) as a population reinforcement tactic is being investigated in a series of five release studies in a 1700 ha Red Squirrel Reserve in Thetford Forest, East Anglia, UK. A soft-release method using a 1 ha pre-release pen is being used. The protocols developed and the results of the first four studies have been described.1 This paper describes the health of the squirrels involved in two of the studies, number three (the experimental study) and number four (the follow-up study). In the third study, 23 red squirrels were translocated from two sites in the north of England to Thetford. The health of each squirrel was examined under inhalational anaesthesia using isoflurane (Isoflo, Mallinckrodt Veterinary, Uxbridge UB9 6LS, UK) prior to release. Lactated Ringers solution was administered subcutaneously and tick infestations were treated with ivermectin (MSD Agvet, Hoddesdon EN11 9BU, UK) diluted in propylene glycol at approximately 200: g/kg body weight in 18 animals. One juvenile male died prior to the induction of anaesthesia but no pathologic lesions were found on post-mortem examination. Nine squirrels were recaptured 18 days after release for a health examination; they were in good physical condition and had maintained body weight (± 20 g). After 33 days in the pre-release pen, one female was found dead and a post-mortem examination did not reveal the cause of death. Four squirrels escaped from the pre-release pen after three days due to vandalism but the remaining 17 squirrels were successfully released into the Reserve. In the follow-up study (number four), which took place 24 days after the third study, eight animals from different sources were placed in the pre-release pen but all died over the following 28 days from a disease associated with parapoxvirus infection before release into the Reserve. Parapoxvirus infection may cause significant mortality in red squirrel populations and the translocation of squirrels may increase the likelihood of epidemics of infection. Proposals for translocations and the reinforcement of populations must take account of the risk of parapoxvirus infection.

LITERATURE CITED

DISEASE EVALUATION OF FREE-RANGING ORANGUTANS (*Pongo pygmaeus pygmaeus*) IN SABAH, MALAYSIA

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Abstract

Comprehensive health information and base-line data on free-ranging animals is necessary to evaluate the impacts of habitat destruction, reintroduction and translocation programs.2 Taking advantage of ongoing translocation efforts to rescue wild orangutans in Malaysia, a health evaluation was conducted on these primates. Between August 1996 and August 1997, 56 wild orangutans were captured and translocated to protected habitat by the Sabah Wildlife Department.4 These animals underwent a complete physical examination and blood, hair and fecal samples were collected. The test panels run on blood samples included hematology, serum chemistry, toxicology, metals, minerals, vitamins and examination for infectious diseases. The latter including the evaluation for viral, parasitic, fungal and bacterial diseases.

The preliminary results in this field report provide, for the first time, critically needed 1) baseline information on free ranging orangutans, 2) a reference for comparison in case of future disease outbreaks, and 3) data for the development of rational protocols to guide existing and developing rehabilitation and release programs.

Basic hematology, serum chemistry, vitamins and serum soluble elements results are presented in Tables 1, 2, 3 and 4 respectively. Positive antibody titers for leptospirosis, flavivirus, alphavirus (arbovirus), rotavirus, adenovirus and respiratory syncytial virus were found in the wild orangutans (Table 5). These pathogens have the potential to affect wildlife population dynamics as well as the local human and livestock population. The prevalence of potential active mycobacterial infections, based on serum Ag85 levels, was low in the free-ranging population.3 *Plasmodium pithec* was seen in < 25 % of all wild orangutans evaluated based on peripheral blood films and a nested-PCR technique with high sensitivity for *Plasmodium* species.1,5 No confirmed cases of *Plasmodium sylvaticum* have been identified in the wild animals sampled. Chlorinated pesticides (aldrin; alpha - BHC; beta - BHC; O, P’ - DDD; P, P’ - DDD; P, P’ - DDE; O, P’ - DDT; P, P’ - DDT; dieldrin; endrin; heptachlor; heptachlor epoxide; lindane (gamma - BHC); and nonachlor) and PCBs were not detected in samples analyzed. Samples analyzed for microfilarial infections were also negative. Further laboratory work is underway and, when completed, the results will be analyzed as they relate to sex or age classes, vector and non-vector borne diseases. Determining the significance of the negative and positive titers and the potential pathogenicity of these agents is imperative. Identifying critical health factors affecting wildlife populations is the first step to understanding the role of
diseases in population dynamics. This knowledge is essential for conservation management decisions. This study provides this information and will spur further research.

ACKNOWLEDGMENTS

The authors thank the Sepilok translocation team, especially Elis Tambing for his hard work in catching wild orangutans; Richard Heberling at the Primate Virus Reference Lab for sample analysis; Mark Eberhart at the Center for Disease Control for vector-borne disease analysis; Renke and Pamela Thye for their support; and the Morris Animal Foundation for supporting laboratory analyses.

LITERATURE CITED

Table 1. Hematology values for free-ranging orangutans.

<table>
<thead>
<tr>
<th>Test</th>
<th>Units</th>
<th>Mean (±SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV</td>
<td>% (n = 46)</td>
<td>32.7 (± 5.2)</td>
<td>22-46</td>
</tr>
<tr>
<td>TS</td>
<td>g/dl (n = 46)</td>
<td>6.8 (± 0.58)</td>
<td>5.2-8</td>
</tr>
<tr>
<td>WBC</td>
<td>×10^3/μl</td>
<td>9.680 (± 1.817)</td>
<td>7.7-12.5</td>
</tr>
</tbody>
</table>

Table 2. Serum chemistry values for free-ranging orangutans.

<table>
<thead>
<tr>
<th>Test (n = 38)</th>
<th>Units</th>
<th>Mean</th>
<th>SD</th>
<th>Test</th>
<th>Units</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUC</td>
<td>mg/dl</td>
<td>118.7</td>
<td>47.5</td>
<td>K</td>
<td>mEq/L</td>
<td>4.55</td>
<td>1.12</td>
</tr>
<tr>
<td>BUN</td>
<td>mg/dl</td>
<td>10.8</td>
<td>13.4</td>
<td>Cl</td>
<td>mEq/L</td>
<td>97.5</td>
<td>15.4</td>
</tr>
<tr>
<td>CREAT</td>
<td>mg/dl</td>
<td>1.4</td>
<td>2.2</td>
<td>A/G</td>
<td>ratio</td>
<td>1.56</td>
<td>.33</td>
</tr>
<tr>
<td>TP</td>
<td>g/dl</td>
<td>6.75</td>
<td>0.63</td>
<td>BUN/CRT</td>
<td>ratio</td>
<td>9.48</td>
<td>7.7</td>
</tr>
<tr>
<td>ALB</td>
<td>g/dl</td>
<td>4.07</td>
<td>0.55</td>
<td>GLOB</td>
<td>g/dl</td>
<td>2.6</td>
<td>0.42</td>
</tr>
<tr>
<td>BILI</td>
<td>mg/dl</td>
<td>0.57</td>
<td>0.54</td>
<td>CO₂</td>
<td>mEq/L</td>
<td>5.2</td>
<td>1.67</td>
</tr>
<tr>
<td>ALK PHOS</td>
<td>U/L</td>
<td>288.9</td>
<td>186.3</td>
<td>LIP</td>
<td>U/L</td>
<td>23.2</td>
<td>22.4</td>
</tr>
<tr>
<td>ALT</td>
<td>U/L</td>
<td>65.9</td>
<td>88.8</td>
<td>AMYL</td>
<td>U/L</td>
<td>187</td>
<td>565</td>
</tr>
<tr>
<td>AST</td>
<td>U/L</td>
<td>246</td>
<td>490</td>
<td>TRIGLYC</td>
<td>mg/dl</td>
<td>71.2</td>
<td>30.4</td>
</tr>
<tr>
<td>LDH</td>
<td>U/L</td>
<td>1424.8</td>
<td>2508</td>
<td>CPK</td>
<td>U/L</td>
<td>2497</td>
<td>3767</td>
</tr>
<tr>
<td>CHOL</td>
<td>mg/dl</td>
<td>161.9</td>
<td>165.9</td>
<td>GGTP</td>
<td>U/L</td>
<td>14.7</td>
<td>10.6</td>
</tr>
<tr>
<td>Ca</td>
<td>mg/dl</td>
<td>8.46</td>
<td>1.32</td>
<td>Mg</td>
<td>mEq/L</td>
<td>1.63</td>
<td>0.41</td>
</tr>
<tr>
<td>PHOS</td>
<td>mg/dl</td>
<td>3.68</td>
<td>1.2</td>
<td>Na</td>
<td>mEq/L</td>
<td>135.5</td>
<td>17.7</td>
</tr>
</tbody>
</table>
**Table 3.** Vitamin levels in free-ranging orangutans (in µg/ml).

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Mean</th>
<th>SD ±</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-tocopherol</td>
<td>2.1</td>
<td>± 1</td>
<td>0.7-4.88</td>
</tr>
<tr>
<td>Retinol</td>
<td>0.41</td>
<td>± 0.18</td>
<td>0.16-0.87</td>
</tr>
<tr>
<td>Gamma-tocopherol</td>
<td>0.27</td>
<td>± 0.12</td>
<td>0.1-0.55</td>
</tr>
</tbody>
</table>

**Table 4.** Serum soluble element values in free-ranging orangutans (in ppm) (n = 33).

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Mean</th>
<th>SD ±</th>
<th>Range</th>
<th>Mineral</th>
<th>Mean</th>
<th>SD ±</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba</td>
<td>0.07</td>
<td>0.01</td>
<td>0.05-0.132</td>
<td>Cr</td>
<td>0.27</td>
<td>0.03</td>
<td>0.22-0.33</td>
</tr>
<tr>
<td>Fe</td>
<td>2.08</td>
<td>1.0</td>
<td>0.547-4.76</td>
<td>Cu</td>
<td>2.09</td>
<td>0.44</td>
<td>1.48-3.37</td>
</tr>
<tr>
<td>P</td>
<td>126.8</td>
<td>27.56</td>
<td>73.2-195</td>
<td>Mn</td>
<td>0.086</td>
<td>0.096</td>
<td>0.056-0.62</td>
</tr>
<tr>
<td>B</td>
<td>1.38</td>
<td>0.16</td>
<td>1.11-1.67</td>
<td>Na</td>
<td>3539</td>
<td>198</td>
<td>3200-4300</td>
</tr>
<tr>
<td>Ca</td>
<td>99.2</td>
<td>8.18</td>
<td>88-125</td>
<td>Co</td>
<td>0.14</td>
<td>0.01</td>
<td>0.11-0.16</td>
</tr>
<tr>
<td>Mg</td>
<td>24</td>
<td>6.9</td>
<td>10.1-44.5</td>
<td>Mo</td>
<td>0.28</td>
<td>0.03</td>
<td>0.22-0.33</td>
</tr>
<tr>
<td>Zn</td>
<td>1.23</td>
<td>0.37</td>
<td>0.5-2.0</td>
<td>K</td>
<td>190</td>
<td>37.7</td>
<td>142-2</td>
</tr>
</tbody>
</table>
Table 5. Virus, Leptospira serovars and other positive antibody titers and microfilaria in free-ranging orangutans.

<table>
<thead>
<tr>
<th>VIRUS</th>
<th>+ %</th>
<th>VIRUS</th>
<th>+ %</th>
<th>Lepto (serovars)</th>
<th>+ %</th>
<th>Lepto (serovars)</th>
<th>+ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRV</td>
<td>0 0</td>
<td>SA 11</td>
<td>10 30 pomona</td>
<td>0 0</td>
<td>tarassovi</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>SIV</td>
<td>0 0</td>
<td>EBV-VCA</td>
<td>34 100 hardjo</td>
<td>2 6</td>
<td>australis</td>
<td>4 1</td>
<td></td>
</tr>
<tr>
<td>STLV</td>
<td>0 0</td>
<td>SIM, V-Z</td>
<td>0 0 icter/cop</td>
<td>4 12</td>
<td>pyrogenes</td>
<td>2 6</td>
<td></td>
</tr>
<tr>
<td>MMR</td>
<td>0 0</td>
<td>Cox B 1-5 (4+) 1b 3b grippo</td>
<td>30 88</td>
<td>brattslava</td>
<td>16 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV</td>
<td>1b 3b</td>
<td>Hepatitis A,C,E a a canicola</td>
<td>0 0</td>
<td>sejroe</td>
<td>2 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EMC</td>
<td>0 0</td>
<td>CHIMPCMV</td>
<td>0 0 ballum</td>
<td>9 27</td>
<td>javanica</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>MPV</td>
<td>0 0</td>
<td>Polio</td>
<td>0 0 wolfi</td>
<td>3 9</td>
<td>szwajzak</td>
<td>1 3</td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td>0 0</td>
<td>B-VIRUS</td>
<td>0 0 automnalis</td>
<td>25 73</td>
<td>saxkoehing</td>
<td>1 3</td>
<td></td>
</tr>
<tr>
<td>Fila</td>
<td>0 0</td>
<td>HSV 1</td>
<td>0 0 bataviae</td>
<td>0 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavivirus A</td>
<td>a a</td>
<td>Hepatitis B,D a a</td>
<td></td>
<td>OTHER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alphavirus A (Arbo)</td>
<td>a a</td>
<td>Adenovirus</td>
<td>3 9</td>
<td>P. pseudomelei d d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flu-A&amp;B</td>
<td>0 0</td>
<td>Para Flu 1,2,3</td>
<td>0 0</td>
<td>Microfilaria</td>
<td>0 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a = Possible positives and/or further analysis in progress; b = positives not significant-possible cross reaction/non specific; SRV = simian retrovirus; SIV = simian immunodeficiency virus; STLV = simian T-cell leukemia virus; MMR = mumps, measles, rubella; EMC = encephalomyocarditis virus; VZV = Varicella-Zoster; SA 11 = rota 1 virus; EBV-VCA= Ebstein-Barr virus; Cox B 1-5 = Coxsackie B; HSV 1,2,3=Herpes simplex; CHIMPCMV = cytomegalo virus; B virus = Parvo virus; MPV = monkey pox; Filo = Ebola like; Flavi = including Dengue and Chikungunya.
MEDICAL MANAGEMENT OF THE FREE-RANGING CALIFORNIA CONDOR

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Abstract

The California condor is an endangered species that has required aggressive medical management in conjunction with captive breeding and release programs to ensure its survival in captivity and the wild. The organization of this management has become increasingly complicated, but it is imperative to the successful medical management of this species. Lead poisoning cases provide an example of how the current medical management approach works.

Introduction

The California condor is a highly endangered species that reached a low population number of 27 birds when they were all placed in captivity to begin an aggressive captive breeding program in 1987. By 1992 sufficient numbers had been reached to begin releasing birds back to the wild. Veterinary involvement with this species began in the 1980’s during hands-on field studies, and has evolved to a very high level to date, due to increased numbers of condors, more sophisticated avian medicine, and a more complex recovery program. This paper will give an overview of the organization of the current medical management of the California condor, both in captivity and in the wild. Lead poisoning scenarios demonstrate how this management is critical to the recovery of these birds.

Organization

Currently, three institutions have captive breeding programs for the California condor: the Los Angeles Zoo in Los Angeles, California, the Zoological Society of San Diego at the San Diego Wild Animal Park in Escondido, California, and the World Center for Birds of Prey in Boise, Idaho. The birds in captivity are medically managed by the veterinarians at these institutions who work together but manage their birds independently. Offspring that are scheduled for release receive a standardized thorough veterinary screening prior to transport to release sites. Birds are often first sent to the Los Angeles Zoo which serves as a “staging” area prior to transport to a release site.

Birds currently fly at three release sites: the Los Padres National Forest in Southern California, the Vermillion Cliffs in the southwest portion of the Paria Plateau north of the Grand Canyon National Wilderness in Arizona, and the Ventana Wilderness area of the Los Padres National Forest in northern California. These birds are managed by four entities: The U.S. Fish and Wildlife Service in southern CA, the Ventana Wilderness Sanctuary in northern California, and the Peregrine Fund in conjunction with the Bureau of Land Management in the Grand Canyon. Released birds are monitored intensively by field biologists and receive veterinary care when necessary.
The position of Veterinary Coordinator has been established, in order to coordinate veterinary care for all release birds and provide veterinary care for the southern California birds. Local veterinarians have been identified and trained to work with immediate problems that may occur in northern California and Arizona. Biologists notify the Veterinary Coordinator when a problem is identified, or if there is an emergency, and the Coordinator notifies the local veterinarian directly. Local veterinarians triage at the site and work with the Veterinary Coordinator to determine how further care will be handled. If needed, birds are transported back to either the San Diego Wild Animal Park or the LA Zoo for long-term care if needed. Minor health problems that can be remedied or monitored in the field are handled by the local veterinarian.

The California Condor Recovery Team is the organization which meets and decides the plan for management of the species. In addition to other team members, the team includes one veterinarian, called the Veterinary Advisor. The Veterinary Advisor works with the Veterinary Coordinator and clinical veterinarians to coordinate veterinary management of the species, and advises the recovery team on any veterinary issues. Veterinary protocols are in the process of being refined, aiding in the uniformity of medical screening and treatment which will be of paramount importance as the program continues to expand.

**Lead Poisoning**

Five deaths in the wild population have occurred since 1980; three resulted from lead poisoning. In the fall of 1997, ten released birds fed on a hunter-killed carcass. Blood sampling and radiographs were taken on birds in the field by trained field biologists. Three birds had levels above 30 µg/dl (157.3 µg/dl, 75.6 µg/dl, and 56.2 µg/dl respectively). All three also had evidence of metal ingestion on radiographs. These three asymptomatic birds were brought into the LA Zoo Health Center for chelation therapy, and were re-released once the levels had come down. The others were monitored in a field pen, radiographed and blood sampled and released without ever being brought in from the wild. At this writing, a released bird was noted to be ataxic and weak while feeding on a carcass, and was captured and brought into captivity for evaluation and treatment. This bird is suffering from lead poisoning with a level at 291.4 µg/dl with no metal on radiographs, and clinical signs of neurologic deficits of the legs and crop stasis. The wild bird presented to the San Diego Wild animal Park in January of 1986 had a blood lead of 420 µg/dl, and died from complications of crop stasis.

**Conclusion**

Even the most skilled veterinarian cannot help an injured or ill animal if field personnel does not identify a problem, locate the animal, and retrieve the animal from the wild if necessary. Arrangements may have to be made to get an animal out of a remote area and into captivity temporarily for treatment. All parties must work together to give the animal the best medical care while keeping in mind the future goal of releasing the animal back to the wild. While these obstacles are extremely challenging, they can be overcome. Excellent communication, organization, and cooperation is necessary to provide the best medical care possible for an endangered species.
ACKNOWLEDGMENTS

The present and past contributions of veterinarians, field personnel, zoo and breeding facility animal care specialists, and supporting staff members, have allowed this program to continue to build on itself and achieve the success it has today.

LITERATURE CITED

ANESTHESIA OF CALIFORNIA SEA LIONS (Zalophus californianus) IN ELEVEN REPRODUCTIVE ROOKERIES OF THE GULF OF CALIFORNIA, MEXICO

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Abstract

The field study was carried out on 13 rookeries in the Gulf of California in Mexico, during the breeding season of 1997 in the months of June and July. A total of 288 (137 males, 151 females) California sea lion pups (Zalophus californianus) were anesthetized for 6-25 min (mean = 12.51, SD = 4.38) with isoflurane. In a period of 20 days, the average for an island was 24 animals (range: 16-29 animals), from an estimated 2-42 days of age, weighing 6-17.4 kg (mean = 10.41 kg, SD = 2.02). The inhalent anesthesia with isoflurane proved to be effective and safe for the immobilization of free-ranging California sea lion pups. The morphometric measurements obtained were more precise than the ones taken when only physical restraint was used.

Introduction

The California sea lion is widely distributed in the Gulf of California in Mexico. The current population for this area is considered to be around 30,000 animals, in 40 rookeries. Thirteen of these are reproductive rookeries, harboring up to 93% of the population during the breeding season. Eighteen rookeries are non-reproductive (7%) and the rest are temporary resting places.1,7

In the last few years in Mexico, research on population dynamics, ecology and interactions of these animals with the fishing industry has intensified. The direct handling of California sea lion pups has been carried out since the 80's using physical restraint, in order to mark, weigh, and measure animals. Direct observations of the quality of the breeding rookeries has suggested that there are significant differences between them.7 However, since the verification of this information requires the safe handling of animals in order to take accurate measurements and samples, chemical immobilization has been required.

As part of a long term study on the ecology, behavior, physiology and epidemiology of the California sea lion, the use of isoflurane as an anesthetic agent for handling a great number of pups (0-6 wk) was evaluated in several colonies from the Gulf of California. The goals were to obtain quick, quality morphometric measurements and biologic samples, and to reduce the negative impact on the breeding rookeries. In recent years, the use of the inhaled anesthesia for marine mammals has gained
popularity, since it provides a quick induction, an ideal state of immobilization, a high margin of safety and a complete and quick recovery from the anesthesia. 

Methods

The field study was carried out on 13 rookeries in the Gulf of California, Mexico. Anesthesia was performed on 11 of them. Transportation to the islands was made possible with the help of a Mexican Navy ship that patrols the area supporting research. The Gulf of California harbors more than 100 islands, all of them with desert climate, and the great majority posses rocky beaches with difficult access, which are preferred by the California sea lions for breeding. The study was carried out during the breeding season of 1997 in the months of June and July. The team arrived at each island at 8:00 a.m. and departed at dusk, in order to continue on to the next rookery.

During the anesthesia, the following was obtained: Morphometric measurements (total length, curvilinear longitude, cranial, axillary, abdominal and pelvic circumference, as well as the thickness of the blubber layer at several levels); body weight; volume of the animal by means of water density immersion; hair samples to study levels of heavy metals; blood (~10 ml) from the jugular vein for hematology and tests against infectious agents. On animals that presented cutaneous vesicles, cultures for viral isolation were collected. Fecal samples were also obtained. Samples that could not be stored were processed on the same day they were collected.

The work team consisted of three persons managing the anesthesia, taking samples and measurements, and two persons capturing, looking after the pups and returning them as required. Each animal was restrained manually and placed in a shaded plastic tub filled with water. A maximum of ten pups were kept while waiting to be handled. For the induction of the anesthesia, the animals were placed on a shaded wooden work table. The animals were masked with a plastic cone attached to an anesthesia machine for small species (Anesco Laboratories). Isoflurane (Forane Abbott Laboratories Limited, Queensborough, Kent, UK) was administered at 5% in oxygen (1-2 L/min) until relaxation was achieved in order to allow endotracheal intubation or sample collection. Only a few animals where intubated. In those cases, a 4-6 mm endotracheal tube (Kendall Gammatron Ltd, UK) was used depending on the animal’s weight. The endotracheal tube was place with direct visualization with a laryngoscope. The isoflurane concentration was adjusted according to responses to stimuli and the presence or absence of palpebral reflex, and mandibular tone. The animals stayed in ventral recumbency except when moved to facilitate the taking of measurements and samples.

For the application of the anesthesia, an Isote 3 (Ohmeda, UK) vaporizer was used, adapted to an anesthesia machine for small species with a 1 L rebreathing bag. The anesthesia equipment weighed 15 kg. Once installed in the place where the anesthesia was to take place, the isoflurane was placed in the vaporizer. The vaporizer was drained at the end of each day.

From the beginning of the anesthesia, all the physical signs were monitored. These included: palpebral reflex, capillary refill time, mandibular tone, breathing by means of the thoracic
movements, and heart frequency, saturation of oxygen and temperature by means of a pulse oximeter with a rectal probe. Once the animal recovered, it remained for a few minutes in a shady place before being returned to the rookery. The animal was observed from a distance.

