MEDICAL MANAGEMENT OF CAPTIVE TAPIRS (*Tapirus* spp.)

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Abstract

Medical and pathology records were reviewed from North American institutions and summarized for analysis. Several anesthetic regimens have been used successfully in tapirs. Tapirs have traditionally been anesthetized with etorphine, but combinations of $\alpha_2$-adrenergic agonists and butorphenol appear to provide some cardiovascular advantages. Common clinical signs of tapirs found in this survey include mandibular swellings, corneal opacities, diarrhea, skin vesicles and epidermal sloughing, chronic weight loss, lameness, colic, nasal discharge, and dyspnea and coughing. Hematologic and serum chemical values were similar to those of other Perissodactyla. The highest mortalities were in the neonatal and adult age groups. The main disease problems identified were noninfectious gastrointestinal disease and infectious respiratory disease. Pneumonia from mycobacterial infections occurred twice in this survey, and stresses the importance of performing tuberculosis monitoring in tapirs. Tapirs should be fed using strategies similar to that of other Perissodactyla, and successful diets usually include forages and nutritionally complete pelleted feeds with minimal produce. Attention to the husbandry concerns following parturition is important for neonatal survival. It is recommended that tapirs be evaluated medically prior to being relocated.

Resumen

Los registros médicos y patológicos de instituciones en Norte América fueron revisados y resumidos para su análisis. Varios procedimientos anestésicos han sido exitosamente usados en tapires. Tradicionalmente los tapires han sido anestesiados con etorfina, pero se ha demostrado que las combinaciones de antagonistas $\alpha_2$-adrenérgicos y butorfenol tienen ventajas cardiovasculares. Signos clínicos comúnmente encontrados al examinar tapires incluyen inflamación mandibular, opacidad corneal, diarrea, vesículas dérmicas, descamación epitelial, pérdida crónica de peso, laminitis, cólicos, descarga nasal, disnea y tos. Los valores hematológicos y químicas serológicas son similares a aquellos encontrados en otros perisodáctilos. Las mortalidades más altas se encuentran en neonatos y en individuos de edad avanzada. Los mayores problemas médicos encontrados son enfermedades gastrointestinales no infecciosas y enfermedades respiratorias infecciosas. Una neumonía por infección micobacteriana ocurrió dos veces durante este estudio y evidencia la importancia del control de tuberculosis en tapires. Se debe alimentar a los tapires siguiendo estrategias similares a las utilizadas con otros perisodáctilos, y las dietas adecuadas usualmente incluyen forrajes y concentrados nutritualmente completos. Los cuidados posteriores al parto son sumamente importantes para la sobrevivencia del neonato. Es recomendable que los tapires sean evaluados médicamente antes de ser transferidos.
Introduction

Of the land megavertebrates, tapirs have received relatively little attention or study in the wild or captivity. Tapiridae is a small family of four species within the single genus *Tapirus*. There are three New World species (*T. terrestris*, the South American, lowland, or Brazilian tapir; *T. bairdii*, the Central American; or Baird’s tapir; and *T. pinchaque*, the mountain or wooly tapir) and one Old World Species (*T. indicus*, the Malayan, saddleback, or Asian tapir). Fossil evidence shows that tapirs have survived nearly unchanged for over nearly twenty million years. The majority of tapirs in captivity are Malayan and South American tapirs. Baird’s tapirs are less common, and mountain tapirs are extremely rare. In the wild, all tapir populations are threatened by habitat loss and hunting. The South American tapir is listed on Appendix II of CITES, and the other three species are listed on Appendix I. The mountain tapir is nearing extinction in the wild.

The body mass of adult tapirs range from 200-400 kg with the female often being larger than the male. The Malayan tapir is the largest species, and the mountain tapir is the smallest. Individuals have lived over 30 yr in captivity. They reach sexual maturity between 2 and 4 yr. Tapirs give birth to usually single, and rarely twin, precocial calves after approximately a 13 mo gestation. Several authors have published reviews that describe the biology and husbandry of the Tapiridae.\(^3,19,20,23\) A bibliography was recently produced which includes citations on the medicine and biology of Tapiridae.\(^15\) The American Zoo and Aquarium Association (AZA) sponsors a Taxon Advisory Group and Species Survival Plans for the Tapiridae.

Anatomic Notes

The internal anatomy of the tapir is analogous to the domestic horse and other Perissodactyla. The guttural pouches of the tapir are similar to those of the horse. They are located in the pharyngeal region, lateral to the hyoid bones.\(^19\) The testes are in the inguinal canals, which are located in the subcutaneous tissues on either side of the penis.\(^13\) Tapirs lack a gallbladder. The tapir is a hindgut fermenter with a relatively small stomach and large cecum and colon. The squamous portion of the stomach is small and is located in the cardia (near the gastroesophageal junction). The kidneys, like those of the horse, are not lobulated. The normal parietal and visceral pleura can be thick and prominent, but only the Malayan tapirs should have adhesions between the lung and chest wall (as in the elephant).\(^18\)

Methods

Medical and pathology records were reviewed from 18 North American institutions. Anesthesia and clinical pathology records were compiled into MedARKS format for analysis. Clinical signs were categorized following review of individual medical records. Pathology findings from 108 cases from 1960-1995 were summarized into a database. Diagnoses were standardized and simplified to avoid confusion arising from different pathologists using different terms to describe the same disease process. Diagnoses were then divided into two categories based on evaluation of the clinical history, gross, and microscopic findings. Primary findings are those that are interpreted as the major factor contributing to the death of the animal. Secondary findings are those that are interpreted as clinically
significant, but not the major contributing factor in the death of the animal. The findings were then sorted by individual, species, age-group, organ system affected, and disease category (infectious or non-infectious) for analysis of trends.

Anesthesia and Restraint

Many tapirs can be habituated to being touched and scratched. Some individuals will even lay down allowing physical examination and venipuncture. Temperaments of individuals vary greatly, however. One should exercise caution when working with any tapir that is being “scratched down” as they are capable of inflicting serious injury with their teeth.

Anesthesia of tapirs over the last two decades has typically been accomplished using etorphine at approximately 10 µg/kg i.m. When etorphine became unavailable during the last several years, alternatives have become necessary. Some veterinarians have chosen to use carfentanil (20 µg/kg i.m.) alone or in combination with xylazine. Preliminary evidence from pulse oximetry monitoring indicates that potent opioids may result in poor oxygen saturation in tapirs. Currently, a promising regimen is a combination of butorphenol (0.15 mg/kg i.m.) and an α₂-adrenergic agonist such as xylazine (0.3 mg/kg i.m.) or detomidine (0.05 mg/kg i.m.). Using this combination on 19 T. indicus and T. pinchaque, bradycardia was seen (30-55 bpm) but relative oxygen saturation measured by pulse oximetry was 90-95%. Good relaxation generally occurred after about 10 min. Ketamine (0.5 mg/kg i.v.) can be given if necessary for further restraint. The effects can be antagonized with yohimbine (0.2-0.3 mg/kg i.v.) and a narcotic antagonist such as naltrexone or naloxone, and recovery is generally rapid, smooth, and complete.

Other combinations such as carfentanil/ketamine/xylazine\(^2\) and xylazine (0.8 mg/kg i.m.) with azaperone (0.8 mg/kg i.m.) followed by ketamine (0.5-1.0 mg/kg i.v.) have also been used successfully in tapirs. Azaperone has caused adverse reactions in horses (CNS excitement, etc), and some caution may be indicated in its use with tapirs. No adverse reactions were seen, however, in over 25 uses recorded from this survey. Direct intravenous induction with xylazine and ketamine can be accomplished on some individuals which are “scratched down” prior to venipuncture. Tiletamine-zolazepam has also been used with some success in tapirs in this survey at approximately 1-2 mg/kg (n=2).\(^4\)

Sedation of tapirs to facilitate introductions or for minor standing procedures has been accomplished with azaperone (1.0 mg/kg i.m.) or less reliably with xylazine (1.0 mg/kg i.m.).\(^1\)

Clinical Problems

Clinical disease problems are summarized in Table 2. These clinical and disease correlates were determined from systematically reviewing medical records.

Clinical Pathology
Blood samples can easily be obtained from the medial saphenous or cephalic veins when the tapir is in lateral recumbency. These veins, however, tend to collapse or spasm while giving injections and during catheterization. The jugular vein is also difficult to catheterize but is available for large blood volume draw.

Analysis of hematology and clinical pathology values from MedARKS summaries showed no major differences between species of tapirs. In general, the values followed similar trends to those of other perissodactyls including horses. In our experience, plasma fibrinogen is particularly important for evaluating the presence of inflammation in tapirs and should be included with any hematologic evaluation. The following are approximate reference ranges for select values:

- Hematocrit: 31-47%
- WBC: 5,000-16,000 cells/µl
- Fibrinogen: 100-400 mg/dl
- BUN: 3-20 mg/dl
- Creatinine: 0.5-1.9 mg/dl
- Glucose: 70-120 mg/dl
- Sodium: 128-145 meq/L
- Potassium: 3.1-4.5 meq/L
- Chloride: 85-110 meq/L
- Calcium: 8.5-12.5 meq/L
- Phosphorous: 3.2-7.0 meq/L

Pathology Findings

There were no apparent differences in disease patterns between species. Overall, noninfectious diseases accounted for two-thirds of the 108 mortalities in this survey, and infectious one-third. The greatest number of mortalities were in the neonatal and adult categories.

Most of the 28 deaths in the neonatal age category were stillbirths or deaths from undetermined causes (n=10), or factors relating to maternal behavior (e.g. maternal neglect and trauma, n=6). In addition, there were three cases of fatal aspiration pneumonia in animals being hand-reared due to maternal rejection. Other significant primary causes of mortality included two cases of accidental drowning, two cases of septicemia (one of which is speculative), one case of necrotizing bacterial enteritis, one case of ceco-colonic tympany, and one case of atresia ani.

In the adult age group, the largest proportion of mortalities fell into the gastrointestinal disease category (15/40), and most of these were noninfectious in etiology. Intestinal volvulus, gastric and colonic impactions, and colonic incarceration accounted for six of the cases. Oropharyngeal abscessation was another important problem, accounting for three cases. Bacterial cultures were seldom obtained in these cases, but the syndrome appears similar to oral necrobacillosis. The primary factors initiating these oral lesions were not clear from this survey, but oropharyngeal trauma would be an important consideration. Acute pancreatic necrosis/pancreatitis was found in three cases, which is a relatively high prevalence compared to domestic species. Eosinophilic enterocolitis was the primary finding in two cases. There was insufficient data to determine whether this condition was analogous to the eosinophilic gastroenteritis described in horses. One case of mandibular osteomyelitis (lumpy jaw) was identified. Infectious GI diseases were relatively uncommon in the adult population, with only one case of Salmonella enteritis identified.
Other significant diseases in the adult age-group included a case of *Mycobacterium bovis* pneumonia, three cases of bacterial septicemia, and a case of myocarditis due to encephalomyocarditis virus infection.

The most significant disease problems identified from the survey, arranged by age category, are summarized in Table 2.

Respiratory diseases were the most important cause of mortality overall. The prevalence of respiratory disease was relatively uniform across the various age-groups, but was uncommon in the aged-adult group. In contrast to the gastrointestinal disease category, most respiratory diseases had infectious etiologies. Most cases were of bacterial etiology, with septicemic/embolic pneumonias predominating over bronchopneumonia. The possibility of underlying viral infection in tapir bronchopneumonia cases has apparently not been investigated. The source of the septicemic/embolic pneumonias was not evident in most cases. There were also two cases of pulmonary tuberculosis due to *Mycobacterium bovis*, and one case of pulmonary coccidioidomycosis. The diagnosis of respiratory disease in Malayan tapirs was confounded by the misinterpretation of pleural adhesions as evidence of pleuritis or pneumonia in a number of instances. Noninfectious respiratory diseases included two aspiration pneumonias which occurred during anesthesia. Three other restraint-related deaths occurred (asphyxia, unexpected death under anesthesia, and hyperthermia during transport).

**Medical Management Recommendations**

**Tuberculosis monitoring**

Tuberculosis has been reported repeatedly in the literature and seen in other animals in this survey. Therefore, it is important to develop reliable diagnostic tests for monitoring tapirs for this disease. However, antemortem diagnostic tests have not been validated in tapirs, and interpretation of results is therefore difficult. Nevertheless testing should be performed particularly prior to relocation of an animal. Tuberculin skin testing using an *M. bovis* antigen (ppd bovis, USDA) in the inguinal region near the nipples may be the preferred site. Cervical skin testing is difficult because of the thick nature of the cervical skin. Tapirs lack a true tail fold, but skin around the perineum can be used.

The BTb test, which includes the lymphocyte transformation and ELISA tests, was developed for testing whole blood of cervids for tuberculosis and may be a useful adjunct to antemortem diagnosis. Again, since this test is not validated for tapirs, the results may not be interpretable. Nevertheless, if results on these tests or others suggest an exposure to mycobacteria, it may be worth pursuing further diagnostics such as comparative tuberculin testing, thoracic radiography, and mycobacterial culture of gastric or tracheal samples.

**Vaccinations**

Diseases for which vaccinations are available have not been documented in tapirs. Some authors
have recommended vaccination for equine encephalitis viruses, tetanus, and other clostridial
diseases.19,24

Pregnancy diagnosis

Pregnancy may be difficult to determine in tapirs using conventional means. Breedings are usually
not observed, and even advanced pregnancy may not be obvious. Pregnancy can be detected by
means of urinary and fecal steroid analysis.4,17 Transabdominal ultrasound has been used to diagnose
and monitor pregnancy in *Tapirus bairdi* (R. Wack, personal communication).

Contraception

Currently, a moratorium has been placed on the breeding of *Tapirus terrestris* in North American
collections. As a result, some institutions have devised methods of contraception for this species.
Castration, melengestrol acetate implants, medroxyprogesterone acetate (DepoProvera, Upjohn)
injections at (2.5-5.0 mg/kg), and altrenogest (Regumate, Hoechst-Roussel) are beginning to be used
in tapirs. Other options including porcine zona pellucida vaccine have not yet been evaluated in
*Tapirus*.

Diet and Nutrition

Due to the similarities of gastrointestinal tract anatomy, the domestic horse is typically used as a
model for all tapir species when developing dietary guidelines. When fed diets of alfalfa or timothy
hay, the comparative digestibility of cellulose by three tapir species and a domestic horse was 41%
and 47%, respectively. Digestibility of hemicellulose by the three tapir species and horses was 45%
and 52%, respectively.8,11,26

Species with a limited stomach volume consume several small amounts of feed frequently over time,
instead of one large quantity within a shorter time period. Horses offered large quantities of food
in a single feeding may demonstrate labored breathing and rapid fatigue.5 Severe overeating has
been implicated as a factor leading to colic, ruptured stomach, or founder in domestic horses. As
a result, it is usually recommended that herbivores with hindgut fermentation be fed two to three
times per day. Frequency of feeding has no apparent effect on digestibility of the feed.12

Tapirs should be fed using strategies similar to those used for other perissodactyls. Their diet
typically includes both forages and pelleted feeds. Hay is the most common forage fed to tapirs,
although browse and pasture may constitute a significant portion of the captive tapir’s diet, based
upon the geographic location of the holding facility and the characteristics of the enclosure. Current
studies examining the feeding behavior and foods selected by free-ranging Baird’s tapirs in Cost
Rica may provide additional insight to the nutrient concentrations in plants selected by wild tapirs.7
Pelleted herbivore feeds, typically based on alfalfa, are most commonly fed to captive tapirs.
Different pellets are formulated to be fed at different rates (e.g., complete feeds versus supplements).
A herbivore pellet formulated for grazing ungulates, which contains 15% crude protein, 0.7% lysine,
and 21% acid-detergent fiber (DMB), along with alfalfa hay (18% crude protein, 30% acid-detergent
fiber) (DMB) has been successfully used for feeding all species of tapirs.

There is some preliminary clinical data which suggest tapirs may have a unique metabolic requirement regarding copper. Mean serum copper in samples collected across all four species of tapirs were 0.21 µg/ml (n=22). Dietary copper concentrations appear adequate, when compared to guidelines for horses.\textsuperscript{22} The interaction of copper with other trace elements, including iron, zinc, sulfur and molybdenum may contribute to these clinically low values. The significance of these serum copper concentrations has yet to be determined.

**Neonatal care**

Limited published information is available regarding the composition of maternal milk in tapirs. A single citation, with no reference to stage of lactation, reports the milk of the Malayan tapir is 15.7% solids. The solids fraction of that sample contained 36.3% crude protein, 21.7% ether extract (crude fat), and 42.0% lactose.\textsuperscript{16} The solids, crude protein, and ether extract concentrations from that reference are relatively higher than the same values for milk from domestic horses.\textsuperscript{27} The carbohydrate (lactose) fraction of the tapir milk sample is relatively lower than that of the horse.

The mean birth weight of female, Malayan tapir calves is 10.1 kg (n=4).\textsuperscript{1} The absolute rate of growth in these mother-reared calves was 1.33 kg/d from 0-29 days. Solids are first consumed at 14 days (range 8-19 days) (n=3). Transfaunation, accomplished by feeding strained feces from normal tapirs, has been useful in our experience to encourage growth of normal flora in young tapirs raised in isolation.

Neonatal examinations can be useful for assessing general health and determining the success of immunoglobulin transfer from the dam. It can be a challenge to collect blood from a struggling newborn tapir. The jugular vein is usually the best site for venipuncture in a neonate. Glutaraldehyde coagulation performed on serum will test for the presence adequate immunoglobulins in tapirs. In cases where the calf fails to nurse, it is often possible to encourage the female to lie down and then place the calf on the nipple.\textsuperscript{3} Neonatal isoerythrolysis has been observed in a Baird’s tapir (R. Wack, pers. comm.). Greater effort needs to be directed toward solving management problems related to failure of the maternal-infant bond, and to identifying causes of stillbirths and early neonatal deaths.

**Pre-shipment procedures - guidelines**

Preshipment testing is recommended for any tapir relocation. Relocation may include captive or free-ranging transfers such as reintroduction, translocation, and relocation from one institution to another. Quarantine of individuals should be performed before exposure to animals at the new location. The risk of disease concern may vary greatly depending on the circumstances, and should be factored into any pre-shipment testing strategy. Zero-risk is a desired but seldom practical goal. Governmental regulations need to be considered, but often are not related to actual disease susceptibility or test validity. Interpretation of results may be difficult in some cases due to lack of scientific validation of the tests for tapirs.
The following are recommended guidelines to aid in decision making by veterinarians together with animal managers and biologists faced with planning the safe transfer of a tapir: 1) fecal sample for parasites particularly nematodes and protozoans, 2) fecal culture especially for *Campylobacter* and *Salmonella*, 3) tuberculin skin testing using ppd bovis intradermally in a soft skin area such as in the inguinal area near the nipples, 4) BTb testing at time of tuberculin testing or reading, 5) blood sample for complete blood count, including fibrinogen, and serum chemistries, 6) vaccination if indicated regionally for tetanus, other clostridial diseases, or equine encephalitis, and 7) a complete physical examination including oral, ophthalmic, and foot pad inspections.

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LITERATURE CITED


### Table 1. Clinical signs with disease correlates commonly seen or previously reported in tapirs.

<table>
<thead>
<tr>
<th>Clinical Sign or Problem</th>
<th>Possible etiologies</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Colic                   | 1. bacterial enterocolitis  
                        | 2. intestinal accidents  
                        | 3. sand impaction      | Important to be able to quickly differentiate medical from surgical problems. Treatment and prevention for sand impaction can include psyllium added to the diet on a routine basis. |
| Corneal cloudiness      | 1. excessive light exposure  
                        | 2. trauma              | Etiology not known. Most common in *T. indicus*. Sometimes associated with corneal ulceration. |
| Death, neonatal         | 1. FPT/septicemia  
                        | 2. hypothermia         | Neonatal mortality will be high unless a suitable birthing environment is available. Neonates born to primiparous females may need assistance in getting to nurse. Male should be removed and pools drained for 1-3 wk after birth. |
| Death, sudden           | 1. encephalomyocarditis virus  
                        | 2. intestinal accidents | Not many other causes of sudden death in healthy tapirs. |
| Dermatitis, general     | 1. sarcoptic mange  
                        | 2. dermatophyte (*Microsporum* spp.) | Both reported in European literature, but not seen in this survey.9,25 |
| Diarrhea, chronic       | 1. inappropriate diet  
                        | 2. Bacterial/protozoal enteritis  
                        | 3. eosinophilic enterocolitis | Minimize fruit in diet. Bacterial enteritis most frequently due to *Salmonella, Campylobacter, Giardia* also may cause diarrhea. Ciliates probably do not. Chronic cases may require repeated fecal cultures and endoscopic GI biopsies |
| Vomiting                | 1. overwear of foot pads  
                        | 2. overactivity during introduction  
                        | 3. hard substrate | Severe pad ulcerations can develop when animals become overactive. A hard substrate or continuously wet concrete can also cause foot problems |
| Lameness, acute         | 1. degenerative joint disease  
                        | 2. chronic foot pad ulcers | DJD common in older animals. Should be differentiated from foot pad disease |
| Lameness, chronic       | 1. molar apical abscess  
                        | 2. mandibular osteomyelitis | Common and difficult to treat successfully. Often becomes chronic problem. Occasionally a cause of death when osteomyelitis leads to septicemic disease. |
| Nasal discharge         | 1. guttural pouch infection  
                        | 2. bacterial rhinitis  
                        | 3. pneumonia | Although nasal discharge may represent only upper airway disease, it may also indicate more serious lower airway disease. See above under dyspnea and coughing. |
| Rectal prolapse         | 1. diet, stress         | 2. unknown | Once a common problem in tapirs, but less frequently seen now. |
| Vaginal discharge       | 1. genitourinary tract infection/estrus  
                        | 2. normal urinary calcium excretion | Best differentiated by urinalysis or cytology |
| Cloudy, chalky urine   | 1. chronic failure  
                        | 2. dental disease         | Pulmonary tuberculosis has been seen repeatedly in captive tapirs, and frequently reported in the literature.26 |
Table 2. Most common primary postmortem diagnoses arranged by group. Numbers indicate total cases for each age category.

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<tr>
<th>Organ System</th>
<th>Disease</th>
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<th>AS</th>
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<td>Aspiration pneumonia</td>
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<td>2</td>
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<td>1</td>
<td>6</td>
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Organ systems: RS=respiratory, GI=gastrointestinal, CV=cardiovascular, GN=general, HP=hematopoietic.

Age groups: N=neonatal (<30d), J=juvenile (1-4yr), AS=subadult (1-4yr), A=adult (4-20yr); AA=aged adult (>20yr); Unk=unknown.

*Intestinal accidents include volvulus, torsion, impaction, foreign objects, and tympany.

**Restraint related deaths include anesthetic deaths, aspiration during anesthesia, hyperthermia, and asphyxiation.

***Includes hemolytic anemias and DIC. These were generally considered secondary, but are included here because they were frequent findings.
IMMOBILIZATION OF FREE RANGING BAIRD’S TAPIR (Tapirus bairdii)

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Abstract

In Corcovado Reserve, Costa Rica, five Baird’s tapirs (Tapirus bairdii) were immobilized, with 1.88 mg of etorphine hydrochloride and 7.7 mg of acepromazine maleate and fitted with radio/telemetry collars. The induction time was 2-3.5 min, and the recumbency time was 3-6 min. The total elapsed time was 29-52 min. The anesthesia was reverted with a triple dose of diprenorphine hydrochloride as compared to the dose of etorphine. The recovery was smooth and complete (recovery 4 min average). The preferable technique for the immobilization was from a tree platform using bait. The blood work was within values compared to captive tapirs, except for eosinophilia due to a heavy load of ecto- and endoparasites. The serology showed positive titers to Leptospira interrogans in 3 animals to 8 different serovars.

Resumen

En la Reserva de Corcovado en Costa Rica, cinco Tapires Centro Americanos (Tapirus bairdii) fueron inmovilizados, con 1.88 mg de Hidrocloruro de Etorfina y 7.7 mg de Maleato de Acepromazina para la colocación de collares de radiotelemetría. El tiempo de inducción fue de 2 a 3.5 minutos, y el tiempo de recumbencia fue de 3 a 6 minutos. El tiempo total de manejo fue de 29 a 52 minutos. La anestesia se revertió con una dosis triple de Hidrocloruro de Diprenorfina comparada con la dosis de Etorfina. La recuperación fue suave y completa (4 minutos promedio). La técnica de elección para la inmovilización fue desde una plataforma en un árbol, usando un sebo. El hemograma y química sanguínea se encontró dentro de los rangos reportados para esta especie en cautiverio a excepción de una eosinofilia marcada causada por una alta carga de endo y ecto parásitos. La serología reveló títulos positivos a Leptospira interrogans en 3 animales a 8 diferentes serovariedades.

Introduction

In Corcovado Reserve, southwest of Costa Rica (Central America), during the month of February, five Central American tapirs (Tapirus bairdii) were immobilized and fitted with radiotelemetry collars. The animals were monitored during the anesthesia and samples were collected for hematology, serology, serum chemistry, rectal cultures, fecal tests, and ectoparasites. The anesthesia was reverted and the animals were monitored during the next 24 hr. The radiotelemetry study
continued for the next 2 yr.

Materials and Methods

Preparation of the immobilization sites

By means of tracking, sites constantly used by tapirs were found (feeding, drinking, and resting sites). Bait (fruits and salt) was placed at the sites and monitored during the next 15 days. The sites were chosen far from deep water holes, since these are used as protection by this species when they are feeling threatened.2,5

The darting platforms which were made with braided ropes and hung from trees were prepared and placed 3-10 m off the ground, depending on the site, and between 3-20 m away from the bait point. The area around each site was recognized and mapped in the event that a darted animal got away from the bait site.

Another technique used was the construction of a corral made of bamboo held together with rope. The corral was 1.5 m high and 15 m in diameter, enclosing the baiting area. Two dropping doors were made at each end of the corral, making sure that they opened in both directions of the tapir’s trail. The doors were held open by means of a mechanism at the middle of the corral that was triggered when the animal stepped on it.

The baiting sites were monitored with infrared sensors to know the exact time the animals were there.

Immobilization

For the immobilization, two persons stayed at the platform from dusk until dawn according to reported tapir activity.2,5 Darts (3 ml, Daninject) were used with a 3-inch collared needle and were projected with an air pistol (Daninject). The neck was chosen as the injection site. Etorphine hydrochloride and acepromazine maleate were used (large animal immobilon c/vet limited). Diprenorphine hydrochloride (large animal revivon c/vet limited) was given as the antagonist. During the immobilization, the following records were kept: induction time, time of recumbency, total elapsed time, time of recovery, respiration rates, pulse and temperature.

Sample collection

After the placement of the radio collars, morphometric measures were taken as well as blood samples, rectal cultures, fecal samples, and ectoparasites. The hemogram was performed at the site with a manual technique. The serum was maintained in liquid nitrogen for later processing of serum chemistry and selected serology tests. The rectal cultures were placed in a conservation medium (Aimes medium) and the fecal samples were placed in a conservation solution (PAF) for later evaluation with the flotation and sedimentation techniques.

Results
The immobilization of four animals was done in a 15 day period and the fifth animal was done on a later visit. The animals preferred the banana bait over other fruits and salt. Four animals were immobilized from the tree platforms and one at the corral. The immobilizations were done between 1900 hr and 0200 hr. The animals were illuminated from the platforms with flashlights without any change at all in their behavior. The closest dart shot was from a 5 m distance and the farthest from a 15 m distance. There was no apparent change in behavior when they received the dart impact, and all animals continued eating the bait. The induction, recumbency, total elapsed, and recovery time as well as their physiological constants are shown in Table 1.

The induction was gradual (2-3.5 min), observing a state where the animal would stand without moving, followed by sternal recumbency (3-6 min). The animals were kept in this position during most of the procedure. They were placed in lateral recumbency at the end of the procedure for blood sample collection and the reversal of the anesthesia (total elapsed time, 29-52 min). There was no need to supplement the anesthesia or administer any other kind of drug during the immobilizations.

The dose used for the first animal was 1.61 mg of etorphine hydrochloride and 6.6 mg of acepromazine maleate. Although a good induction was observed, the animal showed continuous head movement and attempts to stand. Therefore, it was decided to raise the dose for the remaining animals to 1.88 mg of etorphine hydrochloride and 7.7 acepromazine maleate. With this dose, a better immobilization was obtained. It should be noticed that the sex and weight of the animal to be immobilized was unknown.