Results

A total of 288 pups (137 males and 151 females) were anesthetized in a period of 20 days, from an estimated age ranging from 2-42 days, weighing 6-17.4 kg (mean = 10.41 kg, SD = 2.02). Table 1 shows the data obtained during anesthesia. In the cases of the Consagrada and Lobos islands, the pulse rate and oximetry readings are not reported. The inhalation anesthesia allowed the collection of samples and measurements from still animals, enabling the team to obtain blood samples from 80% of the individuals, compared with 40% in previous years when anesthesia was not used. The morphometric measurements were more accurate compared to the ones taken during physical restraint. It was possible to work on 16-29 animals per island (mean = 24 pups), which were anesthetized for 6-25 min (mean = 12.51, SD = 4.38), in an average time of 6 hr of work time per day. The project’s schedule and goals were achieved.

During induction, some animals were apneic for short periods, slowing the induction, and causing a marked bradycardia and a decrease in blood oxygen saturation. All of these animals resumed breathing and returned to the normal physiologic values without any handling. This behavior was observed in animals with a more active temperament. Four animals regurgitated milk during the anesthesia and one of them died after the anesthesia. Another animal died of hyperthermia prior to anesthesia.

Discussion

The isoflurane anesthesia proved to be effective and safe for the immobilization of free ranging California sea lions pups, and allowed us to obtain better data in a situation where work time is limited by tides and climatic conditions.

Part of the study involved estimating the nutritional status and health of the populations by evaluating the physical condition of the pups on different islands. Pup health was also reflected in differences in the average weights, difficulty of handling, difficulty of induction and also the concentration of isoflurane needed for the maintenance of the desired state of anesthesia.

The time of induction varied from 30 sec-3 min, varying depending on the weight and excitement of the animals. The estimated average induction time was 1 min. When endotracheal intubation was performed, the anesthetic planes were maintained with a lower concentration of isoflurane (1.5-2%), and better physiologic values were observed. However, the desire to handle the animals for only a short time and the type of anatomic measurements needed made it more practical to only mask the animals. But since cases of regurgitation and apneas were observed, intubation whenever possible is recommended.
The time of recovery in all animals was less than 1 min. The pulse oximeter readings proved to be practical although not completely effective. Some readings, especially during the apneas were as low as 41% of saturation, and the animals recovered equally fast once breathing restarted.

It was determined that of the two deaths during procedures, one (0.34%) was related to aspiration, and the other one (0.34%) was due to hyperthermia. The former animal was not anesthetized. Due to the geographic location and the time of the year, hyperthermia is the main factor to consider before, during and after the handling of the pups. In some individuals, temperatures were up to 41.5°C. In order to control this, the animals were kept wet and in the shade at all times.4

The use of isoflurane, as compared to halothane, used in other field studies with marine mammal pups, proved to have quicker induction and recovery times. Isoflurane allowed good control of the depth of anesthesia in the pups as reported for adults.2,3 Apnea was more frequently observed as compared to the use of halothane.6

The cost of the anesthetic equipment is justifiable, since a higher number of pups were sampled and more accurate samples and measurements were taken. A total of 300 ml of isoflurane was used, for the 288 pups. During this project, some mature females, weighing up to 120 kg, were anesthetized for the placement of Time-depth Recorder devices, with excellent results. The same anesthetic equipment was used.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the entire personnel of the CICIMAR who participated in the two field trips in the Gulf of California during the breeding season of 1997, in the “Evaluation of the health status of the population of the California sea lion” project. We also thank the Armada of Mexico, especially the crew of the Gunboat C-78 Zamora, who helped carry out the project.

LITERATURE CITED

Table 1. Physiologic and anesthesia parameters from 288 (137 males, 51 females) California sea lion pups in 11 islands in the Gulf of California, Mexico.

<table>
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<th>Respiratory rate (per min)</th>
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<td>Mean</td>
<td>SD</td>
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HYDROCEPHALUS IN WILD RED FOX (Vulpes vulpes) KITS IN NORTHERN VIRGINIA

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Abstract

Hydrocephalus was diagnosed in wild red fox (Vulpes vulpes) kits in four contiguous counties in northern Virginia in 1996-1997. Hydrocephalus has not been previously reported in red fox. The distribution and similar histories of these cases suggest a common etiology. Diagnostic testing indicates a viral cause to be unlikely. Alternatively, these cases of hydrocephalus may be either genetic or environmental in origin.

Introduction

Hydrocephalus is the abnormal accumulation of cerebrospinal fluid within the ventricular system of the brain.4 Clinical signs may include enlargement of the head, prominence of the forehead, divergent strabismus, brain atrophy, seizures, and behavioral or mentation abnormalities. Hydrocephalus can be either acquired or congenital. Acquired forms can be caused by infection, trauma, or tumors. Congenital hydrocephalus can be caused by in utero viral infection, toxoplasmosis, or teratogenic drug or chemical exposure. This report describes a recent, small localized outbreak of hydrocephalus in wild red fox kits in northern Virginia in 1996-1997.

In April 1996, a wild red fox kit found in Loudon Co., VA was brought to a wildlife rehabilitator (APH) who specializes in foxes. The fox had an enlarged head, made frequent, inappropriate vocalizations, and appeared to be blind. The fox was recognized as appearing hydrocephalic, because the rehabilitator had seen a similar previous case in 1986, which had been diagnosed at The Wildlife Center of Virginia as being hydrocephalus. The rehabilitator was presented with a second fox kit with similar clinical signs a few weeks later from Fairfax Co., VA and a third similar kit from Fauquier Co., VA in May 1996.

Methods

Diagnostics were not performed on the first two of these red fox kits. The carcass and blood samples from the third affected red fox kit were submitted for examination to the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia College of Veterinary Medicine. This was the first case of hydrocephalus in red fox ever observed at SCWDS. Subsequently, the rehabilitator and referring veterinarian (VC) started collecting serum samples from unvaccinated, unaffected adult and juvenile red and gray foxes to use as negative controls in a serologic comparison between foxes with hydrocephalus and clinically normal foxes.
No further cases were reported until April 1997, when three more affected red fox kits were found in Fairfax Co., Prince William Co., and Loudon Co., VA. Samples from all three of these foxes were submitted to SCWDS.

Results

External examination and radiographs of the heads of the affected fox kits revealed marked enlargement and doming of the cranium. The cerebral ventricles were markedly dilatated. The cerebral hemispheres were reduced to thin shells, and the surface of the cerebellum was flattened dorsally. Histologically, the cell density of the cerebrum appeared high due to compression of the tissue.

The affected red fox kits had positive serologic titers to canine distemper virus (CDV), canine parovirus (CPV), and canine adenovirus (CA), but negative titers to canine parainfluenza virus (CPI). Samples of brain tissue from the affected red fox kits were negative by fluorescent antibody testing for CDV, CPV, CA, and CPI.

Nine unaffected red and gray fox kits tested from the same area of Virginia had similar serologic profiles. All nine unaffected kits tested had positive titers to CDV, 8/8 tested had positive titers to CPV, 8/9 had positive titers to CA, and 9/9 had negative titers to CPI. Additionally, serum samples from seven unaffected adult red and gray foxes were analyzed. All seven adult foxes had positive titers to CPV, whereas only 3/7 had positive titers to CDV, and only 1/7 had a positive titer to CA.

Discussion

While hydrocephalus is the most common canine congenital anomaly of the nervous system,1 hydrocephalus in foxes is unreported in the literature. Hydrocephalus has been reported to be caused by canine parainfluenza virus in dogs; other viruses known to cause encephalitis in dogs include Aujeszky’s disease, canine distemper, canine herpes, infectious canine hepatitis, and rabies.1 Serologic tests have disclosed antibodies to canine parovirus, canine adenovirus, canine coronavirus, canine herpesvirus, and canine parainfluenza virus in wild red and gray foxes.2 In gray foxes, canine distemper is the most significant mortality factor of all infectious and noninfectious disease.3

The similarity in the ages of the hydrocephalic foxes of this report (less than 3-mo-old) and the close geographic association, found within four adjacent counties, suggests these cases likely share a common etiology. The fox kits of this report were screened serologically for exposure to viral agents associated with encephalitis in domestic canids. Serologically, there were no differences between affected and unaffected fox kits; therefore it is unlikely that viral exposure leading to these positive titers was the cause of the hydrocephalus. Of the viral pathogens screened for in the fox kits of this report, all were negative for canine parainfluenza virus, the only virus confirmed to cause hydrocephalus in domestic canids. The positive serologic titers in the fox kits of this report are most likely due to maternal antibodies. The negative fluorescent antibody test results indicate there was
no active viral infection caused by these viruses at the time of euthanasia. Comparatively, the adult foxes examined in this report had fewer positive titers than did the fox kits, perhaps due to the waning of maternal antibodies over time. However, positive titers in some adult foxes suggest exposure as adults to these viruses without the occurrence of hydrocephalus as a consequence.

These findings suggest that a viral etiology for the hydrocephalus seen in these red fox kits is unlikely. Alternatively, the hydrocephalus seen in these foxes might have a genetic basis present in the breeding subpopulation encompassing these four adjacent counties. An environmental toxin or contaminant present within the geographic limits of these four adjacent counties represents another possible cause, with implications for the human residents of these counties, as all are well populated. The red fox kits produced in northern Virginia in 1998 will be closely watched for reoccurrence of this anomaly.

ACKNOWLEDGMENTS

The authors would like to thank Drs. John Fischer and Michael Teglas of SCWDS for performing the post-mortem examinations on the foxes in this report.

LITERATURE CITED

IMMUNOCONTRACEPTION OF FREE-RANGING AFRICAN ELEPHANTS IN KRUGER NATIONAL PARK, SOUTH AFRICA

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Abstract

In order to seek a more publicly acceptable alternative to the management of African elephants by culling, a test was conducted to determine if a porcine zona pellucida (PZP) vaccination could effectively contracept females of this species. Initially, ovaries were recovered from culled animals and slices were incubated with immunogold-labeled rabbit antibodies against PZP. Significant staining of the elephant zona suggested that PZP would be an effective contraceptive vaccine. In a second experiment, three captive female zoo elephants were inoculated with the PZP vaccine (400 µg PZP + 300 mg RIBI). These non-breeding animals were tractable and blood samples were recovered and assayed for anti-PZP antibodies. Antibody titers (1:500 dilution) peaked (0.75-1.3 OD) at 1-2 mo following the initial inoculation, declined to 0.1-0.34 at 6 mo-1 yr, and peaked again following a third inoculation (0.8-2.3). These data indicated that African elephants would mount a significant antibody response to the PZP vaccine and together with the histochemical study, suggested the vaccine would be a successful immunocontraceptive in this species.

Methods

In October 1996, 100 elephants in Kruger National Park (KNP) were captured by etorphine immobilization from a helicopter. Forty-one were determined to be non-pregnant on the basis of transrectal ultrasound examination conducted by Thomas Hildebrandt and colleagues (Institute for Zoo and Wildlife Research, Berlin). On the strength of the ultrasound determinations, it was found that the helicopter pilot and park veterinarian could identify non-pregnant females with 85-90% accuracy, on the basis of the size of calves with them. Twenty-one non-pregnant adult females received an initial i.m. dose of 600 µg of PZP vaccine emulsified in RIBI adjuvant, and another 20 received only saline and adjuvant. The treated females were fitted with radio-collars and the control animals with colored collars without radios. Approximately 1 mo later the experimental group received a 600 µg PZP-RIBI booster remotely, by darts fired from the helicopter. Eight months after the initial treatment, the treated animals received a second 600 µg PZP-RIBI booster remotely, via darts.

Results
In October 1997, all 20 control cows and 19 of the 21 PZP-treated cows were located, immobilized, and examined for pregnancies by ultrasound. All cows still had their calves at their sides, indicating no adverse behavior reactions to the vaccine. Eighteen of 20 control elephants were pregnant (90%). Three of the 19 treated females were pregnant and the size of the fetus indicated that they were in the early stages of pregnancy at the time of initial PZP treatment and ultrasound examination. Of the remaining 16 treated animals, only six were pregnant (37.5%) and the difference in pregnancy rates between control and treated animals was significant. Antibody titers among the treated elephants, across the 1 yr, were similar to those in the three zoo elephants, with an initial rise after the first booster inoculation, a steady decline during the next six mo, and a significant elevation after the second booster inoculation.

Discussion

These results indicate that the PZP vaccine can contracept African elephants. On the basis of both the zoo and free-roaming elephant antibody titer data, a second trial has been carried out with six additional non-pregnant females, in which the PZP vaccine was given at day 0, day 14, and day 35-42. These animals have been fitted with GPS collars as well. The hypothesis is that the change in the timing of booster inoculations will raise contraceptive efficacy to near 100%. Half of the previously treated and non-pregnant females were given another booster inoculation to test longer-term contraceptive effects and the other half were left untreated, to test reversibility of contraceptive effects.

Data retrieved thus far indicates that 1) PZP vaccination will inhibit fertility in adult female African elephants, 2) inoculations can be given remotely, 3) family group integrity is not affected by PZP vaccination, 4) reversibility of contraceptive action is probable, based on the declining antibody titers, and 5) no abscesses formed at the injection sites. Further studies may provide sufficient data to provide managers of African elephants with a non-lethal and publicly acceptable management tool.
AN INDICATOR OF HUMAN IMPACT: GASTROINTESTINAL PARASITES OF MOUNTAIN GORILLAS (Gorilla gorilla beringei) FROM THE VIRUNGA VOLCANOES REGION, CENTRAL AFRICA

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Abstract

The mountain gorillas in the Virunga Volcanoes of Rwanda, Uganda and Democratic Republic of Congo, seem able to tolerate certain species of gastrointestinal parasites without manifesting signs of sickness. Close contact between people and this endangered population is not new as the fertile Virunga region has supported a high density human population (up to 400 people/km²) for decades. However, in 1994, as a result of the war and genocide in Rwanda, there was a massive increase of human traffic crossing the park, followed by a military presence. Due to the close genetic relatedness of humans and gorillas there is the possibility of anthropozoonotic disease transmission with potentially devastating consequences for this endangered species.

In 1996-1997, a study carried out in Rwanda by the Mountain Gorilla Veterinary Centre and researchers of University of Tennessee, found three intestinal parasites, Trichuris trichiura, Chilomastix sp. and Endolimax nana not previously identified in habituated gorillas, but known to infect humans. Fecal samples (n = 98) were collected from 74 free-ranging gorillas in the Volcanoes National Park with the collaboration of Karisoke Research Center. All samples were examined using centrifugal flotation (zinc sulfate and Sheather’s sugar solutions). Larval cultures were performed to further identify the strongyles eggs seen on flotations. Examination of these samples revealed that 72 of 74 (97%) were infested with strongyles/trichostrongyle-type, 63 of 74 (85%) with Anoplocephala gorillae, 7 of 74 (9%) with Probstmayria sp., one of 74 (1%) with Trichuris trichura, one of 74 (1%) with a Psoroptid mite, and one of 74 (1%) with one unidentified mite. Trichrome stains from 70 of 74 (95%) of gorillas were examined and revealed that 31 of 70 (44%) had Endolimax nana (cysts), 11 of 70 (16%) Iodamoeba buetschlii (trophozoites), 63 of 70 (90%) E. nana and/or I. buetschlii (trophozoites), 31 of 70 (44%) Chilomastix sp. (cysts and trophs), 19 of 70 (27%) Entamoeba hartmanni (cysts and trophs), 14 of 70 (20%) Entamoeba coli (cysts and trophozoites), one of 70 (1%) Entamoeba histolytica (trophozoite), and two of 70 (3%) Giardia sp. These newly identified parasites may indicate an increase in contact with human fecal material.

A comparative study is underway in the Virunga National Park, Democratic Republic of Congo, and we may find further evidence of human impact on the gorillas’ health. The Virunga National Park shelters six habituated gorilla groups, about 24% of the gorilla population in the Virunga region.
Pressures on this protected area were enormous after the establishment of two major refugee camps on the edge of the park in 1994. Human presence in the forest has once again increased significantly with the war in the Democratic Republic of the Congo (formerly Zaire) after October 1997, which is potentially disastrous to the gorilla population.

Thus, there is a need to monitor closely the health of the Virunga population. Specifically, we should periodically assess changes in the spectrum of parasites carried by gorillas and variation in parasite loads. This should assist with the control of potential disease outbreaks and provide additional data for the long-term management of this very endangered primate.
AN OUTBREAK OF SARCOPTIC MANGE IN FREE-RANGING MOUNTAIN GORILLAS (Gorilla gorilla berengei) IN BWINDI IMPENETRABLE NATIONAL PARK, SOUTH WESTERN UGANDA

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Abstract

From August to December 1996 an outbreak of a debilitating skin disease occurred in mountain gorillas in Bwindi Impenetrable National Park in Uganda. All four members of one gorilla group habituated for tourism were clinically affected by the disease, with severity of clinical signs inversely related to age. An infant male gorilla was most severely affected and later died. A juvenile male showed serious manifestations of the disease, but recovered following treatment. The two adult animals in the group, one female and one silverback, showed mild signs, with the female being more severely affected, and recovered following treatment. Sarcoptes scabiei mites were observed on skin scrapings and skin biopsies taken during an immobilization of the juvenile for diagnosis. All animals in the group were treated with ivermectin (Ivomec, Merck & Co., Rahway, New Jersey USA), an anti-parasitic drug, with the exception of the infant who died before treatment could be administered.

The diagnosis of sarcoptic mange was confirmed in the infant with post-mortem skin scrapings and skin biopsy. Treatment with a single 0.2 mg/kg i.m. dose of injectable ivermectin, administered to all surviving gorillas in this group, was successful, and no recurrence of clinical signs was observed within the next year. No other gorilla groups in the park were clinically affected. This is the first recorded incidence of sarcoptic mange in a free-ranging population of mountain gorillas. The source of the mange mite has not yet been confirmed, but we are concerned that it may be from contact with local people, tour groups and ranger guides because scabies is a common disease in human populations around Bwindi. If the source is found to be human, it will result in the need for serious attention to control measures to prevent re-infection of wild mountain gorillas.
CARNIVORE RESEARCH IN SOUTHERN AFRICA: A PROJECT UPDATE

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Abstract

The Chicago Zoological Society and the University of Illinois Zoological Pathology Program in collaboration with the Ministry of Environment and Tourism, Namibia, and the Natal Parks Board, South Africa, have an ongoing in situ project involving a multi-disciplinary study of southern African carnivores in their native habitat. With these and several other collaborators an expansive, in-depth study of multiple carnivore species is in progress to help determine the overall health of these animals. The species being studied are African lion (Panthera leo), spotted-hyena (Crocuta crocuta), African wild dog (Lycaon pictus), cheetah (Acinonyx jubatus), leopard (Panthera pardus), African wild cat (Felis lybica), and the black backed jackal (Canis mesomelus). To gain a thorough understanding of their current physical condition, we are employing a variety of disciplines, including veterinary medicine, immunology, pathology, reproduction, and genetics. We are using these disciplines in several different free-ranging conditions to learn how various management strategies and population structures affect the wild populations. As we more fully understand the consequences of reduced range, increased opportunity for inbreeding, and disease transmission, we can better predict how our management decisions will affect the overall chance of both short and long-term survival. The study also allows for the establishment of baseline information by which to measure following generations of animals. This paper provides an overview of procedures and preliminary results for lions.

Background

In 1992 this project was initiated in the Hluehluwe/Umfolozi Park (HUP) in South Africa to study the health of an isolated population of African lions. This project then expanded into two additional areas of southern Africa, both of which represented different types of management systems and ecologic niches. These included the Etosha National Park (ENP) and the Bushmanland/Caprivi regions in the Republic of Namibia. Comparisons among these three populations allowed for assessment of several different factors concerning the lions’ overall health status.

The populations in the three areas are quite different:

- The lions from HUP (90,000 ha) came from only three founders and were quite isolated from other African lion populations.
- The Etosha lions are a relatively stable group of animals in an area approximately five times larger than HUP. Estimates of the population were near 350 individuals with animals able to move in and out of the fenced park.
The animals from the Bushmanland/Caprivi area have had little human interaction because the area is sparsely inhabited by people.

Our goal was to utilize the two free-ranging populations in ENP and Bushmanland/Caprivi and the intensely managed free-ranging group in HUP to establish criteria by which to compare the strengths and weaknesses of the different management techniques for both free-ranging and captive populations.

Goals

Determine the clinical health of individual animals and relate it to the population’s overall health.
Determine exposure to bacterial and viral diseases.
Determine the cause of death and disease affecting animals that were found dead.
Establish a tissue bank from pathology samples taken.
Determine the semen quality of the males, relate this to overall birthrate and determine if there is seasonal production.
Determine the parentage, subspecies, and genetic variability of each population and compare this between individuals within the population and between populations.

In order to understand some of the interactions between species and possible effects of disease or poor health on one group of animals, the original work expanded to include spotted-hyena, African wild dog, leopard, cheetah, African wild cat, and the black-backed jackal. In order to take more of a complete look at the ecosystem, we felt it important to use the same techniques to evaluate other carnivores in these regions. This would allow us to help determine if one management system favored a particular species and also to evaluate the effects of a potential shift in a population. Management approaches that could increase likelihood of survival include a variety of options such as facilitation of movement of animals across areas of human population where migration would have historically occurred, but is now impossible.

To assess these parameters, we do the following:

General health and baseline medical assessment included performing physical examinations of each animal, collecting blood, performing complete blood counts, serology, serum chemistries, characterizing enteric flora, performing fecal examinations for intestinal parasites, and examining the animals for external parasites.
Evaluation of the reproductive status included examination of the male reproductive tract and collection of semen. Semen was analyzed using standard criteria including volume, concentrations, motility, status, percent normal/abnormal, and pH. The semen of high quality is frozen and stored in liquid nitrogen for future use in reproductive studies. We are analyzing the semen and comparing data between populations and by season.
Genetic analysis has included protein electrophoresis, micro satellite analysis, and DNA fingerprinting. These analyses allow us to determine the amount of inbreeding within and between each population, determine parentage, and determine subspecies.
To perform the tests and procedures outlined above, several collaborators from a variety of fields have been brought together to do these assessments.

Collaborators

This project has several collaborators involved and thus, only the primary people in each region and portion of the study will be listed. Dr. Michael Briggs is the principal investigator for the project and coordinator of studies in the United States. Dr. Robert Murnane, University of Illinois Zoo Pathology Program is implementing the pathology, microbiology, and parasitology portions of the study. Dr. Jacques Flamand was project coordinator for the HUP portion. The project coordinators in Namibia were Kallie Venzke, former control warden of Etosha, Dr. Phillip Stander, Large Carnivore Biologist of Bushmanland Region, and Lue Scheepers, Regional Biologist-Caprivi Strip. Dr. Jean Dubach is the molecular geneticist from Brookfield Zoo performing genetic evaluation, Dr. Jim Evermann at Washington State University is the collaborator regarding disease surveillance, and Dr. Suzanne Kennedy-Stoskopf is evaluating immune functions.

Current Status and Developments

To date, we have completed nine trips to the parks over the 6-yr period and have samples from 270 lions, 45 spotted hyena, 20 jackal, 7 African wild cat, 6 cheetah, 5 African wild dog, and 3 leopards. The areas of study are general physical health, genetics, morphometrics, semen production, disease exposure (serology), immune function, presence of ecto- and endoparasites, description of enteric flora, and pathology. A summary of each area of study follows, including some raw data and findings for lions.

We have performed physical examinations on approximately 120 lions and collected semen samples on 37 males. Ten of these animals were sampled in both the dry and the wet season. We have conducted serologic evaluations on 184 individual lions. These evaluations thus far have only looked for exposure to feline immunodeficiency virus, feline leukemia virus, and canine distemper virus. Genetic samples have been evaluated using DNA fingerprinting, protein electrophoresis, microsatellite techniques as well as mini-satellite techniques. Fecal and hair samples have been collected and analyzed from 48 lions and 16 hyena for metazoan and protozoan parasites. Complete post-mortem evaluations have been done on 14 lions and one hyena, including gross and histologic evaluations. There have been an additional eight histopathology exams performed on lions from the study area. There have been approximately 100 CBC’s and 50 serum chemistry analyses performed.