The reversal agent (diprenorphine hydrochloride) was three times the dose used for the etorphine, administering b i.v. and a i.m. The recovery was smooth and complete (recovery, 4 min standard) and no renarcotizations were observed. After reversal, the animals continued eating the bait, and then disappeared into the forest.

The results from the hemogram, serum chemistry, bacteriology cultures and fecal samples are shown in Table 2. The serology results were positive only for *Leptospira interrogans*. Tapir #1 to serovars *ballum* and *bataviae*, with titer of 1:50; Tapir #3 to *sejroe* and *wolffi* with titer of 1:200 and *tarassovi* with titer of 1:50; and Tapir #4 to *ballum*, *cynopteri*, and *paidjan* with titer of 1:50 and *grippotyphosa* with titer of 1:100.

**Discussion**

The immobilization from a tree platform using bait worked best. The animal should be shot as soon as it starts eating the bait, to assure that it will stay in the same site until the anesthesia has taken effect. There is the risk that the animal will disappear into the jungle after being shot and is then easily lost. Therefore, it is important to take into account the attractiveness of the bait, since it will vary between regions.

In theory, the corral that was used for the immobilization of one of the tapirs should have kept the animal from running into the jungle. In this case, the animal did not try to escape before or during the immobilization. But when it was reverted and had finished eating all the bait, it went right
through the walls and out of the corral with no problem. The size and shape of the tapir makes it difficult to build an enclosure with locally available materials that would contain it.

The immobilizations were adequate for the procedure. It was observed that during the next 12 hr that the animals were followed, they lost the fear of our presence. This was attributed to the acepromazine.\(^3\) Also, the second male presented priapism due to the acepromazine.\(^4\) The penis returned to its normal stage in the following few hours.

The etorphine/acepromazine combination has lost popularity because of the thermal regulation problems,\(^1\) but since the anesthesias were done at night, we did not find this to be a problem. All animals were found in clinically good health, having good body weight. The radiotelemetry study is showing that the animals are doing well at the present time. The hemogram showed an eosinophilia probably due to the heavy load of ecto and endo parasites. The rest of the values for the hemogram and the serum chemistry are within values compared to captive tapirs, although some values were lower than the captive reference range.

LITERATURE CITED

**Table 1.** Basic anesthetic event parameters for five *Tapirus bairdii* field immobilization.

<table>
<thead>
<tr>
<th>Tapir ID number/sex</th>
<th>Est. body mass (kg)</th>
<th>I Dosage (mg)</th>
<th>R Dosage (mg)</th>
<th>Time to first effect</th>
<th>Time to recumbency</th>
<th>Time to standing post-R</th>
<th>Total elapse time</th>
<th>Rectal temp. (C)</th>
<th>Resp. rate (range/min)</th>
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<tbody>
<tr>
<td>1/m</td>
<td>300</td>
<td>1.61</td>
<td>3.22 / 1.61</td>
<td>3.5</td>
<td>5</td>
<td>4</td>
<td>52</td>
<td>37</td>
<td>25-30</td>
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<td>2/f</td>
<td>270</td>
<td>1.88</td>
<td>3.76 / 1.88</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>29</td>
<td>37</td>
<td>20</td>
</tr>
<tr>
<td>3/m</td>
<td>350</td>
<td>1.88</td>
<td>3.76 / 1.88</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>41</td>
<td>12</td>
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</tr>
<tr>
<td>4/f</td>
<td>350</td>
<td>1.88</td>
<td>3.76 / 1.88</td>
<td>3</td>
<td>6</td>
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<td>50</td>
<td>37.4</td>
<td>20</td>
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<td>5/f</td>
<td>270</td>
<td>1.88</td>
<td>3.76 / 1.88</td>
<td>-</td>
<td>4</td>
<td>3</td>
<td>30</td>
<td></td>
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<tr>
<td>mean</td>
<td>308</td>
<td>1.826</td>
<td>3.65 / 1.82</td>
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<td>4.4</td>
<td>3.8</td>
<td>40.4</td>
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<td>± SD</td>
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<td>0.12</td>
<td>0.24 / 0.12</td>
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<td>3.0-4.0</td>
<td>29-52</td>
<td>37-37.4</td>
<td>12-27.5</td>
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</table>

I = Immovilon (each ml contains etorphine hydrochloride 2.45 mg-acepromazine maleate 10 mg)
R= Revivon (diprenorphine)

\( f = \text{female} \)
\( m = \text{male} \)
Table 2. Results from hemogram, serum chemistry, bacteriology cultures and fecal samples.

<table>
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<tr>
<th></th>
<th>TAPIR # 1</th>
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<th>TAPIR # 3</th>
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<td>WBC *10^3/UL</td>
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<tr>
<td>HCT %</td>
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<td>27.5</td>
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<tr>
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<tr>
<td>T. PROT. g/dl</td>
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<td>6.2</td>
<td>6.7</td>
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<td>SEG *10^3/UL</td>
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<td>LYMPHOCYT *10^3/UL</td>
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<td>MONOCYTES *10^3/UL</td>
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<td>0.11</td>
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<td>EOSINOPHIL *10^3/UL</td>
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<td>BASOPHILS *10^3/UL</td>
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<td>URIC ACID mg/dl</td>
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<td>CPK IU/L</td>
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<td>LIPASE U/L</td>
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Calculate values

**PARASITE EXAM**

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** FECAL CULTURES **

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<th>Escherichia coli</th>
<th>Providencia rettgeri</th>
<th>Escherichia coli</th>
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HERPESVIRUS INFECTION IN AN INDIAN TAPIR (*Tapirus indicus*) AND IN A BLACK RHINOCEROS (*Diceros bicornis*): CASE REPORTS
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Zoologischer Garten Berlin, Berlin, Germany

Abstract

This paper represents a report of the death of a female Indian tapir far advanced in pregnancy, and a preliminary report of the death of a male black rhinoceros at the Berlin Zoo in 1995. Either case clearly indicates that a herpesvirus infection is to be made responsible for the death of the animals.

As is generally known, both the Indian tapir and the black rhinoceros are highly endangered species. For this it is of greatest importance to put all emphasis on the breeding of these species in zoological institutions. All the more regretful is the loss of a female tapir far advanced in pregnancy, and that of a breeding male black rhinoceros as has been experienced in the Berlin Zoo in 1995.

Resumen

Este documento presenta el reporte de la muerte de un tapir indio hembra en estado avanzado de gestación, y el reporte preliminar de un rinoceronte negro macho en el Zoológico de Berlín en 1995. En ambos casos claramente se observó una infección por herpesvirus que fue la causa presumible de la muerte.

Como se sabe, el tapir indio y el rinoceronte negro son especies en peligro de extinción. Por lo anterior es muy importante poner un gran énfasis en la cría de estos animales en los zoológicos. Lo más deplorable es la pérdida de un tapir indio en estado avanzado de gestación y el de la pérdida de una cría de un rinoceronte negro reproductor como se ha experimentado en el zoológico de Berlín.

Indian Tapir

After decades, finally in March 1995 a healthy male Indian tapir was born at the Berlin Zoo. In consequence of absence of estrus, of conclusive general symptoms, and on the basis of fecal hormone analysis we had even expected an additional birth from our second female in May that year.

Unfortunately, one morning several days before the expected delivery, the animal became restless, showing symptoms of pain, and forced pressing set in. Little liquid feces, yet no urine was discharged. A rectal and vaginal palpation failed to prove impaction of a fetus in the parturient canal as had been suspected, but rather showed a tightly filled urinary bladder pushing down into the pelvis. The animal was administered a spasmolysant.

Together with colleagues of the Clinic of Animal Reproduction of Berlin Free University and because no improvement was noticeable, several hours later the tapir was immobilized with Immobilon® at a dose of 0.9 ml and with xylazine/ketamine at a dose of 40 mg each.
Catheterization of the urinary bladder proved somewhat problematic since the animal kept pressing intensely. Drainage of the bladder produced 4½ L of sanguineous urine. Parturition clearly was not an issue since the *Cervix uteri* was completely closed, yet a rectal ultrasonic examination proved the presence of a living fetus.

Tentative diagnosis was an acute retention of urine in the bladder, presumably caused by flexion of the urethra. That day the animal was administered intramuscular a long-term penicillin preparation and another penicillin preparation directly into the bladder. The next day the animal’s general condition had substantially deteriorated; motivating it to get on its feet was difficult. Here, for the first time observation was made that the animal could not fully stand up, but with bent hind legs could only take a few steps.

Respiration was accelerated and labored. On rectal palpation the urinary bladder showed to be little filled. A long-term penicillin again was administered and additionally Clenbutyrol® (Ventipulin). The next day the weakened female tolerated an infusion of Sterofundin®, glucose, and calcium into the *Vena saphena* on his hind limb. A few hours later the animal was dead.

The summarized post-mortem protocol reads as follows: Primarily evident pathological changes in the lymphoid tissue are foremost found in the body and organo lymphatic nodes, which to a great extent are clearly enlarged, partially marmoreal with hemorrhagic leaks, partial necrotic disintegration of tissue is seen. The gastro-intestinal mucosa shows ulcerative processes with inflammatory depositions. Massive sub-endocardial hemorrhages in the left ventricle are found. The liver seems enlarged, the hepatic tissue friable. Focal adhesive pleuritis is seen in the lungs. The urinary bladder is empty. Detected in the uterus is a fully developed female fetus, the amniotic fluid appears ocher in color and turbid.

Histological findings in the preparations from the different lymphatic nodes are acute inflammatory processes as well as large necrotic lesions. Inclusion bodies (I.B.) are clearly identifiable in the nuclei. In the large parenchyma hemorrhages and degeneration predominate.

*Escherichia coli* bacteria is observed in the intestine, and *Klebsiella pneumoniae* in the lungs. Virological examination of the organo system concludes EHV-1-herpesvirus infection. To support this diagnosis an examination of the central nervous system should have been conducted but was refrained from, as it had been agreed upon to send the carcass to a museum.

**Black Rhinoceros**

One morning in October 1995 our proven male breeder was found dead in his shed without previously having shown any pathological symptoms. An autopsy was performed the same day with the following findings:

Altogether the animal is seriously emaciated. On the gastrointestinal, hepatic, and spleno mucus lining, hemosiderosis is seen.

The gastro glandular mucosa shows numerous ulcers that appear up to 1 cm in diameter.
The mucosa of the duodenum and jejunum shows chronic hypo-generative atrophy with loss of surface epithelium and partial fusion of the villi. Also, indication of bronchitis and myocardial degeneration is seen.

Bacteriological investigation proves the presence of Escherichia coli in liver, spleen, and intestine, and Klebsiella pneumoniae in the lungs.

Virologically a herpesvirus is identified by means of electron microscope.

Discussion

So far as is known, herpesvirus infection in tapirs has not been reported. Consequently, this is the first diagnosed case of herpesvirus-equi-1 infection in a tapir, underlining this species’ close genetic relation to equids.

Herpesvirus-equi-1 infection in equids has been described previously.¹²

In the Berlin Zoo equine degenerative myoencephalopathy (EDM) was frequently diagnosed in Przewalski horses and zebras. In this connection the strong suspicion of the presence of EHV-1 infection was expressed.³⁵

As a result of the virological findings we have since vaccinated all tapirs with an inactivated vaccine against EHV-1 (Resiquin F®, Hoechst).

Also, very few references exist regarding herpesvirus infection in rhinoceroses.

In the Berlin Zoo, in black rhinoceroses, concurrently three cases were observed of small cutaneous ulcers in great number as well as disturbance of the general state of health.⁴

A biopsy under the electron microscope revealed the presence of herpesvirus-like particles. However, absolute proof of the involvement of a herpesvirus could not be furnished.

In the case at question, organic material was inoculated to an aerobic cultivated hen’s egg, isolating an agent which under the electron microscope was identified as herpesvirus. Whether or not and EHV-1 infection was involved could not be clarified.

Hypo-generative atrophy of the mucosa as is registered after infection with an entero-pathogenic virus, was seen in this individual as well as the other 2 who had died of hemolytic anemia at the Berlin Zoo.⁵

Consequently, necropsies in rhinoceroses should always be performed with regard to possible involvement of viruses in the pathological processes.

LITERATURE CITED


THE POPULATION STATUS OF RHINOCEROSES

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Abstract

Clearly, rhinoceroses are one of the most endangered taxa of “charismatic megavertebrates” in the world. Due to poaching and habitat destruction, nearly all wild rhinoceros populations have suffered dramatic declines and are in crisis. About 12,000 rhinoceroses of all species survive in the wild and over 50% are white rhinoceroses (Ceratotherium simum simum) in South Africa (see Table 1). Currently about 1000 rhinoceroses are maintained in captivity--about 8% of all living rhinoceroses. Again, over 50% of this population is southern white rhinoceroses (see Table 2).

Resumen

Definitivamente, el rinoceronte es uno de los taxones más amenazados entre los megavertebrados carismáticos del mundo. Debido a la invasión y destrucción de su hábitat casi todas las poblaciones de rinocerontes salvajes han sufrido una declinación dramática, encontrándose actualmente en una situación crítica. Cerca de 12,000 rinocerontes de todas las especies sobreviven en estado salvaje y cerca del 50% son rinocerontes blancos (Ceratotherium simum simum) de Sudáfrica. Actualmente cerca de 1,000 rinocerontes son mantenidos en cautiverio (cerca del 8% del total de los rinocerontes). Dentro de esta población el 50% son rinocerontes blancos de Sudáfrica.

Wild Populations

Poaching remains the major threat to wild rhinoceros populations. Additionally, the status of the Sumatran rhinoceros (Dicerorhinus sumatrensis) is further endangered by habitat destruction due to logging. Poaching is driven by the demand for rhinoceros horn, creating prices that rival those of gold on a per ounce basis. The result is a market with economic forces similar to those in the drug trade, e.g., hazards of poaching, even in countries with “shoot-on-site” policies are worth the risk. Currently, the majority of the horn goes to the Asian pharmaceutical trade where it is used in anti-pyretic and anti-inflammatory medications. A smaller market exists in some areas, most notably Gujarat state of India, for rhinoceros horn as an aphrodisiac.

The results of the demand have been dramatic - the 60,000 black rhinoceroses (Diceros bicornis) in 1970 sub-Saharan Africa have been reduced to 2500, the northern white rhino population has been reduced to a mere 31, the Sumatran rhino has been decimated to fewer than 500 on the verge of extinction, and both the Indian and Javan rhinos are under constant and perhaps increasing challenge. The southern white rhino has been the least affected although locally some populations
have been heavily poached.

Currently in the wild, the most numerous black rhinoceros populations are in southern Africa: South Africa (over 1000), Namibia (600+), and Zimbabwe (300). Eastern Africa, most notably Kenya has another approximately 600 black rhinoceroses. The most successful programs for protection of rhinoceroses in East Africa have involved maintaining populations in small, fenced ranches and parks that are heavily protected. In Zimbabwe, rhinoceros protection has also adopted a sanctuary policy on both governmental and private lands. Additionally, Zimbabwe has been a leader in a dehorning program designed to remove the poacher’s primary motive for killing; however, some poaching of even dehorned individuals has taken place.

The southern white rhinoceros populations exist almost wholly in South Africa. What had been the largest population outside South Africa, the 100 animals in Hwange National Park in Zimbabwe, has been severely reduced by poaching. In South Africa, the population has reached 6500+ animals from a low of fewer than 100 in the 1920s. The story of this program, centered at Umfulozi/Hluhluwe Reserves in Natal is one of the classic conservation success stories of the early part of this century. However, the future of this species is also dependent on the stability and protection of one government. At the present time, the northern white rhinoceros (C. s. cottoni) is known to exist in the wild only in Garamba National Park in Zaire. Currently there are 30 animals, up from fewer than 15 individuals 10 yr ago.

Greater Asian one-horned rhinoceros (Rhinoceros unicornis) populations are split between India (~1600) and Nepal (~ 500). Populations continue to increase at a modest rate, although poaching pressures in both countries are significant. In addition to shooting, poaching there has included poisoning and even electrocution (hanging wires down from high tension lines).

In the same genus, is the Javan rhinoceros (Rhinoceros sondaicus). Before 1994, this species was thought to be limited to 50-60 individuals in Ujong Kulong National Park in Indonesia. However, in 1995 evidence arose that there may be a small (estimated at ~10 individuals) remnant mainland population in a remote area of southern Vietnam.

Lastly, is the Asian two-horned or Sumatran rhinoceros. This species is found both on Sumatra and in peninsular (mainland) Malaysia. At approximately 500 kg, it is the smallest of the rhinoceros species. Fewer than 800 individuals exist and they are severely threatened by both poaching and habitat destruction from logging.

At the present time, all trade in rhinoceros horn and other parts is banned under the Convention on the International Trade in Endangered Species (CITES). However, the southern African states (South Africa, Zimbabwe, and Botswana) have argued for a controlled trade feeling that sustainable use is preferable to the present market. That debate is ongoing. An excellent reference for those interested in this discussion is the book, At the Hand of Man, by Raymond Bonner.

Captive Populations

Four of the five recognized species are currently held in captivity (only the Javan rhinoceros
Captive populations of rhinoceroses are managed under the direction of a Global Captive Action Plan (GCAP) and its Global Animal Survival Plan (GASP) established under the World Conservation Union’s (WCU, formerly the IUCN) Conservation Breeding Specialist Group (CBSG).

In these captive programs, each African rhinoceros species is managed as two subspecies: the Eastern (D. b. michaeli) and Southern (D. b. minor) black rhinoceros and the southern (C. s. simum) and northern (C. s. cottoni) white rhinoceros. The two Asian species in captivity, the greater Asian one-horned rhinoceros (Rhinoceros unicornis) is managed as a single species while the Sumatran rhinoceros (Dicerorhinus sumatrensis) is currently being managed more or less as two subspecies (Dicerorhinus sumatrensis sumatrensis and Dicerorhinus sumatrensis harissoni).

One example of a regional management plan would be the North American Species Survival Plans (SSPs) and the individual SSPs are coordinated by the Rhinoceros Taxon Advisory Group (TAG). Each SSP Committee and the TAG designates advisors from the zoo, academic and field biology communities to address specific needs, e.g., veterinary medicine, nutrition, and research (see Table 3). The goal of these captive management plans is to maintain rhinoceros populations that are demographically and genetically viable. All rhino in captivity are considered to be under a demographic imperative, i.e., demographic considerations are more important than genetic concerns in management at this time. The emphasis is on reproduction with the goal of ensuring that the captive populations are self-sustaining. Genetic goals and guidelines are still important where they do not conflict with the demographic ones. In general, the genetic goal is to preserve in the captive populations 90% of the average gene diversity that occurs in the wild populations for 100-150 yr (i.e., 7-10 rhino generations.).

In designing a program to fulfill these goals, each rhinoceros species and subspecies represents unique challenges that will be discussed below. The genealogy of each species is maintained via a studbook. Table 3 lists the location and keeper for each studbook.

Worldwide, there are currently 700-900 African rhinoceroses in captivity (220-225 black and 500-700 white rhinoceroses). The uncertainty derives from incomplete reporting to the studbooks. There are 134 greater one-horned Asian and 21 Sumatran rhinoceroses in zoological parks. One goal of the regional management programs is to develop target populations of 1000 rhino in 10 yr and 1700 in 100 yr distributed among the various species as indicated in Table 4. This will be done primarily through captive reproduction. The allocation of space among species and subspecies will be reconfigured as indicated in Table 4.

Indeed, the spaces occupied by these rhinoceroses are often interchangeable. Thus, one role of the TAG is coordination of efforts and spaces of all rhinoceros facilities in North America.

**Eastern Black Rhinoceros (D. b. michaeli)**

There are 155 definite and 21 probable individuals of the eastern black rhinoceros subspecies in world zoological collections with 68 in SSP facilities in North America. The current world
population has a relatively good founder base. In North American 80% of the population is captive-born. The present rate of reproduction appears adequate for maintaining a stable or slowly increasing population; however, there are several management and demographic problems that are cause for concern. Disease has been a major factor limiting the growth of this population (see next section) and the population has an undesirable age and sex structure. In North America, in any given year, approximately 15% of the adult females give birth (65% of all adult females have produced calves). However, 66% of adult females are greater than 22 yr of age, and presumably past their breeding prime (a recent calf born to a 31-yr-old female at the Sedgwick County Zoo in Wichita, Kansas exceeded the previous record of a 27-yr-old dam).

One impediment to population growth has been prolonged intercalving intervals in captivity.8 It has been estimated that the use of early weaning and other management techniques that allow for earlier introduction of the male, may reduce the intercalving interval to 24-30 mo from the present 40 mo. This situation would approach intercalving intervals seen in the wild. Thus production could be doubled from the present group of proven breeder females. By the year 2000, the African Rhino GCAP recommends expansion of the world population to 200 animals with 90 as the target for the American Zoo and Aquarium Association (AZA) SSP Masterplan population.

Southern Black Rhinoceros (D. b. minor)

Forty-seven animals of the southern subspecies are maintained in captivity with 29 in SSP facilities in North America. The largest populations are in North America and Australia. Of note are several ranch facilities in Texas that hold the southern subspecies as part of a cooperative effort with the AZA’s SSP program. The majority of 43 southern black rhinoceroses that have been imported into captivity over the past 10 yr have been from Zimbabwe with a few from South Africa. A complication of this program has been the death of 30% of the last 20 animals imported from Zimbabwe within 6 mo of arrival (losses after that period appear to be unremarkable). These deaths may have been due to stress and possibly toxic factors such as creosote4 that can be avoided in the future. The population currently exhibits a desirable age distribution with majority of the animals in their breeding prime. Twenty-seven percent of the population is captive-bred and born, and when animals that arrived pregnant are included, approximately 60% of the females are proven breeders. At the present time, it appears the population has a good start towards becoming self-sustaining. As with the eastern subspecies, it is a goal to maintain a 30 mo intercalving interval.8 The target population goal of the Rhino GCAP is 80 individuals by the year 2000 while the AZA SSP Rhino Masterplan recommends that this subspecies be expanded to 50 animals in North America by that time.2

Southern White Rhinoceros (C. s. simum)

Currently there are 500-700 southern white rhinoceroses in captivity worldwide with about 125 in SSP facilities in North America. Although there was a dramatic influx of founder animals from Natal in the 1970s, many of these animals have not bred in captivity and are now growing senescent. The limited founder base is compounded by over-representation of a few individuals (75% of the F1
The most successful captive breeding situations have occurred when a single or several males were grouped with multiple females. Breeding has also taken place in pairs when animals were introduced in adulthood, or when previously platonic pairs were moved to new environments. In an attempt to increase the founder representation of this aging population, preference has been given to moving nulliparous animals to successful breeding groups. The Rhino GCAP has a goal of 515 southern white rhinoceroses in institutions by the year 2000 with 120 as the SSP target for the same time frame.\textsuperscript{2,7}

**Northern White Rhinoceros (C. s. cottoni)**

The status of the captive population of the northern white rhinoceros is ominous.\textsuperscript{2} Only 9 individuals, nearly all of which are 20+ yr of age, are held in captivity. At the present time the status and future of these animals is uncertain. Intensive efforts are underway to evaluate and manipulate these animals reproductively to induce breeding. Additionally, scenarios are being explored to combine some or all of the captive animals, augmented by a few from Garamba National Park, in an environment more conducive to breeding.

**Great Asian one-horned rhinoceros (Rhinoceros unicornis)**

There are 134 greater Asian one-horned rhinoceroses in captivity. This population is primarily based in zoos in Europe, North America and India. In general the population is self-sustaining and the global plan is for 145 in zoological parks by the year 2000 with 50 the designated target for the AZA SSP population. Medical problems of this species in captivity are limited, although they do seem to have a higher incidence of abortion/stillbirth, foot problems and uterine leiomyomas than other rhinoceros species.

**Sumatran rhinoceros (Didermocerus sumatrensis)**

There are currently 21 Sumatran rhinoceroses in captivity. Captive management of this species has been complicated by a high death loss in captivity and a failure of these animals to breed in captivity outside of their range states. The North American population currently consists of 3 animals, all housed at the Cincinnati Zoo. There is a goal of 32 captive Sumatran rhinoceroses in captivity worldwide by the year 2000 to be achieved by reproduction of the current 21. A major initiative for attempted remediation of the poor performance of the captive programs is development of managed breeding centers in native habitat in Indonesia and Malaysia, to which individuals currently in captivity would be repatriated.

**GENERAL MANAGEMENT**

Management of each species is dependent on the characteristics of that species. For example, black rhinoceroses are generally housed in pairs or trios that are often kept separate outside of the breeding season. This is similar to their natural behavior in the wild. In contrast, white rhinoceroses are compatible in herd situations, and predictable breeding only occurs in larger groups of animals. Again, this mimics their wild behavior. Animals in breeding situations should preferably have a large yard, and if not, “run-around” capabilities so that a mate may not be trapped in a “blind”
corner. This is particularly important with black, greater one-horned Asian and Sumatran rhinoceroses in which breeding behavior is often combative.

For all rhinoceros species, adequate space is a must, and the large and potentially destructive nature dictates heavily barred or moated enclosures. Wood treated with creosote or its derivatives should never be used in the construction of rhinoceros enclosure. In colder climates, supplemental heat must be provided if there is prolonged exposure to subfreezing temperatures. When bars are used they should be vertical as horizontal bars present a higher risk of horn breakage if caught under the crossbars.

A manual that reviews the overall management of these species is in preparation.3

**VETERINARY CONCERNS**

Many of the medical conditions of rhinoceroses are similar to those of the horse. One example is the gastrointestinal system. Rhinoceroses can experience colic, up to and including intestinal torsions. A bibliography reviewing rhinoceros veterinary literature exists.6

All rhinoceroses should be checked for gastrointestinal parasites and treated accordingly. In general, once free of parasitic infection, reoccurrence is low. For many species, such as the gastric botfly of rhinoceroses, intermediate hosts may be missing in captive situations. Except for the use of leptospirosis bacterins in black and perhaps greater Asian one-horned rhinoceroses (a leptospiral-induced abortion has been reported in the latter species), vaccinations are not routinely practiced (it should be noted that, although infrequent, apparent adverse reactions to the leptospiral vaccination have included acute weakness and skin sloughing—all affected animals have survived). An ongoing program of vermin control is vital to maintaining sanitation and preventing the spread of disease.

Mammalian tuberculosis has been reported in several species of rhinoceroses and there is no reason to believe that they are not all susceptible. Testing has not been fully standardized, but several black rhinoceros that were infected with *Mycobacterium bovis* responded to the use of PPD bovis in the eyelid. Salmonellosis, including fatalities, has been reported in black rhinoceroses and again, it is prudent to consider all species susceptible.

There is some species-specific variation in the disease problems for rhino in captivity. The diseases of white rhinoceroses are fairly routine and mimic those that would be expected in populations of domestic large animals. However those of the black rhinoceros are much more common and unusual. In the black rhinoceros these include syndromes of hemolytic anemia, mucocutaneous ulcers, encephalomalacia, and fungal pneumonia. The causes of these syndromes are not all well-understood, however promising ongoing research may link apparently variant cellular metabolism in this species (much lower levels of intracellular glucose and the possible use of alternative energy pathways) with the etiologies of these syndromes. In the cases of acute hemolytic anemia in the black rhinoceros, at least 50% of the cases have been associated with *Leptospirosis interrogans* infection. Given that information, vaccination with a 5-way bacterin including the serovars for *icterohaemorrhagica* and *grippotyphosa* have been recommended. Indian rhinoceros have been notable for uterine leiomyomas, foot infections, and a possibly increased rate of stillbirths and
abortions. In at least one case, an abortion was associated with infection with *Leptospirosis interrogans*. Although their captive numbers are small, Sumatran rhinoceroses have been diagnosed with gastrointestinal torsions, sepsis resulting from old snare wounds, uterine neoplasia and poor reproduction.

Anesthesia is relatively safe in all species and either standing or full anesthetic regimens have been employed. These generally are based on the use of reversible narcotics etorphine or carfentanil.

Nutrition of captive rhinoceroses is an ongoing challenge and an area that requires further research. We know that in the wild, black and Sumatran rhinoceros are browsers, white rhinoceroses are grazers, and that the greater Asian one-horned rhinoceroses is generally classified as intermediate, but is mostly a grazer. The challenge arises in trying to present reasonable approximations of these natural diets to captive animals. Current recommendations are for grass hays for grazers and a mixture of grass and lucerne hay to the browsing species. Whenever possible, fresh browse plants should be fed to browsing species. Levels of vitamin E (α-tocopherol) should be monitored as several studies have noted lower levels in captive rather than wild rhinoceroses.