LIONS

Physical examinations: General physical examinations were performed by veterinarians on approximately 120 of the animals immobilized. A variety of maladies have been observed, most of which were apparently traumatic and chronic. Animals have been noted to have a fractured femur, a dislocated shoulder, a dislocated stifle, and a subluxation of the lumbar vertebrae. Trauma to the eyes with lacerations and punctures were noted, as well as many fractured teeth. These animals were
males in dominant roles maintaining prides and only in one case was the animal in poor body condition. There were no signs of respiratory or cardiac diseases present.

**Genetics:** The lions examined to date include animals from HUP, Etosha National Park, Bushmanland, the Caprivi Strip, the Transvaal (Phinda Wildlife Reserve), and captive lions of unknown origin. The results suggest there is not enough genetic distance to classify the Southern, free-ranging groups as separate subspecies. Also, data indicate that in Etosha dominant males are not the exclusive sires in known prides. This reflects the thoughts of the Ministry staff that pride males do not maintain exclusive breeding rights to pride females. Questions have arisen regarding the translocation of lions from Namibia to HUP, but the genetics issue does not appear to merit great concern, since the genetic distance is not consistent with subspeciation. The HUP population has less genetic variability than the Etosha population, but the variation observed is not categorized as “dangerously low.”

**Reproduction:** The semen studies show seasonal production in the lions in both Etosha and HUP. To confirm this, there needs to be a larger sample size of animals sampled during both the dry and the wet seasons. Obtaining enough males in both the wet and dry seasons has been problematic, yet with further study these preliminary results will likely be confirmed. The animals in Etosha and HUP had an increase in both the concentration and quality (status) of the semen in the dry season, September to November, as compared to the wet season, February to April.

More information on seasonal production of semen will be obtained through continued sampling in the Caprivi area and in Etosha. This will also expand the genome bank. The males in Caprivi and Etosha had concentrations of sperm that were up to 1450 times higher with status five times higher than HUP. This does not mean the animals in HUP are infertile, but when compared to reproductive capabilities and mean offspring production in other species, they are less likely to have reproductive success.

**Serology:** The animals have had serologic testing for Feline Immunodeficiency Virus or Feline Lentivirus (FIV), Feline Leukemia Virus (FeLV), and Canine Distemper Virus (CDV). All lions have been evaluated for FIV and FeLV using the ELISA snap kit by IDEXX (IDEXX Veterinary Services, 1 IDEXX Drive, Westbrook Maine, 04092) for screening. Of the 184 tests run, there have been no positives or equivocals reported. Forty animals (22%) were re-tested with the Western Blot and all were confirmed to be negative. There is also a zero incidence of positive animals for FeLV antibodies. The canine distemper antibody titers ranged from 0 to 1:640. Out of 156 lions tested, twenty were positive and 136 were negative. The highest incidence of canine distemper titers was in Etosha and the lowest incidence in HUP and Bushmanland. It was expected that the animals closest to humans with domestic dogs would have a greater chance of exposure, yet these results seem to indicate the opposite. Therefore, the source of exposure warrants further investigation.

**Immunoe function:** Samples were obtained from HUP and Etosha to evaluate the immune function of the animals. Dr. Suzanne Kennedy-Stoskopf is doing this work. Results are pending.
**Ecto/endo parasites:** The most common endoparasites of the Etosha and Caprivi groups were *Taenia* sp. with several animals showing *Strongyloides, Isospora,* and *Sarcocystis.* There was also a few incidences of *Echinococcus* infection. The only ectoparasites seen were *Culicoides* and *Rhipicephalus* sp.

**Enteric flora:** These data also apply to the Etosha and Caprivi animals only. There are several *Bacillus* sp. present but no *B. anthracis,* even during the season when carcasses abound with anthrax. There were also many animals with *Salmonella* sp.

**Pathology:** Multiple gross and microscopic evaluations have been performed in Etosha. Lesions were within the realm of those related to generally healthy animals with no obvious disease processes occurring. Most of the animals necropsied are those which were destroyed due to stock raiding events and were shot by local ranchers. This is functioning mainly to establish a database for the free-ranging population, but unusual and/or significant results are being published as they occur. This was the case with the *Sarcocystis* and gastric spiral bacteria infection, which was reported by Kinsel, *et. al.* Field staff has been trained in necropsy techniques and necropsies are being performed throughout the year by Ministry staff. Histopathology is performed when the tissues are sent to the University of Illinois as part of the ongoing service being provided.

**Summary:** Much of the information which was initially sought has been obtained and there is a good working understanding of the overall status of these populations. Ongoing work continues to update and enhance the original sampling. The researchers continue with the lion work in Namibia with the current focus on lions in Caprivi. Continuing serology, physicals, semen evaluation, and disease surveillance is essential for monitoring the health of the population. It would be of particular interest to observe the changes in the population in HUP should new animals from Etosha be placed in the group. As cubs were produced, the genetically known sire could be determined from the previous samples obtained with the parentage data now held. This would allow for a determination of how the introduction program effects the wild population and could thus allow for a benchmark for further translocations. Also, any changes in sperm production could be evaluated as the population changed.

**ACKNOWLEDGMENTS**

The authors would like to acknowledge the support of the individuals, institutions and agencies which have made this project possible. The Chicago Zoological Society (Brookfield Zoo and SEACON Conservation grants), the Natal Parks Board, Republic of South Africa, the Ministry of Environment and Tourism, Republic of Namibia, the Chicago Board of Trade Conservation Fund, Chicago Zoological Society Board member (anonymous), University of Illinois, College of Veterinary Medicine, Washington State University and the Washington Animal Diagnostic Disease Laboratory, Jacques Flamand, Lue Scheepers, Kallie Venzke, Phillip Stander, Suzanne Kennedy-Stoskoph, Tony Conway, Anthony Maddock, Thomas Spurgeon, Alan Studley, V. Piper Kimball, Olivia Forge, Gwen Myers, and the entire staff of the CZS Department of Animal Health.
HEALTH EVALUATION OF WHITE-LIPPED PECCARY POPULATIONS IN BOLIVIA

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Abstract

During February 1996 and 1997, white-lipped peccary (Tayassu pecari) herds were captured for health evaluations and population dynamics studies at Noel Kempff Mercado National Park, Santa Cruz, Bolivia (Lat. 13°, 35.64’, Lon. 60°, 54.74’), at the base of the northern tip of the Huanchaca escarpment.

The peccary herds were captured in a large pen built around a frequently used salt lick (salitral) in the forest (Fig. 1). Once the peccaries entered the pen, they were darted with a CO2 powered pistol using plastic darts (Telinject, Telinject USA, 9316 Soledad Canyon Rd., Saugus, California USA). The animals were immobilized using a premixed combination of equal parts tiletamine hydrochloride and zolazepam (Telazol, Fort Dodge, Fort Dodge, Iowa USA) using dosages of 80-120 mg for sub-adults and juveniles and 120-150 mg for adults. Some of the peccaries were supplemented with 100 mg i.m. doses of ketamine hydrochloride (Ketaset, Fort Dodge, Fort Dodge, Iowa USA) when they were not immobilized enough for safe handling. Several animals which recovered slowly from anesthesia were given 2-3 mg of flumazenil (Mazicon, Hoffman-La Roche, Inc., Nutley, New Jersey USA) i.m. to reverse the effects of the zolazepam. Thirty two adults (>2 yr), three sub-adults (2 yr) and five juveniles (< 2 yr) were immobilized, individually identified, and examined. Blood and fecal samples collected for analysis.

Basic hematolgy was conducted at the field site, including packed cell volumes, total protein, blood smears and WBC counts. Plasma and serum were frozen in liquid nitrogen and later analyzed for serum chemistry, mineral, metal, polychlorinated biphenyl and chlorinated pesticide levels, and infectious diseases.

No evidence of gastrointestinal parasites was found in fecal samples. All peccaries were heavily infested with ticks (Amblyomma sp.). Chlorinated pesticides (aldrin; alpha-BHC; beta-BHC; O,P’-DDD; P,P’-DDD; P,P’-DDE; O,P’-DDT; P,P’-DDT; dieldrin; endrin; heptachlor; heptachlor epoxide; lindane (gamma-BHC); and nonachlor) and PCBs were not detected in samples analyzed. Tissues from three peccaries (two adult female and one adult male) that died during anesthesia were evaluated histologically. All three had severe diffuse pulmonary edema and congestion. Two
individuals had a multifocal, moderate chronic-active eosinophilic interstitial pneumonia, bronchitis, tracheitis, and pleuritis, and degenerating nematodes were found within sections of the third peccary’s lungs. One peccary had a diffuse severe lymphoplasmacytic epicarditis and multifocal lymphoplasmacytic myocarditis, and another peccary had a chronic-active multifocally extensive severe epicarditis and myocarditis. Two peccaries had diffuse, moderate to severe chronic-active eosinophilic inflammation in the tunica adventitia of aortic tissue. One of these animals also had a chronic-active eosinophilic moderate diffuse splenic capsulitis. No bacteria or parasites were found associated with these heart lesions.

Two of the peccaries had hepatic capillariasis and all three peccaries had a multifocal, moderate chronic-active and eosinophilic hepatitis. The hepatic capillariasis may have been acquired by the ingestion of tissues from decaying or dead rodents on the forest floor. Two peccaries had a multifocal, moderate chronic-active lymphoplasmacytic interstitial nephritis, and one peccary had a multifocal, moderate lymphoplasmacytic interstitial nephritis.

Despite the negative parasite and ova findings on fecal examination, all peccaries had a diffuse, moderate to severe chronic-active eosinophilic enteritis, suggestive of parasitic inflammation. Two peccaries that had gross evidence of intestinal acanthocephalids also had fibrous and granulomatous nodules within the intestinal wall associated with acanthocephalid attachment. Two peccaries had a multifocal moderate to severe chronic-active eosinophilic dermatitis (most likely associated with cutaneous acariasis found during physical examination).

Infectious disease serology results are listed in Table 1. The negative infectious disease tests indicate that the animals have not been exposed to these pathogens in recent times.

*Mycoplasma hyorhinus*, *Hemophilus parasuis*, and *Streptococcus suis*, or other bacterial infections were among the differentials considered for the serosal inflammation seen histologically. Chagas disease and encephalomyocarditis (EMCV) virus were among the differentials considered for the myocardial lesions. However, serologic tests for Chagas disease and EMCV were negative for these individuals. For other herdmates tested, all were seronegative for antibodies to *Hemophilus parasuis* and one animal in the herd had a positive Mycoplasma antibody titer. All herd animals tested had *Streptococcus suis* antibody titers, but these results are equivocal in relation to necropsy findings because most adult domestic swine (*Sus scrofa*) test positive for this commensal organism.

The high incidence of antibody titers to vesicular exanthema of swine virus and the closely related San Miguel sea lion virus suggests that one or more Calyciviruses (which share antigenic qualities) is circulating in the population. Only one out of 14 animals tested in 1996 had positive titers, while 23 out of 26 in 1997 had positive titers. We do not know which strain or strains of Calycivirus is responsible for the observed titers nor their effect on the peccary herds studied.

The high incidence of positive Leptospirosis titers (65%) shows that the majority of the peccaries have been infected with this organism. The interstitial nephritis seen in the animals that died is consistent with Leptospirosis, and one of the peccaries that died did have a positive leptospirosis titer.
However, immunohistochemistry on renal tissue for leptospirosis was negative in all three animals. Leptospira interrogans can cause illness, reproductive abnormalities and sometimes death in almost all mammal species including humans. Repeated abortions or what appears to be infertility are commonly reported in infected females of other species. Therefore, finding evidence of the presence of this organism has relevance to the population dynamics of Bolivian white-lipped peccaries.

ACKNOWLEDGMENTS

We would like to thank the Bolivian national Secretariat for Protected Areas for permission to work in Noel Kempff Mercado National Park, and the National Directorate for the Protection of Biodiversity for help in acquiring the necessary permits. We are extremely grateful to our field assistants Nicolas Tagua and Jose ChuviZa whose dedication and initiative were exemplary. Thanks to F.A.N. (Fundación Amigos de la Naturaleza, Santa Cruz, Bolivia) for the logistic support they provided within the park, and to Lidivet for the use of their blood and tissue storage facilities in Santa Cruz. We thank Sue Rosenberg and Marianne Fitzpatrick of the Wildlife Conservation Society (WCS) and Kirk Stuart of the Michigan State Animal Health Diagnostic Laboratory for their help in blood sample analyses. For the identification of parasites, we would like to thank Dr. Eric Hoberg of the U.S. Department of Agriculture Biosystematic Parasitology Laboratory and Dr. Lance Durden of Southern Georgia University. We would also like to thank Drs. Dennis Martin and Nick Karabatsos of the Division of Vector-Borne Infectious Diseases, Centers for Disease Control for the yellow fever testing. Hoffman-La Roche, Inc. kindly provided the Mazicon for use in this study. Much of this study was funded through the Bolivian Sustainable Forestry Project (BOLFOR), which is financed by USAID and the Bolivian Government.
Table 1. Serologic tests performed, the number of positive animals, and the number of animals tested in the evaluation of infectious disease agent exposure in free-ranging white-lipped peccaries in Noel Kempff Mercado Nat. Park, Santa Cruz, Bolivia.

<table>
<thead>
<tr>
<th>Agent</th>
<th># positive (# tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudorabies virus (Aujezky’s disease)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Porcine parvovirus</td>
<td>0 (40)</td>
</tr>
<tr>
<td>Swine influenza virus</td>
<td>0 (40)</td>
</tr>
<tr>
<td>Transmissible gastroenteritis</td>
<td>0 (40)</td>
</tr>
<tr>
<td>Vesicular stomatitis virus</td>
<td>1 (40)</td>
</tr>
<tr>
<td>Anaplasmosis</td>
<td>0 (40)</td>
</tr>
<tr>
<td>Bluetongue virus</td>
<td>0 (26)</td>
</tr>
<tr>
<td>Johne’s disease (Paratuberculosis)</td>
<td>0 (40)</td>
</tr>
<tr>
<td>Foot and mouth disease</td>
<td>0 (40)</td>
</tr>
<tr>
<td>African swine fever</td>
<td>0 (40)</td>
</tr>
<tr>
<td>Hog cholera</td>
<td>0 (40)</td>
</tr>
<tr>
<td>Rinderpest</td>
<td>0 (39)</td>
</tr>
<tr>
<td>Brucellosis</td>
<td>0 (40)</td>
</tr>
<tr>
<td>Vesicular exanthema of swine</td>
<td>24 (40)</td>
</tr>
<tr>
<td>San Miguel sea lion virus</td>
<td>22 (40)</td>
</tr>
<tr>
<td>Encephalomyocarditis virus</td>
<td>0 (40)</td>
</tr>
<tr>
<td>Leptospirosis (19 serovars)</td>
<td>26 (40)</td>
</tr>
<tr>
<td>Hemophilus parasuis</td>
<td>0 (17)</td>
</tr>
<tr>
<td>Mycoplasma hyorhinus</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Streptococcus suis</td>
<td>13 (17)</td>
</tr>
</tbody>
</table>
**Figure 1.** Diagram of capture pen used for white-lipped peccaries in Bolivia.
CAPTURE AND IMMOBILIZATION OF MUSTELIDS IN BRITISH COLUMBIA

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Abstract

Medium and large-sized mustelids were live-captured and immobilized in winter field conditions in British Columbia. Inventory and population studies of wolverine (Gulo gulo), fisher (Martes pennanti) and badger (Taxidea taxus taxus) required placement of collar and abdominal cavity radio transmitters, measurement of morphometric data, collection of genetic samples and blood for disease surveys. A variety of traps were used for initial capture, including leg-hold, metal barrel, log construction, and Havahart (Ekco Canada, Box 210, Niagara Falls, Ontario, Canada) designs.

Immobilization agents used were zolazepam-tiletamine (Telazol, Fort Dodge, Fort Dodge, Iowa USA) alone, and in combination with isoflurane (Aerrane, Ohmeda Caribe Inc., Guayama, Puerto Rico USA) or halothane (Fluothane, Ohmeda Caribe Inc., Guayama, Puerto Rico USA) gases, and midazolam (Versed, Hoffman-La Roche Inc., Nutley, New Jersey USA) with ketamine hydrochloride (Ketaset, Fort Dodge, Fort Dodge, Iowa USA). Zolazepam-tiletamine was delivered by intramuscular injection to wolverine at doses ranging from 7-15 mg/kg, however, 10 mg/kg was considered most desirable.

Induction times were generally under 5 min, however some animals required second injections, apparently due to incomplete first injections. Analgesia and anaesthesia depth was often inadequate for invasive procedures, such as extraction of premolars, unless the procedure was performed within the first 15 min of the immobilization. Arousal after 45-60 min was common in wolverines given only zolazepam-tiletamine. Isoflurane gas was used to extend the length and depth of anesthesia and improve analgesia for abdominal implant surgery in young and adult wolverines.

Fisher were given zolazepam-tiletamine by intramuscular injection at doses of 9-17 mg/kg. Rectal temperatures were decreased in most fisher immobilized in the field and extended recovery times of over 90 min were noted in a captive group given 12-17 mg/kg.

A limited number of badgers were given zolazepam-tiletamine by intramuscular injection at doses of 7-14 mg/kg for restraint or induction with anesthesia maintenance on halothane gas. Induction was generally under 2 min with routine recovery from the gas anesthesia. Inadequate results in some badgers resulted in a trial dose of midazolam at 0.2 mg/kg and ketamine hydrochloride at 6 mg/kg. The immobilization was considered superior to those previous and recovery was rapid, however, the combination requires further evaluation in this species.
In general, zolazepam-tiletamine was found to be a safe and effective combination in all three mustelid species for minor procedures in field conditions, however, further trials are recommended with more vigorous monitoring.
INFECTIONOUS DISEASE SEROLOGY OF FREE-RANGING ALASKAN PACIFIC WALRUS
(Odobenus rosmarus divergens)

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Abstract

Infection by Leptospira spp., Brucella spp., phocine distemper virus (PDV), caliciviruses, and Influenza A virus have all been documented in free-ranging pinniped populations, with some implicated in significant morbidity or mortality.1-5,7-9,11,13,15 This study investigated the infectious disease exposure status of adult free-ranging Pacific walrus (Odobenus rosmarus divergens) in Alaska. Serology was performed for the five main serovars of Leptospira interrogans (pomona, hardjo, icterohaemorrhagiae/copenhageni, grippotyphosa, canicola), Brucella spp., PDV, influenza A virus, and 26 strains of calicivirus [San Miguel Sea Lion Virus (SMSV) and Vesicular Exanthema of Swine Virus (VES)]. Preliminary data have previously been reported.12

Blood samples were collected from 20 male and 20 female walrus at St. Lawrence and Round Islands, Alaska, during May 1994, 1995, and 1996 and October 1995 and 1996. Walrus ages ranged from 6-31 yr, based on visual counts of lower canine cementum growth layer groups (J. Garlich-Miller, personal communication).6

Leptospira serology was performed by microscopic agglutination and Brucella serology was performed by tube or card agglutination. Serology for PDV and calicivirus (SMSV strains 1, 2, 4-13 and VES strains A48, B51, C52, D53, E54, F55, G55, H54, I55, J56, K54, 1934B, Tillamook, and Walrus)10 were performed by virus neutralization in tissue culture. Sera from 38 walrus were tested for antibodies to influenza A virus by agar gel immunodiffusion (AGID). Subtype specificity of positive sera were determined by hemagglutination-inhibition (HI) tests (Hemagglutinin subtypes H1-H14) and neuraminidase-inhibition (NI) tests (Neuraminidase subtypes N1-N9).

All walrus were negative to four Leptospira interrogans serovars (pomona, hardjo, icterohaemorrhagiae/copenhageni, canicola) but three males sampled on Round Island had low titers (1:100, 1:100, 1:200) to L. interrogans serovar grippotyphosa. All walrus were negative for Brucella. Two walrus (females sampled at St. Lawrence Island) had low PDV titers (1:22, 1:45) which were interpreted as nonspecific, and 25 were negative at a dilution of 1:20. Samples from the remaining 13 walrus sera were toxic at low dilutions but were negative at the lowest dilution (1:20 to 1:80) at which the test could be performed.
Eight walrus (four females and two males from St. Lawrence Island and two males from Round Island) were AGID-positive for Influenza A. Antibodies were specific for hemagglutinin subtype H10, with titers ranging from 1:8 to ≥ 1:32. In addition, two sera had antibodies to neuraminidase subtypes N7 (H10N7); two were positive for subtypes N2, N3, and N7; one was positive for subtypes N2 and N7; one was positive for subtypes N2, N3, N5, N6 and N7; and two were negative for specific neuraminidase antibodies. These results are consistent with exposure of this population to multiple serotypes of influenza A virus.

Seven walrus were positive for one or more strains of calicivirus. Two females sampled at St. Lawrence Island had a titer to VES G55 (1:60, ≥ 1:230); one female sampled at St. Lawrence Island had a titer to VES F55 (1:140); one female sampled at St. Lawrence Island had a titer to VES E54 (1:140); one female sampled at St. Lawrence Island had a titer of 1:57 to both SMSV 12 and VES 1934B; and two males (one each from St. Lawrence and Round Islands) had titers to SMSV 12 (1:180, ≥ 1:230). These results are consistent with a population which has a low level of exposure to several marine caliciviruses.

This study extends the assessment of the Pacific walrus population’s infectious disease exposure status. Although *L. interrogans* serovar *pomona* infection is common in Northern fur seals (*Callorhinus ursinus*) and California sea lions (*Zalophus californianus*), the walrus were infrequently seropositive to *Leptospira* spp. and were seronegative to the known pinniped pathogen *L. interrogans* serovar *pomona*. Three walrus were seropositive to *L. interrogans* serovar *grippotyphosa*, a serovar which has also recently been documented associated with renal disease in Pacific harbor seals and in an asymptomatic California sea lion and elephant seals in a rehabilitation center. *Brucella* spp. can cause abortion and infertility in other species, pinniped PDV epizootics have occurred, and both are emerging diseases in pinniped populations. Atlantic walrus (*Odobenus rosmarus rosmarus*) seropositive to both *Brucella* spp. and PDV have been documented but few, if any, Pacific walrus in this study were seropositive for these pathogens. A number of walrus demonstrated serologic evidence of influenza A infection, a disease which has caused mass mortalities in Atlantic harbor seals (*Phoca vitulina*). Serologic evidence of influenza A infection has also recently been documented in one ringed seal (*Phoca hispida*), but not numerous other pinniped species including walrus, from Alaska. This study demonstrated exposure to caliciviruses in the population, consistent with previous studies in the Pacific walrus, California sea lion, Steller sea lion (*Eumetopias jubatus*), Northern fur seal, and various phocids from the eastern Pacific and Bering Sea.

Populations without previous exposure to infectious agents are more susceptible to significant health consequences when first exposed. The results of our study continue to suggest that the Pacific walrus population has had limited exposure to many of these potentially pathogenic bacteria and viruses. As a naive population they could be adversely affected if these agents were introduced.

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LITERATURE CITED


A VETERINARY ANESTHESIOLOGIST'S VIEW OF ANIMAL PAIN

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Abstract

Pain is a very personal experience. This reality and the fact that we can not communicate with animals about their pain experience has lead to controversy about whether animals do or do not perceive pain. My assumption is that animals do experience pain but that many confounding factors make it difficult for us to assess pain in animals. Given these realities I am optimistic that new methods for assessing pain in animals, such as fecal steroids or species specific pain scales, will help us identify the animal in pain. Furthermore, as we gain a better understanding of the biochemical and cellular mechanisms involved in pain, so too will we gain a better perspective on how to treat pain.

“Dying is nothing, but pain is a very serious matter.” Henry Jacob Bigelow, 1871

What is pain? Pain is defined as an unpleasant sensory experience associated with actual or potential tissue damage, or it is described in terms of such damage. It is often further defined in temporal terms – acute vs chronic. Acute pain, although unpleasant, is usually transient and does not have long-term detrimental effects on an animal. Chronic pain, can have long-term effects on the well-being of the animal: food consumption may be significantly reduced, social interaction with conspecifics may be affected and adversely affect the social status of the animal, and hormonal responses to pain may adversely affect healing processes and the body’s immune responses.

What are the mechanisms of pain and the implications for therapy? The pain an animal experiences when exposed to a noxious stimulus typically has a high threshold, is well localized and transient, and has a stimulus-response relationship similar to that of other somatosensations. This pain has protective value and it is often referred to as physiologic pain. Clinical pain is associated with peripheral tissue damage such that occurs for example during surgery. This pain has three characteristics: it is spontaneous and human patients describe it as dull, burning or stabbing; it is exaggerated in response to an applied stimulus (hyperalgesia); and it can be produced by stimuli that normally would not produce pain (allodynia). Hyperalgesia and hypersensitivity are due to inflammatory mediators and other endogenous substances that are produced at the time of injury or in response to injury. They include hydrogen and potassium ions, bradykinins, prostaglandins, cytokines, norepinephrine, and various tissue growth factors to mention just a few. These substances cause normally high-threshold primary sensory neurons to become hypersensitized (i.e., their threshold for transmitting noxious stimuli is reduced). In addition to this peripheral response, there is a central response where neurons in the spinal cord have an increase in excitability that is triggered by the nociceptive afferent inputs from the periphery as well as the central action of...
endogenous substances such as Substance P, NMDA, and nerve growth factor. This central sensitization can outlast noxious sensory inputs from the periphery.

The realization that clinical pain is a reflection of peripheral input as well as an expression of changes produced in the CNS has lead researchers to direct their investigations at treating both the disease/injury process in the periphery and the changes induced or triggered in the CNS. An interesting and promising approach for treating pain that takes into account the fact that both peripheral and central neural sensitization play key roles in pain perception is preemptive analgesia. The underlying theory of preemptive analgesia is to use drugs and techniques that block the hypersensitizing processes before, during and after surgical trauma. Therefore it is reasoned that if a patient is treated pre-operatively with an opioid, a non-steroidal anti-inflammatory drug (NSAID), and a local anesthetic, then intra-operatively with an analgesic, and finally post-operatively with an opioid and NSAID, then theoretically the patient should be more comfortable in the post-operative period than if treated in a conventional manner. Some studies suggest that preemptive analgesia is effective, but two studies specifically designed to investigate this issue did not demonstrate a benefit of preemptive analgesia in surgical patients. This should not be a surprise given the number of endogenous substances that play key roles in sensitizing peripherally and centrally located neurons and receptors. One should not expect that a single drug, or combination of drugs such as opioids, local anesthetics and NSAID, could block the effects of all of these various substances. Preemptive analgesia is limited at this time by the fact that the fundamental molecular processes involved in pain signaling are not completely understood. Furthermore, there is some question as to whether general inhalant anesthetics block preemptive analgesia. A study that investigated the genetic expression of an immediate early gene “c-fos” (a molecule that may produce molecular memory for painful stimuli) within the spinal cord after painful peripheral stimulation, demonstrated that general anesthesia failed to prevent expression of the gene. However, low concentrations of lidocaine have been shown to have a selective action on nociceptive transmission in the spinal cord that is different and more potent than its local anesthetic conduction blockade in the periphery, an indication that this class of sodium channel blockers may be potentially useful as analgesic agents. The concept of preemptive analgesia has merit and there is a great deal of current research into the biochemical mechanisms involved in central neural hypersensitivity.

**Do animals perceive pain?** The definition of pain contains elements of communication that have made it difficult to assess pain in animals and has lead to controversy as to whether animals do in fact perceive pain as do humans. This controversy has been further complicated by the fact that the way animals respond to pain is not always what we humans expect. Assessing pain in animals is further complicated by large confounding factors such as the differences between acute pain and chronic pain, behavioral differences between domestic and wild animals, the response of the individual as compared to the species as a whole, and prey species versus predator species behaviors. An animal’s behavioral response to pain is the outward manifestation of a number of complex physiologic and emotional interactions. Fear or aggression can affect an animal’s perception of pain, or how an animal responds to pain. The fact that certain emotional states can affect an animal’s sensitivity to noxious stimuli may partially explain the difficulty of assessing pain in animals. For example, an animal’s fear response may cause immobilization that could be misinterpreted as
evidence that the animal is not experiencing pain. Many veterinarians recognize that there is no advantage for a wild animal – either hunted or hunter – to show signs of “disease,” including pain, for to do so attracts unwanted attention. This has been amply demonstrated in birds,\textsuperscript{8,9} and highlights another species-related phenomenon that complicates our ability to assess pain in animals.

All animals do not respond to drugs in a similar manner. Some reasons for this are quite obvious. Analgesic drugs administered orally to a ruminant are not likely to achieve blood concentrations that are pharmacologically effective because of poor absorption from the rumen, rumen pH effects, and the action of rumen microbes. We are also familiar with the so called “morphine mania” associated with doses of morphine and morphine-like drugs in cats that are more appropriate for use in dogs. We recognize now that this response is dose related due to differences in receptor numbers and that appropriate doses of opioids can be used quite effectively in cats both as pre-anesthetics and as analgesics. It also appears that there are species differences in terms of types of opioid receptors. Pharmacodynamic studies in pigeons have demonstrated that these birds have more kappa opioid receptors than mu opioid receptors.\textsuperscript{15} This may explain why birds do not respond to mu agonists like morphine in the same manner as mammals and lends support to the argument that kappa opioids such as butorphanol, may be more efficacious as analgesics in birds than mu agonists.

The process of “thinking” about pain is another confounding factor of pain perception in animals.\textsuperscript{14} It is certainly true that humans can and do think about pain; but what about animals? If animals don’t “think” about pain does that mean there is no pain?\textsuperscript{14} An observer’s interpretation of a behavioral response to pain is often based on the observer’s own responses to pain. The manner in which we respond to pain is how we expect others to respond, including animals. These anthropocentric perspectives of pain are misleading when applied to animals.

These issues are interesting and serve to point out how difficult it is to assess pain in animals, but in my mind there is no question that animals do perceive pain. Extensive research has clearly shown that animals possess the same neural circuitry, neuroreceptors, and neurotransmitters for the sensory component of pain as do people.\textsuperscript{6} So the task for us is not to discuss whether animals feel pain, but to focus on the dilemma of how we can determine when an animal is in pain, how to treat the pain, and what the future holds in terms of new analgesic drugs and therapeutic techniques.

\textbf{What can we do to determine that an animal is in pain?} There are a number of approaches being pursued to try to determine when an animal is in pain. There is increasing interest in assaying blood, urine or feces for hormonal indicators of stress due to any number of causative factors including pain.\textsuperscript{7, 26, 28, 30} Assaying feces for cortisol, or corticosterone in the case of birds, is appealing because specimen collection can be done without stressing the animal. My own preliminary work in this area suggests that this technique may only be effective if one has sufficient baseline data for an individual animal or group of animals that take into account diurnal, seasonal and reproductive fluctuations.

Another technique that is increasingly being applied to assess pain in animals is the development of pain scales, especially ones that are specifically designed for a given animal species. These pain scales take time and effort to design. Terms must be clearly defined to reduce inter-observer
variability and to make the scoring process as objective as possible. The people using the scale must have a good understanding of normal and pain-related behavior for a given animal species.

Some therapeutic options for treating pain. Of real interest to all of us is how to treat pain in the diverse species that are presented to us for care. Realizing that clinical pain involves both peripheral and central mechanisms means that there is no one single silver bullet for treating pain. The best guide for treating pain is that the right drugs should be used at the right time and at the right dose. But what drugs should we use and what techniques? In the last few years we have seen “old” drugs used in “new” ways. Opioids have long been a standard of care for treating pain in a variety of animal species, but their use has not always been practical in zoo settings and certainly not in the wild except for capture purposes. However, the development of fentanyl-impregnated patches that, when applied to the skin, produce sufficient plasma concentrations of fentanyl and analgesia of several days duration have been used in a number of animal species for treating surgical and chronic pain. These patches have been used to treat pain in cats, dogs, and a Southeast Asian pig at the Bronx Zoo.

EMLA® cream is a eutectic mixture of lidocaine and prilocaine that, when applied to the skin, provides superficial, local analgesia. Although this product may reduce the pain associated with catheterization, the requirement that it be applied 45 min or more prior to a superficially painful procedure limits its usefulness in many animals.

The epidural administration of local anesthetics such as lidocaine and bupivacaine, is a well recognized and time-honored technique for providing regional anesthesia extending caudally from the thoracolumbar region. But it was the discovery of opioid and alpha2 adrenergic receptors in the spinal cord that has lead to novel methods for providing pain relief. Veterinarians have injected a variety of opioids and alpha2 adrenergic agonists into the epidural space of animal patients to provide postoperative analgesia. Although most of the reports concerning the epidural injection of morphine concern dogs that have had stifle arthrotomies or thoracotomies, this technique has been used in a cheetah following total hip replacement. Interestingly, morphine injected into the lumbosacral epidural space appears to provide analgesia for patients after thoracotomy, and serves to demonstrate the complex interconnected neural circuitry in the dorsal horn of the spinal cord. Alpha2 adrenergic drugs also have been injected epidurally in a variety of animals including dogs and horses.

Local anesthetics and opioids, primarily preservative free morphine, have been injected into the intra-articular space following surgery of the stifle. While it is the local anesthetics, especially bupivacaine, that provide a great deal of pain relief in the immediate post-operative period, it is the opioids that appear to provide analgesia that lasts as long as 24 hr.

When do we stop treating pain? A difficult question to answer is when to stop treating an animal for a painful condition? The cut off point may be after resolution of the condition for which the animal is being treated; and there are often clear medical guidelines to help us. What about the animal with a chronic painful condition for which therapy does not provide a cure and only provides marginal relief from the pain associated with the condition? When do we consider euthanasia to be the more
humane answer to the animal’s condition? These situations often do not have easy answers. For example, other considerations such as the breeding value of the animal or its genetic value to the species as a whole may influence the decision to keep an animal alive, albeit as comfortable as possible. In these situations my own opinion is that if the decision is repeatedly made to keep an animal alive for reasons unrelated to the well-being of the animal itself and the condition can not be medically corrected, then it is time to consider euthanasia as the better solution for the animal.

LITERATURE CITED

PAIN AND ANALGESIA IN REPTILES AND AMPHIBIANS

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Abstract

In humans, pain is a multifaceted experience with the sensory component (nociception) being only one factor. Higher limbic and cortical faculties modify the nociceptive signal to increase or decrease the pain experience. These may or may not be developed in subhuman animals. Humans that have undergone cortical ablation for treatment of severe chronic pain report an awareness of pain but that the pain no longer bothers them, suggesting that vertebrate species that do not have developed higher cortical faculties may have very different and diminished appreciation of nociceptive sensations.

Electrophysiologic studies conducted as early as 1926 support the morphologic correlation between free nerve endings and painful sensations in non-mammalian vertebrates (such as reptiles and amphibians) . Pain pathways, pain responses, and some effective analgesics have been demonstrated in amphibians and reptiles; however, little information is available to clinical veterinarians regarding appropriate drugs and dosages for potentially useful analgesics. Additionally, clinical indices for pain and relief of pain have not been documented in amphibians and reptiles. It is generally preferable and appropriate to consider procedures accepted as painful in humans to be painful in other animals.

Two processes may represent dual selection pressures that have guided the emergence of nociception across phylogeny. The response to pain that represents a threat of tissue damage generally induces vigorous activity and efforts to escape the stimulus. The response to pain from tissue already damaged is often inactivity. The former is generally easier to study in a laboratory setting while the latter more closely represents pain in a clinical setting. This underscores the philosophy that inactivity must not be interpreted as a lack of pain. Non-mammalian vertebrates demonstrate four responses to nociceptive stimulation which threaten tissue damage: a) a nonspecific flight response, b) a rapid startle reaction, c) an affective response such as vocalization, d) a coordinated reaction such as biting at the source of pain.

There is very little information available regarding pain and analgesia in reptiles. It is frequently assumed that the ranking of animals’ ability to experience pain follows class lines such that Mammalia > Aves > Reptilia > Pisces. Because of their use in the laboratory setting, pain and analgesia in amphibians has been more thoroughly investigated than in reptiles. Evidence for the ability of reptiles and amphibians to experience pain is supported by the presence of appropriate neurologic components with the capacity to elicit and action potential in response to a painful
stimulus, endogenous antinociceptive mechanisms to modulate pain and demonstrable modulation of pain with pharmacologic agents used in other species.

The neuroanatomic components and pathways necessary for nociception have been demonstrated in amphibians. Nociceptive action potentials generally have a long latency from stimulus to discharge and longer duration but lower amplitude compared to action potentials stimulated by light touch. These slow impulses are evoked by injurious stimuli such as application of weak acid to the skin, intense mechanical stimulation, and strong heat but are not seen following a gentle touch.

Neurotransmitters documented as important in pain modulation in mammals occur in reptiles and amphibians, and are believed to function in a similar manner. As an example, gamma amino butyric acid (GABA) is responsible for primary afferent depolarization and presynaptic inhibition in both mammals and amphibians. The presence of serotonin and substance P in reptiles and amphibian primary afferents also supports the similarity though, their specific function has not been completely elucidated.

Amphibians and reptiles possess well developed endogenous opioid systems. Immunohistochemical studies have demonstrated the presence of dynorphins, endorphins, and enkephalins within the central nervous system of reptiles and amphibians. These sites appear to be ubiquitous across the phylogeny. Additionally, in restrained frogs, immobilization stress produces a naloxone reversible analgesia compared with control frogs. This implies that them is endogenous release of opioids in frogs that are restrained.

In any pain test there are two basic components—a noxious input and a behavioral output. The input must exclusively activate the pain fibers and the behavioral output should occur as a result of a specific noxious input. Most common is a motor response occurring shortly after the noxious stimulation. Morphine is an exogenous opioid commonly used to provide analgesia in mammals. It exerts its effect by interacting with a mu receptor whose ligand is an endogenous opioid peptide. Initial studies using a hot plate test or electric shock did not show any analgesic effect of morphine at any doses studied in frogs. An analgesic effect was easily demonstrated with morphine in frogs (10-100 mg/kg s.c.) using an acetic acid test. Application of acetic acid to the skin of the hind leg in frogs induces a spinal wiping reflex which is reproducible and, now, commonly used for testing nociception in hogs. The failure of the hot plate and electric shock to detect opioid analgesia was likely due to using an inappropriate test. Frogs show great variability in their latency for jumping off hot plates and responding to electric shock. Analgesia with morphine in amphibians has little effect on motor activity, feeding or general behavior, but can be blocked with naloxone. High doses of morphine (320-640 mg/kg) produce hyper responsiveness to sensory stimuli similar to the reaction produced in cats given high doses of morphine.

In Nile crocodiles (Crocodylus niloticus) nociception has been studied using a hot plate test, a formalin test where formalin is injected into the hindlimb, and a capsaicin instillation test where capsaicin is instilled onto the eye. The hot plate test was the most reliable. This test was then used to study the analgesic effect of morphine and pethidine in crocodiles. Morphine at 0.05-1.0 mg/kg
intracoelomically induced a significant increase in response latency at all doses studied with a maximum effect observed at a dose of 0.3 mg/kg.\textsuperscript{8} Onset of action was 30-120 min lasting the entire 6 hr of the study. The crocodiles weighed only 619 ± 325 g. Pethidine also induced an increase in response latency at 1-8 mg/kg intracoelomically with a maximum response at 2 and 4 mg/kg (different responses–escape maneuver vs. foot lift). The onset of action was 30-180 min lasting the entire 6 hr of the study. These doses are considerably lower than expected. In mice and rats, doses more than 20 times greater are required to observe increased response times. Additionally, both drugs demonstrated a dose dependent response but also reached a plateau effect.

A tail-flick apparatus was used in a study examining the neural mechanism underlying tonic immobility in green anoles (\textit{Anolis carolinensis}).\textsuperscript{15} Morphine at 5 mg/kg intracoelomically caused a significant increase in the latency of the response. Since the purpose of the study was to study tonic immobility, other doses were not evaluated.

In amphibians, systemic administration of the mu agonists fentanyl, levophanol, methadone, morphine, meperidine and codeine; partial mu agonists buprenorphine; and kappa agonist U50488, nalorphine, and bremazocine produced dose dependent analgesia for at least 4 hr with a gradual increase in effect during the first hour reaching a peak at 60-90 min.\textsuperscript{25} The order of analgesic potency in amphibians was fentanyl > levophanol > U50488 > methadone > bremazocine > morphine > codeine > nalorphine which parallels the potency of mu agonists found in mammals. Kappa agonist potency was not similar to that found in mammals potentially related to the difference in concentration of kappa receptors in amphibians compared to mammals.\textsuperscript{25}

The pharmacokinetics of synthetic opioids differs greatly between amphibians and mammals. Amphibians appear to require higher doses compared with rodents; however, the algesiometric test used are different (hot plate vs. acetic acid).\textsuperscript{25} Endogenous opioids play a role in hibernating in both mammals\textsuperscript{13} and amphibians\textsuperscript{24} Cold adapted frogs returned to room temperature showed a naloxone attenuated increase in nociceptive threshold.\textsuperscript{24}

Mammals, amphibians, and reptiles have the same anatomic and functional characteristics in alpha 2 adrenoceptor mediated analgesia.\textsuperscript{7,22} They produce a dose dependent sedation in mammals; however, 10 mg/kg of dexmedetomidine or of clonidine in frogs provides analgesia but does not produce sedation. Dose dependent analgesia following systemic\textsuperscript{2} and intraspinal\textsuperscript{23} administration of dexmedetomidine, clonidine, epinephrine, and norepinephrine was demonstrated using the acetic acid test. The effects lasted 6-8 hr following systemic administration and 4 hr following intraspinal administration. The relative potency following systemic administration is dexmedetomidine > epinephrine > norepinephrine > clonidine. Following spinal administration, all were equivalent except for clonidine which was not as potent as the others.

Ketamine produces profound somatic analgesia but little visceral analgesia in mammals. A proposed mechanism for its analgesic effects in amphibians may be through one of the opioid receptors since naloxone significantly blocks the effects of ketamine on nerves and skeletal muscle.\textsuperscript{10}
Pain perception in reptiles and amphibians is, therefore, likely analogous to mammals. When performing invasive and painful procedures appropriate anesthesia and analgesia should be administered. Though specific doses have not been established in clinical trials, research indicates a potential benefit to the use of opioids in reptiles and amphibians. Other analgesics such as alpha 2 agonists and ketamine also have analgesic benefits in reptiles and amphibians.

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LITERATURE CITED

ADDRESSING PAIN IN THE AVIAN PATIENT

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Abstract

Introduction

Effective control of pain for the avian patient is a tremendous challenge to the veterinarian. It is easily accepted that birds are able to feel pain, but very little research has been conducted to objectively determine methods to relieve pain in birds. No set of objective criteria has been established to assess pain or the effectiveness of an analgesic agent. Perhaps this accounts for the lack of published information which in turn may account for the infrequent use of analgesic agents in clinical care of birds.

Conditions that cause pain to people can be assumed to be painful to birds. A perplexing component of this approach is that not all people respond to pain in the same manner. Two people can have the same surgical procedure and their individual analgesic requirements can vary greatly. This seems to be true for individual birds as well as variations between species of birds. Prey species may not respond in the same manner as predatory species. Social species may exhibit different behaviors than solitary species. Many birds do not respond to procedures that would be painful to humans with easily interpreted, overt painful behaviors. Some would assume that if the bird does not show painful behaviors such as crying or whining then perhaps their experience of pain is less severe. My concern is that our ability to recognize pain is inadequate and that we continually underestimate our avian patients. Avian behavior changes are subtle such as a slight alteration in posture, a slight restlessness or change in perching position, a decrease in appetite or being quieter than usual. Birds may guard a painful area and decrease their social interactions with the owner or other birds. Rather than looking for obvious changes in behavior we should key in on the absence of normal behavior.

Opioids

Limited studies in chickens suggest that pain perception is mediated by neural pathways and neurotransmitters that are similar to mammals. Studies to evaluate pain in birds (primarily Gallinaceous birds) have been based upon changes in heart rate, increases in blood pressure, vocalizations, attempts to escape, and behavioral changes. Studies performed in pigeons and chickens suggest that opiate receptors are present in birds Early avian opioid studies described the effects of morphine administered to chickens and it was determined that dosages greater than 10-30 times a mammalian dose were needed to produce analgesia. Subsequent studies using different strains and ages of chickens yielded conflicting information. Testing methods varied using several different forms of noxious stimuli. The diversity of testing methods permit only one
conclusion: opioid effects are variable depending on the species, strain and route of administration.\textsuperscript{16} Drug discrimination trials have been used to investigate the stimulus properties of opiates and pigeons were a common animal used in these studies. Pigeons can identify several opioid drugs such as buprenorphine, oxymorphone and fentanyl as being more like morphine than saline.\textsuperscript{10} Other pigeon studies concluded that birds could not discriminate between μ and κ agonists and therefore both opioid receptors were thought to have a similar mechanism of action.\textsuperscript{14} A recent study with chickens tested both a μ agonist and a κ agonist and found that both drugs similarly decreased isoflurane anesthetic concentrations in a dose-dependent manner when administered to chickens.\textsuperscript{7}

Buprenorphine is a partial agonist that avidly binds to μ opioid receptors but activates them less than morphine does which results in a long duration of analgesia in mammals. Pigeons can discriminate buprenorphine for up to 120 hr, yet phamakokinetic studies in the African gray parrot show a rapid metabolism and excretion of the drug within 8 hr (Paul-Murphy, unpublished data). Current studies evaluating buprenorphine in African gray parrots indicate that even at extremely high i.m. dosages (0.2 mg/kg) the analgesic effect is minimal.\textsuperscript{19}

Mixed agonist-antagonist opioids are the most common drugs used in veterinary medicine for prolonged pain relief. Mixed agonist-antagonists are characterized by agonist activity at opioid κ receptors and minimal or antagonist effects at opioid μ receptors. In the pigeon forebrain, 76\% of the total opioid receptors are κ.\textsuperscript{17} If other species of birds are like the pigeon, then these κ agonist drugs may be more effective than μ opioid agonists. Butorphanol is a mixed-agonist-antagonist drug. The use of butorphanol as an analgesic was recently studied in psittacines by determining its isoflurane sparing effect. After administration of 1 mg/kg butorphanol tartrate intramuscularly (i.m.), the effective dose 50 (ED 50) of isoflurane was decreased in cockatoos and African gray parrots but was not significantly changed in blue-fronted Amazon parrots. Current studies evaluating butorphanol in awake African gray parrots at 1.0 mg/kg determined that 50\% of the birds showed an analgesic response.\textsuperscript{19} Mixed agonist-antagonist have a low therapeutic ceiling in mammals such that the analgesic effect is maximum at a specific dosage and higher dosing will not improve efficacy. The therapeutic dosage of butorphanol for many species of bird is not yet determined, but empirical dosages recommended for psittacines range from 1-4 mg/kg. In my experience, this dosage level will cause recumbence and respiratory depression in buteos.