**RESEARCH**

In North America, under the direction of the Taxon Advisory Group’s Research Advisory Group (RAG), a comprehensive, coordinated research program is being developed to address many of the problems in the captive management of these species. A list of scientific advisors is included in Table 3. Included in this effort will be a program to address health, reproduction, behavior, genetics and husbandry of these species. Reproductive studies will include better methods of cycle determination, semen collection, and possibly artificial insemination and embryo transfer. If successful, the latter two procedures may in turn, prove useful in moving genetic material not only between zoological parks, but also from zoos to the wild, and even from park to park in the wild.

**Summary**

The very survival of most rhinoceroses as a species and/or subspecies is challenged by poaching and habitat destruction. Continued and improved methods of protection in field are necessary to maintain these remnant populations. In addition, despite the challenges that each of these species/subspecies presents in establishing viable and self-sustaining populations in captivity, progress is being made. Recent animal moves and pairings, plus management changes will hopefully result in increased reproductive rates and more equal genetic representation for these populations.

It seems reasonable to note that the survival of black rhinoceroses in eastern Africa has been in small, managed parks; preserves that contain isolated, protected populations. Thus, as wild populations become more threatened, the demographic, management and genetic principles of captive breeding programs become important to their survival as well. As captive populations become larger through regional management programs, and many wild populations become more fragmented and isolated, the management techniques will grow more similar and offer increased opportunities for interaction between the wild and captive communities.
LITERATURE CITED


Table 1. World populations of rhinoceroses. 2

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<th>Species</th>
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Table 2. Rhinoceros populations under intensive management ex situ or in situ on both global and regional levels current numbers 1995.*

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*Numbers are current through 1 January 1996 for North America; 1 January 1995 for all other continents.*

Note: Numbers in this Table differ from totals provided in the International Studbooks. Numbers in this Table have been differentiated as Definite (DFNT) and Possible (PSBL). Definite are from places that have reported to the International or Regional Studbooks. Possible are animals registered as living in the International Studbooks but for which their institution has not communicated with the Studbook Keeper. Numbers have also been slightly modified based on personal knowledge of T.J. Foose.

T.J. Foose - 1 February 1996
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>African Rhino</td>
<td>239</td>
<td>61</td>
<td>300</td>
<td>242</td>
<td>20</td>
<td>262</td>
<td>26</td>
<td>28</td>
<td>54</td>
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<tr>
<td>Asian Rhino</td>
<td>33</td>
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<td>33</td>
<td>48</td>
<td>0</td>
<td>48</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>All Rhino Taxa</td>
<td>272</td>
<td>61</td>
<td>333</td>
<td>290</td>
<td>20</td>
<td>310</td>
<td>27</td>
<td>28</td>
<td>55</td>
</tr>
</tbody>
</table>

*Numbers are current through 1 January 1996 for North America; 1 January 1995 for all other continents.

Note: Numbers in this Table differ from totals provided in the International Studbooks. Numbers in this Table have been differentiated as Definite (DFT) and Possible (PER). Definite are from places that have reported to the International or Regional Studbooks. Possible are animals registered as living in the International Studbooks but for which their institution has not communicated with the Studbook Keeper. Numbers have also been slightly modified based on personal knowledge of T.J. Foose.

T.J. Foose - 1 February 1996
Table 3. Coordinators and advisors to the North American regional management plans.

<table>
<thead>
<tr>
<th>Rhinoceros Taxon Advisory Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coordinator: Robert Reece, The Wilds</td>
</tr>
<tr>
<td>Program Officer: Dr. Tom Foose, The Wilds and IRF</td>
</tr>
<tr>
<td>Research Coordinator: Dr. Evan Blumer</td>
</tr>
<tr>
<td>Veterinary Advisor: Dr. Eric Miller, St. Louis Zoo</td>
</tr>
<tr>
<td>Nutritional Advisor: Dr. Ellen Dierenfeld, Wildlife Conservation Society</td>
</tr>
<tr>
<td>Reproduction Advisor: To be named</td>
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</table>

<table>
<thead>
<tr>
<th>Black Rhinoceros SSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coordinator (eastern subspecies): Edward Maruska, Cincinnati Zoo, Cincinnati, Ohio</td>
</tr>
<tr>
<td>Coordinator (southern subspecies): Dr. Don Farst, Gladys Porter Zoo, Brownsville, Texas</td>
</tr>
<tr>
<td>Veterinary Advisor: Dr. Eric Miller, St. Louis Zoo</td>
</tr>
<tr>
<td>Pathology Advisor: Dr. Richard Montali, National Zoo</td>
</tr>
<tr>
<td>Nutritional Advisor: Dr. Ellen Dierenfeld, Wildlife Conservation Society</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>White Rhinoceros SSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coordinator: Michael Fouraker, Fort Worth Zoo</td>
</tr>
<tr>
<td>Veterinary Advisors: Dr. Michael Briggs, Chicago Zoological Park and Dr. Doug Page, Jacksonville Zoo</td>
</tr>
<tr>
<td>Pathology Advisor: Dr. Robert Murname, Chicago Zoological Park</td>
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</table>

<table>
<thead>
<tr>
<th>Greater Asian One-horned Rhinoceros</th>
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</thead>
<tbody>
<tr>
<td>Coordinator: Mike Dee, Los Angeles Zoo</td>
</tr>
<tr>
<td>Veterinary Advisor: Dr. Scott Citino, White Oak Conservation Center</td>
</tr>
<tr>
<td>Pathology Advisor: Dr. Don Nichols, National Zoo</td>
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<table>
<thead>
<tr>
<th>Sumatran Rhinoceros</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veterinary Advisor: Dr. Mark Campbell, Cincinnati Zoo</td>
</tr>
<tr>
<td>Pathology Advisor: Dr. Linda Lowenstein, Zoological Society of San Diego</td>
</tr>
<tr>
<td>Nutrition Advisor: Dr. Ellen Dierenfeld, Wildlife Conservation Society</td>
</tr>
</tbody>
</table>
Table 4. Rhinoceros populations under intensive management ex situ and in situ on both global and regional levels target numbers 1995.

<table>
<thead>
<tr>
<th>RHINO TAXON</th>
<th>WORLD</th>
<th>AFRICA</th>
<th>ASIA</th>
<th>AUSTRALASIA</th>
<th>EUROPE</th>
<th>N. AMERICA</th>
<th>C.&amp;S.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target 10/50/100 Years</td>
<td>Target 10/50/100 Years</td>
<td>Target 10/50/100 Years</td>
<td>Target 10/50/100 Years</td>
<td>Target 10/50/100 Years</td>
<td>Target 10/50/100 Years</td>
<td>Target 10/50/100 Years</td>
</tr>
<tr>
<td>Eastern Black</td>
<td>200/240/240  10 ?  40/40/40</td>
<td>0</td>
<td>0</td>
<td>65*/100/100 +fndr?</td>
<td>90/90/90</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Southern Black</td>
<td>80/160/400  50</td>
<td>0</td>
<td>20*75/250 + 6 fndr</td>
<td>0</td>
<td>50/80/80</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Sthwstrn Black</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nthwst Black</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Northern White</td>
<td>?</td>
<td>?</td>
<td>0</td>
<td>0</td>
<td>?</td>
<td>?</td>
<td>0</td>
</tr>
<tr>
<td>Southern White</td>
<td>515/525/500  0</td>
<td>150/??</td>
<td>45*/125/250 + 30 fndr</td>
<td>200/??</td>
<td>120*/120/120 + 10 fndrs</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Indian/Nepalese</td>
<td>145/250/250  0</td>
<td>55/80/80</td>
<td>0</td>
<td>40*/80/80 +?fndrs</td>
<td>50/90/90</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Indian (Java)</td>
<td>?</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Indian (Vietnam)</td>
<td>?</td>
<td>0</td>
<td>?</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>?</td>
</tr>
<tr>
<td>Mainland Sumatran</td>
<td>12/40/100**  0</td>
<td>12/40/50</td>
<td>0</td>
<td>(50)**</td>
<td>0</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Sumatran Sumatran</td>
<td>12/40/100**  0</td>
<td>12/40/50</td>
<td>0</td>
<td>0</td>
<td>10/20*/50 +10 fndr</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Borneo Sumatran</td>
<td>8/25/100**  0</td>
<td>8/25/50</td>
<td>(50)**</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>African Rhino</td>
<td>795/925/1140  60</td>
<td>140</td>
<td>65/200/500</td>
<td>220</td>
<td>290</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Asian Rhino</td>
<td>177/355/550  0</td>
<td>230</td>
<td>(50)**</td>
<td>130</td>
<td>140</td>
<td>?</td>
<td></td>
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<tr>
<td>All Rhino Taxa</td>
<td>1000/1300/1700  60</td>
<td>277/300/400</td>
<td>65/200/500</td>
<td>305/300/300</td>
<td>320/400/430</td>
<td>?</td>
<td></td>
</tr>
</tbody>
</table>

* The “+ number” indicates that the indicated target includes the acquisition of this number of new founders which are thus included in the target total.

** A desirable target if and when husbandry of this species can be mastered and sufficient founders for ex situ populations can be produced by captive propagation programs within range states.

T.J. Foose - 1 February 1996
FUNGAL PNEUMONIA IN BLACK RHINOCEROS (*Diceros bicornis*)

Martha Weber, DVM, R. Eric Miller, DVM*
St. Louis Zoological Park, 1 Government Drive, St. Louis, MO 63110-1396, USA

Abstract

Fungal pneumonia is an uncommon disease entity in domestic mammals, occurring principally in animals with severe primary disease or in animals that are immunocompromised. A survey of U.S. institutions holding black rhinoceros identified eight animals that had fungal respiratory disease at the time of death. The predominant fungus involved was *Aspergillus* spp. All black rhinoceros with fungal pneumonia had concurrent disease, including some with hemolytic anemia and/or mucocutaneous ulcers. Premortem diagnosis and treatment in the rhinoceros are challenging, making this a condition that may prove difficult to manage.

Resumen

La neumonía fúngica es una enfermedad poco común en mamíferos domésticos, ocurriendo principalmente en animales con una enfermedad primaria severa o en aquellos animales que están inmunodeprimidos. Un estudio de instituciones de Estados Unidos que tienen rinocerontes negros identificó ocho animales que tenían una enfermedad respiratoria fúngica en el momento de la muerte. El hongo predominantemente involucrado fue *Aspergillus* spp. Todos los animales con neumonía fúngica tenían otra enfermedad concurrente, incluyendo, en algunos casos, anemia hemolítica y/o úlceras mucocutáneas. El diagnóstico antemortem y el tratamiento en el rinoceronte representa un desafío, ya que esta condición puede ser difícil de manejar.

Introduction

Black rhinoceros in captivity are affected by a number of unusual diseases that have been previously described, including hemolytic anemia, mucocutaneous ulcers, and encephalomalacia. The etiologies of these diseases are not fully understood and at this time treatment is largely supportive, consisting of variable courses of antibiotics, corticosteroids, and nutritional supplements. A review of necropsy results from black rhinoceros showed that a seemingly large number of animals that had died or were euthanatized had evidence of invasive pulmonary fungal disease at necropsy. As fungal pneumonias are rare in domestic animals, including equids, a survey was sent to veterinarians at U.S. institutions holding black rhinoceros asking for information regarding occurrence of pulmonary fungal disease in their collections. Information was also requested regarding the use of corticosteroids in black rhinoceros at these institutions because of the potential immunosuppressive effects of these drugs and because of one previously reported case of a black rhinoceros that died of a systemic
mycotic infection after a long course of corticosteroid therapy.4

Results

Thirty surveys were sent out; at the time of writing 22 institutions had responded. Between the years 1980-1994 eight animals were reported to have had evidence of fungal respiratory disease at necropsy or on histopathology. During the same time period the North American Regional Studbook for black rhinoceros lists 57 animals greater than 1 yr of age that died, an incidence of 14%. Seven of the eight animals died between 1988-1994, a time period in which 28 black rhinoceros greater than 1 yr-old died, an incidence of 25%. All rhinoceros with fungal respiratory disease were affected with concurrent disease (anemia, mucocutaneous ulcers, tuberculosis). Corticosteroid use in the affected animals was sporadic, with two animals reported to have been on long-term steroid therapy immediately prior to death. Institutions reporting steroid use in black rhinoceros generally used corticosteroids as a single dose as an adjunct to anesthesia or as part of a short-term therapeutic regime. Most affected animals had been treated with broad spectrum antibiotics.

The most common findings on gross necropsy were multifocal firm nodules distributed throughout the lung lobes. Some nodules were foci of mineralization or fibrosis while others contained purulent debris. One animal had mats of fungi present in the trachea but no other reported gross evidence of fungal pneumonia. Microscopic lesions included extensive pulmonary necrosis with granulomatous inflammation, fibrosis, and mycotic emboli. Most fungi seen on histopathology were morphologically determined to be Aspergillus spp. These fungi (Aspergillus spp.) were also the organisms most frequently cultured; one animal had concurrent infections with Mucor spp. and Aspergillus spp.

Discussion

Systemic fungal infections in most animals are rare and fungal pneumonia is infrequently reported.6 One retrospective study examined necropsy results from 7,020 horses and confirmed 19 cases of fungal pneumonia, an incidence of 0.27%.5 In the above study some cases may have been missed on histopathologic examination as fungal pneumonias are often localized. However, there is still an apparently increased incidence of fungal pneumonia in black rhinoceros when compared to their most closely related domestic species. Possible explanations include increased environmental exposure to fungi, increased incidence of severe disease, and/or an inherent immunologic abnormality. The change in incidence from 14% to 25% between 1980-1994 and 1988-1994 is most likely due to improved institutional record keeping and more intensive postmortem diagnostic testing.

The majority of domestic mammals with fungal pneumonia have a serious primary disease such as enterocolitis, organ failure, neoplasia, or septicemia.6 Others are predisposed to infection by the use of corticosteroids or broad spectrum antibiotics. All the black rhinoceros identified in this survey had severe concurrent disease. Most had suffered bouts of mucocutaneous ulceration and many had had episodes of hemolytic anemia. Two animals were determined to be infected with Mycobacterium spp. The affected rhinoceros may have
been immunocompromised by their concurrent disease, however further studies are necessary to evaluate the black rhinoceros immune system and response to stress. The use of corticosteroids is not definitively related to the presence of fungal pneumonia but caution should still be exercised with the use of long-term or high-dose corticosteroid in sick rhinoceroses. Due to the alteration of bacterial flora, the use of broad spectrum antibiotics may also be an area of concern.

Premortem diagnosis of fungal pneumonia in black rhinoceros is difficult. *Aspergillus* spp. are ubiquitous in the environment and tracheobronchial lavage of normal horses can reveal the presence of fungal hyphae, either free or within mononuclear cells. Radiography is not likely to be a useful option because of the size of the animals. Percutaneous lung biopsy may miss a site of infection due to the localized nature of fungal pneumonias. Serology has been shown to be of questionable value. Normal horses can have high titers against *Aspergillus* spp. due to environmental exposure, and animals that are immunosuppressed may not develop effective antibody titers.

Systemic antifungal agents such as amphotericin B, ketoconazole, miconazole, or itraconazole have been considered for treatment. These agents have not been especially effective in humans with invasive pulmonary aspergillosis and the cost of long-term therapy with these drugs in black rhinoceroses would be prohibitive.

At the present time, invasive fungal pulmonary disease appears to occur with unusually high frequency in black rhinoceroses. The association with other severe diseases, the difficulty of premortem diagnosis, and the lack of effective and available therapy combine to create a clinical challenge. It is important to be aware of fungal pneumonia as a potential complicating factor when treating a sick black rhinoceros.

**ACKNOWLEDGMENTS**

This project was supported by a grant from Mallinckrodt Veterinary Inc. The authors wish to thank the following participating institutions: Bass-El Coyote Ranch, Cheyenne Mountain Zoo, Cincinnati Zoo, Columbus Zoo, Dallas Zoo, Denver Zoo, Detroit Zoological Institute, Fort Worth Zoo, Fossil Rim Wildlife Center, Gladys Porter Zoo, Lincoln Park Zoo, Metro Washington Park Zoo, Miami Metrozoo, Milwaukee County Zoo, National Zoo, Oklahoma City Zoo, Potter Park Zoo, Riverbanks Zoological Park, San Francisco Zoo, White Oak Conservation Center, Wildlife Conservation Society, Zoological Society of San Diego.

**LITERATURE CITED**

IS IMPAIRMENT OF OXIDANT NEUTRALIZATION THE COMMON DENOMINATOR AMONG DIVERSE DISEASES OF BLACK RHINOCEROSSES?

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UCLA Hematology Research Laboratory, Department of Pathology and Laboratory Medicine, UCLA School of Medicine, Los Angeles, CA 90095-1732, USA

R. Eric Miller, DVM
St. Louis Zoological Park, St. Louis, MO 63110-1396, USA

Stephen W. Renner, MD
Department of Pathology and Laboratory Medicine, Veterans Affairs Medical Center, Los Angeles, CA 90073, USA

Abstract

Several disparate diseases of high morbidity and mortality have had a major impact on the captive breeding program for African black rhinoceroses (Diceros bicornis). These include a mucocutaneous ulcerative disorder, congenital leukoencephalomalacia, susceptibility to fungal pneumonia, and acute episodic hemolytic anemia. The latter appears to be related to some of the unusual metabolic features of rhinoceros erythrocytes, which differ radically from most other mammals, particularly in regard to ATP generation and antioxidant metabolism. Strategies based on these red cell characteristics have been developed to prevent or treat the hemolytic syndrome. Comparative studies of red cell biochemistry, enzymology and metabolism indicate that extremely low catalase activity is the principal feature distinguishing the black rhinoceros from three other species which are rarely, if ever, affected by these disorders. A hemorrhagic diathesis, perhaps initiated by infectious agents, may represent an additional new, nonhemolytic anemia syndrome. We postulate that relative catalase deficiency and/or other impairments in neutralization of reactive oxygen species in diverse tissues, (such as leukocytes, vascular endothelium, neural and connective tissue elements), may represent a common abnormality responsible for any or all of these disorders.

Resumen

Varias enfermedades similares, de alta morbilidad y mortalidad, han tenido un gran impacto en el programa de crianza en cautiverio del rinoceronte negro africano (Diceros bicornis). Estas enfermedades incluyen un desorden ulcerativo mucocutáneo, leucoencefalomalacia congénita, susceptibilidad a la neumonia micótica y episodios de anemia hemolítica agudos. Este último trastorno parece estar relacionado con algunas de las características metabólicas poco usuales de los eritrocitos de los rinocerontes, los cuales difieren radicalmente de la mayoría de los mamíferos, particularmente en relación con la generación de ATP y el metabolismo anti-oxidante. Estrategias basadas en las características de estas células rojas han sido desarrolladas para prevenir o tratar el síndrome hemolítico. Estudios comparativos de los eritrocitos en cuanto a su bioquímica, enzimología y metabolismo indican que la baja actividad de la catalasa es el principal rasgo distintivo del rinoceronte negro con las otras tres especies que, muy excepcionalmente, son afectadas por esas enfermedades. Una diástasis hemorrágica, posiblemente iniciada por un agente infeccioso, puede representar un nuevo y adicional síndrome de anemia no hemolítica. Nosotros proponemos que esa relativa deficiencia
Overview

Captive African black rhinoceroses (*Diceros bicornis*) are commonly affected by several clinically disparate diseases of unknown etiology, often with high mortality. As recently reviewed by Miller,3-4 these include acute episodic hemolytic anemia, mucocutaneous ulcerative disease, fungal pneumonia and leukoencephalomalacia, none of which affects other rhinoceros species to any significant degree. Studies at the UCLA Hematology Research Laboratory have focused on the hemolytic syndrome that has now been documented in at least 47 instances affecting 39 animals with a 75% mortality rate. Hemolytic anemia occurs suddenly as a primary disease in otherwise healthy rhinoceroses, often associated with exposure to drugs or chemicals, and it also occurs as a secondary complication in other disorders, including infection and ulcerative disease. Since this hemolytic syndrome has become the leading cause of death within the captive population, extensive investigations have been undertaken to determine its etiology. These have effectively excluded autoimmune mechanisms,1 erythrocyte membrane defects or hemoglobinopathies.2 Studies of the metabolic capacities of rhinoceros erythrocytes, however, have revealed a number of extraordinary differences compared to other mammalian red cells.5,7,9,11,13 Some of these unusual features, either alone or in combination, might be responsible for premature hemolysis, since they appear to reflect a pattern of impaired red cell capacity to neutralize oxidant compounds and free radicals that are generated during many physiological and most pathological processes.5,7,9-12

One of the most unusual biochemical characteristics of rhinoceros erythrocytes initially observed was a dearth of high-energy phosphate that is essential for many metabolic reactions, ATP concentrations being only 2-5% of those found in human and other mammalian red cells.5,11,13 This led to an hypothesis that ATP deficiency might be the biochemical lesion responsible for premature hemolysis in black rhinoceroses under oxidant duress.2 Rationale for that hypothesis derived from the known dependence of mammalian red cells on phosphorylation of glucose by ATP and diversion of its product through an ancillary metabolic pathway, the hexose monophosphate shunt (HMPS), in direct response to the cells’ need to neutralize peroxides and reactive oxygen species. Additionally, an extremely common deficiency of the first enzyme of that pathway, glucose-6-phosphate dehydrogenase (G-6-PD), produces a hemolytic syndrome in humans that is clinically identical to that affecting black rhinoceroses.5 That hypothesis, however, has now been effectively refuted by collaborative studies with Prof. Eric H. Harley of the University of Cape Town in which we have demonstrated complete independence between HMPS flux rates and intracellular ATP concentrations.

Nonetheless, extremely low reserves of ATP in rhinoceros red cells may be the proximate cause for eventual failure of the membrane cation pump with consequent water influx and cell lysis. Studies in humans have shown a direct correlation between serum phosphate and red cell ATP concentrations, and hypophosphatemia with decreased intracellular ATP has been associated with premature hemolysis both in humans and in several rhinoceroses. This has led to a
rationale for phosphate supplementation to increase endogenous ATP concentrations in rhinoceros erythrocytes as a preventive measure or for therapeutic intervention. At the Oklahoma City Zoo, intensive parenteral phosphate infusions in one black rhinoceros during an acute hemolytic episode were associated with progressively increasing red cell ATP, cessation of hemolysis, and return of hematocrit to normal levels (45%) from a nadir of 16%. At the Dallas Zoo, high-phosphate dietary supplements in another rhinoceros with severe mucocutaneous ulcerative disease were associated with increased red cell ATP levels and no evidence of hemolysis. Similar supplementations have been attempted in other animals with a variety of disorders, and elevated ATP concentrations have been documented.

These experiences substantiate conclusions drawn from in vitro experiments and illustrate the importance of avoiding or correcting hypophosphatemia to prevent hemolysis resulting from further depletion of marginal reserves of red cell ATP. Since glycolysis is critical to ATP generation in mammalian erythrocytes, avoidance of any condition that inhibits glycolysis, such as acidosis, also constitutes an important preventive measure. As we have previously stressed, the most effective prevention remains an avoidance of agents known to increase the potential for oxidant production. These include several classes of drugs, such as sulfonamides, antimalarials, sulfones, nitrofurans, chloramphenicol, acetanilid, and possibly vitamin C and vitamin K analogues, as well as a number of chemical compounds, particularly those containing cyclic hydrocarbons such as naphthalene and phenols, and especially creosote, which may have direct hepatotoxic effects as well as potential capacity to initiate hemolysis. Given the hemolytic effects of certain plants, such as wild onion, oak and red maple leaves in horses and other animals, the possibility of similar effects in rhinoceroses must be considered in design of captive diets. Additionally, the frequent association of Leptospirosis infection with earlier cases of hemolytic anemia supports a continuing recommendation for vaccination, although occasional adverse reactions to the vaccine have been observed.

Recent Studies

Recently, we have had an opportunity to study specimens from three black rhinoceroses at zoos in Denver, Fort Worth, and Fossil Rim with a number of clinical and laboratory findings in common that may represent a new non-hemolytic anemia syndrome, possibly a hemorrhagic diathesis. Each had profound loss of red cell mass with packed cell volumes as low as 13%, but laboratory data and clinical observations did not support a hemolytic process or external blood loss. The possibility of internal hemorrhage was supported by low plasma proteins and pronounced swellings involving legs, shoulders, chest and neck, but there was no evidence of a coagulopathy by conventional criteria. In one of these, Dr. Richard Montali of the National Zoo observed histopathologic evidence of a vasculitis with endothelial damage and diapedesis into soft tissue interstitia. The anemia component was eventually self-limited in all three rhinoceroses, as they responded to careful clinical management of their primary conditions (laminitis, post-partum infection). Therapy included phosphate supplementation as necessary to maintain normal or elevated serum phosphate and red cell ATP concentrations. This group of rhinoceroses is the subject of an intensive collaborative investigation to determine whether they share a common etiology, perhaps with infectious initiation.

Discussion
Our studies of comparative red cell metabolism have now been extended to include all extant species except the Javan rhinoceros, and similarities among them are far more common than differences. All have comparably low amounts of erythrocyte ATP, for example, supporting our experimental evidence that low ATP alone cannot account for hemolytic tendencies in black rhinoceroses. The most significant metabolic difference that we have so far observed between the black rhinoceros and other species is in their relative activities of red cell catalase, black rhinoceroses having by far the lowest (<2-5 %, compared to humans). Since catalase is perhaps the singularly most important enzyme in antioxidant metabolism, and its activity in mammalian erythrocytes exceeds all other enzymes by several orders of magnitude, it becomes tempting to postulate that its relative deficiency sets the black rhinoceros apart in terms of susceptibility to oxidant stress and the consequent induction of acute hemolytic crises. 

It is also tempting to go one step further: since enzyme activities in erythrocytes often reflect their corresponding activities in other tissues, the possibility of catalase deficiency or otherwise defective antioxidant metabolism in vascular endothelium, leukocytes, neural and connective tissue elements, etc., should be considered when investigating other disorders affecting this species: namely, mucocutaneous ulcerative disease, impaired immunity with susceptibility to unusual infections, congenital leukoencephalomalacia, and a possibly new syndrome of hemorrhagic diathesis resulting in nonhemolytic anemia. It seems intuitively improbable that so many severe, clinically disparate disorders could occur with such prevalence in a single species without being related by some common etiology or mechanism. Collaborative studies, focused on catalase and other enzymes crucial to neutralization of reactive oxygen species, continue to test this hypothesis.

ACKNOWLEDGMENTS

Portions of these studies were supported by the American Association of Zoological Parks and Aquariums Conservation Endowment, the Bass Foundation, a Fulbright Senior Research Fellowship from the Council for International Exchange of Scholars, the International Rhino Foundation, the Morris Animal Foundation, and the Zoological Society of Cincinnati.

LITERATURE CITED


REPRODUCTIVE APPLICATIONS OF TRANSRECTAL ULTRASONOGRAPHY IN CAPTIVE AFRICAN RHINOCEROS, AND THOUGHTS ON IN SITU USE

Robin W. Radcliffe, DVM* and Steven A. Osofsky, DVM
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Abstract

The utilization of transrectal ultrasonography for research on and management of captive southern black (*Diceros bicornis minor*) and southern white (*Ceratotherium simum simum*) rhinoceroses has proven beneficial via the elucidation of normal and abnormal reproductive function in female rhinos without the need for sedation. Examinations were facilitated by the use of a “free-stall” chute that allows the rhino to choose its own response to the process. This report highlights the types of information gained, and the potential management repercussions. Reproductive work to date in the black rhinoceros has focused on gestational monitoring, using the Aloka 500V console with a hand-held 5.0 MHz linear array transducer. Weekly exams have allowed for the documentation of: fetal heart rate, fetal mobility/orientation within the uterus, and various measurements of fetal parts. In white rhinos, a 5.0 MHz convex array transducer has proven most successful, and has been used to elucidate the estrous cycle of one white rhinoceros female, as well as to document early embryonic loss in this female. Equipment modifications to facilitate ovarian examinations in the white rhino include a custom 10 foot transducer cable and an extensor. The interovulatory interval in the subject white rhinoceros averaged 33 days (n=2). A number of similarities to the horse were documented in the white rhino female. These included the formation of two ultrasonically distinct luteal structures in an approximately even ratio, the formation of anovulatory hemorrhagic follicles, the identification of intrauterine fluid collections in late diestrous as an indication of endometritis, and similar estrus behaviors. In relation to observed breeding behavior, ovulation was documented to occur within 24 hr post-breeding. Concurrent fecal hormone assays confirmed ultrasonographically identified reproductive cycle dynamics. However, delineating the precise timing of ovulation, differentiating between early pregnancy and a nonpregnant luteal phase, and identifying early embryonic loss would have proven difficult using fecal hormone assays without ultrasound. By scanning rhinos opportunistically, managers of free-ranging rhinos in a variety of contexts may obtain practical information while simultaneously enhancing understanding of the causes of infertility in captive specimens, the type of two-way information exchange the conservation community strives for.

Resumen

La utilización de la ultrasonografía transrectal para la investigación y el manejo en cautiverio del rinoceronte negro del Sur (*Diceros bicornis minor*) y del rinoceronte blanco del Sur (*Ceratotherium simum simum*) ha traído beneficios mostrando lo normal y anormal del aparato reproductivo de los rinocerontes hembras sin necesidad de sedación. Los exámenes fueron facilitados por el uso de una manga de manejo denominada “free-stall”, que permite al rinoceronte elegir su propia reacción para el proceso. Este reporte destaca los tipos de información obtenida y las potenciales repercusiones de su manejo. El trabajo reproductivo hecho en el rinoceronte negro se ha basado en el control de la gestación utilizando un aparato de consola Aloka 500V con un trasdutor manual lineal de 5 MHz.

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Exámenes semanales permitieron la documentación de los siguientes parámetros: frecuencia cardíaca fetal, movilidad y orientación fetal en el útero, y la medida de partes fetales. En rinocerontes blancos fue más exitosa la utilización del transductor convexo de 5 MHz, el mismo que se ha utilizado para poner en evidencia el ciclo estral en un rinoceronte blanco hembra, así como para documentar una pérdida embrionaria temprana en esta hembra. Las modificaciones al equipo para facilitar el examen de los ovarios en el rinoceronte blanco incluyeron el empleo de 3 metros de cable para el transductor y un extensor. El intervalo interovulatorio en el rinoceronte blanco fue de 33 días (n= 2). Se documentaron algunas similitudes encontradas en el caballo y en el rinoceronte blanco. Estas similitudes incluyen la formación de dos estructuras lúteas ultrasónicamente distintas en proporción similar, la formación de foliculos hemorrágicos anovulatorios, la identificación del fluido intra-uterino recolectado en el di-estro tardío como un indicador de endometritis y un comportamiento estral similar. En relación con la conducta observada, se concluyó que la ovulación ocurre dentro de las 24 hrs. después de la cópula. Los ensayos de una hormona fecal confirmaron la dinámica del ciclo reproductivo identificado por el ultrasonido. Sin embargo, la determinación del tiempo preciso de la ovulación, diferenciación entre preñez temprana y fase lútea de no gestación, como la identificación de pérdidas embrionarias tempranas han sido complicadas utilizando las pruebas de hormona fecal sin el auxilio del ultrasonido. Al examinar rinocerontes oportunísticamente, los manejadores de rinocerontes silvestres en una amplia variedad de contextos pueden obtener información práctica mientras simultaneamente se incrementa el conocimiento de las causas de infertilidad en especímenes en cautiverio. Este es el tipo de intercambio de información mútua por el que la comunidad conservacionista se esfuerza.

**Introduction**

Ultrasoundography is a tool being applied in captive management to resolve some of the basic mysteries surrounding rhinoceros reproduction. Managed breeding decisions can finally be based on objective reproductive assessments of individual animals instead of conjecture. This technology can be taken into the field setting where it could provide valuable information about the reproductive functioning of wild rhinos as well. At its most basic level, opportunistic ultrasonography at the time of capture could provide insights into the effects of the translocation process on embryo/fetal viability during different stages of gestation. For an animal producing one offspring at a time with a long inter birth interval, this information could prove valuable as rhinos are by necessity becoming more painstakingly managed in parks, reserves, conservancies, sanctuaries, and intensive protection zones throughout Africa.

The following case studies highlight the application of this tool in the captive setting, the types of information gained, and the potential management repercussions.

**Black Rhinos**

Reproductive work to date in the southern black rhinoceros (*Diceros bicornis minor*) at the Fossil Rim Wildlife Center has focused on gestational monitoring. In black rhinos, the Aloka 500V console used with a hand-held 5.0 MHz linear array transducer has proven most successful.
Case Study I

An approximately 12-yr-old female southern black rhinoceros was captured in Zimbabwe, held in a boma for several months, and then transferred to the Fossil Rim Wildlife Center in Texas, U.S.A. in April of 1992. Upon arrival at Fossil Rim, this female was aggressive in nature and remained apprehensive in the presence of humans. Starting in January of 1995, a full-time caretaker began intensive conditioning of the rhino to allow hands-on examinations with the hopes of eventually performing transrectal ultrasound evaluations without sedation. The conditioning process involved exposing the female to long hours of human contact along with visual, tactile, and auditory stimuli, including the intermittent playing of a radio to add background noise to her normal environment.

The positive conditioning process began with food such as apples and sweet potatoes as a reward for tolerating the proximity of people. This soon expanded to the application of human touch on different areas of the rhino’s body at the time of feeding. Over a period of several months, the rhino began to trust her human caretakers enough to facilitate twice daily examination and treatment of a potentially serious hoof crack. The conditioning process was facilitated by the use of a “free-stall” chute that was designed to allow the rhino to choose its own response to the process. The rhino was never restrained physically or chemically for the purposes of conditioning, examination, or treatment. Starting in July of 1995, the female was exposed to daily rectal examination in the chute without chemical restraint. Within 2 wk the application of transrectal ultrasound was successful, again without sedation. The fetal ultrasonographic images obtained correlated well with a breeding date approximately 11 mo earlier.

Case Study II

Another female southern black rhinoceros, 5 yr of age, is part of ongoing research designed to document normal fetal dynamics throughout gestation in this species. This female also had a history of a foot problem that was being treated in the free-stall chute. Separation from the male was necessary in order to facilitate daily therapy. An ultrasound exam at 57 days post-breeding confirmed an early pregnancy, thus making separation of the pair for medical management more feasible. The continuation of weekly exams over time has allowed for the documentation of: fetal heart rate, fetal mobility/orientation within the uterus, and various measurements of fetal parts. As sufficient data is collected to document and chart fetal dimensions such as eye diameter or skull length over time as has been done in the horse, gestational age charts can be developed for the rhino. Ultrasonic monitoring of the fetus also proved beneficial following a Type-I hypersensitivity reaction this rhino cow exhibited in response to routine leptospirosis vaccination. Despite the severe effects noted (serosanguinous fluid exuded from various skin sites, fever), the fetus appeared unaffected based on ultrasonographic visualization.

The management implications of this work are obvious regarding captive rhinoceros propagation. The conditioning process not only allowed for the transrectal ultrasound examinations, but enabled successful treatment and monitoring of medical problems in previously intractable rhinos. In the first case, a decision to postpone immobilization of the black rhino female for more aggressive treatment of the hoof crack was based partly on ultrasonographic confirmation of late-term pregnancy. Furthermore, documentation of the stage of pregnancy facilitated dietary modifications to match the nutritional demands of late gestation. A healthy calf was born in December, 1995 to the older cow,
and the birth of the younger cow’s first calf is anticipated in January - February, 1997. Foot problems have resolved in both females.

**White Rhinos**

The potential applications of transrectal ultrasound in large nondomestic animals have recently been recognized.1-4 This technology has been used to elucidate the estrous cycle of one of Fossil Rim’s southern white rhinoceros (*Ceratotherium simum simum*) females, as well as to document early embryonic loss in one female. The early embryonic loss is believed to have been caused by a uterine infection and, like endometritis in the horse, was characterized by intrauterine fluid collections in late diestrous.

In white rhinos, the Aloka 500V console used with a 5.0 MHz convex array transducer has proven most successful. Equipment modifications to facilitate ovarian examinations in the white rhinoceros include a custom 10 foot transducer cable and an extensor. The extensor, formed of PVC pipe reshaped via thermal manipulation, is required in order to consistently image the left ovary, which is beyond the operator’s unassisted reach in an adult white rhino.

The rhinoceros belongs to the order Perissodactyla or ‘odd-toed’ ungulates which includes the horse and tapir. The equine species was used as a model for interpretation and evaluation of ultrasonographic information in this study2,3 and this comparative approach has proven essential to a greater understanding of rhinoceros reproductive biology. The following is an outline of important reproductive events documented to date through utilization of transrectal ultrasound in white rhinos in the captive setting:

1) The interovulatory interval in the subject white rhinoceros averaged 33 days (n=2).

2) A number of similarities to the horse were documented in the white rhino female. These included the formation of two ultrasonically distinct luteal structures in an approximately even ratio, the formation of anovulatory hemorrhagic follicles, the identification of intrauterine fluid collections in late diestrous as an indication of endometritis, and similar estrus behaviors.

3) Maternal recognition of pregnancy appeared to occur both times there was early embryonic loss on or before day 28, with subsequent persistence of the luteal phase for 10-11 wk. This suggests that maternal recognition of pregnancy in the white rhinoceros occurs prior to 28 days of gestation.

4) In relation to observed breeding behavior, ovulation was documented to occur within 24 hr post-breeding. Obtaining this information is a requisite step prior to the application of advanced reproductive techniques such as artificial insemination in the rhinoceros.

5) Concurrent fecal hormone assays confirmed ultrasonographically identified reproductive cycle dynamics (Czekala, personal communication), and this type of correlation helps validate both methodologies. Without ultrasound, however, delineating the precise timing of ovulation, differentiating between early pregnancy and a nonpregnant luteal phase, and identifying early embryonic loss would prove difficult. Estrous cycle lengths of approximately 10 wk, identified
via fecal EIA alone, have been reported elsewhere as the expected normal in this species, and may be inaccurate based on the findings in this study. In situ and ex situ managers should, if at all practical, monitor rhinos with noninvasive (and less expensive) fecal assays. It is suggested, however, that ultrasound “spot-checks” could yield additional, complementary information.

**In Situ Use**

The potential applications of this work in rhino range states remain open to debate. The ability to determine pregnancy status could have management repercussions regarding how an immobilized female rhino would be handled during and following capture/translocation. The stresses associated with immobilization, transport, and boma confinement can result in abortion in a wide variety of species, including rhinos; the detection of an embryo/fetus could potentially change the course of action regarding boma management or translocation, for example. Data collection from field scanning of females could provide managers with a measure of a rhino population’s reproductive health. This would facilitate sound management decisions, differentiating between populations which could sustain translocation of individuals to other areas and those populations requiring more intensive conservation efforts. Fertility problems are certainly bound to be more prevalent in captive situations than in the wild; information gleaned from wild animals could help zoos tease apart environmental, social, as well as nutritional factors that may be contributing to reproductive failure in captivity.

Detecting cyclicity and the corresponding stage of an estrous cycle of a female rhino on one ultrasound exam would be difficult, but this has been done in the equine species based on size and echogenicity of the corpus luteum. Since both the rhinoceros and the horse, as perissodactylids, share a common evolutionary history, it seems reasonable to look for similarities in their reproductive biology as part of ongoing research efforts.

**Thoughts for the Future**

The utilization of transrectal ultrasonography for research and for management of captive black and white rhinoceros has proven beneficial via the elucidation of normal and abnormal reproductive function in female rhinos without the need for sedation. This technology can be taken into the field setting where it could provide valuable information about the reproductive functioning of wild rhinos as well. The authors are not suggesting that wild rhinos be subjected to immobilization simply to be scanned ultrasonographically. This would be an inappropriate use of financial and technological resources in most contexts. It may, however, be worth integrating a 10-15 min scanning procedure into some capture and translocation protocols already in place for a variety of reasons. By scanning rhinos opportunistically, managers of free-ranging rhinos in a variety of contexts may obtain practical information while simultaneously enhancing understanding of the causes of infertility in captive specimens, the type of two-way information exchange the conservation community strives for. It may be worth considering the selective application of this tool as an adjunct when questions regarding fecundity and fertility arise in free-ranging populations that are, by default, requiring more and more intensive management in the face of a plethora of human-related pressures. In short, the selective application of transrectal ultrasonography could help shape management decisions that underlie the maintenance of healthy conservation units both in situ and ex situ.
ACKNOWLEDGMENTS

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LITERATURE CITED

AZAPERONE FOR STANDING SEDATION IN ASIAN ELEPHANTS (*Elephas maximus*)

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Abstract

Azaperone was used for standing sedation of four Asian elephants (*Elephas maximus*) in 93 trials at Dickerson Park Zoo (DPZ). Procedures including surgical artificial insemination, semen collection, and routine foot trimming were completed while utilizing azaperone as a sedative. All procedures were performed within an elephant restraint device. Azaperone has proven to be a safe and reliable drug for facilitation of routine health and reproductive-related procedures in captive Asian elephants when administered at 0.30 mg/kg. The procurement of azaperone in the United States has been difficult due to changing manufacturing and distribution procedures. The utilization of an Investigational New Animal Drug permit from the Food and Drug Administration is described, to facilitate procurement of azaperone from Canada for use in the United States.

Resumen

La azaperona fue utilizada para lograr sedación sin recumbencia en 4 elefantes asiáticos en 93 pruebas en el Parque Zoológico Dickerson. Los procedimientos que se realizaron mientras los animales estaban sedados con azaperona incluyeron inseminación artificial quirúrgica, recolección de semen, y recorte rutinario de las uñas. Todas las actividades se realizaron dentro del sistema de contención de elefantes. La azaperona administrada a una dosis de 0.30 mg/kg ha demostrado ser una droga segura y confiable que facilita el manejo de elefantes asiáticos para exámenes de rutina y procedimientos reproductivos. La obtención de la azaperona en los Estados Unidos ha sido difícil debido a cambios en los procedimientos en su fabricación y distribución. Se describe la utilización de un permiso para la Investigación de Nuevas Drogas en Animales, dependiente de la Oficina de Administración de Drogas y Alimentos (FDA), que facilita la importación de azaperona de Canadá para su uso en Estados Unidos.

Introduction

Elephant handlers have traditionally applied the so called “free contact” method of elephant control for providing care to captive elephants. The inherent dangers of this free contact method with some individuals in the elephant population are reflected in the numbers of elephant related injuries and occasional deaths of handlers. These dangers, along with the controversy concerning discipline of dominance controlled elephants, have encouraged adoption of “restricted contact” for some individual elephants and programs of captive elephant management. In 1988, DPZ began a program of restricted contact with mature bulls and selected individual cows, with the installation of a moving wall elephant restraint. This conventional restraint provided twice daily confinement for basic care of Onyx, the zoo’s intractable bull Asian elephant. This care included periodic administration of xylazine for standing sedation during trimming of problem toenails. Xylazine was the drug used in each of the eight foot trimming sessions. However, xylazine proved ineffective during these sessions.
due to the bulls frequent arousals when stimulated by activities related to routine foot care.

Xylazine was not considered for use in the zoo’s elephant artificial insemination (AI) project. Persistent urine dribbling under the influence of xylazine precluded its use for semen collection. Xylazine’s attributed inhibition of reproductive hormone secretion in females of other species and its profound changes in uterine motility made its application questionable. An additional problem was the prolonged drowsiness (up to 48 hr) following xylazine administration and yohimbine reversal. The negative aspects of xylazine necessitated finding an alternate method for sedating elephants.

In May 1992, completion of the zoo’s latest elephant restraint device ushered in a new era of captive elephant management at DPZ. The prototype restraint was designed and built for manipulation of elephants in standing and lateral recumbency. The effectiveness of the elephant restraint has been greatly enhanced by the situational use of the sedative tranquilizer azaperone in intractable individuals. Azaperone is classified as a neuroleptic tranquilizer of the butyrophenone series of tranquilizers. The proprietary product Stresnil contains 40 mg/ml of azaperone. Azaperone has been reported by Kock to produce good sedation in various age groups in doses ranging from 30 mg for babies, 120 mg for juveniles, to 760 mg for adult elephants. Azaperone has been used in African elephants as reported in MedARKS records submitted and summarized by Page. In the seven elephants of known weights the mean dosage was 0.10 mg/kg (range 0.06 to 0.15 mg/kg). A calming effect is produced through central nervous system depression. In swine a wide dosage range may be used (2-40 mg/kg). Azaperone is a relatively nontoxic, short acting drug that is active for 2-3 hr and nearly eliminated in 16 hr. It is approved and has been marketed in the United States for use in pigs to relieve stress and minimize introductory aggression by Pittman-Moore Company. Recently they have suspended producing and marketing Stresnil in the United States. However, Janssen Pharmaceutica of Mississauga, Ontario, Canada has gained the manufacturing rights and Stresnil is being distributed by the Upjohn Company (Animal Health Division, Orangeville, Ontario, Canada L9W 3T3; (519) 941-1030, FAX (519) 941-1074). To be able to import azaperone a Notification of intent to import a new animal drug(s) or an investigational new animal drug substance (INAD) must be obtained from the Food and Drug Administration’s Center for Veterinary Medicine. Once the application is returned and approved by the Center for Veterinary Medicine, notification of an INAD# is received and the drug can be ordered. The INAD application can be requested by contacting:

Dr. Marcia Larkins HFV112
Center for Veterinary Medicine
Food and Drug Administration
7500 Standish Place
Room 319
Rockville, MD 20855
(301) 594-1612 or 0614.

Methods

The use of azaperone as an elephant sedative was evaluated in 93 trials in a group of four sexually mature Asian elephants. The study animals included two bulls (ages 32 and 17 yr), and two cows (ages 32 and 21 yr). All the animals were housed at DPZ during the study period.
Occasions to use the drug included foot trimming and semen collection of bulls and surgical AI with subsequent follow-up care of cows. Semen collections were performed using electroejaculation or manual stimulation techniques. The rotational capabilities of the restraint positioned the bulls into left lateral recumbency for the foot trimming sessions.

Weights of the animals were determined using a scale built into the restraint, allowing accurate dosing. Intramuscular injection of azaperone was administered via hand syringe in the triceps muscle using a 2-inch catheter needle. The bulls were injected while confined in the restraint. The cows were normally injected in a stall adjoining the restraint and permitted to enter on their own during the latter stages of induction. Intravenous injections of azaperone were avoided due to transitory excitement tendencies reported when this class of drug is administered in this manner. Following azaperone injection, each elephant was closely monitored to determine the time of initial effect, time of maximum effect, and total duration of effect. The initial effect was recorded at the time of first perceived change of the elephant’s normal behavior or posture. Maximum effect was recorded at the time frame of least response to external stimuli. The duration of effect was defined as the point in time when the drug’s effects were no longer detected.

The degrees of sedation were rated as good, fair or poor. They were determined by the ability to begin and complete a particular procedure in relation to amount of interference from the elephant involved. A rating of good was given when the elephant demonstrated no or minimal response from the procedures stimuli. A fair rating was given if the response was noteworthy, but did not hinder the procedure. A poor rating resulted if the elephant’s response resulted in an aborted or abbreviated procedure.

**Results and Discussion**

The dose range of azaperone utilized in this study was 0.017 - 0.046 mg/kg. The lower dose or half dose was given due to a temporary shortage of azaperone. The half dose provided adequate depth of sedation but effects were short term. The higher range was given to attain profound sedation required for electroejaculation of a bull Asian elephant. A normal dose range from our experience has been determined to be between 0.024 and 0.038 mg/kg for use in minor surgical procedures. Azaperone dosages within this range have provided a safe and reliable standing sedation for restraint of confined Asian elephants.

The sedative effects of azaperone on elephants in 93 trials were rated as good in 81 trials, fair in 12 trials, and poor in none. The calming initial effects of azaperone on the elephant could be seen in 10 to 15 min following injection. In most cases, appetite seemed to increase during this time. Maximum effect was attained in 15 to 25 min. Maximum effects were characterized by: a stuporous or somnolent mental state often accompanied by snoring, an unwillingness to move or respond to stimuli, diminished bowel movement, distended or relaxed penis or clitoris. No tendencies or desires to lie down have been noted. Maximum effects rapidly diminished after approximately 2 hr. Total duration of effects was approximately 3 hr. Repeated daily administration of azaperone during two to six day periods demonstrated no residual effects.

During the induction phase azaperone appears to have sensitizing effects on the elephants’ response to stimuli, particularly noise. Any stimulation or activity tends to prolong induction time particularly
with an intractable or nervous elephant. Consequently it is important to have a quiet environment
during induction until maximum effect is achieved.

Azaperone appears to diminish aggressive behavior in bull Asian elephants. A highly aggressive bull
in musth becomes very placid, and tolerant of stimulation once maximum effects of azaperone have
been reached. Azaperone and the restraint system combined provides the capability to perform a
wide variety of procedures regardless of the animal’s normal intractable nature. Some of the potential
uses for azaperone would include tusk trimming, minor surgery, and other routine treatments.

Two abnormal responses were shown from the same cow during azaperone induction. The episodes
of confused or hallucinatory behavior were responses to mild stimuli. Although these experiences
were isolated, for safety considerations the drug is administered only under controlled circumstances
whereby direct keeper/elephant contact is not required. The reasons for these episodes have not been
determined and call for further investigation. Once maximum effect has been attained, no behavioral
problems have been noted.

Conclusions

Azaperone at a dose of 0.030 mg/kg (range 0.017- 0.046 mg/kg) has proven to be safe and reliable
in providing standing sedation for both male and female Asian elephants within a restricted contact
protocol. Azaperone has shown no residual effects. Recovery is smooth and rapid. Azaperone
provides effective sedation in a small dose (120-160 mg or 3-4 ml). The maximum effect is long
enough for most procedures to be completed and no inclination for recumbency has been noted.
Elephants under the influence of azaperone should be managed only in a restricted contact situation.
Sedated animals show diminished aggression, even males in heavy musth. Azaperone has proven
its value for routine utilization for intractable elephants within these guidelines.

LITERATURE CITED

441.
FOOT CARE IN ASIAN ELEPHANTS USING A ROTATING ELEPHANT RESTRAINT DEVICE

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Abstract

Foot care for elephants is an area that many veterinarians often become involved with only when individuals are not responsive to normal foot care provided by keepers or when intractable elephants require veterinary attention for sedation to enable access to the animal for treatment. The use of a rotating elephant restraint is described and the methods for foot treatment that are useful in the normal care of elephants with or without the use of a rotating elephant restraint.

Resumen

El cuidado de los pies de los elefantes es un área donde muchos veterinarios se ven involucrados solamente cuando los animales no responden al cuidado normal que les proporcionan los cuidadores, o cuando elefantes intractables requieren de atención veterinaria para su sedación, y de esta forma poder llevar a cabo su tratamiento. Se describe el uso de un sistema de contención rotatorio, y los métodos para el tratamiento de los pies que son útiles en el cuidado normal de los elefantes, con o sin el uso del sistema de contención rotatorio en elefantes.

Introduction

Foot care is the most common procedure performed on captive elephants.² Constant evaluation of the condition of each elephant’s feet is essential for responsible animal care. In free contact, elephants are trained to present their feet for regular examination and trimming. In the care of intractable animals, the routine examination and care of feet can become a nearly impossible task, or is delayed until the individual is more willing to cooperate in its care. The use of a rotating restraint device at Dickerson Park Zoo has improved foot care of intractable elephants and treatment when appropriate is easily monitored and administered. By securing all four legs in the elephant restraint and rotating the elephant to its side at about an 80° angle access to all four feet is possible. Tilting, what was the floor, to provide a safe working surface, provides room for two groups to work simultaneously on both the front and rear feet. This reduces the time needed for foot care and many times increases the level of care and evaluation of foot conditions compared to traditional methods. Normal foot care is well-reviewed and discussed by Fowler¹ and excellent information is found in Medical Management of the Elephant² regarding the occurrence and management of foot problems reported in elephants. Many elephant programs have developed excellent foot care protocols and no one protocol is superior to another as long as the elephant’s feet are examined and treated in a conscientious and timely manner.

Discussion
At Dickerson Park Zoo foot care has evolved with exposure to ideas presented by others and adaptation to meet our own needs. The procedure for trimming elephant feet at Dickerson Park Zoo is similar to the procedure described for the Audubon Park Zoo. Excess sole or pad is removed using Swiss cutting knives and abrasive pads on an electric right angle grinder. This procedure leaves relatively smooth weight bearing surfaces. An elephant’s weight is borne on the sole of the foot. The toenails don’t actually carry any of the elephant’s weight. Toenails should be trimmed so that the bottom edge doesn’t bear any weight when the elephant’s foot is weight bearing. With careful attention the abrasive pad on the electric grinder can be utilized for toenail trimming or a hoof rasp for shortening the nail length. An X-acto knife is used for trimming the edges of the cuticles and for probing and trimming the areas of suspected necrotic tissue. Once a necrotic area is located the tract of necrotic tissue is completely removed. This may necessitate removal of nail or sole to the depth of the corium of the foot. Any necrotic debris is flushed with saline or saline/hydrogen peroxide solution. Care must be used in using a hydrogen peroxide solution to assure that the debris can be flushed out of the wound freely to prevent driving bacteria and other contaminants deeper into the tissues. Following thorough flushing, treatment with topical iodine as an antiseptic is liberally applied and allowed to soak. Antibiotic or antiseptic ointment treatment is initiated twice daily to prevent further bacterial growth and the normal growth of the sole or toe to fill in the defect. If abscesses are not able to be completely flushed and exposed, then systemic antibiotic treatment is instituted. In cases that sole or toenail infections cannot be opened for flushing and treatment, soaking the foot in a tub with hot Epsom salts, softens the tissues and makes treatment at a later session possible. If protection of the foot from environmental contamination is needed, then specially constructed boots are fitted to keep the foot dry or to provide continuing treatment with ointments during the hours the elephant has access to outside yards. Many designs of boots and bandages have been applied to elephant feet. We are currently using a vinyl canvas boot constructed by a local canvas and awning company. The design provides a complete covering of the sole and the edge of the foot normally in contact with the ground. A rim of canvas extends up about four inches from the ground with straps sewn around the perimeter at right angles to accommodate two circumferential straps at ankle height used to secure the boot to the elephant’s foot. This design allows ointments or antiseptics to be applied to areas needed. One benefit of this boot is the foot can remain dry and relatively free from environmental contamination. These foot care procedures are utilized in all of our elephants but were nearly impossible in our mature bulls. With the use of the rotating elephant restraint three elephant keepers can trim and treat all four feet in about 1 hr. The elephant is comfortable, and securely restrained so that all foot care can be safely performed. While not all elephants need to be restrained in this manner, those few that do require extraordinary restraint, can benefit from its use.

LITERATURE CITED

HIPPOPOTAMUS TRAINING: IMPLICATIONS FOR VETERINARY CARE

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Abstract

The implementation of training programs involving captive exotic species has proven to be an extremely useful tool in the veterinary field. Many species have been successfully trained to cooperate for veterinary procedures, lessening stress to the patient and danger to those performing veterinary and related procedures.1,2 Some species present more challenges to providing veterinary care than others. This is often the case in working with the hippopotamus (Hippopotamus amphibius).

At The Toledo Zoo a 37-yr-old male Nile hippopotamus had a history of an intermittent draining fistula on the underside of its lower right mandible. Several discussions on how to deal with this case took place to attempt to devise a way to avoid dangerous immobilization procedures while diagnosing and treating the problem. Finally, a veterinary/keeper brainstorming session ended with the development of an idea to incorporate the existing holding facility hydraulic gates into a hippo restraint device (Figs. 1 and 2). The existing holding facility gates were subsequently modified at a relatively low cost and the training experiment began. Over the course of a few months the hippo was given varying doses of azaperone (400-800 mg i.m.) and while sedated was coaxed into the Hippo Restraint Device (HRD). Using this restraint technique, part of the mandible was successfully radiographed with a portable x-ray unit, the fistula was surgically opened, debrided, cultured and flushed with an antibacterial solution. The hippo then acclimated to the HRD well enough to allow daily flushing treatments without sedation and this continued until the infection was cleared.