\textbf{Non-Steroidal Antiinflammatory Agents}

NSAIDS act by inhibiting cyclooxygenase and the production of prostaglandins. There are several categories of NSAIDS but few have been investigated in birds. A study of analgesic agents using pigeons tested 5 mM solutions of naproxen, ibuprofen, ketoprofen, piroxicam and acetaminophen and found that only naproxen was significantly effective against ingestion of chemical irritants.\textsuperscript{3} High levels of aspirin were fed to chickens to assess affects on fertility and embryo health.\textsuperscript{18} Phenylbutazone and indomethacin were tested in chickens to assess the inhibitory effects on anaphylactic responses. A few drugs have published dosages based on empirical use such as acetylsalicylic acid (aspirin) @ 5.0 mg/kg p.o., t.i.d. or 325 mg per 250 ml drinking water, flunixin
meglumide @ 1.0-10.0 mg/kg i.m., s.i.d., ketoprofen @ 2 mg/kg i.m. and meclofenamic acid @ 2.2 mg/kg p.o., s.i.d. Phenylbutazone has been used in raptors @ 20 mg/kg p.o. and 3.5-7.0 mg/kg q 8 hr in psittacines.21

A common side effect of NSAIDS is gastrointestinal ulceration caused by direct mucosal irritation and inhibition of prostaglandin synthesis. High dosages of flunixin meglumide (10 mg/kg) have been reported to cause regurgitation and tenesmus in budgerigars.2 Prolonged used of flunixin meglumide has caused frank blood in the feces of birds which cleared after cessation of the treatment.4

Renal ischemia and tissue damage is a serious complication of flunixin meglumide in birds. Test groups of Bobwhite quail were given 0.1 and 32.0 mg/kg daily i.m. for 7 days and all birds had histologic evidence of renal damage. Lesions consisted of acute necrotizing glomerulitis, gout tophi in the renal tubules and visceral gout. The severity of the lesions were directly correlated to the dosage of flunixin meglumide.15

Local Anesthetics

Local anesthetics block ion channels which interrupts the transmission of pain impulses. Local anesthetic blocks can prevent central sensitization when used preoperatively. Lidocaine can be used safely in birds at dosages below 4 mg/kg. Overdosage has been reported to cause seizures in small birds.

LITERATURE CITED

ASSESSMENT OF PAIN IN CAPTIVE AND FREE-RANGING DUCKS AFTER INTRA-ABDOMINAL TRANSMITTER PLACEMENT

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Abstract

Little information is available on avian pain perception, but it is generally accepted that birds are able to feel pain. Often analgesics may be withheld because the presence and severity of pain is not recognized. In small animals, our familiarity and close relationship with these animals often facilitates the recognition of pain. In addition, veterinarians are less familiar with the normal behavior of waterfowl and prey animals may be less likely to display overt pain-associated behavior. In comparison to pet species, wild avian species do not respond positively to human contact. It is therefore necessary to devise different methods of assessing pain in these species.

Free-ranging waterfowl are captured routinely to gain information about their biology and physiology. Often studies rely on radiotelemetry because it provides excellent information on movements, behavior and survival. Intra-abdominal transmitters provide more reliable data than externally mounted transmitters, but surgery is required for radio transmitter placement. Pain from surgery or direct pressure from intra-abdominal radio transmitters may alter behavior and decrease food intake, which may result in lowered reproductive success. Garrettson found that blue winged teal (Anas discors) recovering from intra-abdominal radio transmitter surgery had progressive weight loss over 1 wk. Pain may also alter the normal responses of a bird to its environment, which may increase susceptibility to predation. Behavioral changes may lead to disruption of pair bonds, alter nest site choice or reproductive success. Modification of normal behavior may result in erroneous interpretation of data if unrecognized subtle changes occur in the physiology, behavior, or well-being of the bird.

Short holding times are beneficial to reduce stress, prevent pair bond disruption, or reduce detrimental effects of egg cooling when females are not incubating. Non-sedative analgesia is desirable since suitable conditions for safe recovery may be impossible to provide, therefore, research is directed at non-steroidal anti-inflammatory drugs (NSAIDs) and local anesthetics. Administration of analgesics prior to surgery reduces pain experienced by an animal more effectively than if the analgesic had been administered post-operatively. Therefore, a single pre-emptive dose may provide adequate analgesia following intra-abdominal transmitter placement.

To determine efficacy of a NSAID, ketoprofen, a sham surgery for implanting radio transmitters was done in captive mallard ducks (Anas platyrhynchos). Groups of two ducks (one male and one female) were chosen randomly to have either an incision (surgery) and ketoprofen (0.5, 1, 2, 5, 7.5 or 10
mg/kg) or no surgery (control) and an equal volume of saline. One male and one female mallard received each dose and treatment and control animals were randomized completely. Two ducks were anesthetized with isoflurane simultaneously to allow birds to recover at the same time. Surgery involved a skin and muscle incision on the ventral abdomen to mimic placement of an intra-abdominal transmitter and tissues were closed with 4.0 absorbable suture.

Ducks were acclimatized to the pen for 3 days prior to the experiment and post-operatively birds were returned to the same pen as soon as possible after recovery. All birds were monitored using video recording for 24 hr prior to the experiment (control) and 48 hr post-operatively. Time spent performing various behaviors including comfort movements (stretching, flapping wings, preening, etc.), preening of surgical site, resting, swimming, standing, walking and feeding were recorded. Birds were assessed at 6 hr and daily for 7 days post-operatively. Body weight, incision site, hydration status and flight distance were assessed at 24, 48, 72 and 168 hr. Flight distance was defined as the distance between the observer and duck when the duck moved as the observer entered the room. All birds were euthanatized 7 days after surgery and a complete necropsy and histologic examination was performed to ensure that no gastrointestinal or renal damage occurred. Histology results will not be discussed here. Seventy hours of video tape for each group was viewed as 4-hr video tape segments which were chosen and viewed randomly by an observer blind to the treatments. Behaviors (listed above) were recorded at 30-sec intervals. Data were analyzed using a change point test and repeated measures ANOVA.

All ducks stood, appeared active and clinically normal when observers entered the room. Flight distance could not be assessed as ducks stood against the far wall before they could be visualized. Palpation of the surgical site did not elicit wound guarding behavior or vocalizations and all birds struggled during handling. Preliminary results revealed that at lower doses of ketoprofen (0.5-2.0 mg/kg), the most useful index of pain following surgery was reduced locomotion (walking, swimming) and time spent resting in a sitting position was increased. Preening of the ventral abdomen (surgical site) increased in all ducks but the control group. This may have been because feathers were removed from the surgical site or a true response to inflammation. Comparisons made by video taping pre- and post-operatively were more effective for determining responses to pain than physical examination or observations done while observers were in the room. Conclusions about the effective dose of ketoprofen must be drawn with caution. Captive and field studies are ongoing to further define an effective dose for ketoprofen. No gross lesions were visible on necropsy. In addition, evaluation of pathologic effects must be completed to ensure safety when using single doses.

As part of another study, the efficacy of a local anesthetic (bupivicaine) was investigated using male ruddy ducks (*Oxyura jamacensis*) implanted with 18-20 g radio transmitters. The research was conducted near Minnedosa, Manitoba (50°10'N, 99°47'W). Forty male ruddy ducks were divided randomly into one of two groups: (a) leg banded only and (b) surgical implantation of an intra-abdominal radio transmitter during propofol anesthesia.15 Birds that had surgery had 2.0 mg/kg of bupivicaine injected into the surgical site prior to surgery. Ducks were observed, through a spotting scope, 6 hr post-operatively for 20 min. Aspects of behavior were recorded as comfort movements...
(stretching, flapping wings, preening), preening of surgical site, resting in cover or in the open, swimming, foraging and out of sight every 30 sec. Data were analyzed using a multivariate analysis of variance (MANOVA) to control simultaneously for several dependent behavior variables. Univariate ANOVAs were used to identify which aspects of behavior were significant. Results were considered significant at \( P < 0.05 \).

Nineteen ducks that had surgery were easily located because they were resting motionless on floating vegetation or land, and had a puffed up appearance. One implanted male was killed by a red-tailed hawk (\textit{Buteo jamacensis}) approximately 6 hr after surgery. Males with leg bands only were more difficult to locate and only 8 ducks in this control group were observed. There was an overall significant difference in behavior between implanted and leg banded only ducks (MANOVA, \( df = 6,24, P = 0.035 \)). Implanted males spent significantly less time feeding and swimming and more time resting in the open than leg banded only males (univariate ANOVA, \( df = 1, 29, P = 0.001, 0.013 \) and \( 0.002 \), respectively). In contrast, leg banded only males spent more time out of sight, as they were difficult to locate, and when resting, spent less time in the open. In addition, a female ruddy duck (as part of a different study), recaptured 5 days after surgery, had a weight loss of 50 g. Ruddy ducks are adapted highly to their aquatic lifestyle and resting on land is considered an unusual behavior. Results from this study suggest that the surgical procedure produced discomfort and abnormal behavior post-operatively which may also make ducks more susceptible to predation. Local anesthesia using bupivicaine at the dosage in this study is likely not adequate for pain control after surgical placement of intra-abdominal transmitters. Secondary inflammatory effects may contribute to altered behavior in ruddy ducks and weight loss seen in the female ruddy duck.

Evaluation of responses of captive wild ducks was best done through video taping. In the field, ducks showed similar behavioral responses to a painful procedure as captive ducks. A knowledge of normal time budgets and diurnal behavior for the species is also important. Additional experiments are needed to further characterize behavioral changes associated with pain in waterfowl and evaluate preemptive analgesic efficacy.

ACKNOWLEDGMENTS

This research was supported by the Canadian Wildlife Service, Delta Waterfowl Foundation, Ducks Unlimited Institute for Wetland and Waterfowl Research, and the Wildlife Health Fund, University of Saskatchewan. I would also like to thank my supervisor Dr. Alex Livingston and Dr. Robert Brua for their assistance in writing this abstract. Special thanks to Marnie Cooper and Lise Tellier for technical support during these studies.

LITERATURE CITED

USE OF A FENTANYL TRANSDERMAL PATCH FOR POST-SURGICAL ANALGESIA IN A MEXICAN GRAY WOLF (Canis lupus baileyi)

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Abstract

A Mexican gray wolf (Canis lupus baileyi) underwent four orthopedic surgeries over a period of 3 yr. Knowledge of this wolf’s normal behavior suggested inadequate analgesia and significant stress in the first two surgeries. A fentanyl transdermal patch (Duragesic®-Jansen Pharmaceutical) was used for analgesia after the next two surgeries. Post-surgical behavior suggested the wolf received acceptable analgesia from the fentanyl patch for 72 hr. The patch provided analgesia and reduced stress to the wolf by minimizing animal handling.

The mere presence of humans, let alone handling by humans, evokes a profound stress response in wild animals. Compounded by pain from surgery and/or trauma, the end physiologic and psychologic response can be detrimental to healing and even survival. Minimizing both stress and pain will improve recovery. Suffering animals seldom eat well and generally wild animals are very suspicious of odd smelling food making oral pain medications unpredictable. Repeated analgesic injections relieve pain, but also increases the stresses due to handling which ultimately degrades the relationship of the animal with the caregiver.

The efficacy of pain medications is subjective and is usually based on behavioral observations. Variations in body posture, wakefulness and response to interaction with caregiver have been described as the most reliable evaluations of pain. Wild animals innately hide disabilities and those highly debilitated by trauma and/or surgery cannot express painful behaviors. Considering that conditions that cause pain in people will cause pain in animals, behavioral evidence of pain should not be a requirement for intervention. This case report describes post-surgical pain control in a Mexican gray wolf (Canis lupus baileyi) with minimal animal handling using a fentanyl transdermal patch.

Fentanyl is an opioid analgesic drug with agonist activity. Opioids in general are short acting and provide analgesia with few adverse effects. The most frequent side effects are constipation and sedation but respiratory depression and bradycardia can occur. Fentanyl is a class II narcotic and requires accurate record keeping in accordance the Controlled Substance Act. As a pure agonist, fentanyl may be inactivated when used in combination with agonist-antagonists, such as butorphanol.

The fentanyl transdermal patch has been used in people for chronic cancer pain and has seen increasing use in companion animals.
The fentanyl transdermal system (Duragesic, Jansen Pharmaceutica, Titusville, NJ) is an adhesive patch that allows constant rate, dermal absorption of the drug from a reservoir across a release membrane. The patch is applied to clipped, clean, dry skin in an area where the animal will not chew or scratch it. Water will not affect absorption but direct heat, solvents or alcohol may increase absorption of the drug. Patches are available in four sizes: 25, 50, 75, 100 µg. A Duragesic-50 is designed to provide 50 µg/hr for 72 hr but individual variations occur. Recommended use is 25 µg patch in dogs and cats less than 10 kg, 50 µg patch in 10-20 kg dogs, 75 µg patch in 20-30 kg dogs and 100 µg patch in dogs over 30 kg.

In dogs blood levels of fentanyl have been reported at a mean of 1.6 ng/ml, which corresponds with therapeutic levels reported in humans. Lag time to the steady state was reported at up to 24 hr after application of the patch. Mean elimination half-life was reported at 1.39 hr after patch removal. In cats absorption of fentanyl from a patch applied to the lateral thorax or abdomen was more rapid and blood levels lasted longer, but were lower than those reported in dogs.

A female Mexican gray wolf underwent four orthopedic surgeries over a period of 3 yr. The surgeries were humeral osteotomy, two carpal arthrodeses and amputation of a forelimb. Pain control in the humeral osteotomy and first carpal arthrodesis was intravenous butorphanol intraoperatively at a dose of 0.1 mg/kg, followed by oral butorphanol at a dose of 0.4 mg/kg. Pain control was successful in the humeral osteotomy but not in the first arthrodesis. Failure of pain control following the first arthrodesis was based on observations. The wolf did not eat well, completely rejected food that contained a pill (antibiotic and butorphanol) and was described by the keeper as irritable and unsociable. Posture was difficult to evaluate due to physical limitations.

The first arthrodesis failed and a second, more aggressive arthrodesis was performed. Immediately after induction of general anesthesia the fentanyl patch was applied. Hair was clipped on the back of the neck. A Duragesic 50 was applied to the skin. The patch was manually held in place for 1 hr. The patch was covered with a soft bandage to help keep it in place. Pre-emptive pain control for this surgery was intraoperative morphine at 0.5 mg/kg i.v. and the same dose immediately postoperatively. The wolf was anesthetized every 72 hr to change the fentanyl patch and the leg bandage. A total of three patches were used. One year later a fentanyl patch was again used for pain control after amputation of a forelimb; the same application procedure was used. Morphine was not used intra- or post-operatively for the amputation.

Behavioral observations, made by the same keeper in all instances, were subjective but remarkable. The wolf was alert within 2 hr of the second arthrodesis and 1 hr of the limb amputation. The animal consumed a full meal within 12 hr of each surgery and even consumed food containing an antibiotic tablet. The animal’s caregiver described it as minimally irritable and nearly as sociable as prior to the surgeries. Specifically the wolf snorts, snaps, growls, urinates and defecates when it is uncomfortable. When comfortable, the wolf responds submissively to the keeper and does not snort, snap and growl and does not defecate but occasionally urinates.
Other factors could contribute to the improvement in post-surgical behavior when the fentanyl transdermal patch was used in this Mexican gray wolf. Our overall subjective impression was improvement in the level of pain. The keeper’s knowledge of the wolf’s normal behavior was essential to reaching this conclusion. Ease of fentanyl administration, minimal animal handling and timely return to food consumption were significant advantages hastening the healing process.

Ideally the fentanyl patch should be applied 24 hr prior to surgery allowing drug levels to reach steady state. Since the manufacturer suggests increased rate of drug absorption may occur if direct heat is applied to the patch, we maintained direct contact with the patch on the wolf to enhance absorption. This avoids extra pre- and post-surgical handling. Intraoperative morphine was considered necessary to provide pre-emptive analgesia for the second arthrodesis. The wolf’s behavior suggested this approach was acceptable. Agonist-antagonist analgesics such as butorphanol should be avoided as they may inactivate the fentanyl.

The fentanyl patch appeared to provide adequate analgesia for the suggested 72 hr. The soft bandage covering the patch was well tolerated. The patch may contain residual fentanyl after 72 hr and should be disposed of properly to avoid exposure to other animals or children. The manufacturer recommends flushing the patch down the toilet.

Using a class II narcotic requires meticulous record keeping which is not unreasonable. We keep only a few patches on hand and obtain them from a local pharmacist. The cost is $21 for a Duragesic 50 μg/hr patch in our area. At an average cost of $7 per day and with minimal animal handling, the fentanyl transdermal patch provided acceptable post-surgical analgesia in a Mexican gray wolf.

LITERATURE CITED

DETERMINING DOSAGES FOR ANTI-INFLAMMATORY AGENTS IN ELEPHANTS

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Abstract

Introduction

Choosing an appropriate drug and dosage is difficult for veterinarians due to a lack of research in elephant pharmacology. Clinical application of drug use in elephants for safe, reliable, and effective results necessitates the establishment of a treatment response curve or blood concentration profile for each individual drug and species (African vs. Asian). There is some evidence of a possible species (African vs. Asian) difference, but poorly documented. Because of the difficulty in obtaining accurate pharmacokinetic information, it is more common to select a drug dosage and frequency interval by using what has been effective for other species, specifically the cow and horse. With this strategy, the potential for dosage and treatment errors increases alarmingly as the size (weight) disparity between the animals treated increases.6

Where treatment monitoring with serum concentrations of the drug is difficult to obtain, extrapolation of treatment regimens between species of extraordinary size difference may be done by metabolic scaling to establish drug dosage rates and frequency intervals. The principle of metabolic scaling of pharmacokinetic parameters is based on the well established scaling of physiologic processes across animals of various sizes.

The goals of this paper are to cover what anti-inflammatories are currently used now with Asian and African elephants in North America zoos, review standard equine doses, and discuss metabolic scaling attempts.

Methods

A survey was sent to forty zoos in the United States, that hold two or more elephants, to determine how zoo veterinarians currently use anti-inflammatories in both African and Asian elephants. The median dosages and treatment intervals were determined for the most commonly used anti-inflammatories from the twenty zoos that responded. Of the respondents, ten held African elephants, and ten held Asian elephants. Although not asked about in the survey, it is assumed the reported dosages are mainly reflective of use on adult elephants.

Standard equine dosages were obtained.4 These represent referenced dosages that are commonly used for horses, and have been determined by pharmacokinetic studies.
Allometric (metabolic) scaling involves the concept of the relationships of organic function and systems to body size. There is a direct relationship between metabolic rate and size within the five major taxa of animals. These taxa include placental mammals, marsupial mammals, passerine birds, nonpasserine birds, and reptiles. Many biologic parameters have been measured and demonstrate a logarithmic, linear relationship with body weight.

Some examples include: cardiac output, capillary density, kidney filtration rate, and oxygen consumption. Metabolic scaling for drugs is based on body weight converted to metabolic size. The uptake, distribution, and elimination of a drug depends on physiologic processes, which scale allometrically. This concept has been used in human medicine for over 30 yr, and more recently in veterinary nutrition and anti-neoplastic drugs. Metabolic scaling and calculation worksheets have been covered by other authors, and I refer the reader to the following references. Specific minimum energy cost (SMEC) doses and treatment intervals were calculated for a 3200 kg elephant using the horse as a model species and referenced equine dosages.

A literature search was done, but no published articles were found for anti-inflammatory use in elephants.

Results

Results are presented in Table 1.

Discussion

Dosages for anti-inflammatories used in elephants were close to reported equine levels. Although ibuprofen is used with elephants, there is not an established dosage for equines to use as a model species in metabolic scaling. There are no reported pharmacokinetic studies with anti-inflammatories in elephants, and is an area in need of research to allow clinicians to more accurately use this class of drugs. Phenylbutazone use has the most potential for adverse side effects of the three drugs compared. Currently it is being used by zoo veterinarians at a treatment interval much shorter than predicted by metabolic scaling and could be a health risk if used as such on a chronic basis.

Dosages for polysulfated glycosaminoglycans (Adequan®), glycosaminoglycan enhancers (Cosequin®), acetaminophen, aspirin, butorphanol and ketofen were reported in the survey, but by only one source each and were not included in the comparisons.

Metabolic scaling appears to determine dosages that have excessively long treatment intervals, but pharmacokinetic studies are needed to confirm this. The difference can be related to the following biologic functions: biotransformation of the drug, tissue receptor sites, plasma protein binding, enzyme systems, and drug distribution. Drugs that are minimally biotransformed most likely will be therapeutically effective at metabolically scaled dosages. At this time, zoo veterinarians are not routinely using metabolic scaling formulas to calculate elephant drug dosages.
Due to the difficulties of giving oral or intramuscular medications, the potential to limit their use in elephant care is real. Anti-inflammatories are useful for their properties of reducing soft tissue swelling and providing analgesia. Both of these aspects are difficult to assess clinically in a large, relatively inactive animal. The field of pharmacology research in elephants can be helped with several collaborative projects between zoos that hold multiple individual elephants.

LITERATURE CITED


<table>
<thead>
<tr>
<th></th>
<th>Zoo veterinarians</th>
<th>Equine formulary</th>
<th>Metabolic scaling *</th>
<th>Pharmacokinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flunixin</td>
<td>1.0 q 24 hr</td>
<td>1.1 q 12/24 hr</td>
<td>0.7 q 40 hr</td>
<td>None</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.5-4 q 24 hr</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>1-2 q 24 hr</td>
<td>4 q 12 hr</td>
<td>2.5 q 40 hr</td>
<td>None</td>
</tr>
</tbody>
</table>

All dosages are in mg/kg.
* Metabolic scaling dosages based on a 3200 kg elephant.
ANALGESIA IN NONHUMAN PRIMATES

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Abstract

The purpose of this report is to review current literature and clinical experience with use of analgesic agents in nonhuman primates. The use of analgesics in animals has received much attention in recent years, especially in laboratory animals where pain management is mandated by law and regulations.9,10,13,14 Successful management of pain has been shown to help maintain normal physiologic and cardiovascular stability, decreasing postoperative recovery time.3,14 Assessment of pain in animals is difficult, especially in non-domestic species that may have evolved to protect themselves by showing minimal overt evidence of pain or discomfort. While there are attempts at general guidelines for assessment of pain in animals including nonhuman primates, it is best to consider that procedures and injuries that cause pain in humans cause similar levels of pain in animals.2,4,10,12 A literature review of analgesic use in nonhuman primates includes work done on specific analgesics and their receptors, but there are few controlled studies of clinical assessment of pain, control of pain, or side effects of analgesics in nonhuman primates. Agents, dosages and indications have been extrapolated from human and domestic animal literature.

Common situations or procedures where analgesics are required in nonhuman primate medicine include management of perioperative and post surgical pain (cesarean section or other laparotomy, orthopedic surgery, digit amputation, etc.), minor surgical and diagnostic procedures such as laceration repair, biopsies, dental and ophthalmic procedures, and management of pain associated with disease (including osteoarthritis) or trauma. Nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen, opioids, and corticosteroids are the most commonly used analgesic agents in nonhuman primate medicine.1,4,7,9,14,15 The use of alternative methods for achieving analgesia in nonhuman primates has not been well studied, although the reversal of acupuncture induced analgesia with naloxone was reported.6 There are a number of anesthetic agents that provide little or no analgesia beyond the period of general anesthesia. These include inhalant anesthetics and propofol. When these agents are used for procedures, an analgesic agent must be given perioperatively. Ketamine and α2-adrenergic agonists like xylazine and medetomidine do have some analgesic properties, but in general these agents do not provide adequate analgesia for anything more than minor procedures and cannot be used post-operatively due to their anesthetic properties.8,16

NSAIDs and acetaminophen are excellent choices for relief of mild to moderate pain such as minor surgical procedures and premenstrual anorexia and pain. Increasing doses will eventually result in a limit on their maximum analgesic effect.11 Acetaminophen has good analgesic properties, but is not anti-inflammatory like the true NSAIDs. While gastrointestinal upset and bleeding from platelet
dysfunction are possible side effects of this class, there are few reports of these occurring in clinical cases in nonhuman primates. At Wisconsin Regional Primate Research Center one of the newer veterinary NSAIDs, carprofen, has been used successfully in rhesus monkeys in the treatment of severe osteoarthritis that is no longer responsive to aspirin. Liver and kidney parameters are being followed through carprofen therapy. After 6 mo there is no evidence of toxicity. NSAIDs can be effectively combined with other agents to produce better analgesia. Acetaminophen/codeine and flunixin/buprenorphine have been reported to be effective combinations for mild to moderate postoperative pain in great apes.