The initial successes using the HRD prompted keepers to incorporate its use in the daily gating routines of the hippos. The hippos are now held in the HRD on a daily basis without coaxing and stand without sedation for minor inspections and manipulations, using food treats as incentive. Many minor veterinary procedures can now be performed on the hippos while restrained in the HRD. Exams have been done of the eyes, ears, teeth, mouth, genitals, mammary glands, skin, legs, underside and various soft tissue structures palpated. Tusk trims, body measurements, tissue biopsy of an oral mass, treatment of skin problems, saliva collection for pregnancy testing, and collection of milk from mammary glands have been accomplished. Other veterinary related procedures planned include obtaining body temperatures and weights. Ideas are also being explored for blood collections and heart auscultation.

The HRD has also been used for the attachment of a CritterCam, an underwater camera developed by Greg Marshall of National Geographic Television. Toledo Zoo Keeper Stephen Krueger designed, fitted and fabricated a harness to hold the CritterCam in place on the back of a female hippo. The hippo was desensitized to wearing the harness and then released into the zoo’s Hippoquarium where successful video taping of her male pool-mate occurred.

The HRD combined with training and desensitization techniques have proven to be excellent and inexpensive tools for assisting in the safe and effective veterinary care of the hippopotamuses at The
Resumen

El uso de programas de entrenamiento en especies exóticas en cautiverio ha resultado ser una herramienta sumamente útil en el campo veterinario. Se han entrenado muchas especies con un gran éxito para que cooperen con diversos procedimientos veterinarios, aminorando el stress del paciente y el peligro que estos ofrecen a quienes intervienen en dichos procedimientos y otros manejos relacionados.1,2. Unas especies presentan más desafíos para el veterinario que otras. Este es el caso en el manejo del hipopótamo.

Un hipopótamo del Nilo macho de 37 años de edad del Zoológico de Toledo presentó un historial clínico con una fístula intermitente en la porción submandibular derecha. Se tuvieron varias discusiones de cómo tratar este caso evitando procedimientos de inmovilización peligrosos mientras se diagnosticaba y se trataba el problema. Finalmente, durante una sesión de animaleros y veterinarios se ideó convertir las puertas hidráulicas del área de manejo en un sistema de sujeción para hipopótamos. Las puertas fueron modificadas a un relativo bajo costo y se inició el experimento de entrenamiento. Durante el curso de algunos meses el hipopótamo fue sedado con dosis variables de azaperona (400-800 mg i.m.) y mientras estuvo sedado fue guiado al sistema de sujeción para hipopótamos (HRD). Utilizando esta técnica de sujeción, se obtuvieron exitosamente placas radiográficas de una parte de la mandíbula con un equipo portátil de rayos-x, la fístula fue quirúrgicamente abierta, debridada, se realizaron los cultivos necesarios, y se lavó con solución antibacteriana. El hipopótamo se habituó al sistema hasta permitir los tratamientos de lavado diariamente sin requerir sedación. Los lavados fueron constantes hasta que la infección desapareció.

El éxito inicial usando el sistema HRD indujo a los animaleros a incorporar su uso en las rutinas diarias de encierro de los hipopótamos. Ahora los hipopótamos se mantienen en el HRD diariamente sin tener que ser forzados, así como también a permanecen quietos sin sedación para realizar manipulaciones e inspecciones menores, utilizando recompensas alimenticias para incentivarlos. Varios procedimientos veterinarios se pueden verificar mientras los animales se encuentran en el sistema de sujeción. Se han llevado a cabo exámenes en ojos, oídos, piezas dentarias, hocico, genitales, glándulas mamarias, piel, extremidades, vientre y se han palpado varias estructuras de tejidos blandos. Se les han limado los colmillos, se han efectuado mediciones corporales, biopsias de tejidos de una masa oral, tratamiento de problemas en piel, colecta de muestras de saliva para diagnóstico de gestación, así como de muestras de leche de las glándulas mamarias. Otros procedimientos veterinarios relacionados que se planean son la obtención de temperatura y peso corporales, y se explora la posibilidad de colectar muestras sanguíneas así como la auscultación cardíaca. El sistema HRD también ha sido utilizado para fijar un sistema de video subacuático llamado “CritterCam”, desarrollado por Greg Marshall de la compañía de TV de National Geographic. Esta cámara se montó en un arnés diseñado y construido por Stephen Krueger, animalero del Zoológico de Toledo, misma que se colocó en el dorso de una hembra de hipopótamo. La hembra fue condicionada al uso del arnés y luego liberada en el hipoacuario para obtener exitosamente video de su cópula con el macho.

El sistema HRD con el entrenamiento y técnicas de desensibilización han probado ser herramientas...
excelentes y económicas para contribuir a la seguridad y el cuidado veterinario efectivo de los hipopótamos en el Zoológico de Toledo.

LITERATURE CITED

Figure 1.

Hippo holding stalls and pools. This diagram shows a hippo entering the Hippo Restraint Device (HRD) through a hydraulic gate which was incorporated as part of the HRD. See Figure 2 for details of the HRD.
Figure 2.

Detailed views of the Hippo Restraint Device. a) Side view: showing the parallel hydraulic gates and 4.5" dia. pipes that were incorporated to make the HRD. b) End view: showing a hydraulic gate with the pipes arranged on supports that are attached within the framework of the gate. The pipes are free to slide horizontally, in tandem, when either gate is opened. The long pin through the end of each pipe prevents them from slipping out of place. The pipe supports are removable and can be placed in any of the six spaces in the framework of either gate.
HIPPOPOTAMUS UNDERWATER BEHAVIOR AND COMMUNICATION

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Abstract

The hippopotamus (Hippopotamus amphibius) is the second largest terrestrial mammal in the world; however, very little information can be found in the literature about the ecology, behavior and communication of this species. Although the hippopotamus is considered a terrestrial species it may be more correct to refer to it as an amphibious species, living in social groups (herds) in the aquatic environment and foraging solitarily in the terrestrial environment at night. Hippopotamuses are highly adapted to the aquatic environment and it is interesting to note that this may have evolutionary implications in light of recent genetic studies indicating that hippopotamid artiodactyls are close relatives of cetaceans.3 The amphibious nature and aquatic environment of hippos has presented field researchers with practically insurmountable obstacles. Knowledge of hippopotamus behavior and communication in the aquatic environment was especially lacking until recent ground breaking research, by Dr. William Barklow (Framingham State College in Massachusetts), that has provided evidence of hippopotamuses emitting a variety of sounds in the aquatic environment, possibly used as a form of communication and in conjunction with specific underwater social behaviors.1,5 This is being further investigated with captive hippopotamuses in the controlled environment of a zoo exhibit that provides unimpeded underwater observations and no extraneous animal sounds.

Since 1989, Barklow has studied hippopotamus schools in their natural habitat and has analyzed recorded water surface behaviors, above water vocalizations and underwater vocalizations. Hippopotamuses apparently make elaborate vocalizations like click-trains (a series of rapid click sounds similar to those used by cetaceans for echolocation) and other sounds that are transmitted in the aquatic medium only.2 Also, these animals may be able to produce and process sound in three environments: air, underwater and air/underwater simultaneously (amphibious communication); thus suggesting that hippopotamuses may have a more advanced communication system than before imagined. An interesting addition to this is that a more highly advanced communication system often indicates a more sophisticated social structure.

The dilemma that is faced with studying and recording hippopotamuses in their natural habitat is that the water is too murky to observe underwater behaviors and that the hydrophones not only record hippo sounds, but all sounds of the other animals (i.e., crocodiles, fishes, frogs, turtles, etc.) in the aquatic habitat as well. This makes it difficult to discern which sounds are emitted by hippo and which are not. The Toledo Zoo’s Hippoquarium (a 360,000 gallon, filtered water, glass viewing, naturalistic exhibit) has provided a unique opportunity to investigate hippopotamus underwater behavior and communication in a controlled environment (i.e., no sounds produced by other animals and clear underwater viewing through glass and filtered water).

This type of study had never been attempted before. It is providing evidence for specific hippopotamus sounds made in the aquatic environment and possibly what they may mean in regard to communication and related behaviors. The first clear recording of underwater hippo sounds from the hippos (August 1994) in The Toledo Zoo’s Hippoquarium was a significant addition to Dr.
Barklow’s work. From just this one recording he was able to discern many sounds in his underwater recordings from the wild to be hippo sounds rather than fish or crocodile sounds as he originally thought. Audio/video recordings have revealed that click train sounds can be produced with the mouth agape and expelling no air. Underwater courtship with ritualistic tusk clashing sounds of hippos has been documented and confirmed in the Hippoquarium. The courtship behavior occurs when the female is apparently in estrus (monthly) and involves underwater sparring and tusk clashing between the male and female while they gyrate around and chase one another. Copulation usually occurs after approximately 15 min of this behavior and may take place a few times per day.

Analyzing underwater hippopotamus behaviors and sounds from audio/video recordings is ongoing and a study of the morphology of hippo hearing and sound production is currently underway. Dr. Darlene Ketten, an otolaryngologist at Harvard Medical School, is studying a newborn hippo head from a baby that died 20 min after birth at The Toledo Zoo. The study will concentrate on adaptations for aquatic and amphibious hearing involving the inner ear and associated structures. An experiment to determine if the hippos are using click trains for echolocation is planned as well as hearing tests involving task discrimination training to determine if hippos can hear underwater sounds in the amphibious and submerged positions.

Resumen

El hipopótamo (Hippopotamus amphibius) es el segundo mamífero terrestre más grande en el mundo; sin embargo hoy en día es poca la información en la literatura sobre la ecología, conducta y la comunicación de esta especie. Aunque el hipopótamo se considera una especie terrestre, probablemente es más correcto referirnos a él como una especie anfibia, que vive en grupos sociales (manadas) en medios ambientes acuáticos y que, durante la noche, es un solitario forrajero en el medio ambiente terrestre. Los hipopótamos están altamente adaptados al medio ambiente acuático. Es importante hacer notar que este hecho tiene implicaciones evolutivas muy interesantes, ya que estudios recientes indican que estos artiodáctilos hipopotámidos pueden estar relacionados con los cetáceos. La naturaleza anfibia y el medio ambiente acuático de los hipopótamos ha ocasionado que los investigadores tengan grandes obstáculos para sus estudios. El conocimiento de la conducta y la comunicación en el medio acuático es prácticamente desconocida hasta recientes investigaciones realizadas por el Dr. William Barklow (Universidad Estatal Framingham en Massachusetts), que nos han proporcionado evidencia que los hipopótamos emiten una variedad de sonidos en el medio acuático posiblemente usados como una forma de comunicación y con conductas sociales específicas bajo el agua. Por otro lado se encuentran realizando investigaciones con hipopótamos en ambientes controlados en cautiverio en donde se pueden obtener observaciones bajo el agua sin ningún otro sonido de otros animales.

Desde 1989, Barklow ha estudiado grupos de hipopótamos en su medio natural y ha analizado, mediante grabaciones, conductas en la superficie del agua, vocalizaciones en el agua y vocalizaciones bajo el agua. Los hipopótamos al parecer elaboran vocalizaciones como rápidos clicks, que son sonidos similares a los utilizados por los cetáceos para la ecolocación, y otros sonidos que son transmitidos en el medio acuático solamente. Estos animales son capaces de producir y procesar sonidos en tres diferentes ambientes: aire, bajo el agua y en el aire/y bajo el agua simultáneamente (comunicación anfibia), por lo que se sugiere que los hipopótamos tienen un sistema de comunicación
mucho más avanzado de lo que nos imaginamos. Algo muy interesante de este sistema avanzado de comunicación es que nos indica una alta y sofisticada estructura social.

El dilema a que se enfrenta el estudio y grabación de los hipopótamos en su hábitat natural es que el agua es demasiado turbia para observar conductas bajo el agua y los hidrófonos no sólo registran los sonidos de los hipopótamos, sino los de otros animales (eje. cocodrilos, peces, ranas, tortugas, etc..) que se encuentran en el mismo medio acuático. Esto hace muy difícil discernir cuales son los sonidos emitidos por los hipopótamos y cuales no. El exhibidor de hipopótamos del Parque Toledo (con 360,000 galones de agua filtrada, vitrina subacuática, y ambientación natural) ha proporcionado la oportunidad única de investigar la conducta y la comunicación del hipopótamo en un ambiente controlado (aquí no hay sonidos producidos por otras especies y el agua está clara).

Este tipo de estudios no habían sido realizados con anterioridad. El estudio proporciona datos específicos de sonidos de hipopótamos hechos en un medio acuático, y posiblemente cuales de estos sonidos pueden significar algo importante respecto a la relación de la conducta y comunicación. La primera grabación de sonidos bajo el agua de hipopótamos en el Zoólogico de Toledo, fue en 1994, y fue muy importante para el trabajo de Barklow. Sólo de esta grabación él pudo separar y discernir los sonidos emitidos en estado silvestre. Grabaciones de audio y de video han revelado que este sonido se puede producir con el cierre de la boca y sin expeler el aire. El cortejo bajo el agua se realiza de forma ritual a través de chasquidos de colmillos, mismos que se han documentado y confirmado en el hipocuario de Toledo. La conducta de cortejo ocurre cuando la hembra está en estro aparente (mensualmente). La pareja comienza una aparente pelea bajo el agua y sus colmillos chasquean mientras la hembra y el macho giran uno alrededor del otro. La cópula ocurre después de aproximadamente quince minutos de esta conducta, y se puede observar varias veces durante el día.

El análisis de las conductas del hipopótamo bajo el agua y sus sonidos a través de videos y grabaciones de audio continúan y un estudio de la morfología del oído del hipopótamo y la producción de los sonidos esta actualmente llevándose a cabo. La Dr. Darlene Ketten, una otolaringologista de la Escuela Medica de Harvard, esta estudiando un hipopótamo recién nacido que murió veinte minutos después de su nacimiento en el Zoológico de Toledo. El estudio se concentra en las adaptaciones acuáticas y anfibias del oído interno y las estructuras asociadas. Este experimento pretende determinar si los hipopótamos usan estas secuencias de sonidos agudos y cortos para su ecolocación, así como realizar otras pruebas del oído que pretenden determinar si los hipopótamos pueden oír sonidos bajo el agua en posiciones sumergidas o de anfibios.

LITERATURE CITED

Clinical Aspects of a Neonatal Care Program

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Abstract

Recently, zoos have begun to see themselves as important players in the race to save species and protect ecosystems. Captive breeding programs in zoos are key components in endangered species management. It is important that any captive endangered species breeding program also have a plan in place to provide critical neonatal support care when natural rearing is not possible. Institutions who accept the responsibility of breeding captive endangered species also commit to building a staff whose skills and expertise are essential to successful rearing of these species.

The normal development and eventual socialization of any hand-reared animal is solely dependent on the quality and consistency of care received from well-trained, professional handlers. Adequate staffing is the basis for a well-defined neonatal care program. The developmental stages of young animals dictate the skill criteria required to provide adequate care. Most animals progress through three generalized developmental phases following birth: neonatal phase, growth phase, and the weaning phase. Four levels of care may be utilized in designing a system which takes into account the stages of development outlined above (Table 1). Handrearing teams should be identified from a pool of trained professionals with skills to match the required care criteria for a neonate at specific developmental levels. Table 2 illustrates some of the basic skill criteria which should be required of staff participating in handrearing programs.

Handrearing may not be the only option when it appears an animal has been rejected. It may be possible to successfully replace an infant with its natural parent. The last option for any rejection scenario would be handrearing. Once this decision is made, housing and equipment become the next step to implementing a handrearing plan. Each zoo must consider its resources and intentions when housing an animal being handreared. Animals with little or no thermoregulatory capability will require supportive heat. As part of the hygiene protocol, the choice of which disinfectant to use depends on several factors. Accurate scales, mixing and measuring devices, and food preparation equipment such as ovens, stoves and refrigerators should be dedicated to the neonatal care facility.

Balanced nutrition contributes to normal physiological development contributing to an animal’s weight gain, coordination and development of the immune system. It is well-documented that mammals receive immunoglobulins by passive transfer in utero and/or postpartum in maternal colostrum. It is also believed that birds provide initial immunity for offspring through crop formula contents. It is important to attempt to provide immunoglobulins in a usable form to a neonate who may not have received them naturally. Caloric needs can be calculated using the formula for basal metabolic rate presented in Fig. 1. Milk intake varies somewhat predictably according to taxonomic group. Among typical zoo species expected intake per day is 10-18% body weight in ungulates, 12-25% in carnivores, and approximately 20% in primates. In birds the task for providing a balanced
neonatal formula is even more challenging.

Every animal reaches key levels of development which can act as markers for tracking normal development. Knowing these stages and identifying them in growing animals is an important part of assessing the success of a nursery program. Animals exhibit normal development through predictable weight gain and an increase in feeding initiative. Normal limb development, mobility, coordination, dentition (mammals), vision, and pelage or plumage are also used as important indicators of normal (or potentially abnormal) development.

The weaning process is generally automatic and in most carnivores usually starts when the canine teeth have erupted at about the eighth week of age. Although many behavioral characteristics are genetically controlled, environmental factors can affect the quality of much of an animal’s psychological development. The object of a handrearing program is to produce a physically and psychologically productive adult. Controlling imprinting is likely the biggest challenge a neonatal program will face. We can attempt to reduce inaccurate imprinting but we cannot eliminate imprinting entirely. Through environmental manipulation natural behaviors may be encouraged or discouraged. A successful neonatal program should seek to provide an enriching environment, not a sensory deprived one. When and where appropriate, animals should be raised with conspecifics. To strengthen normal psycho-social development it is necessary to be creative with naturalistic environmental stimuli which encourage normal behaviors.

A well-designed and managed neonatal care unit is essential to the operation of any zoo participating in endangered species breeding programs. The role of and the information gained by a well-run neonatal care program is valuable from a comprehensive ecological point of view.

**Resumen**

Recientemente los zoológicos se han empezado a considerar como protagonistas importantes en la carrera para salvar especies y proteger ecosistemas. Los programas de crianza en cautiverio en los zoológicos son claves importantes en el mantenimiento de especies amenazadas. Es importante que cualquier programa de crianza de especies en cautiverio también tenga un plan en operación para proveer cuidados neonatales de emergencia, cuando la crianza natural no es posible. Las instituciones que han aceptado la responsabilidad de la crianza en cautiverio de especies amenazadas, también tienen el cometido de formar un equipo humano con la destreza, habilidad y experiencia para la crianza exitosa de estas especies.

El desarrollo normal y eventual socialización de cualquier animal criado artificialmente únicamente depende de la calidad y consistencia del cuidado recibido de criadores profesionales bien entrenados. Un equipo de trabajo adecuado es la base para un programa de cuidados neonatales bien definidos. Las etapas de desarrollo de animales jóvenes dictan un criterio práctico para proporcionar un cuidado adecuado. El progreso de muchos animales es a través de tres etapas generalizadas de desarrollo después del nacimiento: fase neonatal, fase de crecimiento y fase de destete. Cuatro niveles de cuidados pueden ser utilizados para diseñar un sistema que tome en cuenta las fases de desarrollo descritas (Tabla 1). Los equipos de crianza deben elegirse entre un grupo de profesionales capacitados con la habilidad requerida para el cuidado del neonato según el nivel específico de
desarrollo. La Tabla 2 ilustra algunos criterios básicos de habilidad que deben ser requeridos por el personal participante en los programas de crianza.

La crianza artificial no puede ser la única opción cuando hay un animal que ha sido rechazado; puede ser posible una reintegración con éxito con sus congéneres. La última opción para cualquier rechazo sería la crianza artificial. Una vez tomada la decisión, el alojamiento y el equipo serían los próximos pasos para implementar el plan de crianza. Cada zoológico debe considerar sus recursos e intenciones cuando alojan a un animal que está siendo criado a mano. Los animales con poca o nula capacidad de termoregulación requerirán una fuente de calor. Como parte del protocolo de higiene, la opción del desinfectante que se va a usar depende de varios factores. Balanzas exactas, los implementos para medir y mezclar y el equipo para la preparación de los alimentos como estufas, hornos y refrigeradores deberán de ser destinados a las unidades de cuidados del neonato.

Una nutrición balanceada contribuye a un desarrollo psicológico normal, contribuyendo a que el animal tenga ganancia de peso, coordinación y desarrollo del sistema inmune. Es bien conocido el hecho que los mamíferos reciben inmunoglobulinas por transferencia pasiva en el útero y/o postparto por medio del calostro. También se cree que las aves proporcionan una inmunidad inicial a sus crías a través del contenido del buche. Es importante intentar proporcionar inmunoglobulinas de una manera fácil para el recién nacido que no las ha recibido de forma natural. Las necesidades calóricas puede ser calculadas usando la fórmula del metabolismo basal representadas en la figura 1. Los promedios de ingestión de leche varían de acuerdo al grupo taxonómico. Entre las especies típicas de los zoológicos se espera un promedio por día del 10 al 18% del peso corporal en ungulados, del 12 al 25% en carnívoros y de aproximadamente 20% en primates. En aves la labor para proporcionar una fórmula balanceada para neonatos es aún más desafiante.

Cada animal alcanza un nivel clave de desarrollo, el cual puede actuar como indicador para un desarrollo normal. Conocer estas etapas e identificarlas en animales en crecimiento es una parte importante de la evaluación del éxito de un programa de crianza. Los animales muestran un desarrollo normal a través de una ganancia de peso predecible y también en un incremento en su iniciativa para alimentarse. El desarrollo normal de sus miembros, movilidad, coordinación, la dentición (mamíferos), la visión y la condición del pelo ó pluma también son usados como indicadores importantes de un desarrollo normal (o potencialmente anormal).

El proceso de destete es automático generalmente, y en la mayoría de los carnívoros empieza cuando brotan los dientes caninos que salen alrededor de la octava semana de edad. No obstante que muchas características del comportamiento son genéticamente controladas, los factores ambientales pueden afectar la calidad del desarrollo psicológico de muchos animales. El objeto de un programa de crianza artificial es el de obtener un animal adulto física y psicológicamente productivo. El control de la impronta es probablemente el mayor desafío que un programa para neonatos enfrentará. Podemos intentar reducir una impronta incorrecta pero no podemos eliminarla totalmente. A través de manipulaciones externas, las conductas normales pueden ser alentadas o desalentadas. Un programa exitoso para neonatos debe buscar el proporcionar un ambiente externo enriquecido, provisto de diversidad sensorial. Cuándo y dónde se pueda, los animales deben ser criados con sus congéneres. Para fortalecer el desarrollo psicosocial normal es necesario ser creativo con estímulos ambientales naturales que favorezcan conductas normales.
Una unidad de cuidados neonatales bien planeada y manejada es esencial en las operaciones de cualquier zoológico participante en los programas de crianza de especies amenazadas. El papel de y la información generada por el buen desempeño de un programa de cuidados neonatales son muy valiosos desde el punto de vista ecológico.

**Introduction**

Within the last 150 yr, human influence has contributed more than any other event in history to the accelerating rate of species decline worldwide. Each day conservationists are aware of more and more species teetering on the brink of extinction. Some statistics estimate that one species of plant or animal is lost every 60 sec.

Recently, zoos have begun to see themselves as important players in the race to save species and protect ecosystems. For this to be successful, it is critical that each institution evaluate its collection plans, reproductive goals, and education mission. Through collaborative collection management, interdisciplinary involvement in the preservation of ecological systems and expansive education programs zoos may contribute directly to species survival.

Captive breeding programs in zoos are key components in endangered species management. It is important that any captive endangered species breeding program also have a plan in place to provide critical neonatal support care when natural rearing is not possible. There would likely be no argument that an animal reared naturally by its own parent or parents and within its own species group is better off both physically and psychologically than if reared by hand. In most cases such an infant receives proper parental care, balanced nutrition and the opportunity to develop into a socially normal adult.

Unfortunately, problems may occasionally arise that make it impossible for a newborn to be reared naturally. It is important that institutions who accept the responsibility of breeding captive species also commit to building a staff whose skills and expertise are essential to successful rearing of these species. The normal development and eventual socialization of any hand-reared animal is solely dependent on the quality and consistency of care received from well-trained professionals. Animal care personnel must address the importance of staffing and project management in the design and implementation of a neonatal care program. This should include staff training, initial response, meeting nutritional requirements, monitoring developmental stages and controlling socialization and imprinting.

**Staffing**

Adequate staffing is the basis for a well-defined neonatal care program. The developmental stages of young animals dictate the skill criteria required to provide adequate care. Most animals progress through three generalized developmental phases following birth: neonatal phase, growth phase, and the weaning phase. The intensity and duration of these stages will vary from species to species. The neonatal phase is defined as the period of initial stabilization after birth or hatching. At this stage husbandry must meet critical physiologic needs by providing proper temperature, humidity, nutrition, immunization and hygiene. In the growth phase an infant is basically physiologically stable but still dependent upon at least one parent for protection or total nutritional support. The weaning phase in
development is characterized by the period following initial reduction of milk or crop formula and an increase in the consumption of adult food items. Adjustment to a solid diet may contribute to an increase in susceptibility to infection or physiological stress making this a very critical phase in development.

Four levels of care may be utilized in designing a system which takes into account the stages of development outlined above. These levels are characterized by the intensity of monitoring required, the amount of time involved in caregiving and the number of handlers recommended for maximum consistency (Table 1).

Animals requiring Level 1 Care have a medical condition which outweighs their neonatal status. These animals are a veterinary rather than a husbandry concern. Animals which fall into the category of Level 2 Care would be altricial young which are sensitive to changes in environment, stimulation or handling. Some examples are carnivores, primates, psittacines and some ungulates. Semi-precocial young which are not so affected by environmental changes would require Level 3 Care. Some examples include spheniscids, raptors, most ungulates and older, more stable neonates from higher care levels. Precocial or relatively independent young would require Level 4 Care which primarily involves only basic husbandry. This would include waterfowl, gamebirds, and some precocial mammals.

Handrearing teams should be identified from a pool of trained professionals with skills and experience to match the required care criteria for a neonate at specific developmental levels. Table 2 illustrates some of the basic skill criteria which should be required of staff participating in handrearing programs. Once a trained staff of neonatal caregivers is in place and is able to mobilize when a handrearing situation arises, the next consideration is one of project management.

**Initial Response**

Handrearing may not be the only option when it appears an animal can not be parent-reared. When a newborn is rejected, rather than immediately initiating the process of handrearing, some other alternatives might be considered. For example, it may be possible to successfully replace an infant with its natural parent to allow normal rearing to take place. This has been accomplished with primates whose rearing instincts involve clutching behaviors. The staff at the San Francisco Zoo have successfully used a technique of chemically immobilizing postpartum females, placing the neonate on them and allowing the dam to recover. The reintroduction success rate with primates has been over 60% involving species such as Francois langur (*Presbytis francoisi*), Patas monkeys (*Erythrocebus patas*) and Emperor tamarin (*Saguinus imperator*). Ideally the parent and young should remain part of an established group but if group dynamics will not allow a reintroduction to take place the parent and young may need to be isolated and housed separately.

With some birds and mammals the use of surrogates or cross-fostering to conspecifics or to other species for brooding may be a second option for consideration. In this case, only supplement feeding would be necessary. Domestic chickens and ducks have successfully brooded and reared rare land and water birds. Domestic livestock have provided environmental stimulation for many species of exotic ungulates in captivity although precautions should be taken to avoid introducing domestic animals which may carry organisms potentially pathogenic to native or exotic species. Another
possibility is supplemental feeding with natural parent (primates, ungulates, birds).

The last option for any failure of parental care would be handrearing. Once this decision is made, housing and equipment become the next step to implementing a handrearing plan. It may be possible to return the offspring to the parental group at the earliest opportunity for socialization purposes.

**Housing and Equipment**

Each zoo must consider its resources and intentions when housing an animal being handreared. Does the facility have space and equipment necessary to house and raise the animal? Will the animal be on public display? If so, how will the message be presented to the public viewing the animal in an artificial environment? How will the presentation maintain the integrity of the animal in the eyes of the public? If the animal is being raised for potential release, how will imprinting and socialization be controlled?

Animals with little or no thermoregulatory capability will require supportive heat. Incubators should have internal thermometers with which to monitor temperature, thermostat control, malfunction alarm systems, adjustable humidity control, smooth interior and secure door systems. It may also be valuable to have ports for nebulization should vaporized medications or supplemental humidity or oxygen be required. Many hospitals are willing to donate incubators no longer in use in their pediatric wards. Other incubators, specifically designed for veterinary use, can also be easily integrated into a nursery program. Some of these may have particularly desirable features such as the ability to separate the housing and heating units allowing the housing unit to be submerged for cleaning and disinfecting.

Other temperature regulating equipment used for more precocial neonates includes heating pads and lamps. Precautions should be taken when using these heat sources so that curious animals do not injure themselves by touching or chewing. It is also recommended that Teflon coated heating equipment be avoided in facilities which handle birds because of the risk of toxicity if the Teflon is overheated.

As part of the hygiene protocol, the choice of which disinfectant to use depends on several factors. Targeted pathogens may not be sensitive to all disinfectants and not all disinfectants kill all types of organisms. Some are more broad spectrum but may also be more toxic. Others may be very specific. Consider what it is that needs to be disinfected (countertops, glassware, bedding, etc.), and choose a chemical appropriate for that use. Choose disinfectants which are safe for the species of animal that may be exposed to them. For example, phenols such as Lysol® are toxic to cats.

Accurate scales, mixing and measuring devices, and food preparation equipment such as ovens, stoves and refrigerators should be dedicated to the neonatal care facility. When in operation, the nursery facility should be treated as a quarantine area. This assures that the equipment is there when needed, and that quality and hygiene have been maintained.

**Nutrition**

Balanced nutrition contributes to normal physiological development which includes weight gain,
musculoskeletal integrity and development of the immune system. It is well-documented that mammals receive immunoglobulins by passive transfer in utero and/or postpartum in maternal colostrum. It is also believed that birds provide initial immunity for offspring through crop formula contents. It is important to attempt to provide immunoglobulins in a usable form to a neonate who may not have received them naturally.

Neonatal primates and carnivores appear to receive much of their antibodies transplacentally. Ungulates receive these critical antibodies shortly after birth through ingestion of colostrum. Although not as much is known about transferred immunity in birds, it is accepted that some species benefit from receiving the crop contents of their parent(s) shortly after hatching. This is particularly true of Columbiformes whose “crop milk” is highly specialized. It also appears to be important in psittacines and some insectivoruous birds.

Ungulates can receive colostrum artificially in several ways. The most effective method is by collecting milk from the dam within 12-24 hr of parturition and feeding it to the neonate. If it is not possible to deliver maternal colostrum to a neonate, colostrum from the same or a closely related species may be used. Other options would be to collect and store fresh domestic cow, goat, or equine colostrum from a local disease free facility or to use a commercial freeze dried bovine powdered colostrum. Immunoglobulins may also be provided by administering serum or plasma from adult to neonate either p.o., i.v., i.p., or s.c. This procedure is often used for primates and in conjunction with the oral administration of colostrum in ungulates.

General developmental nutrition comes in the form of milk formulas for mammals and various formulas for birds. In some cases it is recommended that an electrolyte solution containing 5% dextrose or less be fed for the initial one to three feedings. This will help the neonate to stabilize physiologically and reach normal body temperature prior to the introduction of formula or maternal milk.

Caloric needs can be calculated using the formula for basal metabolic rate presented in Fig. 1. In this system, Large Birds are defined as those whose full body size is greater that 100 g and Small Birds are those whose full body size is less than or equal to 100 g. Recording an animals weight and calculating caloric needs and consumption are useful in monitoring growth.

Milk intake varies somewhat predictably according to taxonomic group. Among typical zoo species expected intake per day is 10-18% body weight in ungulates, 12-25% in carnivores, and approximately 20% in primates. Many commercial milk replacements are produced for mammals. Most attempt to mimic the concentrations of fat, protein and carbohydrate found in maternal milk. Some examples are presented in Table 3. Commercial products shown here are rated as most effective to least appropriate for carnivores, primates and ungulates. There are also occasions when nutrients in commercially available milk will not be complete for certain species. The maternal milk of cats in the genus Panthera (with the exception of Panthera leo) contains 43-49.3% protein, at least 20% more than other carnivores. If raised on commercial KMR or Esbilac cats often develop alopecia and slower general development. By adding egg yolk protein to the commercial formula the protein level is raised better meeting the requirements for this genus.

In birds the task for providing a balanced neonatal formula can be even more challenging. Nestling
diets often differ greatly from adult diets. Many passerines who, as adults are primarily seed eaters, are fed almost exclusively insect matter as nestlings. The protein requirements may be as much as 50% more in chicks than in adult birds. Some species, such as raptors, cannot digest complex carbohydrates. Other nutrient requirements may differ greatly from those of mammals as well. Birds require vitamin D₃ while most mammals require vitamin D₂ (New World primates are an exception, requiring vitamin D₃). The key to meeting the nutritional requirements of a chick in a handrearing environment is first to know as much about the natural history of the species as possible. Then it is important to provide a high quality and varied diet. Variety will contribute to the overall nutritional balance of any chick formula.

If the formula being given to a neonate is balanced, additional vitamin and mineral supplements should not be necessary. If there is a question with regard to some element which may be lacking or the overall balance of a diet then a high quality pediatric vitamin/mineral supplement may be used. It is important to distinguish between avian and mammalian supplements which may be very different in content.

Establishing a well-balanced nutrition regimen will contribute significantly to the normal physical and psychological development of a young animal. Monitoring and documenting these developmental stages is an important part of a well-managed neonatal care program.

**Development and Weaning**

Every animal reaches key levels of development which can act as markers for tracking normal development. Knowing these stages and identifying them in growing animals is an important part of assessing the success of a nursery program. Most developmental stages are very species specific, others more general. Adequately monitoring the normal growth of a young animal depends on thorough research and planning for the appearance of physical and behavioral characteristics expected for that species. A minimum number of handlers whose knowledge is built from experience will know best what to expect and when. Keeping detailed developmental records for each individual creates a solid reference for future neonatal projects.

Animals exhibit normal development through predictable weight gain and an increase in feeding initiative. A caution for those animal’s whose initiative is strong and who will beg for food even when full: overfeeding can cause more complications and is harder to correct than underfeeding. Caretakers should monitor the size of a nestling’s crop and the distension of a mammal’s abdomen prior to any feeding and be reluctant to feed if it appears to be 10% or more full.

Normal limb development, mobility, coordination, dentition (mammals), vision, and pelage or plumage at the predicted age are also used as important indicators of normal development. A poor feeding response from a young ungulate may indicate a more serious systemic problem such as hypothermia or hypoglycemia. In most arboreal primates the development of hand-eye coordination is essential at an early age.

The weaning process is generally automatic and in most carnivores usually starts when the canine teeth have erupted at about the eighth week of age. In addition, animals should specifically develop...
behavioral patterns which characterize that species. Vocalizations, facial expressions and meaningful posturing all develop as significant species dependent behaviors.

All of these criteria can be used as cues to monitor normal development in a young animal based on a program which allows normal development and adjustment to take place. It is important to neither push an animal too fast nor hold it back. The process of weaning presents an excellent illustration of this point. During weaning, flexibility and reliable observations become critical. Forcing an animal to rely on solid foods before it is developmentally ready can increase stress and contribute to serious metabolic problems. In psittacines the ability of the crop to process solid food increases gradually and sour crop is a major problem in birds who have been forced prematurely onto adult diets. Likewise, keeping an animal on an infant formula longer than it should can result in metabolic problems. Esbilac, although used extensively as one of the best large cat formulas, can cause cataracts if fed over an abnormally prolonged period.

Knowledge of and planning for normal development in handreared animals is not isolated to physiology. The psychological development of a young animal is at least as important as physical development and determines that animal’s level of socialization and adjustment as a member of its species.

Socialization and Imprinting

Although many behavioral characteristics are genetically controlled, environmental factors can affect the quality of much of an animal’s psychological development. Since the object of a handrearing program is to produce a physically and psychologically normal adult, the commitment must be made to provide enriching environmental stimulation which promotes healthy development. A neonatal program should begin with a secure, consistent environment and lead to developing self confidence and independence of the animals in its charge.

Controlling imprinting is likely the biggest challenge a neonatal program will face. All animals enter critical phases where they learn who and what they are. In handrearing we must accept some compromises to imprinting. We can attempt to reduce inaccurate imprinting but we cannot eliminate imprinting entirely. It is the object of most neonatal programs to encourage correct species specific psycho-social behavior away from human dependence.

Through environmental manipulation natural behaviors may be encouraged or discouraged. A successful neonatal program should seek to provide an enriching environment, not a sensory deprived one. Different species imprint and respond differently to varying environmental changes. Understanding these differences will allow us to design individual programs for specific species.

Young ungulates and carnivores identify early with smell, touch and vocalization. Primates which usually cling to parents’ fur, respond strongly to tactile as well as auditory stimulation. Something as simple as fur rolls or padding may approximate a parenteral limb on which to cling. Nestling birds respond to tactile, visual and auditory cues. Beak stimulation prior to feeding or the presence of a feather duster which encourages brooding may contribute positively to natural stimulation during critical developmental periods. Natural sounds, smells, perching and bedding will all enhance the naturalistic environment of the developing neonate.
When and where appropriate animals should be raised with conspecifics. If a collaborative arrangement can be made between zoos to rear similar species together, much of the human dependence associated with rearing isolated individuals may be avoided. The animals will grow while depending upon each other. Many ungulates can be returned to the herd after being trained to a whistle or clicker. In response to the stimulus, the calf will go to the fence to accept a bottle.

There are also cases where surrogates must be used to rear an animal. Puppets or models are often used to simulate the presence of a parent during feeding. These techniques are particularly useful when behaviors are learned from a parent. An example of how this process can contribute to normal development is with Andean condors (*Vultur gryphus*) raised at the San Francisco Zoo. Chicks fed solely by a female condor puppet had no problem in recognizing adults, each other or their prey. It became evident, however, that the chicks had failed to learn how to process their food for eating since the puppet had no feet and the food was always presented in bite sized pieces. Condors stand on their prey and tear it apart with their beak prior to eating it. The chicks did learn the behavior but much later than they might have if we had been prepared.

To strengthen normal psycho-social development it is necessary to be creative with naturalistic environmental stimuli which encourage normal behaviors. These can be as simple as providing logs or mulch in which primates can forage or as challenging as teaching a polar bear to swim. The object is to create independence and success as an adult.

**Conclusion**

The importance of a well-designed and managed neonatal care unit is essential to the operation of any zoo participating in endangered species breeding programs. As zoos guide the captive management of endangered species and become activists in conservation programs, the role of and the information gained by a well-run neonatal care program is valuable from a comprehensive ecological point of view. Professional staffing, adequate response resources and comprehensive systems for nutrition and psycho-physiological development will define the success of any handrearing program. Zoos have the potential to become leaders in resource management. A comprehensive endangered species plan which includes a commitment to neonatal care and development will help to propel this objective forward. And it is with this is mind that zoos and their colleagues may realize their common goal: worldwide conservation.

**LITERATURE CITED**

Table 1. Levels of coverage needed for neonatal care based on general developmental levels.

<table>
<thead>
<tr>
<th>LEVEL OF CARE</th>
<th>CARE CRITERIA</th>
</tr>
</thead>
</table>
| Level 1 Care  | *24 to 15 hr care  
*Neonate requiring intensive physiological monitoring and/or support therapy  
*Maximum number of caregivers should be seven or fewer  
*Sensitive to multiple handlers |
| Level 2 Care  | *24 to 15 hr care  
*Neonate requiring moderate physiological monitoring and/or therapy  
*Maximum number of caregivers should be seven or fewer  
*Sensitive to multiple handlers |
| Level 3 Care  | *24 to 15 hr care  
*Not requiring support therapy  
*Maximum number of caregivers should be ten or fewer  
*Not sensitive to multiple handlers |
| Level 4 Care  | *8 hr day care only  
*Not requiring support therapy  
*Maximum number of caregivers should be 15 or fewer  
*Not sensitive to multiple handlers |
Table 2. Criteria for handrearing teams covering specified care levels.

<table>
<thead>
<tr>
<th>TEAM 1: RESPONSIBLE FOR LEVEL 1 CARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Must be able to perform at skill levels of Teams 2 and 3, and:</td>
</tr>
<tr>
<td>(2) i.v. catheter with line and volume maintenance;</td>
</tr>
<tr>
<td>(3) Draw blood;</td>
</tr>
<tr>
<td>(4) Perform basic blood screening.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TEAM 2: RESPONSIBLE FOR LEVEL 2 CARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Must be able to perform at skill level of Team 3, and:</td>
</tr>
<tr>
<td>(2) Tube feed (as therapy);</td>
</tr>
<tr>
<td>(3) Give injections;</td>
</tr>
<tr>
<td>(4) Administer subcutaneous fluids.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TEAM 3: RESPONSIBLE FOR LEVEL 3 AND 4 CARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Read and follow instructions accurately;</td>
</tr>
<tr>
<td>(2) Maintain records and charts accurately;</td>
</tr>
<tr>
<td>(3) Read a thermometer accurately;</td>
</tr>
<tr>
<td>(4) Perform temperature, pulse, and respiration evaluation;</td>
</tr>
<tr>
<td>(5) Work with standard dilutions (percent and ratio);</td>
</tr>
<tr>
<td>(6) Understand metric weight and volume measurements;</td>
</tr>
<tr>
<td>(7) Stimulate for urination and defecation;</td>
</tr>
<tr>
<td>(8) Administer oral medications;</td>
</tr>
<tr>
<td>(9) Check hydration and mucous membrane color;</td>
</tr>
<tr>
<td>(10) Interpret and report stool consistency and appearance;</td>
</tr>
<tr>
<td>(11) Understand concepts of hygiene and disinfection;</td>
</tr>
<tr>
<td>(12) Understand the operation of nursery equipment;</td>
</tr>
<tr>
<td>(13) Tube feed as husbandry.</td>
</tr>
</tbody>
</table>
Table 3. Commercial milk products and applications.

<table>
<thead>
<tr>
<th>Product</th>
<th>Large Felids</th>
<th>Other Carnivores</th>
<th>Primates</th>
<th>Ungulates</th>
</tr>
</thead>
<tbody>
<tr>
<td>KMR (Pet Ag)</td>
<td>X₂</td>
<td>X₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esbilac (Pet Ag)</td>
<td>X₁</td>
<td>X₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Similac (Ross)</td>
<td></td>
<td></td>
<td>X₄</td>
<td></td>
</tr>
<tr>
<td>Enfamil (Mead-Johnson)</td>
<td>X₁</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isomil (Ross) (Soy base)</td>
<td>X₃</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat Milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canned</td>
<td>X₂</td>
<td>X₅_all</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kid Replacer (Purina)</td>
<td></td>
<td></td>
<td>X₅_all</td>
<td></td>
</tr>
<tr>
<td>Sheep Milk</td>
<td>X₅</td>
<td></td>
<td>X₅</td>
<td></td>
</tr>
<tr>
<td>Equine Fo-Lac (Purina)</td>
<td>X₅</td>
<td></td>
<td>X₅</td>
<td></td>
</tr>
<tr>
<td>Bovine Calf Replacer (Purina)</td>
<td>X₅</td>
<td></td>
<td>X₅</td>
<td></td>
</tr>
<tr>
<td>Evaporated Milk (not recommended--)</td>
<td>---_---</td>
<td>---_---</td>
<td>---_---</td>
<td>---_---</td>
</tr>
</tbody>
</table>

Note: 1, 2 etc...recommended preference

Figure 1. Formula for recommended caloric intake based on taxonomic group, age and health status.

\[
\begin{align*}
\text{Mammals:} & \quad 70_a(W)^{0.75} \text{(status factor)} = \text{kcal offered/24hr} \\
\text{Large Birds:} & \quad 78.3_a(W)^{0.75} \text{(status factor)} = \text{kcal offered/24hr} \\
\text{Small Birds:} & \quad 129_a(W)^{0.75} \text{(status factor)} = \text{kcal offered/24hr} \\
\end{align*}
\]

\text{Status Factor:} \quad \text{Healthy Adult} = 2 \\
\text{Sick Adult} = 3 \\
\text{Healthy Juvenile} = 4 \\
\text{Sick Juvenile} = 5 \\

W=\text{Body Weight in kg.} \\
a=\text{taxonomic classification constant}
Abstract

Marine World Africa USA and The Aquarium for Wildlife Conservation in New York successfully recovered seven orphaned walrus calves (Odobenus rosmarus divergens) from the Alaskan waters of the Bering Sea during the annual spring migration in May of 1994. Three animals were transported to the New York facility, and four were brought to Marine World in Vallejo, California. The Marine World animals have had no significant health problems during the first 2 yr, and have increased their body weight by over 500%. The calves were raised on a powdered animal milk replacer (Multi-milk) as their sole diet for 8 mo, at which time clams, herring, squid and white bait fish were slowly added to the diet. The animals have been trained to allow routine blood sampling, and their blood parameters have been followed. After weaning from the milk formula, some animals had lower-than-expected calcium levels and were started on calcium supplements.

Factors that appear to be most significant to the consistent good health of these animals include the facility design and maintenance; nutritional, social and psychological management; staff training and involvement; and a well-planned program of preventive veterinary care.

Resumen

Mundo marino Africa-USA y el Acuario para la conservación de la Vida Silvestre en Nueva York rescataron con éxito a siete crías huérfanas de morsa (Odobenus rosmarus divergens) de las aguas del mar de Bering en Alaska durante la migración anual de primavera, en mayo de 1994. Tres animales fueron transportados a las instalaciones de Nueva York y cuatro fueron llevados al Mundo Marino, en Vallejo, California. Los animales del Mundo Marino no han tenido problemas significativos de salud durante los primeros años y han incrementado su peso por encima del 500%. Las crías fueron criadas con un sustituto de leche en polvo para animales (Multi-milk) durante 8 meses como su única dieta; en el momento propicio fueron añadidos lentamente a la dieta mariscos, moluscos, arenques, calamares y pescado blanco. Los animales fueron entrenados para permitir llevar a cabo una rutina de muestreo sanguíneo y se le ha dado seguimiento a sus parámetros. Después del destete de la fórmula, algunos animales tuvieron los niveles de calcio más bajos de lo esperado, por lo que se les adicionó con suplementos de calcio.

Los factores que parecen ser los más significativos en la buena salud de estos animales incluyen el buen diseño y mantenimiento apropiado de las instalaciones, la buena alimentación así como el buen manejo social y psicológico; el equipo humano bien entrenado y comprometido, y un programa de medicina preventiva bien planteado.
NUTRITIONAL ASPECTS OF HANDREARING OKAPI (Okapia johnstoni)

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Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513, USA

Abstract

Since 1985 Brookfield Zoo has successfully raised seven okapi calves of which three were handreared using the protocol from the Okapi SSP. The SSP Handrearing Protocol was developed based on background data available from prior okapi handrearing experience (a calf in 1960), okapi milk data, giraffe milk data, and ungulate handrearing experience. For all seven calves weekly body mass data were calculated. For the three handreared calves extensive data were calculated for growth rate including percent body mass gain, energy consumption related to growth rate, and formula consumption on a body mass basis. Information allows for comparison of mother reared to handreared calves for monitoring growth rates. The three okapi calves (1.2) were handreared because of maternal/calf conflict. The formula was 46.25% Evaporated milk, 46.25% water, and 7.5% Esbilac (as-fed). It is recommended that the formula be offered at 9-12% of the animal’s body mass for approximately 4 wk. At that point the calf should be fed a minimum of 6% of its body mass. Dilutions of the final formula are used to adapt the animal and its gastrointestinal tract to the final formula. The formula feedings should be divided evenly among five or six feedings in a 12 hr day since it has been observed that mother reared okapi calves usually suckle in daylight hours. The first of the three handreared calves was handreared beginning day 2. This calf was offered a 50:50 mixture of Evaporated milk and water for week 1; a mixture of 48.78% Evaporated milk: 48.78% Water: 2.44% Esbilac for week 2; a mixture of 47.50% Evaporated milk: 47.50% Water: 5% Esbilac for week 3; the final formula mixture from week 4 until weaning. Based on the performance of the first calf, the formula was modified for the next two. The second calf, was handreared beginning day 3. The second calf was offered a formula mixture of 48.125% Evaporated milk: 48.125% Water: 3.5% Esbilac for week 1 and the final formula mixture from week 2 until weaning. The third calf was handreared beginning in her third week. She was offered the final formula mixture until she was weaned. All the okapi calves were offered formula divided evenly among 6 feedings in the 12 hr daylight period. The formula also was supplemented with a multi-vitamin and an iron supplement. Other foods including herbivore pellets, assorted vegetables, and alfalfa hay were available at all times. Additionally, fresh water was available ad libitum. The first calf consumed the highest percent body mass of all the calves, but they all followed a similar trend. The first calf’s formula consumption declined (4.5-1.4% body mass) around week 14 to 15 and was attributed to illness. In the beginning the second calf had a lower formula consumption than the first calf due to illness (8% vs 13% body mass). Once her health improved, her consumption increased. The third calf did not experience any decrease in consumption due to illness but did have some decreases in consumption due to immobilization for cast changes for a broken leg. Overall the third calf consumed somewhat less per body mass, but all three calves followed similar trends. Decline in the first calf’s energy intake reflects his lowered intake due to illness. Overall it appears that the second calf consumed the most energy per kg body mass. Since energy intake is directly related to formula intake the curves are similar once full formula strength was consumed. While the third calf appeared to consume the lowest formula as a percent body mass and energy per kg body mass she had a higher body mass gain. All three handreared calves appeared to gain body mass consistently. All four mother reared calves showed similar trends to each other in body mass gain. All calves had similar body mass gain
curves regardless of how they were reared. Overall the SSP Handrearing Protocol for okapi has proved successful. Data collected provide optimal growth rates for both mother reared and handreared okapi calves as well as a range of expected energy intakes.

Resumen

Desde 1985 el zoológico de Brookfield ha criado con éxito siete crías de Okapi, de los cuales tres fueron criados usando el protocolo del plan de supervivencia de especies del Okapi. El protocolo SSP para crianza a mano fue desarrollado basándose en la información previa de las experiencias en la crianza de Okapis (una cría en 1960), datos de composición de leche de Jirafa y de Okapi y de la experiencia en crianza de ungulados. La masa corporal de las siete crías fue calculada semanalmente. Para tres crías los datos fueron más extensos, calculando la tasa del crecimiento, incluyendo el porcentaje de ganancia de peso, consumo de energía relacionada con el promedio del crecimiento y consumo de la fórmula en base a la masa corporal. La información permite la comparación entre la crianza materna con la artificial para el monitoreo de rangos de crecimiento. Tres crías de Okapi (1.2) fueron criadas a mano a causa de un conflicto entre la madre y la cría. La fórmula usada fue 46.25% de leche evaporada, 46.25% de agua y 7.5% de Esbilac. Es recomendable que la cantidad ofrecida sea del 9-12% del peso corporal por 4 semanas aproximadamente. A este punto la cría debería consumir por lo menos un 6% de su masa corporal. Las diluciones de la fórmula final son utilizadas para adaptar al animal y a su tracto gastrointestinal a la fórmula final. La fórmula de alimentación debe ser dividida entre cinco y seis tomas en 12 horas luz, desde que se observó que la madre amamanta las crías en horarios diurnos. El primero de los tres becerros criados artificialmente empezó a ser criado al segundo día. A este becerro se le ofreció una mezcla 50:50 de leche evaporada y agua por una semana; una mezcla de 48.78% de leche evaporada, 48.7% agua y 2.44% Esbilac en la segunda semana; y una mezcla de 47.50% de leche evaporada, 47.50% de agua y 5% de Esbilac para la tercera semana. La última fórmula fue ofrecida desde de la cuarta semana hasta el destete. Basándose en los resultados obtenidos con la primera becerra, la fórmula fue modificada para los otros dos animales. El segundo becerro empezó su crianza al día tres y le fue ofrecida una fórmula de 48.125% leche evaporada, 48.125% agua y 3.5% Esbilac para la primera semana y la fórmula final desde la segunda semana hasta el destete. El tercer becerro empezó su crianza hacia su tercera semana de vida. A esta cría se le ofreció la fórmula final hasta que fue destetada. A todos los Okapi se les dividió la fórmula ofrecida en seis comidas durante 12 horas (durante el día). La fórmula también fue suplementada con multivitaminas y hierro. Otros alimentos, incluyendo pellets para herbívoros, vegetales adecuados y alfalfa seca estuvieron disponibles ad libitum. El primer becerro obtuvo el mayor porcentaje de masa corporal de todas las crías, pero todos siguieron una tendencia similar. El consumo de la primera fórmula declinó alrededor de la semana 14-15 y fue atribuido a enfermedad. Al principio el segundo becerro tuvo un consumo más bajo de la fórmula que el primero debido a enfermedad (8% contra 13% de masa corporal). Una vez que su salud mejoró, el consumo se incrementó. El tercer becerro no experimentó ninguna baja de consumo por enfermedad, pero tuvo algunos decrementos debido a inmovilizaciones para cambiar la férula de una pata fracturada. Por encima de todo, el tercer becerro consumió un poco menos con relación a su peso corporal, pero las tres crías tuvieron tendencias similares. La baja en el consumo de energía en el primer becerro parece reflejar su bajo consumo por enfermedad. El segundo becerro consumió más energía por kg de masa corporal. El consumo de energía está directamente relacionado al consumo de la fórmula, ya que las curvas de consumo de energía son similares una vez que
consumieron fórmula completa. Mientras que el tercer becerro consumió la fórmula más baja en relación a la masa corporal y menos energía por kilo de peso, tuvo una mayor ganancia de peso. Todos los becerros criados a mano ganaron masa corporal consistentemente. Los cuatro becerros criados por su madre mostraron tendencias similares en ganancia de peso. Todas las crías tuvieron curvas similares de ganancia de peso indistintamente de como fueron criados. En general, el protocolo de la SSP de crianza artificial para el Okapi tiene éxito probado. Los datos obtenidos proveen rangos óptimos de crecimiento para ambos tipos de crianza del Okapi, así como un rango de consumo de energía esperada.

**Introduction**

Okapi, first discovered in 1901, inhabit the forest in the Northeast area of Zaire. The closest relative to this somewhat shy sensitive animal is the giraffe. Although population status is not certain, research is being conducted to estimate okapi numbers. Okapi have been in captivity since 1919. Captive populations always have been small making sound management strategies vital to the long term success of the population.

Since the captive population is relatively small, development of improved husbandry techniques and protocols have proven integral to the successful reproduction and maintenance of okapi in captivity. While it is preferable for okapi to naturally raise their own offspring, there are circumstances where the risk to either calf or dam necessitates handrearing. Given the importance of successful handrearing to calf survival and thus to the okapi population, the established protocol must have a scientific basis. The purpose of this paper is to present data supporting the Species Survival Plan (SSP) handrearing protocol.

Since 1985 Brookfield Zoo has successfully raised seven okapi calves of which three were handreared. For the three handreared calves extensive data were calculated for growth rate with percent body mass gain, energy consumption related to growth rate, and formula consumption on a body mass basis. For all seven calves weekly body mass data were calculated. This information allows for comparison of mother reared to handreared calves for monitoring growth rates.

**Handrearing**

The three okapi calves (1.2) were handreared because of dam/calf conflict. The first two calves were separated from their dam within the first two days of life, while the last calf was separated 3 wk after birth. Each calf nursed prior to separation from the dam and presumably received colostrum. All three calves have the same dam.

**Diet**

The Protocol

The SSP protocol contains information about feeding, formulas, behavior, normal defecation (first defecation usually not before 21 days, but 30-60 days is not unusual), and weaning procedures. For specific information please refer to the SSP protocol. The SSP Handrearing Protocol was developed
based on background data available from prior handrearing experience (1960), okapi milk data, giraffe milk data, and ungulate handrearing experience. The final formula was 46.25% evaporated milk, 46.25% water, and 7.5% Esbilac (refer to Appendix) (as-fed). There were several steps which vary concentrations to the final formula. The nutrient content of the formulas are outlined in Table 1.

If the calf is separated from the dam before it was able to receive the mother’s colostrum, it should receive domestic cow colostrum. This colostrum should be diluted 50:50 with water. At 48 hr after birth the calf should be offered an initial formula with 48.125% evaporated milk, 48.125% water, and 3.5% Esbilac (percent as-fed). At day 9 until weaning, the calf should be offered the final formula.

Quantity of formula to feed should be adjusted for optimum growth rate based on information from mother reared calves. It is recommended that the formula be offered at 9-12% of the animal’s body mass until approximately 4 wk. At that point the animal should be fed a minimum of 6% of its body mass. Dilutions of the final formula are used to adapt the animal and its gastrointestinal tract to the final formula. The formula feedings should be divided evenly among 5 or 6 feeding in a 12 hr day since it has been observed that mother reared okapi calves suckle only in daylight hours.