The pure opioid agonist morphine is the standard by which analgesics are measured. Synthetic opioids like oxymorphone, and agonist-antagonists like butorphanol and buprenorphine are effective analgesics in nonhuman primates. They are frequently used perioperatively with or followed by NSAIDs upon recovery. Opioids are very useful in the postoperative period after major surgery. Complications include CNS and respiratory depression, although oxymorphone and buprenorphine are reportedly less depressive than other opioids. Epidural morphine is reported to provide good analgesia for up to 24 hr in macaques. There is anecdotal evidence that fentanyl patches are very useful in providing long-term post-operative analgesia in macaques. They are kept in place with jackets (D. Clemmons, Covance Labs, Madison, WI, personal communication).

Not technically classified as analgesics, corticosteroids do provide anti-inflammatory properties that reduce pain due to inflammation and/or steroid responsive tumors. Concurrent use with NSAIDS is contraindicated due to the increased risk of gastrointestinal upset and ulceration.

In conclusion, the use of analgesic agents is an important aspect of primate medicine and surgery. Analgesic agents and dosages are presented in Table 1, however it must be stressed that dosages require clinical evaluation and may need to be revised.

LITERATURE CITED
Table 1. Agents useful in nonhuman primate analgesia.

<table>
<thead>
<tr>
<th>Class of agent</th>
<th>Agent</th>
<th>Dosage (mg/kg)</th>
<th>Route</th>
<th>Interval</th>
</tr>
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<tbody>
<tr>
<td>NSAID</td>
<td>Acetaminophen &lt;sup&gt;1&lt;/sup&gt;</td>
<td>5-10</td>
<td>p.o.</td>
<td>q 6 hr</td>
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<tr>
<td></td>
<td>Aspirin &lt;sup&gt;8&lt;/sup&gt;</td>
<td>5-20</td>
<td>p.o., rectal</td>
<td>q 4-6 hr</td>
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<td></td>
<td>Ketorolac &lt;sup&gt;15&lt;/sup&gt;</td>
<td>15-30 mg/macaque or baboon</td>
<td>i.m.</td>
<td>initially, then 10-15 mg/animal q 8 hr</td>
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<tr>
<td></td>
<td>Ketoprofen &lt;sup&gt;Washington Primate Center&lt;/sup&gt;</td>
<td>5</td>
<td>i.m.</td>
<td>q 6-8 hr</td>
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<tr>
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<td>Carprofen &lt;sup&gt;Wisconsin Primate Center&lt;/sup&gt;</td>
<td>2-4</td>
<td>p.o., s.c.</td>
<td>q 12-24 hr</td>
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<tr>
<td></td>
<td>Flunixin meglumine &lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.3-1</td>
<td>s.c., i.v.</td>
<td>q 12-24 hr</td>
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<td>Opioid</td>
<td>Buprenorphine &lt;sup&gt;16&lt;/sup&gt;</td>
<td>0.01-0.02</td>
<td>i.m., i.v.</td>
<td>q 8-12 hr</td>
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<td>Butorphanol &lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.1-0.2</td>
<td>i.m.</td>
<td>q 3-4 hr</td>
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<td>Oxymorphone &lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.03-0.2</td>
<td>s.c., i.m., i.v.</td>
<td>q 6-12 hr</td>
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<td>Fentanyl &lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.05-0.1</td>
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<td>Pentazocine &lt;sup&gt;5&lt;/sup&gt;</td>
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<td>i.m.</td>
<td>q 4 hr</td>
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<td>Morphine &lt;sup&gt;15&lt;/sup&gt;</td>
<td>0.1</td>
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<td>Naloxone &lt;sup&gt;16&lt;/sup&gt;</td>
<td>0.01-0.05</td>
<td>i.m., i.v.</td>
<td></td>
</tr>
</tbody>
</table>
OCCUPATIONAL INJURIES AND ILLNESSES REPORTED BY ZOO VETERINARIANS IN THE UNITED STATES

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Abstract

This study was undertaken to identify the prevalence of occupational injuries and illnesses associated with the zoo veterinarian medical practice. A 14-page comprehensive survey was mailed to all 565 United States members of the American Association of Zoo Veterinarians (AAZV) to identify the frequency of physical injuries, radiation exposures, chemical exposures, allergic or irritant reactions, infections, and use of preventive measures. The response rate was 55.8% (315/565). Results are summarized in Tables 1-7 and Figure 1. Significant findings include major animal-related injury (61.8%, major animal-related injury is defined as one for which medical treatment was required either by a physician or self-administered, excluding those requiring only topical antibiotics), back injury (55%), necropsy injury (44.1%), adverse formalin exposure (40.2%), animal allergy (32.2%), zoonotic infection (30.2%), and insect allergy (14.2%). We also found that sex, length of experience, and practice type affected the number and type of incidents encountered in practice. Females were more likely than males to report a zoonotic infection, insect allergy, and an adverse exposure to anesthetic gas, formalin, and disinfectants/sterilants. Zoo veterinarians with more years of experience were more likely to receive a major animal-related injury and associated hospitalization, back injury and associated lost work time, adverse anesthetic gas exposure, and a positive skin test for tuberculosis. Full-time zoo veterinarians were more likely than other practice types to report back injury and inadequate knowledge of occupational hazards. Results are compared to hazards reported by veterinarians working in other fields. The frequency of injuries reported demonstrates a greater need for comprehensive health and safety program for zoo veterinarians. In addition to the requirements mandated by the Occupational Safety and Health Administration in 29 Code of Federal Regulations Part 1910, summaries of prudent safety and industrial hygiene practices are provided.
Table 1. Percentage of respondents exposed to specific agents from needle sticks.

<table>
<thead>
<tr>
<th>Needle exposure agent</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No injection of fluid</td>
<td>71.3</td>
</tr>
<tr>
<td>Animal blood</td>
<td>58.4</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>52.3</td>
</tr>
<tr>
<td>Vaccines</td>
<td>51.6</td>
</tr>
<tr>
<td>Immobilizing agents</td>
<td>17.2</td>
</tr>
<tr>
<td>Other¹</td>
<td>9.3</td>
</tr>
</tbody>
</table>

¹Types of other exposure agents were not reported.

Table 2. Percentage of respondents that reported major animal-related injuries.

<table>
<thead>
<tr>
<th>Animal-related injury</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal bite</td>
<td>55.3</td>
</tr>
<tr>
<td>Kick</td>
<td>29.0</td>
</tr>
<tr>
<td>Scratch</td>
<td>22.9</td>
</tr>
<tr>
<td>Other¹</td>
<td>16.4</td>
</tr>
<tr>
<td>Knocked over</td>
<td>14.9</td>
</tr>
<tr>
<td>Stepped on</td>
<td>12.0</td>
</tr>
<tr>
<td>Crushed</td>
<td>9.5</td>
</tr>
<tr>
<td>Horn wound</td>
<td>6.9</td>
</tr>
<tr>
<td>Insect bite</td>
<td>3.6</td>
</tr>
</tbody>
</table>

¹Other injuries included talon punctures and lacerations, shoulder separation, finger dislocation, broken tooth, fractured wrist and nose, fractured hand, hand trauma, hernia, herniated disk, contusion from fluke, and cervical fracture.

Table 3. Percentage of respondents that reported a necropsy-related injury and the percentage that reported medical treatment for at least one of those injuries.

<table>
<thead>
<tr>
<th>Necropsy-related injury</th>
<th>Injuries/illnesses reported</th>
<th>Injuries/illnesses requiring medical treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knife wound</td>
<td>87.0</td>
<td>46.7</td>
</tr>
<tr>
<td>Infection</td>
<td>18.7</td>
<td>78.3</td>
</tr>
<tr>
<td>Chemical exposure</td>
<td>9.8</td>
<td>33.3</td>
</tr>
<tr>
<td>Other¹</td>
<td>8.9</td>
<td>72.7</td>
</tr>
</tbody>
</table>

¹Other injuries/illnesses reported included bone splinters, serum sickness, injuries from incinerator explosion (injuries unknown), eye trauma, and zoonotic exposure (psittacosis, plague, M. bovis, and rabies).
Table 4. Prevalence of adverse exposures to specific pesticides.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Skin Reaction (n = 279)</th>
<th>Respiratory Reaction (n = 279)</th>
<th>Other Reaction (n = 279)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrethrins</td>
<td>2.2%</td>
<td>2.9%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Carbamates</td>
<td>1.1</td>
<td>1.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Organophosphates</td>
<td>1.1</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Other</td>
<td>0.7</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 5. Prevalence of adverse exposures to specific disinfectants/sterilants.

<table>
<thead>
<tr>
<th>Disinfectant/Sterilant</th>
<th>Skin Reaction (n = 278)</th>
<th>Respiratory Reaction (n = 278)</th>
<th>Other Reaction (n = 278)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde/Paraformaldehyde</td>
<td>4.3%</td>
<td>6.1%</td>
<td>4.3%</td>
</tr>
<tr>
<td>Phenolics</td>
<td>2.9</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Chlorine bleach</td>
<td>4.0</td>
<td>5.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Iodine complexes</td>
<td>3.2</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Quaternary ammonium</td>
<td>2.5</td>
<td>1.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ozone</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ultraviolet radiation</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>4.3</td>
<td>0.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Other</td>
<td>0.4</td>
<td>0.4</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 6. Number of zoonotic infections reported among respondents.

<table>
<thead>
<tr>
<th>Zoonosis</th>
<th>Infection</th>
<th>Sero positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td>Count</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>Amoebiasis</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Pinworms or hookworms</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Strongyloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Scabies</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Ringworm or other superficial fungal infection</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Psittacosis</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Shigella</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Erysipelas</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcosis</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Hepatitis A, B, other</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Herpesvirus B</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Lyme disease</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Rocky Mountain Spotted Fever</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Giardia</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Other</td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 7. Prevalence of vaccinations among zoo veterinarian respondents.**

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabies</td>
<td>277</td>
<td>77.3%</td>
</tr>
<tr>
<td>Tetanus</td>
<td></td>
<td>93.9</td>
</tr>
<tr>
<td>Polio</td>
<td></td>
<td>62.5</td>
</tr>
<tr>
<td>Measles</td>
<td></td>
<td>46.9</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td></td>
<td>25.3</td>
</tr>
<tr>
<td>Rocky Mountain Spotted Fever</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Yellow Fever</td>
<td></td>
<td>23.8</td>
</tr>
<tr>
<td>Typhoid Fever</td>
<td></td>
<td>19.1</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>9.7</td>
</tr>
</tbody>
</table>
Figure 1. Percentage of respondents who followed various hygiene practices.

- Manually restrain animals during x-ray: (15.5, 19, 19.4, 46)
- Wear protective clothing, ie lead apron: (88, 5.2, 2.4, 4.4)
- Wear radiation film badge: (54.8, 12.4, 4.8, 28.4)
- Shower before coming home after handling animals: (2.9, 4.4, 4.0, 88.7)
- Wash hands before eating at work: (91.4, 7.2, 1.1, 0.4)
- Remove coveralls before eating: (29.4, 13.3, 7.9, 30.8, 18.6)
- Wash hands after handling animals: (84.9, 14.3, 0.7)
- Wear gloves when handling hazardous chemicals: (43.7, 31.0, 8.3, 9.7, 7.2)
- Wear gloves when doing necropsies: (94.6, 2.5, 0.4, 0.4, 2.2)
- Wear appropriate personal protective equipment: (53.9, 39.5, 4.8, 1.8)
RADIATION EXPOSURE IN THE VETERINARY WORKPLACE

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Abstract

The diagnostic use of x-ray irradiation, if done without regard to proper radiation safety practices, has the potential to cause significant deleterious biologic effects. The major effects are the induction of fatal cancers, genetic effects passed on to children, and developmental defects in the fetus when irradiated in utero. The major sources of radiation exposure to the veterinary worker are the primary x-ray beam, scattered radiation from the patient, and leakage radiation from the x-ray tube housing. The principle means of radiation protection are time, distance, and shielding: keep the time of exposure to radiation as short as possible; increase your distance from the source of radiation; place shielding material between you and the source of radiation. Annual radiation dose limits for occupational workers, set by state health codes and the United States Nuclear Regulatory Commission, are established to “limit the probability of occurrence of random carcinogenic and genetic effects” and “to prevent completely the occurrence of deterministic effects.” In addition to these limiting dose values, it is expected that the working conditions are such that the radiation dosage is kept As Low As Reasonably Achievable. If strict adherence is made to the safe practice of radiography, the probability of radiation induced injury is negligible.

Introduction

Not long after the discovery of x-rays in 1895, their use in evaluating anatomic changes caused by disease processes was well recognized. The first paper on veterinary radiography was written in 1896. At the same time the damaging effects of large radiation doses were becoming known. In 1896, 23 cases of radiodermatitis were reported. In review articles of 1911 and 1914, 54 cancer deaths attributable to radiation and 198 radiation induced malignancies were reported. The first American death attributed to radiation exposure occurred in 1904. The cause for the morbidity and mortality associated with radiation was due to the complete absence of any protection for the radiation worker, be they the scientist, physician or assistant. Very long exposures times were needed to adequately expose the "plates," and so patients were exposed to high doses. The first official action taken to investigate methods to reduce radiation exposure occurred in England in 1921.

Knowledge of the effects of excessive human radiation exposure comes from the study of the health of early radiologists, survivors of the atomic bombs, nuclear power plant accidents, radium dial watch painters, people treated with radiation for ankylosing spondylitis, and numerous other groups. Data from experimental exposure of animals have also been extrapolated to the human. The radiation dose and dose rate to these groups range from acute high dose exposure to chronic lower dose exposure. Because of the knowledge gained from the studies, it is obvious that radiation exposure entails some
risk of harmful effects. The changes in radiation practice that have occurred in the medical and veterinary profession have focused on the reduction of radiation exposure delivered to the patient and that received by the radiation worker. In the veterinary profession we use x-rays primarily to aid us in making diagnoses and to sequentially evaluate the response to treatments. By the nature of our patients we are much more likely to need manual restraint than our human counterparts. We thus are chronically exposed to radiation of low doses.

Methods of Injury

X-rays, being a form of ionizing radiation, causes cellular damage by altering critical molecular structures, principally DNA. The alteration in the DNA can be sublethal and be repaired causing no acute or long term effect or can be sublethal and upon repair, induce a mutation in the cell that leads to the development of cancer months to years later. The radiation damage can also be lethal. If only a small cluster of non-dividing cells are killed there may be no recognizable effect. However, if the cells destroyed are critical to maintaining that cell line then the absence of this cell line, will be expressed. The most significant concern generated for the chronic low dose exposure is the late effects.

There are two classes of late effects as related to exposure dose–stochastic and deterministic (nonstochastic) effects. Stochastic effects are random effects for which any dose, however small, carries with it a probability of producing the effect. The effect will either occur or not occur. The probability that the effect will occur increases as the cumulative radiation dose increases. However, the severity of the effect is not related to the dose. That is, the effect will not be more severe if the dose were higher. The stochastic effects are the genetic effects and carcinogenesis.

Results of Injury

The deterministic effects are somatic effects that increase in severity with increasing dose in affected individuals. The effects are caused by damage to an increasing number of cells and amounts of tissue. These effects are basically degenerative and the best known examples are cataracts, organ atrophy and tissue fibrosis. The deterministic effects have a threshold dose below which the effect will not occur. Above the threshold dose the severity of the effect increases with increasing radiation dose.

A major question is how much radiation exposure is acceptable or safe? That’s the question to which there is no definite answer. Ample scientific evidence indicates that any dose of radiation poses some possibility of causing a damaging effect. If the damaging effect involves a non-critical cell/organ system then it may never be realized by the individual. Mathematic interpolation of data indicates that for the stochastic effects there is no known threshold dose below which there is no risk of the effect occurring. This forms the basis for the establishment of the radiation protection standards. The exposure dose levels set by these standards are intended to make the environment of the radiation workplace such that the stochastic effects are never likely to be a problem. The risk of the stochastic effects still exist but if exposure dose is kept as low as possible then the risk is reduced. At the same
time, the dose limits are set below the threshold for the deterministic effects. It must be kept in mind however, that the radiation dose equivalent is cumulative.

The NCRP dose limits are all subject to the concept of ALARA—as low as reasonably achievable. Ideally occupational exposure would be zero. Since that is not possible, the facilities and equipment should be designed and used so that exposure to personnel is minimal. No unnecessary exposures should be allowed.

The Keys to Radiation Exposure Reduction

Distance
The intensity of radiation exposure is dramatically reduced as the distance from the source is increased. This follows the Inverse Square Law—the exposure rate from an x-ray source is inversely proportional to the square of the distance from the source. This means that if the distance from the x-ray tube is doubled, the exposure is reduced to 1/4 of the initial dose rate. This forms the basis for the recommendation that manual restraint of animal patients be done as infrequently as possible. The use of mechanical restraining devices and/or chemical restraint is recommended to keep humans away from the primary beam and should be the preferred choice.

Time
Assuming that the radiation is leaving the x-ray tube at a constant rate, the total dose equivalent received depends on the length of time exposed. Thus the amount of radiation received can be controlled by the time of exposure. Using exposure times as short as possible will reduce the radiation exposure received.

Shielding
Even though radiation interacts with any type of material and is reduced in amount by these interactions, certain materials are more efficient in absorbing radiation. These are the best materials to be used for shielding. The purpose of shielding is to attenuate the x-ray beam so that either none or extremely small amounts of radiation will reach the person or area being shielded. For the x-ray energies of diagnostic radiology, lead has been the shielding material of choice. Lead aprons and gloves should be worn by anyone assisting in the x-ray procedures. Gloves should be worn by anyone who is manually restraining the patient or holding the x-ray cassette. Preferably the x-ray cassette is not being held by hand but rather it is placed within a cassette holder that has a rod or arm to position the holder’s hands farther away from the primary beam. Gloves should be worn even if a cassette holder is used. The lead aprons and gloves are intended to protect the individual from scattered radiation and not the useful or primary x-ray beam. Aprons and gloves of 0.5 mm lead equivalency are recommended.

Shielding of the x-ray facility’s walls, ceiling and floor must also be considered when there is any likelihood of access to the adjacent areas by either employees or the general public. Such shielding can be in the form of lead or various thickness of other construction materials to achieve a specified lead equivalency.
Rotation
Rotation of personnel who assist in radiography. It is advisable, if possible, to have several assistants trained to make radiographs. This then serves to divide the radiation exposure among individuals so that no one individual will bear the entire burden of cumulative exposure.

Plan of radiographic procedure
Plan your radiographic procedures. Radiation exposure can be significantly reduced if good technical quality is accomplished the first time. This is improved by the establishment of technique charts and careful positioning of the patient. Each time a retake is done, radiation exposure is increased.

Equipment
It is not uncommon for veterinarians to purchase older used x-ray machines. In general, older x-ray machines have an increased risk of poor and unsafe performance. It is suggested that any newly acquired older x-ray machine be inspected by a radiation physicist for radiation output and leakage potential. Generally the state Department of Public Health inspects veterinary radiation facilities and should be contacted for inspection services.

With older machines it is usually necessary to use a long exposure time to achieve the necessary film density. Radiation exposure time and thus, radiation exposure dose, can be reduced by using the rare earth film screen systems instead of the older calcium tungsten systems. The rare earth systems require less radiation exposure to achieve the needed film blackening.

Most radiation exposure received by those participating in making radiographs comes from scattered radiation from the patient. Anything that can be done to reduce the amount of scattered radiation produced will reduce the exposure to the personnel. A very efficient method of reducing scatter radiation is to reduce the surface area that is exposed. This is accomplished by using x-ray beam limiting devices. These can be in the form of exchangeable cones or cylinders or best by an adjustable collimator. The cones and cylinders must be changed when a change in field size is desired. This is often not done and instead the largest one is affixed to the tube. The adjustable collimator allows for an infinite number of rectangular to square sized fields to be easily “dialed” in. Most adjustable collimators come with a visible light that corresponds with the primary x-ray beam. As light is illuminated onto the patient you are able to selectively expose only the area that needs to be evaluated. This results in a reduction of scattered radiation to the operators and improves image quality as well.

Personnel monitoring
The use of radiation dosimeters is strongly recommended for any person who participates in any routine manner in the making of radiographic exposures. The primary use of the dosimeters is to have a record of exposure dose. This record shows whether exposures are below the regulated dose limits. Most of the radiation safety codes indicate that a film dosimeter be worn if the individual has the potential to receive 1/10 of the annual limit. Without any monitoring there is little way of knowing that potential. For many people the fear of the unknown is great. An accurate
documentation of the exposure received can go a long way to allay such fears. The dosimeters can help detect a leakage of radiation from a machine that may otherwise go unnoticed. Having personnel exposure records may be essential in defending a potential violation investigation. Periodic review of these exposure records can help to revise radiation practices to further reduce personnel exposure, i.e., adhering to the ALARA component of the regulations.

**Personnel training**

Many veterinary practices do not hire assistants that are trained in the use of x-rays. Thus it your responsibility to train these individuals as to the safe use of x-rays. It is important to discuss with them the potential deleterious effects of exposure to ionizing radiation and how this exposure can be minimized. The use of high school or junior high school students, under the age of 18, to assist in making radiographs (i.e., in the actual setting in which the exposure is made) is strongly discouraged.

**What About the Employee of Child Bearing Age or the Pregnant Employee?**

There are two major issues to this concern. First, the hazard of radiation exposure to the employees of child bearing age carries a risk of genetic effects, however this has been estimated to be very minimal. At a low radiation dose rate the testes are much more radiosensitive than the ovary. The differences between the sexes is so pronounced that for practical purposes at a low dose rate, almost all of the radiation-induced genetic burden in a population is carried by the males. To avoid radiation exposure to the gonads proper use of shielding (i.e., wearing aprons) must be followed. When proper lead aprons are worn, the exposure to the gonadal area should be negligible.

The second issue is the pregnant employee. The most radiosensitive tissues or organs are those that have a high mitotic rate. Such is the case of the embryo and the fetus during the majority of its development. The effects of radiation on the unborn child are of great concern. The classic effects are:

1. Death induced by relatively small doses before or immediately after implantation of the embryo (0-9 days). This results in spontaneous abortion and the pregnancy may go unrecognized. If exposure during this time does not cause embryonic death, the embryo usually develops into a normal fetus with no residual effects.

2. During the period of organogenesis (10 days-6 wk), the radiation effects will usually be those of malformations. The central nervous system is particularly sensitive in the human embryo since the CNS is developing slowly throughout this period of organ development. Some temporary intrauterine growth retardation can be seen but there is usually recovery from this. If the radiation dose is high enough, death can occur but it will usually occur in the neonatal period.

3. Irradiation during the fetal period (6 wk-term) can result in permanent growth retardation. There is a low risk of general malformations but a persistent high risk of CNS malformations.
Death can be caused by irradiation during this phase but the dose required gradually approaches the adult dose in the late fetal stages.

4. In utero irradiation results in an increased risk of development of fatal and non-fatal childhood cancers. Risk estimates indicate an overall risk of a 50% increase over the natural incidence. The risk appears to be greater if the irradiation occurred during the first trimester.

The safest procedure for the employee actively trying to become pregnant or who is pregnant would be to avoid any and all exposure to ionizing radiation. This may be possible by reassignment of duties. Exposure can be greatly decreased by rotation of employees who assist in radiography. Neither of these may be possible. In such instances, it is recommended that the woman be provided with a lead apron that wraps totally around the body and thus protects from inadvertent exposure to the trunk from any direction. Two personnel monitoring devices should be worn: one at the collar level and one at the waist level under the apron. The latter dosimeter is used to estimate any radiation dose to the fetus as a consequence of irradiation of the mother. Since the embryo/fetus is considered to be a member of the general public, it is restricted to a maximum permissible dose of 5 mSv for the entire 9-mo gestation period and a monthly dose not in excess of 0.5 mSv.