Handreared calves

The first calf, Ndura (1.0, born 1989), was handreared beginning day 2. It was assumed he received colostrum while with his mother. The calf was offered a 50:50 mixture of evaporated milk and water for week 1: a mixture of 48.78% evaporated milk: 48.78% water: 2.44% Esbilac for week 2: a mixture of 47.50% evaporated milk: 47.50% water: 5% Esbilac for week 3: the final formula mixture from week 4 until weaning. Based on the performance of Ndura, the handrearing formula was modified for the next two calves. The second calf, Sefini (0.1, born 1990), was handreared beginning on day 3. The calf was offered a formula mixture of 48.125% evaporated milk: 48.125% water: 3.5% Esbilac for week 1 and the final formula mixture from week 2 until weaning. The third calf, Sudi (0.1, born 1995) was handreared beginning in her third week. Sudi was offered the final formula mixture until she was weaned. The formula nutrient values are presented in Table 2.

All the okapi calves were offered formula divided evenly among 6 feedings in the 12 hr daylight period. Water used in making the formula was boiled and refrigerated before use. To avoid possible diarrhea caused by lactose intolerance, Lactaid (refer to Appendix) was added as per package directions. The formula also was supplemented with a multi-vitamin (refer to Appendix) and an iron supplement (refer to Appendix).

Other foods including herbivore pellets, assorted vegetables, and alfalfa hay were available at all times. Fresh water was available ad libitum.

Intake

Formula intake as a percent of body mass for the handreared calves is shown in Figure 1 (as-fed). Because of the quantity of formula Ndura consumed (13% body mass) initially, he consumed the highest percent of body mass over the other calves, but they all followed a similar trend. As Figure 1 shows Ndura’s formula consumption declined (4.5-1.4% body mass) around week 14 to 15. This
decline was attributed to illness. At first, Sefini had a lower formula consumption than Ndura due to her illness (8% vs 13% body mass). Once her health improved, her consumption followed Ndura’s trend. Sudi did not experience any decrease in consumption due to illness but did have some decreases in consumption due to immobilizations for cast changes to repair a fractured leg.

Figure 2 shows energy consumption per kg body mass (as-fed). Overall Sudi consumed somewhat less per body mass, but all three calves followed similar trends. Decline in Ndura’s energy intake reflects his lowered intake due to illness. Overall it appears that Sefini consumed the most energy per kg body mass. Since energy intake is directly related to formula intake the curves are similar once full formula strength was consumed. Ndura and Sefini consumed similar energy levels for weeks two and three.

**Body Mass Gain**

Figure 3 outlines the body mass gain for handreared calves. While Sudi appeared to consume the lowest formula as a percent body mass and energy per kg body mass she had a higher body mass gain. All three handreared calves appeared to gain body mass consistently. Sefini and Ndura had very similar body mass gain curves.

Figure 4 provides the body mass gain data for the four mother reared calves. All four calves showed similar trends in body mass gain with Kuamba consistently being heavier and Kenda consistently being lighter.

Figure 5 provides body mass gains for all calves regardless of how they were reared and shows all calves had similar gains. In general, Kuamba (mother reared) was the heaviest in body mass with Sudi (handreared) next. Sefini, a handreared calf, was the lightest for weeks 4 through 14, but Kenda, a mother reared calf had the lowest body mass from week 21 thru week 35.

**Discussion**

Overall the SSP Handrearing Protocol for okapi has proved successful. Brookfield Zoo has successfully handreared three calves in the last 7 yr (1989, 1990, 1995). Two of the calves have grown to be reproductive okapi; Sefini successfully gave birth and reared her calf and Ndura has successfully sired his first offspring both in 1994. All animals continue to be healthy. Ndura and Sefini have been transferred to other institutions and have successfully integrated with other adult okapi. Data collected provide optimal growth rates for both mother reared and handreared okapi calves as well as a range of expected energy intakes.

**LITERATURE CITED**

food limitation in a rain-forest herbivore?


Table 1. Nutrient content of handrearing formulas.

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<tr>
<th>Formula</th>
<th>Energy, kcal/100 ml</th>
<th>Fat</th>
<th>Protein</th>
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Table 2. Nutrient content of the handrearing formulas used at Brookfield Zoo.

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Figure #1

Formula Intake as Percent of Body Mass for Handreared Calves
(as-fed)

Intake % Body Mass

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Ndura  Sefini  Sudi
Figure #2

Energy Intake per Kilogram Body Mass for Handreared Calves

(kg-fed)

Weeks Old

kcal/kg Body Mass

Ndura  Sefni  Sudi

1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20  21  22  23  24  25  26  27  28  29  30  31  32  33  34  35  36  37  38  39  40  41  42  43  44  45  46  47
Figure #3

Body Mass Gain for Handreared Calves

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- Ndura
- Sefini
- Sudi
Appendix

Carnation Evaporated Milk  
Carnation Company  
Los Angeles, CA 90036

Esbilac  
Pet-Ag, Inc. (Division of Milk Specialities)  
30 W 432 Route 20  
Elgin, IL 60120-9527

Poly-vits (multi-vitamin)  
(Distributor)  
Major Pharmaceutical Corp.  
Chicago, IL 60612

Fer-in-sol  
Meade Johnson Nutritional Division  
Bristol-Myers Company  
Evansville, IN 47721

Lactaid  
Lactaid, Inc.  
P.O. Box 111  
Pleasantville, NJ 08232
VITAMIN D RELATED DISEASE IN INFANT PRIMATES

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Abstract

Vitamin D deficiency is a well-known cause of disease in primates. Prior to the discovery that New World primates could only poorly utilize oral vitamin D, rickets, secondary nutritional hyperparathyroidism and “cage paralysis” were regularly diagnosed in these species. In spite of the current knowledge of vitamin D requirements, vitamin D deficiency disease still occurs.1

Primates that do not have access to UV light require a dietary source of Vitamin D. Vitamin D produced in the skin due to exposure to UV light or supplied in the diet is hydroxylated in the liver to 25-hydroxy vitamin D (25OHD). 25OHD is subsequently hydroxylated into the active metabolite 1,25-dihydroxy vitamin D (1,25(OH)2D) in the kidney. The active metabolite reacts at specific receptor sites to regulate the gastrointestinal absorption of calcium and phosphorus. Vitamin D is a steroid hormone and regulates gene transcription of specific mRNA species in its target sites.3 The mechanism for the end-organ resistance to vitamin D in New World primates is also seen with other steroidal hormones.2

Infant primates that are mother-reared indoors are at risk for vitamin D deficiency. Milk is a poor source of vitamin D and infants that do not have exposure to UV light will not produce vitamin D in their skin. Nursery reared infants may also be at risk for metabolic bone disease. The use of soy based formulas due to intolerance of milk-based formulas is associated with an increased risk of vitamin D and calcium malabsorption, and subsequent metabolic bone disease. Soy based formula should be supplemented with oral vitamin D preparations.

Vitamin D deficiency produces alterations in the metabolism of calcium and phosphorus that include hypophosphatemia due to secondary hyperparathyroidism and, in more severe deficiencies, frank hypocalcemia and rickets. Clinical signs of rickets include hypotonia, muscle weakness, and in severe cases, tetany. Weight bearing produces bowing of long bones.4 Radiographic signs of rickets include widening and fraying of the metaphyses of long bones. Bone density is decreased, but this must be evaluated carefully since significant trabecular bone may be lost before visible decrease is noticed in the cortical bone.5 Vitamin D deficiency is associated with pneumonia, although the mechanism is not known. Serum biochemical abnormalities include low serum phosphorus, low or normal serum calcium and elevated alkaline phosphatase. 25OHD levels are low in vitamin D deficiency, while circulating 1,25 (OH)2D levels may be normal or elevated due to increased renal hydroxylation until all 25OHD is depleted. Parathyroid hormone levels are increased secondary to hypocalcemia.

Treatment of rickets in mother-reared infant primates is complicated by the desire to leave the infants with their mother. Mothers may be trained to allow individual oral supplementation of the infants.
It is also possible to give long term supplementation with injectable an vitamin D preparation (calciferol in oil). It is important to measure 1,25(OH)\textsubscript{2}D levels in addition to 25OHD levels to differentiate between various deficiency states. Response to treatment can not be accurately assessed using only 25OHD measurements. 1,25(OH)\textsubscript{2}D may rise to high levels following treatment while 25OHD levels are still low.

The understanding of the clinical and biochemical profile of metabolic bone diseases in infant non-human primates will allow specific therapy and improve the clinical outcome of affected primates.

**Resumen**

La deficiencia de la vitamina D es una causa muy común de enfermedades en primates. Antes de descubrir que los primates solo pueden utilizar la vitamina D\textsubscript{2} pobremente por vía oral, el raquitismo, el hiperparatiroidismo nutricional secundario y la parálisis del tren posterior eran frecuentemente diagnosticadas en estas especies. A pesar de que los requerimientos de vitamina D se conocen bien, la deficiencia de vitamina D aún continúa presentándose.

Los primates que no tienen acceso a la luz UV requieren una fuente de vitamina D. La vitamina D se sintetiza en la piel por la exposición de los rayos UV o por suplementos en la dieta y es hidroxilizada en el hígado a 25-dihidroxivitamina D (25OHD) y esta a su vez hidroxilizada convirtiéndola en un metabolito activo 1,25-dihidroxivitaminaD [1,25(OH)\textsubscript{2}D] en el riñón. El metabolito activo reacciona con un receptor específico para regular la absorción gastrointestinal del calcio y fósforo. La vitamina D es una hormona esteroide y regula la transcripción de los genes de especies mRNA específicas en sus sitios blanco. El mecanismo de resistencia órgano-terminal a la vitamina D en primates del nuevo mundo se ha visto también con otras hormonas esteroides.

Los primates infantes que son criados por sus madres en interiores tienen el riesgo de tener deficiencia de vitamina D. La leche es una fuente pobre en vitamina D y los infantes que no tienen exposición a los rayos UV no tendrán vitamina D en su piel. Los animales criados artificialmente pueden también tener el riesgo de una enfermedad ósea metabólica. El uso de fórmulas basadas en la soya por intolerancia a los productos lácteos, está asociado a un incremento en el riesgo de mala absorción de vitamina D y calcio, y en consecuencia habrá una enfermedad metabólica ósea. Las fórmulas de soya deben de ser suplementadas con vitamina D oral.

La deficiencia de vitamina D produce alteraciones en el metabolismo del calcio y del fósforo que incluyen una hipofosfatemia debida a un hiperparatiroidismo secundario y, en deficiencias más severas, hipocalcemia y raquitismo. Los signos clínicos del raquitismo son: hipotonía, debilidad muscular y en casos severos tetania. La carga del peso produce arqueamiento de los huesos largos. Los signos radiológicos del raquitismo incluyen desgaste y ensanchamiento de la metáfisis de los huesos largos. La densidad del hueso disminuye, pero esto debe ser evaluado cuidadosamente, ya que el hueso trabecular puede perderse antes de que se note una disminución visible en el hueso cortical. La deficiencia de vitamina D se asocia con neumonías, a pesar de que el mecanismo no es conocido. Las anormalidades bioquímicas del suero incluyen, bajos niveles de fósforo, niveles de calcio bajos a normales y una elevación de la fosfatasa alcalina. Los niveles de 25OHD son bajos en la deficiencia de vitamina D, mientras que los de 1,25 (OH)\textsubscript{2}D circulante pueden ser normales a elevados debido...
al incremento de la hidroxilación renal hasta que todo el 25OHD es agotado. Los niveles de la hormona paratiroides están aumentados por causa secundaria a la hipocalcemia.

El tratamiento del raquitismo en los primates infantes criados por su madre es complicado por el deseo de dejar a las crías con sus madres. Las madres deben ser entrenadas para que permitan una suplementación oral individual a sus crías. También es posible darles una suplementación prolongada con vitamina D inyectable. Es importante medir los niveles de 1,25(OH)₂D en adición con los niveles de 25OHD para diferenciarlos entre varios estadios de deficiencia. La respuesta al tratamiento no puede ser evaluada con exactitud efectuando solamente mediciones de 25OHD. La 1,25 (OH)₂D puede alcanzar niveles altos después de un tratamiento mientras que los niveles de 25OHD se conservan aún bajos.

El conocimiento del perfil clínico y bioquímico de las enfermedades óseas metabólicas en primates no humanos infantes permitirá una terapia específica y mejorará los resultado clínicos en primates afectados.

**LITERATURE CITED**


COMPARISON OF IN VITRO TESTS FOR EVALUATION OF PASSIVE TRANSFER IN GIRAFFE

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Abstract

Failure of passive transfer (FPT) with secondary complications is a leading cause of morbidity and mortality in neonatal ungulates. Development of a breeding program for captive animals requires a large commitment of resources. Enhancing survivorship of offspring is often critical to the success of the program. Frequently, the decision to remove an animal for hand-rearing occurs after secondary complications, such as pneumonia, diarrhea, and septicemia, become evident. The time and materials required for a favorable prognosis are significantly increased once clinical signs occur. Therefore, better methods for evaluating an infant’s status are critical to making the appropriate decision for intervention. Early detection of FPT allows for appropriate prophylactic measures to reduce the risk of secondary infections. In domestic ungulates, methods of assessing immunoglobulin levels include measurement of total protein, electrophoretic protein fractionation, zinc sulfate turbidity, sodium sulfite precipitation, glutaraldehyde coagulation, and radial immunodiffusion. Although glutaraldehyde coagulation, zinc sulfate turbidity and total protein measurements have been used in exotic ruminants to assess passive transfer, other methods of estimating immunoglobulin levels and their association to other tests and medical history have not been reported. The purpose of this study was to compare commonly available tests for evaluation of humoral immune status in giraffe as an aid to early detection of FPT.

Data were obtained using serum samples from giraffe that were born or maintained at Busch Gardens Tampa. The sample population included hand- and dam-reared neonates, and adults. Each sample was tested using previously described methods. These included: 1) protein electrophoretic fractionation by a commercial laboratory (Smith-Kline); 2) refractometric measurement of total protein; 3) zinc sulfate turbidity measured spectrophotometrically; 4) glutaraldehyde coagulation (Bova-S, VMRD, Inc., Pullman, WA); and 5) sodium sulfite turbidity assessed visually. Comparisons between tests were standardized using a bovine control (immunoglobulin level determined by radial immunodiffusion).

Using least squares regression analysis, a linear relationship existed between total protein (TP), gamma globulin (glob), and estimated immunoglobulin (Ig) levels based on zinc sulfate turbidity (r>0.85, p<0.001 for all comparisons) (Figs. 1-3). Using values similar to those described for domestic ruminants, cutoff points were chosen to assess passive transfer. FPT was defined in these assays as: 1) total protein <6.0 g/dl; 2) gamma globulin <0.5 g/dl; 3) estimated immunoglobulin level <1000 mg/dl; 4) glutaraldehyde coagulation test negative; or 5) no visually detectable turbidity at 15% sodium sulfite. Sample values were segregated based on these criteria and mean values compared. Means were significantly different (p<0.01) for each of the comparisons (Table 1). A positive correlation was observed between samples that were designated as resulting in FPT based
on immunoglobulin level (<1000 mg/dl) and those determined to result in FPT based on all other tests used (total protein, gamma globulin, glutaraldehyde coagulation, and sodium sulfite turbidity). The majority of young animals tested were selected because they were believed to have an increased likelihood of FPT based on clinical history.

The relationship between age and immunoglobulin level was examined using samples obtained from giraffe with a broad range of ages. Samples from animals less than 1 mo of age were distributed between both categories (FPT and adequate passive transfer as determined by the criteria established above). Statistical analysis indicated that animals less than 1 mo of age were as likely to fall into either category based solely on age (i.e., the criteria were not biased for age). Calves with adequate passive transfer had values comparable to those of clinically normal adults. Retrospective examination of the medical histories showed a strong statistical association between animals designated as having FPT and those that were hand-reared based on clinical assessment (Table 2).

In summary, using previously described methods for assessment of passive transfer in domestic ruminants, criteria were established to predict the likelihood of FPT in captive giraffe. Although the sample size was relatively small, a statistically significant correlation was observed between each of the tests. Use of at least two of the criteria would be expected to increase the accuracy of these predictions. Reliable methods for early detection of FPT allows for appropriate intervention (such as removal for hand-rearing) and enhances neonatal survivorship.

**Resumen**

La falla en la transferencia pasiva inmunitaria (FPT) con complicaciones secundarias es la causa principal de la morbilidad y mortalidad en ungulados neonatos. El desarrollo de programas de crianza para animales en cautiverio requiere de un gran cometido de recursos. El incremento de la supervivencia de la descendencia es un punto crítico para determinar el éxito del programa. Frequentemente la decisión de retirar un animal para criarlo artificialmente ocurre después de complicaciones secundarias como: neumonía, diarrea y septicemia muy evidentes. El tiempo y los materiales necesarios para un pronóstico favorable se incrementa significativamente una vez presentados los signos clínicos. Por lo tanto los mejores métodos para evaluar el estatus de la cría son críticos para tomar la decisión adecuada para intervenir. La detección temprana por medio de la FPT seguida de medidas profilácticas apropiadas reducen el riesgo de infecciones secundarias. En ungulados domésticos, los métodos para determinar los niveles de inmunoglobulinas incluyen: cantidad de proteínas totales, proteínas fraccionadas por electroforesis, turbidez del sulfato de zinc, precipitación de sulfito de sodio, coagulación de glutaraldehído e inmunodifusión radial. Aunque la coagulación de glutaraldehído, turbiedad del sulfato de zinc y la cantidad de proteínas totales han sido usados en rumiantes exóticos para evaluar los niveles de FPT, otros método para la estimación de los niveles de inmunoglobulina y su asociación con otras pruebas e historias médicas no han sido reportados. El propósito de ese estudio fue comparar las pruebas comúnmente disponibles para la evaluación de la inmunidad humoral en jirafas como una ayuda temprana para detectar la FTP.

Los datos fueron obtenidos usando el suero de las muestras provenientes de jirafas que nacieron en el Busch Gardens, Tampa. Se incluyeron muestras de animales criados artificialmente, criados con sus madres y animales adultos. Cada muestra fue examinada usando los métodos previamente
descritos que incluyen: 1) fracción proteínica y electroforesis (en laboratorios comerciales); 2) proteína total (medición refractomérica); 3) turbidez de sulfato de zinc (por medio de electroforesis); 4) coagulación de glutaraldehido (Bova-S, VMRD, Inc., Pullman, WA); y 5) turbidez de sulfito de sodio. Las comparaciones entre las pruebas fueron estandarizadas usando un control de bovino (niveles de inmunoglobulinas determinados por inmunodifusión radial).

Usando un análisis de regresión, una relación lineal entre la proteína total (TP), gammaglobulina y los niveles estimados de inmunoglobulinas (Ig) basados en la turbidez de sulfato de zinc (r>0.85, p<0.001) se obtuvo para todas las comparaciones (Figs.1-3). Utilizando valores similares a los descritos para rumiantes domésticos, los puntos más separados fueron escogidos para medir la transferencia pasiva. FPT fue definida en estos ensayos como: 1) proteína total <6.0 g/dl; 2) gammaglobulina < 0.5 g/dl; 3) nivel estimado de Ig < 1000 mg/dl; 4) coagulación glutaraldehido negativo; 5) turbiedad visual detectable al 15% del sulfito de sodio. Los valores de las muestras fueron segregados basados en estos criterios y sus valores medios comparados. Los valores medios fueron significativamente diferentes (p< 0.01) para cada una de las comparaciones (Tabla 1.). Una correlación positiva fue observada en las muestras que fueron designadas como resultantes en la FPT basadas en el nivel de inmunoglobulinas (< 1000 mg/dl) y aquellas determinadas en los resultado de la FPT basados en otras pruebas utilizadas (proteína total, gammaglobulinas, coagulación de glutaraldehido y turbiedad del sulfito de sodio). La mayoría de los animales jóvenes examinados fueron seleccionados porque se creyó que tenían la probabilidad de un incremento de FPT basado en la historia clínica.

La relación entre edad y niveles de inmunoglobulina fue determinada examinando muestras obtenidas de jirafas con un amplio rango de edades. Las muestras de animales de menos de un mes de edad fueron distribuidos en ambas categorías (FPT y adecuada transferencia pasiva determinada por el criterio ya establecido). El análisis estadístico indicó que los animales de menos de un mes de edad fueron propicios a caer dentro de cualquier categoría basada solamente en la edad (los criterios no fueron influenciados por la edad). Además, crías con una adecuada transferencia pasiva tuvieron valores comparables con la de los adultos clínicamente normales. Un examen retrospectivo de las historias clínicas mostraron una fuerte asociación estadística entre animales designados a tener FPT y aquellos que requirieron de crianza artificial después de una evaluación clínica (Tabla 2).

En resumen, utilizando los métodos descritos previamente para evaluar la FPT en rumiantes domésticos, se establecieron los criterios para predecir la probabilidad de FPT en jirafas en cautiverio. Aunque el tamaño de las muestras fue relativamente pequeña, estadísticamente fue significante la correlación observada entre cada una de las pruebas. Usando al menos dos de los criterios, podría esperarse el incrementar la exactitud de estos pronósticos. Los métodos seguros para una detección temprana de FPT permiten una intervención apropiada (como decidir la separación para la crianza artificial) y aumentan la supervivencia neonatal.

ACKNOWLEDGMENTS

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LITERATURE CITED
Table 1. Comparison of mean values using established criteria for FPT.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Number of samples</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP &gt;6.0 g/dl*</td>
<td>8</td>
<td>7.6 g/dl</td>
<td>1.0</td>
</tr>
<tr>
<td>TP &lt;6.0 g/dl</td>
<td>13</td>
<td>5.2 g/dl</td>
<td>0.3</td>
</tr>
<tr>
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<td>8</td>
<td>2196 mg/dl</td>
<td>1120</td>
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<td>13</td>
<td>506 mg/dl</td>
<td>243</td>
</tr>
<tr>
<td>Glob &gt;0.5 g/dl*</td>
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<td>1.9 g/dl</td>
<td>0.9</td>
</tr>
<tr>
<td>Glob &lt;0.5 g/dl</td>
<td>12</td>
<td>0.2 g/dl</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* Statistically significant at p<0.01, Student’s t-test.

Table 2. Comparison of hand-reared versus dam-reared calves < 1 week of age.

<table>
<thead>
<tr>
<th>Criterion</th>
<th># Hand-reared</th>
<th># Dam-reared</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP &lt; 6.0 g/dl*</td>
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<td>0</td>
</tr>
<tr>
<td>TP &gt; 6.0 g/dl</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Ig &lt; 1000 mg/dl*</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Ig &gt; 1000 mg/dl</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Glob &lt; 0.5 g/dl*</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Glob &gt; 0.5 g/dl</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Bova-S negative*</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Bova-S positive</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

* Statistically significant at p<0.01, Student’s t-test.
Figure 1. Ig vs Gamma Globulin

Y = -0.09 + 0.0009X, r=0.86
p<0.001, Student’s t-test
Fig. 2 TP vs Ig

Y = -3034.2 + 683.8X, r=0.85
p<0.001, Student's t-test
Fig. 3  TP vs Gamma Globulin

Y = -4.07 + 0.82X, r=0.90
p<0.001, Student’s t-test
ANALYSIS OF AFRICAN ELEPHANT MATURE MILK IN EARLY LACTATION AND FORMULATION OF AN ELEPHANT CALF MILK REPLACER

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Abstract

Mature milk samples (n=5) were collected from one African elephant (Loxodonta africana) during early lactation for analysis of nutrient composition. Total solids averaged 11.32% and were significantly lower than previously reported for African elephants. Lactose averaged 2.79% (24.6% dry matter basis), which was also significantly lower than previously reported and indicates African elephants are a low-to-moderate lactose species. Bovine milk contains 1.5-2 times this level, and human milk replacers contain 2.5 times this level of lactose on a dry matter basis. This could represent a significant cause of diarrhea when human milk replacers are used in African elephant calves. Milk fat averaged 4.38% (39% dry matter basis) and ranged from 3.51-5.32%. Protein levels averaged 2.3% (20% dry matter basis). Ash levels averaged 0.53% (4.7% dry matter basis). Vitamin A levels ranged from 28-171 IU/100 g (249-1361 IU/100 g dry matter basis) and vitamin D ranged from 22-69.8 IU/100 g (196-693 IU/100 g dry matter basis). Vitamin E ranged from 0.33-0.88 µg/ml, with the cow supplemented on a diet of 8,000 IU per day. Calcium levels averaged 37.8 mg/100 g (334 mg/100 g) and ranged from 28-43 mg/100 g (257-431 mg/100 g dry matter basis); phosphorus averaged 18.8 mg/100 g (166 mg/100 g dry matter basis) and ranged from 15.9-20.8 mg/100 g (143-204 mg/100 g dry matter basis). The calcium:phosphorus ratio averaged approximately 2:1.

An African elephant calf milk replacer was formulated based on the mature milk analysis of early lactation. The general makeup included: total solids (11.5%), fat (5%), lactose (2.5%), protein (3.3%), ash (0.52%), calcium (65 mg/100 g), phosphorus (42 mg/100 g), vitamin A (75 IU/100 g) and vitamin D (46 IU/100 g). Vitamin E is supplemented separately as 2 IU/kg body weight micellized natural tocopherol (Stuart Products) to insure bioavailability. The milk replacer is produced starting with bovine skim milk powder and bovine whey protein concentrate, mixed to provide the milk proteins necessary in the milk replacer. Fat is then added using a fat premix and coconut oil (coconut oil is approximately 25% of the total fat supplied). A mixture of mineral and vitamin premix completes the formula. The final formulation maintained lactose on the low end of the milk analysis range (20-26% dry matter basis), to minimize the risk of a lactose-induced diarrhea. Protein and fat were maintained at the high end or slightly above the range in the milk analysis to accommodate the lower lactose and still maintain a total solids of approximately 11.5%.

Resumen

Cinco muestras de leche madura fueron colectadas de una hembra de elefante africano (Loxodonta africana) durante una lactación temprana para un análisis de sus elementos nutricionales. El promedio de sólidos totales fue del 11-32% y fue significativamente bajo en relación a lo reportado anteriormente en esta especie. El promedio de lactosa fue de 2.79% (24.6% base materia seca) y también fue significativamente bajo a lo ya reportado e indica que el elefante africano es una especie con niveles de lactosa de bajos a moderados. La leche de bovino contiene 1.5-2 veces este nivel y...
los sustitutos de leche para humanos contienen 2.5 veces más este nivel de lactosa en base a materia seca. Esto podría representar una causa importante de diarrea cuando los sustitutos de leche para humanos son usados en crías de elefante africano. El promedio de grasa es de 4.38% (39% b.m.s.) y tiene un rango de 3.51%-5.32%. El promedio de nivel de proteína es de 2.3% (20% b.m.s.). Cenizas 0.53% (4.7% b.m.s.). La vitamina A tiene un rango de 28-171 UI/100 g (249-136 UI/100 g b.m.s.) y la vitamina D un rango de 22-69.8UI/100g b.m.s. (156-693 UI/100g b.m.s.). La vitamina E va de 0.33 a 0.88 µg/ml cuando la hembra lactante es suplementada en su dieta con 8,000 UI/día. El promedio de niveles de calcio es de 37.8 (334 mg/100g) con un rango de 28-43 mg/100g (257-431 mg/100 g b.m.s.). El promedio de fósforo es de 18.8 mg/100 g (166 mg/100 g b.m.s.) con un rango de 15.9-20.8 mg/100 g (143-204 mg/100 g b.m.s.). En promedio, la relación calcio-fósforo es de aproximadamente 2:1.

Un sustituto de leche para cría de elefante africano fue formulado basándose en el análisis de la leche madura de la lactación temprana. La composición general incluye: sólidos totales (11.5%), grasa (5%), lactosa (2.5%), proteína (3.3%) cenizas (0.52%), calcio (65 mg/100g), fósforo (42 mg/100g), vitamina A (75 UI/100g) y vitamina D (46 UI/100g). La vitamina E se suplementa en forma de tocoferol natural micelizado (Productos Stuart), en dosis de 2 UI/kg de peso, para asegurar su biodisponibilidad. El sustituto de leche se produce empezando con leche en polvo descremada de bovino y con suero proteínado concentrado, mezclado para proporcionar las proteínas de leche en el sustituto. La grasa es entonces añadida usando una premezcla de grasa y aceite de coco (aproximadamente el 25% del total de grasa suplementada). Una mezcla de premezclas minerales y vitaminas complementan la fórmula. La formulación final conservó la lactosa en el límite bajo del rango de la leche analizada (20-26% b.m.s.), para minimizar el riesgo de una diarrea inducida por lactosa. La proteína y la grasa se mantuvieron en el límite alto o ligeramente arriba del rango del análisis de la leche para mantener la lactosa baja y seguir conservando los sólidos totales en aproximadamente 11.5%.