Discussion

The x-ray imaging is invaluable tool to veterinary diagnostics. Equipment costs are affordable by any veterinary practice. Through some unfortunate early exposures to high levels of radiation we know of serious consequences of the misuse of radiation. Federal and state standards have been set to ensure that the benefits derived from the use of x-rays are not outweighed by the risks of its use. The key to the safe use of x-rays is to put into practice as many of the safety recommendations as possible. Even though annual dose limit standards have been established it is imperative to practice the use of ionizing radiation in such a manner as to keep the exposure to yourself and your employees “as low as reasonably achievable.” Blatant disregard for radiation safety is intolerable!

LITERATURE CITED


Table 1. Occupational exposure limits recommended by the National Council on Radiation Protection and Measurements (NCRP) (1987).

<table>
<thead>
<tr>
<th>Occupational Exposures:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Annual Effective Dose Equivalent - Whole body</td>
<td>50 mSv*</td>
</tr>
<tr>
<td>b. Lens of eye</td>
<td>150 mSV</td>
</tr>
<tr>
<td>c. Dose Equivalent to all others (red bone marrow, breast, lung, thyroid, bone surfaces, gonads, skin and extremities)</td>
<td>500 mSV</td>
</tr>
<tr>
<td>d. Cumulative dose equivalent</td>
<td>10 mSV × age</td>
</tr>
</tbody>
</table>

Education and training (under 18):

| a. Annual Effective Dose Equivalent              | 1 mSV  |
| b. Dose equivalent to the lens of eye, skin, extremities | 50 mSV |

Public:

| Continuous exposure                             | 1 mSV  |
| Infrequent exposure                             | 5 mSV  |

Embryo & Fetus:

| Total (for 9 mo)                                | 5 mSV  |
| Monthly                                         | 0.5 mSV |

* Sv = Seivert, 1 mSv = 10⁻³Sv  1 Sv = 100 rem
HAZARDS OF ANESTHETIC GASES

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Abstract

That portion of the fresh anesthetic gas delivered through the breathing circuit which is not metabolized by the patient and exits through the pop-off valve is called waste anesthetic gas. This waste anesthetic gas has been associated with numerous health problems in individuals that chronically inhale polluted air: hepatic disease, renal disease, immunosuppression, bone marrow depression, abortion, infertility, birth defects, cancer, pruritus, and a wide range of Central Nervous System (CNS) disturbances. It is advisable to minimize or eliminate exposure to anesthetic pollution for two reasons: 1) health hazard, and 2) National Institute of Occupational Safety and Health (NIOSH) requirements. This paper will outline the hazards and requirements for waste anesthetic gas.

Introduction

The first report of health hazards associated with anesthesia personnel was in 1967.20 Vaisman’s survey of 15% of the Russian anesthesiologists practicing at that time revealed a high incidence of spontaneous abortion among the females and headache, fatigue, irritability, nausea, and pruritus in the entire group.

Cohen et al.3 found an increased incidence of miscarriage among operating room nurses. Further reports recorded increased frequency of spontaneous abortion, congenital defects, and cancer in female anesthesiologists and nurse anesthetists.2,5,10 But, a conflicting report published in 1979 found no significant incidence of reproductive abnormalities in female operating room workers.8 The National Institute of Occupational Safety and Health (NIOSH) initiated a study in the mid-1970s.6 The study identified an increased prevalence of congenital anomalies in offspring of male and female operating room personnel, increased spontaneous abortions in female workers but not in the wives of male workers. Women developed more hepatic and renal disease while men developed only hepatic disease. Women operating room workers also had a higher incidence of leukemia and lymphoma.

In 1977, NIOSH made recommendations concerning exposure levels and monitoring methods.6 The recommended standards were arbitrarily set at 2 ppm for halogenated agents when used alone, 0.5 ppm when halogenated agents are used with nitrous oxide, and 25 ppm for nitrous oxide. Exact toxic levels were never determined. A subsequent study in the United Kingdom from 1977-1984 surveyed 11,520 female medical school graduates working in hospitals. Anesthesiologists were not found to have an increased risk of infertility, spontaneous abortion, congenital abnormalities, or cancer.13
Recently, the American Society of Anesthesiology sponsored a review of previously published studies. An increased incidence of spontaneous abortion in women working in the operating room was suggested but the accuracy of the data was questioned. Further prospective studies were suggested to determine if trace anesthetic levels in the air breathed by operating room personnel leads to adverse health outcomes. Currently, with scavenging and other safety measures in place, it does not appear that there are proven hazards to personnel working in the operating room.

Veterinarians are usually considered to be at less risk than human operating room personnel because veterinarians spend less time per day in this potentially polluted environment. But, veterinarians tend to perform quite a few mask and box inductions plus monitor recovering animals confined in small cages and stalls. Thus, veterinarians may offset limited time of exposure by excessive levels of exposure.

Surveys of veterinary private practice and institutional surgery rooms revealed that breathing levels as high as 20-30 ppm were occasionally found but levels were usually around 4-5 ppm. This is about double the NIOSH maximum recommendation but less than the 10 ppm found in most human operating rooms. Scavenging these waste gases made dramatic reductions possible. One surgery suite at the University of Georgia decreased from 36.6-0.85 ppm with active scavenging.

Recovery rooms are also important for veterinarians because our patients are frequently placed into small cages or stalls to wake up and their exhaled gases are not efficiently removed. A study was done at Cornell University measuring halogenated anesthetic levels at the cage door of recovering dogs. It took about 2 hr for halothane levels to decrease below the NIOSH maximum recommendation. Relatively large animal with large tidal volumes (5 L) in small recovery stall, and a veterinarian or technician sitting at the head could lead to significant exposure levels.

Methods of Reducing Pollution and Exposure

1. Collection pop-off valves
Old pop-off valves have numerous holes that open directly into the room. Collection pop-off valves direct all the out-flow to a single port (19 mm) and this type of pop-off should be installed on all machines. Nonrebreathing circuits have a lot of waste anesthetic gas and bag-tail-end valves can be used for gas collection if the circuit does not have a collecting port. Most old ventilators cannot be retrofitted with collection devices and need to be replaced with models that have collection ports.

2. Interface
An interface needs to be positioned between the disposal system and the pop-off valve. The interface contains positive (+10 cm H$_2$O) and negative (-0.5 cm H$_2$O) pressure relief valves so the animal will not be harmed if the disposal system malfunctions. A reservoir is an essential part of the interface also. This reservoir bag accepts large volumes of gas that exceed the disposal system capacity to immediately remove.

3. Disposal system
Disposal systems vary and include active vacuum, ceiling or wall vents, direct passive connections to the outside, and charcoal filters. Venting to the floor is unacceptable. Passive systems have no pumps or fans and rely on the pressure difference from the pop-off to the outside air for the movement of gas. Large bore tubing (clothes drier venting) reduces resistance and short distances to the outside wall are preferred. Connections to a non-recirculating room vent is sometimes possible. But, most building air handling systems are recirculating. It may be necessary to install a separate exhaust fan that connects to the attic or outside wall. Charcoal filters are effective if low flows are utilized and careful records are kept on utilization. Because of their expense and short effective life (12-15 hr), they should only be used when transporting animals or as a temporary solution. Active evacuation systems develop a negative pressure and exhaust to the outside atmosphere. Central surgical vacuums or dedicated systems for anesthetic gases can be used.

4. Leak detection
Other than the gas coming out of the pop-off, there are several other sources of leakage from anesthetic machines. Always pressure test the machine and circuit for leaks before usage. Gas leaks around the soda lime canister are very common. The machine circuit should be maintainable at 30 cm H₂O with an oxygen flow of less than 250 ml/min or the pressure drop should be less than 5 cm H₂O in 30 sec.

Endotracheal tube cuffs are another common source of pollution. Check the cuffs ahead of time and allow time for slow leaks to be apparent.

5. Inductions with inhalants
Avoid mask or chamber inductions if possible. When necessary, perform these procedures in large, well ventilated rooms.

6. Recovery
Let animals breath 100% oxygen on the anesthetic machine for the first 5-10 min of recovery. Frequently empty the reservoir bag into the scavenging system and refill with the flush button.

7. Filling vaporizers
Use a bottle adapter to fill vaporizers and fill the vaporizers when very few people are around (end of the day).

Monitoring Anesthetic Gases

Monitoring exposure levels can be done with badges which are worn for a day and then sent out for analysis. They are expensive to use routinely but may be valuable for pregnant employees or reoccurring situations that may be considered hazardous.
Summary

There is definite potential for health hazards with exposure to anesthetic gases and chronic exposure to anesthetic pollution should not be ignored. Install a scavenging system, maintain the anesthetic machine, and educate your employees on the safe administration of anesthetic gases.

Legally, employees have a “right to know” what hazardous chemicals they may use or encounter in the workplace and know how to protect themselves from the adverse effects of these chemicals. The anesthetic gases are considered hazardous chemicals and fall under the OSHA Hazard Communication Standard (HCS). This federal law, which was enacted in 1988, requires employers to keep a list of hazardous chemicals in the workplace, maintain a file of Material Data Safety Sheets (MSDS), and train their employees in the safe and proper use of these hazardous chemicals.

LITERATURE CITED

FIELD MANAGEMENT OF INADVERTENT CARFENTANIL (WILDNIL™)/ETORPHINE (M99™) HUMAN EXPOSURE

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Abstract

Carfentanil and etorphine are synthetic opiates with a clinical potency 10,000 times that of morphine. They have a morphine-like analgesic mode of action and produce rapid immobilization following intramuscular injection. Carfentanil and etorphine are to be used only by a licensed veterinarian for the immobilization of free ranging animals. Etorphine has been reported to cause dizziness, nausea, and coma in a 41-yr-old man after a needle scratch.¹ Immediate life threatening effects of an exposure include seizures, coma, respiratory depression and respiratory arrest. Large doses of naloxone, an opioid antagonist, have been recommended as a reversal agent for these exposures.² Longer acting opioid antagonists are now also available for reversal. A human exposure treatment protocol (Table 1) and antidote kit (Table 2) are recommended for all sites using these potent opiates.

LITERATURE CITED

Table 1. Treatment protocol for carfentanil / etorphine human exposure.

Synthetic opiates should only be used by groups of two or more. At least two people in each group should be experienced in establishing i.v. access and trained in Basic Life Support. The carfentanil / etorphine antidote kit should be readily available.

1. Initiate respiratory support (ambu bag or mouth to mouth) as needed.
2. Notify zoo personnel and the Health Safety officer to call 911. DO NOT LEAVE THE PATIENT UNATTENDED.
3. Open the antidote kit.
4. Establish i.v. access with one 23-ga butterfly. If the patient is awake and talking, observe only.
5. If the patient is losing consciousness and i.v. access is unavailable, draw 10 ml (10 mg) of naloxone into a 10 ml syringe with a 20-ga needle. If the patient is symptomatic give it i.v. push into any visible vein under the tongue or inject it i.m. into the shoulder or thigh. Repeat 10 ml (10 mg) naloxone dosing as quickly as possible as many times as needed.
6. If the patient is losing consciousness and i.v. access is available, draw 30 ml of naloxone from 3 vials via a 20-ga needle into a 30 ml syringe. Administer the 30 ml (30 mg) of naloxone i.v..
7. Continue to repeat the naloxone dosing until the patient wakes up and is able to talk. Many doses may be required.
8. Transport the patient by rescue squad to an emergency room.
9. Send this protocol and all unopened vials of naloxone with the patient to the emergency room.
10. Call the Regional Poison Center

Table 2. Antidote kit for human narcotic exposure.

Whenever carfentanil / etorphine is used an antidote kit should be readily accessible. Each kit will contain the following items:

- Naloxone 1 mg/ml in 10 ml multi-dose vials (#15)
- 23-ga i.v. butterfly (#3)
- 30-ml syringe (#2)
- 10-ml syringe (#2)
- 20-ga needle (#4)
- Tourniquet, alcohol swabs, gauze pads, tape, oral airway, ambu bag, pocket mask
- Carfentanil / etorphine protocol
- Poison Center phone sticker
EXPRESSION OF VP2 cDNA OF INFECTIOUS BURSAL DISEASE VIRUS IN A BACULOVIRUS EXPRESSION SYSTEM

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Abstract

A transfer vector, defined as pAcUW31-VP2, was constructed by insertion BamHI fragment (1.1kbp) of VP2 cDNA of infectious bursal disease virus (IBDV) strain CJ-801 into BamHI cloning site of baculovirus expression vector (pAcUW31). A contransfection was carried out with the purified pAcUW31-VP2 DNA and wildtype baculovirus (AcMNPV.LacZ) DNA that was linearized by Bsu361 digestion. Plaque-assays were conducted and 10 white plaques were picked for further purification after dual staining with X-gal and neutral red. A VP2 cDNA probe was prepared. Dot hybridization analysis of the 10 putative recombinant viral DNA using the probe indicated that 7/10 were positive. One of the recombinants (1/7) was amplified in sf9 and the viral DNA was extracted from infected cells. Southern blot hybridization results demonstrated that the BamHI fragment of VP2 was inserted into the baculovirus genome. Direct immunofluorescence technique confirmed that the protein of interest was present in the cytoplasm and nuclear of the sf9 infected with the recombinants. The result indicated that the recombinant protein could react with the specific antibody. The molecular weight of the expressed protein was about 40kDa determined by SDS-PAGE. In Western-blotting experiment, the protein did not react with the specific antibody, while in dot-ELISA, the native expression product can react with it.

To evaluate the protective properties of the recombinant protein, five groups (10 birds each) of SPF chickens were vaccinated with the expression protein and challenged with the virulent IBDV strain CJ-801. The results showed that the chickens vaccinated were partially protected against the infection with the virulent IBDV. Failures to obtain complete protection maybe account for an insufficient amount of recombinant protein used for immunization.
PANSTEATITIS IN BOAT-BILLED HERON CHICKS

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Abstract

The fat-soluble vitamin, vitamin E, is a biologic antioxidant that protects cellular membranes from lipid peroxides and free radicals. The clinical appearance of hypovitaminosis E is extremely variable and may include: steatitis, myopathy, hemolytic anemia, encephalomalacia, paralysis, paresis, tremors, ataxia, torticollis, exudative diathesis and/or reproductive problems such as decreased hatchability or fertility.1-6

Pansteatitis due to vitamin E deficiency occurred in three 10-wk-old boat-billed heron chicks (Cochlearius cochlearius) at Knoxville Zoological Gardens despite daily vitamin supplementation. The heron diet was prepared in layers and offered free choice. Silversides fish (Menidia menidia) and a vitamin-mineral supplement were placed on top of a commercial bird of prey diet and dry dog food. Chicks presented weak and lethargic. Physical findings included emaciation, yellow-brown subcutaneous nodules, a firm distended coelom, erosions covered by a diphtheritic membrane along the roof of the mouth, and yellow-white, submucosal pharyngeal nodules. Although all adult boat-billed herons behaved normally, mild to moderate amounts of subcutaneous fat were found in all birds. One adult also had a slight distended, firm coelom. Clinical pathology revealed heterophilic leukocytosis, anemia, hypoproteinemia and low plasma alpha-tocopherol levels (1.94 μg/ml-2.14 μg/ml). Dierenfeld reported a mean plasma alpha-tocopherol level of 9.53 μg/ml ± 0.96, (reported range: 7.9-14.2 μg/ml) in six clinically normal, captive boat-billed herons.2 Two of three chicks died. Necropsy revealed coeloms distended with a mass of yellow-brown to golden-yellow firm, nodular fat. The fat surrounded many abdominal organs as well as the heart and compressed the diameter of the gastrointestinal tract significantly. The chicks also had granulomatous pneumonia and airsacculitis due to Aspergillus fumigatus.

LITERATURE CITED

CARBON MONOXIDE AND CARBOXYHEMOGLOBIN IN CAPTIVE ASIAN (Elephas maximus) AND AFRICAN (Loxodonta africana) ELEPHANTS

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Abstract

Carbon monoxide (CO) is the leading cause of poisonings in the United States and cause 3500-4000 deaths per year. Its pathology is related to tissue hypoxia and cerebral edema. The mechanism of this hypoxia is due to the high affinity CO has to bind with hemoglobin molecules. This affinity can be from 240-300 times stronger than the affinity for oxygen depending on the species. The national ambient air quality standard (NAAQS) has recognized that in most individuals, CO in low levels is non-toxic and has established exposure levels for people that permit up to 9 ppm over 8 hr or 25 ppm over 1 hr as levels that will result in carboxyhemoglobin (COHb) of less than 2%. Sub-acute levels (less than 20% COHb) may cause such symptoms as inattentiveness and gastroenteritis. Acute toxicity can be seen at levels around 20% COHb. Clinical signs include headache, nausea, chest pain, and irritability. At levels approaching 40-60% stupor, coma, and death are the possible outcomes.

Chronic toxicity can cause such conditions as polycythemia, neuropsychiatric disorders, cardiac toxicity, and fetal effects. In veterinary medicine the most common presentation of CO toxicity is seen in farrowing houses, with stillbirths and perinatal deaths being significant. Carbon monoxide has widespread effects on the mammalian fetus. These include teratogenicity, neurologic disorders, reduced birth weights, and an increased incidence of stillbirths. These effects are the result of two mechanisms; the direct effect of CO on the fetus and the hypoxic stress placed on the fetus prior to CO diffusion across the placenta. Experimental evidence in domestic animals has shown that a level of 9% COHb in the maternal circulation will effectively reduce the fetal oxygen blood content by 21%, equivalent to a 41% loss of hemoglobin or blood flow.

The 1995-1996 AZA Report on Conservation and Science reported that over 30% of newborn elephants are either stillborn or die within the first 30 days of life. It is known that elephant blood has the highest affinity for oxygen of any terrestrial mammal. Elephant hemoglobin has several amino acid substitutions that enhance oxygen binding, which enhance CO binding as well. Elephant myoglobin has also been demonstrated to have approximately six times greater affinity for CO than human myoglobin. Many elephants spend an extended amount of time indoors during the winter months in North America and Europe. While ambient levels of CO may be in the range acceptable for people, they may provide a source of exposure to more sensitive hemoglobin for months at a time. If these sub-acute levels (~10%) are found consistently in confined elephants, it appears possible that any fetus present may be undergoing some level of hypoxic stress. It may even be possible that fetuses are lost before pregnancy was detected. This could play a role in defining irregular estrus cycles. A lot of questions remain regarding CO in elephants: what is the half-live of bound CO to maternal hemoglobin, what do 2,3-bisphosphoglycerate (2,3 – BPG) levels do with
elevated COHb, could CO have role as a chemical messenger similar to nitrous oxide, can it have a role in uterine leiomyomas. It is recommended that all elephant holding facilities examine their heating equipment and consider this especially in their efforts to breed elephants.

LITERATURE CITED

FATTY ACID COMPOSITION OF WHITE ADIPOSE TISSUE IN CAPTIVE RHINOCEROS

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Abstract

Fatty acid nutrition is intimately tied with general health, immune function, and reproduction in other species, but has not been investigated in detail in the rhinoceros. Limited information on fatty acid composition in native rhinoceros browse is currently available. Data that do exist suggest that native browses contain higher concentrations of alpha-linolenic acid [C18:3(\(n\)-3)] than linoleic acid [C18:2(\(n\)-6)], which vary with plant part, species, and season analyzed. In general, ingestion of seeds and kernels favors linoleic acid intake, while ingestion of leaves favors alpha-linolenic acid. Diets fed to captive rhinoceros are heavily dominated by grains (in pelleted feeds) and dried forages compared with fresh browses, which may affect fatty acid status.

We assayed fatty acids in rhinoceros white adipose tissue samples (\(n = 8\)) that were obtained at necropsy and stored frozen (-70°C) by gas chromatography using standard analytic techniques. Results of this analysis are detailed in Table 1.

Typically, animals feeding on diets with appropriate levels of C18:2 have depot fats containing ≥ 5% linoleic acid. Five of eight samples analyzed contained linoleic acid concentrations below that threshold, indicating possible linoleic acid-deficient diets. All the low-linoleic acid samples were obtained from browsing rhinoceros species (Sumatran rhino, Dicerorhinus sumatrensis, \(n = 1\); black rhino, Diceros bicornis, \(n = 4\)). Further, only two of the rhino white adipose tissue samples contained detectable alpha-linolenic acid (C18:3) concentrations, another essential fatty acid found in natural browses.

These preliminary data indicate that fatty acid nutrition of rhinoceros needs to be evaluated in much greater detail. Captive diets of browsing rhino species should be analyzed (and possibly supplemented) to ensure adequate levels of both essential fatty acids, in proper proportions.

ACKNOWLEDGMENTS

Funding for this study was supplied by the International Rhino Foundation. Adipose tissue samples came from the Rhinoceros TAG frozen tissue bank maintained by the St. Louis Zoological Park.
Table 1. Fatty acid concentrations in rhinoceros white adipose tissue (expressed as a % of total fatty acids), obtained from captive animals at necropsy.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C16:1</th>
<th>C18:0</th>
<th>C18:1</th>
<th>C18:2</th>
<th>C18:3</th>
<th>C20:1</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Diceros bicornis</em></td>
<td>6</td>
<td>1.5 ± 1.4</td>
<td>28.3 ± 5.2</td>
<td>4.6 ± 1.8</td>
<td>6.8 ± 1.8</td>
<td>44.0 ± 8.6</td>
<td>9.6 ± 7.4</td>
<td>1.4 ± 0.6</td>
<td>1.7 ± 1.3</td>
</tr>
<tr>
<td><em>Rhinoceros unicornis</em></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

\(n = 2\) for *Diceros bicornis*; \(n = 2\) for *Rhinoceros unicornis*.
MANAGEMENT OF A RECTAL PROLAPSE IN A FREE RANGING MOUNTAIN GORILLA IN BWINDI IMPENETRABLE NATIONAL PARK, UGANDA

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Abstract

In December 1997, an apparent third degree rectal prolapse was observed in an active and otherwise healthy looking juvenile female mountain gorilla in Mubare tourist group in Bwindi Impenetrable National Park (BINP), Uganda. The juvenile gorilla was observed with a 3- 4-cm diameter mass protruding 10-12 cm from the anus. The juvenile would occasionally squeeze the mass, expressing liquid from the distal lumen, but was active. The mass appeared to be slightly dehydrated. A tentative diagnosis was made and preparations were made for an intervention to further assess and possibly treat the condition. The gorillas of BINP are managed as a population, and the condition was considered to be life-threatening to this individual gorilla, but not to the rest of the group, consisting of 16 members, or to the population.

While preparing for an intervention, observations continued on this gorilla, but within the next 24 hr, the prolapse was not seen again. Forty hours later a small portion of rectum was seen protruding from the anus, a condition which would be described as a first degree rectal prolapse and therefore not serious. Following this case, the BINP field staff reported having observed similar conditions on more than one occasion in two other juvenile gorillas straining to defecate. These conditions reportedly resolved without treatment approximately 20 min after first being observed. The decision to intervene was canceled, and the gorilla was monitored for further signs which did not occur in the next 24 hr. To date no subsequent health problems have been reported in this animal.

Although further investigation is warranted, the limited information available at present would suggest that periodic pronounced rectal prolapse occurs in young gorillas of BINP and spontaneously resolves without serious health effects for the individual. This case study highlighted the fact that there is very limited information on disease conditions in mountain gorillas, therefore staff training in observation and accurate reporting is essential for successful field diagnosis and subsequent management of cases.
DOSAGE TRIALS USING MEDETOMIDINE AS AN ORAL PREANESTHETIC AGENT IN CHIMPANZEES (*Pan troglodytes*)

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Abstract

Current techniques for restraint of chimpanzees (*Pan troglodytes*) require the use of remote injection delivery systems. This method can be difficult to execute on an intelligent, moving target and it is inherently stressful to the animals and personnel involved. This study was undertaken to determine if medetomidine (Domitor, Pfizer, Exton, PA 19341, USA) could be effectively used as an oral preanesthetic agent for chimpanzees immobilized with ketamine (Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501 USA). The study involved 14 animals at the Uganda Wildlife Education Centre (UWEC), two animals at the Detroit Zoo, and one animal at the Knoxville Zoo. Nine animals received 50-100 g/kg of medetomidine orally in marshmallow creme prior to immobilization by ketamine injection. A control group of eight animals received medetomidine with ketamine as a single intramuscular injection. Reversal of the medetomidine was performed at the end of the procedure using 200 g/kg atipamezole (Antisedan, Pfizer, Exton, PA 19341 USA). Animals who received oral medetomidine were monitored for depth of sedation every 5 min for 30 min prior to ketamine injection. Preliminary findings showed that chimps at UWEC receiving 75 g/kg medetomidine orally were noticeably sedate after 30 min. These chimps would move more slowly, lie down more, and sometimes close their eyes. None became completely immobilized by the premedicant alone. Increasing the dose to 100 g/kg did not show a noticeable increase in depth of sedation. The three chimps from U.S. zoos who received oral medetomidine showed little to no visible sedation, even at the 100 g/kg dosage. While the chimps from U.S. zoos had become accustomed throughout their lives to a yearly routine of fasting followed by a stressful encounter with a blowpipe, many of the UWEC chimps were young and had not required previous immobilization; only a few were adults with previous experience with blowdarting. It appears that medetomidine can be used as an effective oral premedicant in chimpanzees if the individual animals are not already stressed before the drug is administered.

ACKNOWLEDGMENTS

This study was supported by a grant from the Columbus Zoo Conservation Fund. The authors would like to thank the staff of the Uganda Wildlife Education Centre, the Detroit Zoo, and the Knoxville Zoo for their assistance with this project.
DEVELOPING A HIPPO SKIN TISSUE BIOPSY DART

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Abstract

To further our understanding of hippo (*Hippopotamus amphibius*) biology, skin tissue samples were collected from free ranging hippos to obtain DNA for a population genetics study. This required the development of a biopsy dart tip specifically designed for hippo skin. Samples of skin were collected from individual hippos in intact herds during the day by using a biopsy dart attached to a crossbow bolt. The bolt, projected from a crossbow, was retrieved via a fishing reel mounted on the crossbow with the line attached to the bolt carrying the biopsy dart. This method of collecting skin tissue samples has previously been used successfully with free ranging whales.1,4

A whale skin tissue biopsy dart tip of simple design and successfully proven in the field on free ranging whales1 was tested on three hippos exhibited in the Toledo Zoo’s Hippoquarium (a 360,000 gallon, filtered, underwater viewing, naturalistic exhibit) at a range of 25-30 m. Animals darted at the Toledo Zoo were individually monitored for any adverse affects to health (i.e., infection of biopsy site) by close visual inspection in the Hippo Restraint Device in the hippo holding facility at the Toledo Zoo.2 No adverse reactions were observed. Several unsuccessful tests using the whale biopsy dart tip and modifications of this simple design were carried out. Hippo skin is composed of a thin epidermis and thick dermis layer, averaging 3.5 cm thick 20-80 cm from the midline of the back,3 which is highly fibrous and tough. The characteristic punch, bounce and tear mechanism of the whale biopsy dart tip simply would not work with hippo skin. A new design with movable parts that would snap and hold a piece of skin tissue upon impact was developed (Fig. 1) and tested on the hippos at the Toledo Zoo. The dart tips successfully excised adequate amounts (0.25-0.5 cc) of skin tissue needed for extracting and analyzing DNA.

The parts for 66 dart tips were fabricated in a machine shop and were assembled in the field. All of the darts were used in the field and some were reused after disassembly, cleaning and reassembly. The following is a description of how the biopsy dart tip works:

1. The bolt with the attached biopsy dart tip is projected from a crossbow towards the target.
2. The four pointed tip of the dart cuts into the skin and penetrates to the depth of the stop ring.
3. The force of the bolt pushes the steel plunger ring forward shattering the acrylic ring (in place to prevent the plunger from pushing the cutting blade down the blade track when the bolt is projected from the crossbow) and allowing the plunger to push the stainless spring steel blade down the curved blade track.
4. The blade snips off the portion of skin inside the dart tip and closes the dart tip opening with the sample held in place.
5. The dart tip pops out of the hippo skin with the rebound of the impact.
6. The bolt with the attached biopsy dart tip and sample are retrieved with the fishing reel and attached fishing line.

The samples were then removed from the biopsy darts and placed in containers with preservatives and stored for future analysis.

The collection period was carried out during the beginning of the dry season (June-August 1997) in Kruger National Park located in South Africa. We sampled hippo herds located in narrow, shallow and accessible stretches of the Olifants and Letaba rivers at a minimum darting range of 20 m and a maximum of 45 m. When possible, hippos were darted on their sides or backs while they rested on beach areas, however; after the initial volley of beach shots the hippos took cover in the water usually leaving only the nape of the neck and/or back of the neck/head area for a target. This required much greater accuracy to hit the target and thus increased the percentage of missed shots. The majority of tissue samples collected were from hippos darted in the water. Care was taken to dart hippos only when they were facing away from the researcher, so as to prevent injury to the eyes, ears or nostrils.

Our sampling of five herds (three of them with adjacent territories along the Olifants River) resulted in 56 individual samples (Table 1).

The basic design and the results of field testing of the hippo skin biopsy dart were successful and may be applicable for use with other thick skinned mammals such as the rhinoceros (W. Karesh, The Wildlife Conservation Society, New York, NY, personal communication).

Additional Notes

The crossbows used were both made by HUNTER’S Manufacturing Company, Inc. The Huntmaster Advantage model with 68 kg draw weight was used for the tests at the Toledo Zoo and the 458 Magnum Treestand with a 75-kg draw weight was primarily used in the field.

The spool on the open-face spin cast fishing reel (mounted on the top front end of the crossbow just behind the bow limbs) was filled with either 23 or 27 kg test SpiderWire, high tensile strength microfilament braided fishing line, tied to a double loop of 36 kg SpiderWire attached to the butt end of the bolt. The leader of the line was laid down in the groove below the bolt track from front to back. All knots in the SpiderWire were secured with super glue.

A safety line, in the event of bolt breakage upon impact, was attached from the loop on the butt end of the bolt to the dart tip.

ACKNOWLEDGMENTS
Disney’s Animal Kingdom for primary funding of this project. Apex Design and Manufacturing Inc. for funding, designing and fabricating of the biopsy dart and the crossbow fishing reel mounts. Kruger National Park for providing lodging and field logistics. The Toledo Zoo for allowing us to test the biopsy darts on the hippos exhibited in the Hippoquarium. Alex Krajcirovic for mechanical engineering of the biopsy dart. Moira Brown for advice concerning the design of biopsy darts and delivery systems. William Karesh, Timothy Reichard and Wynona Shellabarger for advice on the design of biopsy darts. The employees of Cleland’s Outdoor World for expert advice on all matters about crossbows and archery. Finally, we thank our partners, Mary Beth McConnell, Lori-Ann LeBlanc and Patricia Stilwill for their support and invaluable assistance with this project.

LITERATURE CITED

Table 1. Results of crossbow shots and hits of hippos with the skin biopsy dart.

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hits retrieved with tissue</td>
<td>56</td>
</tr>
<tr>
<td>Hits retrieved with no tissue</td>
<td>7</td>
</tr>
<tr>
<td>Hits - line or bolt broke (lost sample)</td>
<td>11</td>
</tr>
<tr>
<td>Total hits</td>
<td>74</td>
</tr>
<tr>
<td>Missed shots</td>
<td>179</td>
</tr>
<tr>
<td>Total shots</td>
<td>253</td>
</tr>
</tbody>
</table>

Percentage of total shots that were hits = 29%
Percentage of total shots retrieved with tissue = 22%
Percentage of retrieved hits with tissue = 89%

Figure 1. Hippo skin tissue biopsy dart: full view and cutaway. Dart weight = 25 grams.
INTESTINAL CRYPTOCOCCOSIS IN A COMMON MARMOSET (*Callithrix jacchus*)

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Abstract

Cryptococcosis is a deep mycosis of animals and human beings produced by *Cryptococcus neoformans*. This organism has two varieties that differ in epidemiologic and clinical aspects. *C. neoformans var neoformans* has a worldwide distribution and has been isolated from bird droppings (mainly pigeon’s excreta), fruits, vegetables, soil, and sawdust of tropical trees.12 *C. neoformans var gattii* mostly occurs in tropical and subtropical areas and has been isolated from red gums (*Eucalyptus camaldulensis* and *E. tereticornis*), although other environmental sources are also possible.15 In human beings, *C. neoformans var neoformans* usually affects immunocompromised hosts, whereas *C. neoformans var gattii* has been mostly isolated from immunocompetent hosts.16

Cryptococcosis has been sporadically reported in captive nonhuman primates.11,14 especially in Old World species.1,4,11 Cryptococcosis has been a remarkable problem in tree shrews (*Tupaia tana* and *Tupaia minor*) and elephant shrews (*Macroscelides proboscides*) at a zoo,20 and has also been documented in other captive colonies of tree shrews.7 There are two reports of spontaneous cryptococcosis in New World monkeys, one case in a squirrel monkey (*Saimiri sciureus*)14 and two cases in Geoffroy’s tamarins (*Leontocebus geoffroyi*).18 This paper describes the unusual pathologic findings of naturally-occurring cryptococcosis in a common marmoset (*Callithrix jacchus*) involving the intestines and lymphatic vessels.

A 5-yr-old female pet common marmoset was submitted for necropsy with a 1-mo history of wasting. The day before death it ate apparently well but had abdominal distension. It was housed in a greenhouse with a 7-yr-old female. Red gums were not present in the enclosure. There were not pigeons or other birds in the greenhouse, but the animals escaped and potentially had access to free-living birds during some days before the death of this marmoset. The diet consisted of a cereal mix, fruits, bread and mealworms.

At necropsy, diffuse hemorrhagic enteritis was observed. Sections of intestine, liver, kidneys, and brain were fixed in 10% buffered formalin and embedded in paraffin, cut at five μm, and routinely stained with hematoxylin and eosin. Special stains included periodic acid Schiff (PAS), and Mayer’s mucicarmine (MM). Microscopically, the most prominent lesion consisted of transmural enteritis with full-thickness necrosis of the mucosa, fibrin deposition and infiltrates of mixed inflammatory cells, mainly neutrophils but also macrophages. There were numerous intralesional spherical to oval yeast-like cells that were 4-15 μm in diameter and surrounded by a wide clear halo. Their capsule stained positively with PAS and MM stains, that gave them a spinous aspect. Both single budding
by a narrow base and chains of three or four budding yeasts were present. Intestinal and mesenteric lymphatic vessels had granulomas attached to their wall and lined by endothelium. These granulomas had intralesional yeasts and caused total or partial occlusion of the affected vessels. Other findings were: focal granulomatous and necrotizing hepatitis without intralesional yeasts and with pleomorphic mononuclear cells, neutrophils and megakaryocytes within sinusoids; and marked mesangial nephropathy.

This is the second report of naturally-occurring cryptococcosis in callitrichids. The diagnosis was based on the morphologic and staining features of the intralesional yeasts found in this marmoset. *Cryptococcus* is the only pathogenic fungus with a mucopolysaccharide capsule that stains positively with mucin stains such as MM.3

The marked involvement of the intestine in this case of spontaneous cryptococcosis in a marmoset is unusual because the intestine is not a common target for cryptococcosis in animals and human beings.3 There were not either gross lesions in the lungs or microscopic findings in the brain. Pulmonary and central nervous system lesions are the most common findings in spontaneous cryptococcosis in nonhuman primates and human beings.1,3,11,14,16,20 Disseminated disease involving the lungs, spleen, intestine and mesenteric lymph nodes was reported in two Geoffroy’s tamarins with spontaneous cryptococcosis; intestinal lesions consisted of “patchy denudation of the mucosa with networks of fibrils and scattered yeasts.”8 Takos subsequently failed to reproduce intestinal lesions in three Geoffroy’s tamarins inoculated orally with *C. neoformans*, yet it was isolated from the gastrointestinal tract of all tamarins.19 *C. neoformans* has been sporadically detected as an opportunistic enteric pathogen in Acquired Immunodeficiency Syndrome (AIDS) patients with diarrhea.10

Inhalation of contaminated dust is expected to occur in most cases of cryptococcosis, and is followed by pulmonary involvement with subsequent hematogenous spread that causes disseminated infection with a marked predilection for the brain.3,12 However, another points of entry, such as the skin and gastrointestinal tract, may also play a role in some cases.3,13,19 According to the pathologic findings in this marmoset, infection was probably acquired by the oral route. The source of infection could not be identified. There was no evidence for the involvement of red gums in this case. However, *C. neoformans* var *gattii* may have another, yet unrecognized sources.5,15 *C. neoformans* var *gattii* has been isolated from domestic species such as horses, cats and dogs,9,13 and also zoo animals such as koalas and a kiwi with evidence of involvement of *Eucalyptus* trees in the transmission.6,9,17

The necrotizing and suppurative aspect of the intestinal mucosal lesions in this marmoset is characteristic of the initial response to *C. neoformans* in healthy, noncompromised individuals.3 These findings suggest that cryptococcosis might occur as a primary intestinal infection in callitrichids. However, concurrent or underlying intestinal or systemic diseases in this marmoset could not be ruled out. The immunologic status of this marmoset is unknown, and the only concurrent disease identified at necropsy was marked mesangial nephropathy, that is a common finding in captive callitrichids.2
Intravascular granulomas with intralesional cryptococci similar to those seen in the intestinal lymphatic vessels of this marmoset have been described in rodents experimentally inoculated intravenously with *C. neoformans*.21

**ACKNOWLEDGMENTS**

The authors are grateful for assistance with histologic studies provided by Pere Losada and Blanca Pérez. We appreciate the helpful comments on this case by Dr. Manfred Brack (Deutsches Primatenzentrum, Göttingen, Germany) and Dr. Richard Montali (National Zoological Park, Washington DC USA).

**LITERATURE CITED**


USE OF GEOGRAPHIC INFORMATION SYSTEMS TO INVESTIGATE DISEASE TRANSMISSION IN ZOO ANIMALS

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Abstract

Geographic Information Systems (GIS) are computer based mapping programs for visualizing and analyzing data. Using GIS to investigate disease in zoo animals can increase knowledge about complex disease transmission patterns. The ability to identify and predict these patterns can help with both research and animal health management. In three studies at Brookfield Zoological Park, a GIS (ArcView) was used to map and analyze spatial patterns to test hypotheses about disease epidemiology. The overall goal of this project was to examine the use of GIS as a visualization tool to study disease transmission within zoos and to develop recommendations to help predict and prevent disease at Brookfield and other zoos.

In the first study, risk factors were studied for seropositivity to Canine Distemper Virus (CDV) among zoo cats. Potential for contact between cats and raccoons was of principle interest, although age, species, origin, and contacts with other animals were also possible risk factors/confounders. Eighty-seven zoo cats were tested for CDV using the serum-neutralizing test. No cats had been previously vaccinated for CDV. Aerial photographs of the zoo were digitized and imported into the GIS. All feline housing and holding enclosures were identified on the GIS coverage. Seropositive and seronegative animals were mapped to their enclosures. Information on each animal’s sex, age, and history was assessed and linked into the GIS database. Based on this information, risk factors were compared between seropositive and seronegative animals using spatial analysis and multivariate logistic regression. Animals in outdoor locations were at significantly higher risk of CDV infection. Other significant covariates included sex, age and species. Seropositive cats were present in all feline-housing locations at the zoo, regardless of proximity to woods, water, trash bins and other habitat features.

The second study mapped the movements of eight reptiles diagnosed, post-mortem, with mycobacteriosis. Each animal’s enclosure history was tracked from 1978 to the date of death. A map of the interior of the Reptile House was digitized and temporospatial movements of infected animals were mapped. This information was used to identify other reptiles which shared contact with diseased animals, to quantify the likelihood of potential exposure, and to distinguish high risk enclosures.
The third study examined the spread of poxvirus lesions among pinnipeds housed in four pools. Eight animals were diagnosed with pox lesions. The objective of the study was to determine the method of viral transmission. Potential means included water-borne spread through a common filtration system, animal-to-animal spread and fomite transmission. Keeper and veterinary records beginning from 1993 were used to date the first appearance of pox lesions in each affected animal. The four pinniped pools were mapped. Each animal was assigned to its inhabited enclosure and disease transmission patterns were determined.

Geographic Information Systems provide an effective way to understand, observe and summarize patterns of disease transmission in zoos. Disease clustering, transmission, animal movement, and high risk individuals can be identified with the powerful aid of a GIS. This technology will be useful to animal health researchers, especially as it becomes more widely used in zoos, by departments such as facilities planning and guest services.

ACKNOWLEDGMENTS

The authors wish to thank the keeper staff and laboratory personnel, who assisted in the gathering of these data and processing of the serum samples; Mark Jocelyn, at the Illinois Natural History Survey, for GIS assistance; and the Howard Hughes Medical Institute for its support.
MEDICAL MANAGEMENT OF EPILEPSY IN A PUMA (Felis concolor)

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Abstract

A neutered male, 51 kg, 3.5-yr-old puma (Felis concolor) presented in February 1995 with episodic grand mal seizures 5 mo after arrival to the Seneca Park Zoo in Rochester, New York. CBC, serum chemistry panel, blood lead assay, blood ammonia assay, serum IgG and IgM levels and conjunctival scraping for canine distemper virus, serum strychnine assay, hemobartonella screen, serum titer evaluations for toxoplasma, FIV, FeLeuk, FIP, urinalysis, and CSF analyses including canine distemper titer as well as bacterial culture and cytology were noncontributory to an etiologic diagnosis.

Three treatment options were considered: phenobarbital (Lilly Corporate Center, Indianapolis, Indiana 46285 USA) 2.5 mg/kg p.o., b.i.d., diazepam (Hoffmann-LaRoche, Inc., 340 Kingsland Street, Nutley, New Jersey 07110 USA) 0.16-0.33 mg/kg p.o., t.i.d. or potassium bromide 8-10 mg/kg p.o., b.i.d.1 Phenobarbital was chosen for proven efficacy and safety in cats.1,4 Phenobarbital (80 mg), given orally twice per day in a food item, controlled seizure frequency to no more than once per month. Trough serum phenobarbital level was 44 μg/dl at 80 mg p.o., b.i.d. maintenance dosage. This phenobarbital serum level was slightly above the normal range reported for medicated, domestic cats (15-40 μg/dl).2 Liver enzymes (alkaline phosphatase, SGPT, SGOT and LDH) remained within normal range of values.3 After 2.7 yr of phenobarbital therapy (80 mg p.o., b.i.d.), the puma presented ataxic and semi-responsive 2 hr after the morning treatment. The serum phenobarbital level was elevated at 72 μg/dl.2 Serum chemistry values including liver enzymes were not elevated. Reduction of the phenobarbital dosage eliminated the ataxia and sedation but failed to prevent epileptic seizure frequency to no more than once per month. A change in treatment to diazepam (5 up to 35 mg p.o., b.i.d.) alone or in combination with phenobarbital failed to control seizures. In February, 1998, magnetic resonance images of the brain demonstrated absence of organic disease or structural defects, the most common cause of seizures in domestic cats.5 Potassium bromide was initiated at 5 mg/kg p.o., b.i.d. and changed after 3 wk to 15 mg/kg p.o., s.i.d. Potassium bromide appears, thus far, effective at controlling seizures in the puma at once daily dosing and without the sedation observed with phenobarbital. Steady state, potassium bromide serum levels will be collected after 4 mo of treatment.

Summary
Epilepsy is a treatable disorder in exotic felids but requires monitoring for adverse effects of therapy, keeping treatment options flexible and fostering communications with zoo staff and visitors.

LITERATURE CITED

GUIDELINES FOR CHEMICAL USE IN THE ANIMAL AREAS OF DISNEY’S ANIMAL KINGDOM

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Abstract

The objective of this project was to review and reduce an initial list of chemicals used by Disney’s in-house Pest Management Department for specific use in animal areas at Disney’s Animal Kingdom, a unique theme park. Subsequently, these chemical use guidelines could be applied to the already existing animal areas on Disney property, the Tri-Circle-D Ranch, the Living Seas and Discovery Island. With 170 chemical products currently used on Walt Disney World’s 42,000 acres, a list needed to be developed that was more manageable from an animal care, safety, product application and monitoring standpoint. The list of 170 chemical products which included fungicides, herbicides, insecticides, rodenticides and wetting agents was reviewed over a 3-mo period. The criteria used in the review included toxicity data and LD 50’s, non-target species effects, environmental hazards, active ingredients, method of application and reason for use. Based on the accrued information, including Material Safety Data Sheets, toxicology studies and reports, various resources on chemical use and discussions with the Manufacturers of the products, a master list of approximately 70 products was developed along with a Standard Operating Procedure for use and method of application in the animal areas. A notification system was also developed for communication between the animal caretakers and the pest management technicians. A computerized tracking system was implemented to record the location where each chemical was used, what time it was used, the reason for use and other pertinent information. This system reduced the number of chemical products being used in the animal areas and made available an ongoing documented history that could be utilized for review of chemical use in order to maintain the highest standards of animal care and husbandry.
KYPHOSCOLIOSIS IN SANDTIGER SHARKS (*Odontaspis taurus*)

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Abstract

Abnormal spinal curvatures have been noted in captive sandtiger shark (*Odontaspis taurus*) populations but incidence and etiology are unknown. A preliminary survey addressing these questions was sent to eighteen institutions listed as exhibiting sandtigers. Fifteen institutions responded, thirteen indicated that one or more of their sandtigers had developed spinal deformities and twenty-one cases were identified. There were no other shark species from these collections reported to have spinal abnormalities.

Clinical presentation was the development of a conspicuous hump or spinal curve in the region between the pectoral fins and the cranial dorsal fin. In cases where the severity of the deformity worsened, additional clinical problems developed including anorexia, difficulty in maintaining body position and obtundation. Radiographs, magnetic resonance imaging (MRI) and/or computed tomography (CT) studies on six euthanatized animals revealed kyphoscoliosis with either single or multiple subluxations or compressed vertebral bodies. Histologic examination of skeletal tissue revealed cartilaginous proliferation and degeneration. Moderate to severe degeneration and fibrosis was noted in the epaxial muscle from affected areas.

All sandtigers were wild-caught, using gill nets, long-line, pound nets or trawls. The average age (based on size) at onset of the condition for fourteen animals was between 2-4 yr (60-160 cm). One additional animal was noted to have developed the deformity at approximately 8 yr of age (240 cm). Length of time the affected animals were on exhibit prior to onset of the condition encompassed a broad range including several months to several years. Both males and females were affected. Diets consisted of herring, capelin, squid, mackerel and bonita and all diets were supplemented with vitamins, although amounts and types varied. There was an enormous variation in feeding schedules but there was a slight tendency for affected sandtigers to have been fed more frequently and at a higher percentage of body weight. Minimal growth information was provided.

Numerous etiologies have been suggested including nutritional (dietary excess vs. essential ingredient deficiencies), musculoskeletal disease, and trauma. More detailed information needs to be collected including growth data of both captive and wild populations, incidence of skeletal lesions in recently collected animals, food analysis and nutritional requirements, evaluation of collection and transport.
methods, as well as physiologic and biomechanic comparisons (intervertebral ligament structure and composition, muscle and skeletal development) with other species.

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