Introduction

The successful hand-rearing of any mammalian species is dependent upon providing a milk replacer which closely resembles the nutrient makeup of the dam’s natural breast milk. Milk is the sole food for infants and is therefore necessary in order to provide 100% of the required nutrients for growth. Normal growth and development requires absorption and assimilation of these nutrients.

The makeup of natural milk is extremely complex. The major components of milk are water, fat, sugar, protein, and ash. Milk varies significantly in composition from species to species in the basic general nutrient components as well as the more detailed analysis (amino acid levels, fatty acid levels, minerals, etc.). Milk fat is composed of fatty acids. At least 167 fatty acids have been identified in human milk. Bovine milk has 437 fatty acids. The milk sugar is almost exclusively lactose, a disaccharide composed of a glucose molecule and a galactose molecule, and is the predominant carbohydrate in milk. This can range from 2% in the rabbit to 7% in the human. Proteins of milk include curds (casein), and whey proteins (lactalbumins). When milk clots or curdles, the casein is transformed into an insoluble calcium caseinate-calcium phosphate complex. This is the major calcium source in milk, and may represent a significant source of calcium in the formulation of a milk replacer. Casein has a species-specific amino acid composition. Milk is highly digestible. The true
digestibility of the major milk components of same species milk approaches 100%.\textsuperscript{10}

Natural milk contains components which are species specific, including immunoglobulins, cellular components (leukocytes), and nonspecific antimicrobial factors. The immunoglobulins in milk are distinct from serum. The main immunoglobulin in serum is IgG. The main immunoglobulin in milk is IgA, primarily in the form of secretory IgA. The lowered incidence of enteric and respiratory infections seen in breast-fed versus formula raised human infants has been recognized.\textsuperscript{6} It should not be surprising, then, that calf diarrhea is a common problem in milk replacer calves when the milk source is from another species. Use of a milk replacer which is significantly different from natural mother’s milk can quickly lead to acute diarrhea, chronic diarrhea, malabsorption, developmental problems, and death.

There are a number of variables which can affect the composition of milk. These variables are very significant in the sampling procedures of milk, interpretation of results, review of previous studies in the literature, and formulation of a calf milk replacer. Below is a list of some of the major variables affecting milk composition:

- Species Variation
- Individual Variation
- Stage of Lactation
- Nutritional Status of the Cow
- Disease
- Time of Day Milking
- Course of Milking
- Milk Handling by staff
- Milking Interval (length of time from previous milking)

Previous studies on African elephant milk are very limited. Many of the variables affecting milk composition and variables in milk sampling procedures were not addressed in the reports. The reliability of the results is not clear.

Analysis of milk replacers, for comparison to milk analysis, is complicated by a lack of uniformity in nutrient measurements. Calf milk replacers are delivered and analyzed in a powder form, rather than as fed or dry matter basis; liquid human milk replacers report some nutrients in g/100 cal rather than percentage; milk analysis is usually reported in percent rather than on a dry matter basis. By not adjusting for total solids (dry matter basis), the real nutrient composition could vary significantly in milk reports.

Case Report

A male African elephant calf was born on November 3, 1995, at the Oakland Zoo. The calf sustained muscle damage at some point within the first 5 hr of birth and was pulled from the cow for physical examination and supportive care. Initially, he was unable to stand or walk on his own without assistance. Attempts that evening were unsuccessful to return the calf to his mother. The calf was subsequently hand-raised.
A second African elephant calf was born November 21, 1995, to another cow. He never stood, and radiographs revealed a luxated left coxofemoral joint, which occurred within the first 3 hr of birth. The second calf was euthanatized.

Following the death of the second elephant calf, the second cow continued to lactate for the next twelve days. She was observed sucking on her teats, possibly due to discomfort associated with full mammary glands. By withdrawing milk from the mammary glands, she was able to keep lactating during this period. This allowed staff the time to prepare for a rare opportunity to milk an actively lactating African elephant cow, for analysis and milk supplementation for the first calf.

During this period, the first calf initially received a human milk replacer (Enfamil) as a calf milk replacer. This was replaced by a calf milk replacer designed specifically for African elephant calves (Grober, Inc.), based on previous literature.

The objective of this study was to analyze African elephant milk through the first 3 ½ mo of lactation and to develop a suitable calf milk replacer for the first calf.

Milking began on the 13th day of lactation. The milking process began with a warm compress first applied to the mammary gland. The handler would slip under the cow to apply an extra-large cup over the teat, connected to a Madela Classic human breast pump. The handler massaged the gland to encourage milk let-down. The process would take approximately 15 min for each gland. Milking was completed by manually expressing any remaining milk, to completely empty the mammary gland.

Initially, the cow was milked three times daily over a 10 hr period. This yielded approximately 950 cc in a 24 hr period. On day 65, the milking was reduced to twice daily. The milk yield decreased to 600-750 cc daily on this schedule. The milk composition did not appear to change due to the decrease in milking frequency.

Milk samples were refrigerated or frozen. Samples were delivered to two different dairy labs for comparison (DFL Laboratories, California Department of Food and Agriculture). Samples were also sent to the International Wildlife Conservation Park (Bronx Zoo), for Vitamin E and retinol assays. Cow serum samples were drawn on the same day as the milk samples, for serum Vitamin E assay and to correlate serum levels with milk levels.

Results

Elephant milk appears grossly like skim milk, with a grey tint to the overall white color.

The results of the milk assays are included in Table 1.

Discussion

The results of the milk analysis were significantly different from the literature in several components, and this would directly modify the formulation of an African elephant calf milk replacer. Care was
taken to completely empty the mammary gland, to allow for any changes in nutrient levels which may occur during the milking process (especially fat). The samples obtained throughout the day were pooled, to represent a 24 hr sample for composition.

The primary individual milk nutrient levels are discussed below.

**Total Solids**

Total solids of African elephant milk were consistently lower than previously reported. Total solids averaged 11.32%. The range of total solids of 10.06 to 12.71 is more in line with most artiodactyls, and was approximately 60% of previous values mentioned in the literature (previous literature values ranged 17-21%). This significantly affects the calculation of concentrations of all other components on a dry matter basis. The lower concentration milk was supported clinically with palatability for the elephant calf. The first calf milk replacer was very cream-like at 20% total solids; the calf preferred the revised milk replacer at 11%, and would drink the replacer much more readily.

**Fat**

Milk fat levels averaged 4.38% (39% dry matter basis) and ranged from 3.51 to 5.32% (34.9 - 41.8% dry matter basis). Previous reports have described much higher levels of milk fat, ranging from 9.3-15.1%.

Results in this study indicate a higher percentage of fat in elephant milk compared to bovine milk on a dry matter basis (39%:28%) (See Table 2).

**Lactose**

Lactose levels averaged 2.79% (24.6% dry matter basis). Lactose levels in this study were 41% to 68% of levels previously reported in the literature. This indicates that African elephants are a low to moderate lactose species.

Of greater importance, human milk replacers have been recommended for African elephant calves and contain 7% lactose (52-58% dry matter basis), which is 2-3 times higher than elephant milk (See Table 2). Essentially over half of the total solids in human milk replacer is sugar. Bovine milk contains 1 ½ - 2 times the level of lactose found in African elephant milk. Lactose is a major concern in formulation of a milk replacer because excess lactose can be a major cause of neonatal diarrhea, due to the osmotic influence in the intestine, as well as alterations of the bacterial flora of the bowel. This makes human milk replacers a poor choice for an African elephant calf milk replacer.

**Protein**

Milk protein levels were consistent throughout the study. Protein levels averaged 2.3% (20.5% dry matter basis) and ranged from 2.0-3.1 %.

**Ash**

Ash content averaged 0.53% (4.7% dry matter basis) and was consistent over time. The level generally agrees with previous studies.
Vitamin A
Vitamin A levels ranged from 28-171 IU/100 g (249-1361 IU/100 g dry matter basis).

Vitamin D
Vitamin D levels ranged from 22-69.8 IU/100 g (196-693 IU/100 g dry matter basis).

Vitamin E
Vitamin E levels averaged 0.62 µg/ml and ranged from 0.33-0.88 µg/ml α tocopherol in the milk. The cow diet is supplemented daily with 8,000 IU of micellized vitamin E (Stuart Products), resulting in serum levels of 0.49-0.78 µg/ml (average 0.63 µg/ml) (See Table 1). The milk and serum samples were intentionally taken on the same day, to correlate milk levels of α tocopherol with the serum levels. The serum levels in the cow are comparable to a report of serum vitamin E levels of wild African elephants. Although the cow serum levels are in the normal range for wild African elephants, the milk levels of vitamin E for wild elephants has not been determined and the milk levels in this study cannot be verified as being within a normal range.

Calcium/Phosphorus
Calcium levels averaged 37.8 mg/100 g (334 mg/100 g dry matter basis) and ranged from 28-43 mg/100 g (257-431 mg/100 g dry matter basis). Phosphorus averaged 18.8 mg/100 g (166 mg/100 g dry matter basis) and ranged from 15.9 to 20.8 mg/100 g (143-204 mg/100 g dry matter basis). The calcium:phosphorus ratio averaged approximately 2:1.

A comparison of African elephant milk to bovine milk and human milk (the basis for many milk replacers), is included in Table 2.

Formulation of a Calf Milk Replacer

The results of the African elephant milk analysis allowed for the development of an African elephant calf milk replacer based on mature milk of early-to-mid lactation. The formula criteria included the following:

<table>
<thead>
<tr>
<th>Component</th>
<th>As Fed</th>
<th>Dry Matter Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Solids</td>
<td>11.5%</td>
<td>------</td>
</tr>
<tr>
<td>Fat</td>
<td>5%</td>
<td>42%</td>
</tr>
<tr>
<td>Lactose</td>
<td>2.3-2.7%</td>
<td>20-24%</td>
</tr>
<tr>
<td>Protein</td>
<td>3.3%</td>
<td>29%</td>
</tr>
<tr>
<td>Ash</td>
<td>0.52%</td>
<td>4.5%</td>
</tr>
<tr>
<td>Calcium</td>
<td>65 mg/100 g</td>
<td>570 mg/100 g</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>42 mg/100 g</td>
<td>364 mg/100 g</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>75 IU/100 g</td>
<td>650 IU/100 g</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>46 IU/100 g</td>
<td>400 IU/100 g</td>
</tr>
</tbody>
</table>
Vitamin E: Provided 2.0 IU/kg body weight or 250 IU daily. This was provided separate from the calf milk replacer mix.

The final formulation maintained lactose on the low end of the milk analysis range (20-26% dry matter basis), to minimize the risk of a lactose-induced diarrhea. Protein and fat were maintained at the high end or slightly above the range in the milk analysis to accommodate the lower lactose and still maintain a total solids of approximately 11.5%. Little short-term risk occurs with fat and protein levels near the high normal range.

The formula is provided in a powdered form. The milk replacer is produced starting with bovine whey protein concentrate and bovine skim milk powder, mixed to provide the 29% milk proteins necessary in the milk replacer. Fat is then added as coconut oil, tallow, and lard, to achieve the desired overall fat level in the formulation. Coconut oil was used as a fat source to insure the bioavailability of fat, described in a report of handrearing African elephants by Daphne Sheldrick in Kenya. The coconut oil represents 25% of the total fat portion. A mixture of mineral and vitamin premix completes the formula. The vitamin E is not included in the mix, but is added as a liquid (Stuart Products) to insure bioavailability. An interesting finding noted by staff included the observation that, although the calf would readily consume the special milk replacer, he could still distinguish the taste of real elephant milk from milk replacer. He clearly preferred the taste of true elephant milk.

The formulation of the calf milk replacer was refined three times. This process of feeding and refining the replacer resulted in additional observations on several nutrients.

Total Solids
As previously mentioned, palatability increased after the total solids were reduced from 20% (the original milk replacer) to 11.5% (revised milk replacer).

Lactose
The original milk replacer contained 7.95% lactose, which was comparable to lactose levels in human infant milk replacers. Although the intermittent diarrhea could not be directly attributed to excessive lactose, a glycosuria resulted and continued until the lactose level was reduced to 2 1/2% (20% dry matter basis). This supports the importance placed on avoiding human infant formulas (Enfamil, SMA, etc.), which contain 7% lactose (50-60% dry matter basis).

Fat
The calf was evaluated for fat absorption of the coconut oil and overall fat premix. Fecal fat analyses were consistently negative, indicating complete absorption of fat. Serum triglyceride levels were 4-5 times higher than juvenile African elephants fed primarily a hay diet in a previous study and the cholesterol level was twice the level reported in the same study. Although these higher levels are probably normal for a calf, this also suggests that the bioavailability is adequate.

Vitamin E
A liquid micellized natural tocopherol (Stuart Products) is mixed into the milk, providing 250 IU total per day. Supplementation of 2.0 IU/kg body weight resulted in calf serum vitamin E levels
approximately six times higher than the adult level (calf 4.08-4.95; cow 0.78 µg/ml).

ACKNOWLEDGMENTS

The author would like to thank the following people for their assistance on this project: Ellen Dierenfeld (Bronx Zoo/International Wildlife Conservation Park); Jim Oosterhuis, DVM (San Diego Wild Animal Park); Bo Lonnerdahl (University of California, Davis); Colleen Kinzley (Oakland Zoo); John Macy (Calif. Dept. of Food & Agriculture); Daphne Sheldrick (Nairobi National Park); Trish Arzaga (DFL Laboratories); Jelle Vanderkrift (Grober Inc.); Andre Roy (Grober Inc.); Joanne Jasson, RN (Kaiser Permanente); Fritzi Drosten, RN (Kaiser Permanente) and Olle Hernell.

LITERATURE CITED

Table 1. Analysis of mature milk in early lactation of the African elephant.

<table>
<thead>
<tr>
<th></th>
<th>(n)</th>
<th>Average</th>
<th>Average Dry Matter Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Solids</td>
<td>(7)</td>
<td>11.32%</td>
<td>-</td>
</tr>
<tr>
<td>Fat</td>
<td>(7)</td>
<td>4.38%</td>
<td>38.70%</td>
</tr>
<tr>
<td>Protein</td>
<td>(7)</td>
<td>2.32%</td>
<td>20.50%</td>
</tr>
<tr>
<td>Lactose</td>
<td>(7)</td>
<td>2.79%</td>
<td>24.60%</td>
</tr>
<tr>
<td>Ash</td>
<td>(7)</td>
<td>0.53%</td>
<td>4.70%</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>(5)</td>
<td>115 IU/100 g</td>
<td>1016 IU/100 g</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>(3)</td>
<td>38.6 IU/100 g</td>
<td>341 IU/100 g</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>(3)</td>
<td>0.62 µg/ml</td>
<td>-</td>
</tr>
<tr>
<td>Calcium</td>
<td>(6)</td>
<td>37.8 mg/100 g</td>
<td>334</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>(5)</td>
<td>18.8 mg/100 g</td>
<td>166</td>
</tr>
</tbody>
</table>

Analysis by:

DFL Laboratories
1548 Cummins Drive
Modesto, CA  95358-6412

California Department of Food and Agriculture
Sacramento, CA

Bronx Zoo/Wildlife Conservation Park
185th Street & Southern Boulevard
Bronx, NY   10460-109
Table 2. Comparison of African elephant milk to bovine milk and human milk.

<table>
<thead>
<tr>
<th></th>
<th>African Elephant</th>
<th>Bovine (Holstein)</th>
<th>Human</th>
<th>African Elephant</th>
<th>Bovine (Holstein)</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Solids</td>
<td>11.3</td>
<td>12.2</td>
<td>12.4</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Fat</td>
<td>4.38</td>
<td>3.5</td>
<td>3.8</td>
<td>39</td>
<td>28.7</td>
<td>30.6</td>
</tr>
<tr>
<td>Protein</td>
<td>2.3</td>
<td>3.1</td>
<td>0.9</td>
<td>20</td>
<td>25.4</td>
<td>7.2</td>
</tr>
<tr>
<td>Lactose</td>
<td>2.79</td>
<td>4.9</td>
<td>7</td>
<td>24.6</td>
<td>40</td>
<td>56.4</td>
</tr>
<tr>
<td>Ash</td>
<td>0.53</td>
<td>0.7</td>
<td>0.2</td>
<td>4.7</td>
<td>5.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>

All units in %

Dry Matter Basis
AVIAN VETERINARY WORK IN TANZANIA

John E. Cooper, DTVM, MRCPath, FIBiol, FRCVS  
National Avian Research Center, P O Box 45553, Abu Dhabi, United Arab Emirates

Abstract

Tanzania is a large, but poor, country in East Africa. It has an impressive avifauna which is of considerable economic importance. Some species of wild bird damage crops while others can transmit disease to domestic livestock and humans. On the other hand, wild birds bring Tanzania much needed foreign exchange; tourists and others visit national parks and other areas to see wild animals, including birds, and some indigenous avian species are trapped and exported overseas as part of the wild bird trade. On the domestic front free-living birds are often trapped for food - especially members of the Galliformes and Passeriformes.1

During 2 yr in Tanzania, the author was able to participate in wide-ranging veterinary work with birds (Table 1). A new avian medicine course for veterinary students was started and the syllabus for this encompassed wild birds as well as domestic species.4 The former included pets and wild bird casualties. Clinical and pathological examination was an integral part of teaching students in addition to providing advice and assistance to owners.23 One important welfare and conservation project concerned the wild bird trade and how best to raise standards of trapping, handling and veterinary care. Advice and training were also given to those involved in the capture or killing of birds for food or because they are pest species. Research projects included studies on the interaction between free-living and domestic birds, particularly in village environments, and the implications to the health of both birds and humans, especially individuals who are immunocompromised by HIV infection, malaria, other diseases or malnutrition. Additional programs of research covered diseases of guineafowl (Numida meleagris), ostriches (Struthio camelus) and Indian house crows (Corvus splendens) (Table 2).

The work in Tanzania concentrated on certain “core species” (Table 3), chosen either because of their intrinsic importance in Tanzania or because they served as useful and readily available models for teaching or research.

Much remains to be learned about the causes of morbidity and mortality of East African birds and this is a fertile field for collaboration between the veterinary profession and others. There is a particular need for a stronger partnership between Tanzanian veterinarians and those in the West.

Resumen

Tanzania es un país grande pero a la vez pobre del Africa oriental. Tiene una avifauna impresionante, de una importancia taxonómica considerable. Algunas especies de aves silvestres dañan las cosechas mientras que otras pueden transmitir enfermedades al ganado doméstico y a los humanos. Por otro lado las aves silvestres traen a Tanzania las divisas extranjeras que tanto necesita; turistas y otros visitantes de los parques nacionales y otras áreas para observar animales salvajes, incluyendo aves, y algunas de aves indígenas son capturadas y exportadas a ultramar como parte del comercio de
animales salvajes.

En el frente doméstico, las aves que viven en libertad se capturan para obtener alimentación especialmente los miembros de la familia galliformes y passeriformes. Durante dos años en Tanzania, el autor fue capaz de participar en un amplio rango de trabajo veterinario con aves. Un nuevo curso de Medicina Aviar para estudiantes de Veterinaria fue iniciado y los fundamentos encaminados a las aves silvestres así como a las especies domésticas. El curso incluyó mascotas y aves silvestres en sus casos clínicos. El examen clínico y patológico fue parte integral de la enseñanza a los estudiantes, en adición se dio un importante beneficio y el proyecto de conservación concerniente al comercio de aves silvestres y cómo establecer un mejor estándar de captura, manejo y atención veterinaria. Asesoría y entrenamiento se dieron también a quienes se involucran en la captura o caza de aves para alimentarse o por controlar especies nocivas.

Los proyectos de investigación incluyeron estudios sobre la interacción entre vida silvestre y aves domésticas, particularmente en aldeas y sus alrededores y las implicaciones de salud de ambos; pájaros y humanos, especialmente individuos quienes están inmunocomprometidos por infección por VIH, malaria, otras enfermedades y mala nutrición. Programas adicionales de investigación cubrieron enfermedades de la gallina (Numida maleagris), avestruces (Struthio camelus) y cuervos indios (Corvus splendens) (tabla 2).

El trabajo en Tanzania se concentra en ciertas especies (tabla 3), elegidas por su intrínseca importancia en Tanzania o porque estas sirvieron como útiles y rápidos modelos disponibles para la enseñanza e investigación. Resta mucho por aprender sobre las causas de morbilidad y mortalidad de las aves del Africa Oriental y este es un campo fértil para la colaboración entre la profesión veterinaria y otras. Existe una necesidad particular de una fuerte asociación entre veterinarios de Tanzania y del Occidente.

LITERATURE CITED

### Table 1. Categories of veterinary work in Tanzania.

1. Routine diagnosis and treatment (including clinical and post-mortem diagnosis)
2. Teaching of veterinary students, veterinary assistants, birdkeepers and others.
3. Welfare work - free-living birds captured for food, for export or as part of pest control.
   - Husbandry of captive birds.
4. Research projects.

### Table 2. Research projects.

- Management and diseases of captive ostriches *(Struthio camelus)*
- Management and diseases of captive and free-living guineafowl *(Numida meleagris)*
- Parasites and diseases of the Indian house crow *(Corvus splendens)*
- Blood parasites of wild and domestic birds
- The interaction between wild and domestic birds and its relevance to health, particularly in the village environment.
- Student projects, e.g. on bursa of Fabricius
Table 3. Avian veterinary work--core species and their significance.

<table>
<thead>
<tr>
<th>Species</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic fowl <em>(Gallus domesticus)</em></td>
<td>Important food sources. Routine diagnostic work and treatment. Student projects both in clinic and in situ.</td>
</tr>
<tr>
<td>Domestic duck <em>(Anas platyrhynchos)</em></td>
<td>As above</td>
</tr>
<tr>
<td>Muscovy duck <em>(Cairina moschata)</em></td>
<td>As above</td>
</tr>
<tr>
<td>Domestic goose <em>(Anser anser)</em></td>
<td>As above</td>
</tr>
<tr>
<td>Domestic turkey <em>(Meleagris gallopavo)</em></td>
<td>As above</td>
</tr>
<tr>
<td>Domestic pigeon <em>(Columba livia)</em></td>
<td>As above but also projects on spread of infectious diseases, especially between captive and free-living birds.</td>
</tr>
<tr>
<td>Domestic guineafowl <em>(Numida meleagris)</em></td>
<td>As above</td>
</tr>
<tr>
<td>Ostrich <em>(Struthio camelus)</em></td>
<td>Occurs naturally in the wild in Tanzania: an important species in national parks and elsewhere. Economic and cultural significance. Increasingly being “farmed” (ranched) commercially. Many veterinary and welfare implications.</td>
</tr>
<tr>
<td>African grey parrot <em>(Psittacus erithacus)</em></td>
<td>Occur in the wild--both naturally and introduced-- but often declining in numbers. Kept as pets and in collections. Exported lovebirds <em>(Agapornis spp.)</em> and others (bird trade). Many veterinary and welfare implications.</td>
</tr>
<tr>
<td>Passerines (e.g. weavers, Ploceidae)</td>
<td>As above but status of many in the wild and other wild species remain uncertain. Some are highly significant pests of crops.</td>
</tr>
<tr>
<td>Indian house crow <em>(Corvus splendens)</em></td>
<td>An introduced species, spreading in some areas. A scavenger that is believed to transmit diseases. Trapped in large numbers; thus live and dead birds are readily available for study. Used in research projects and also to teach students the principles of working with wild birds.</td>
</tr>
<tr>
<td>Birds of prey (raptors)</td>
<td>Occur in the wild, possibly declining in some (Falconiformes and Strigiformes) areas. Important environmental sentinels. Some species kept in captivity, others found sick or injured: care of birds in both groups provided an opportunity to teach students and to develop a database of values and samples.</td>
</tr>
</tbody>
</table>
SPECIES SURVIVAL PLAN HEALTH ASSESSMENT OF CAPTIVE THICK-BILLED PARROTS (Rhynchopsitta pachyrhyncha) IN THE UNITED STATES

Nadine Lamberski, DVM
Riverbanks Zoological Park and Botanical Garden, PO Box 1060, Columbia, SC 29202-1060, USA

Abstract

The thick-billed parrot (Rhynchopsitta pachyrhyncha) Species Survival Plan (SSP) requested all holding institutions evaluate the health of their collections in late 1995. The primary objective was to assess the health of the captive thick-billed parrot population and screen for the presence of infectious diseases. Of the 24 institutions holding thick-billed parrots in the United States at that time, 15 institutions (62%) participated in the health assessment. Sixty-four birds from a total population of 105 (61%) were evaluated between 31 August 1995 and 20 March 1996. Thirty-five of the 64 birds evaluated were males (55%) and 29 birds were females (45%). Thirty-two male birds ranged in weight from 275.0-381.3 g with an average weight of 323.0 g. Twenty-seven female birds ranged in weight from 265.0-354.0 g with an average weight of 306.6 g. Six male birds had evidence of self-inflicted feather damage as did 5 female birds.

CBC’s and selected chemistries from 61 birds were compared and were found to be very similar to the reported reference intervals for these values in this species. The average plasma protein in 61 birds was 2.7 g/dl ranging from 2.0-3.9 g/dl. The average albumin in 41 birds was 1.1 g/dl ranging from 0.7-1.6 g/dl. The average plasma calcium in 61 birds was 7.7 mg/dl ranging from 5.5-10.5 mg/dl. The average phosphorus in 49 birds was 2.5 mg/dl ranging from 0.8-5.4 mg/dl.

Fecal direct smears, flotations, and trichrome stains from 35 birds were negative for ova and endoparasites. The frequency and types of microbial isolates from choanal and cloacal aerobic cultures were summarized (Table 1). Staphylococcus/Micrococcus spp., Streptococcus spp., and Corynebacterium spp. were the organisms most frequently cultured from choanal swabs. Escherichia coli, Enterococcus spp., and Streptococcus spp. were the organisms most frequently isolated from cloacal swabs. Salmonella spp. was not cultured from any of the 46 choanal or 61 cloacal swabs. Fifty-eight birds tested negative for chlamydia and for psittacine beak and feather disease. Cloacal swabs from 56 birds were negative for polyomavirus. Pacheco’s disease virus (PDV) serology results from 30 birds were evaluated in this study. These birds represent 29% of the SSP population. Of the birds tested, 7 (23%) tested positive for PDV antibodies.

In conclusion, the most significant finding from the physical examination and laboratory analysis of 61% of SSP thick-billed parrots was the presence of neutralizing antibodies to Pacheco’s disease virus. The clinical significance of a positive or negative result is questionable in a single-bird household; however, the results can have serious implications for birds managed as a single population. The overall impact these results may have on the captive population and on release efforts needs to be considered in light of the seroprevalence of PDV antibodies in free-living thick-billed parrots as well as the effect viral shedding may have on sympatric species. Efforts are currently underway to confirm if antibody positive birds are truly latently infected with PDV. Isolating the virus from these birds is necessary in order to study its virulence patterns in thick-billed parrots as well as other species.
Resumen

El Plan de Supervivencia de Especies (PSE) de las Cotorras Serranas (*Rynchopsitta pachyrhyncha*) requirió la información que todas las instituciones poseían hasta 1995 sobre la salud de sus colecciones. El objetivo primario fue el estimar la salud de la población cautiva de cotorras serranas y la detección de enfermedades infecciosas. De las 24 instituciones poseedoras de cotorras serranas en los Estados Unidos, 15 instituciones (62%) participaron en el estudio. Sesenta y cuatro aves de una población total de 105 fueron evaluadas conjuntamente entre el 31 de agosto de 1995 y el 20 de marzo de 1996. Treinta y cinco de las 64 aves evaluadas fueron machos (55%) y 29 hembras (45%), treinta y dos aves machos oscilaron en su peso desde 275.0 g a 381.3 g con un promedio de peso de 323.0 g. Veintisiete aves hembras oscilaron desde 265.0 g a 354.0 g con un promedio de peso de 306.6 g. Seis aves machos se auto-inflingieron daños en el plumaje, así mismo 5 aves hembras presentaron la misma conducta.

El recuento sanguíneo completo (CBC) y la química sanguínea seleccionada de 61 aves fueron comparadas y se encontró que fueron muy similares los intervalos de referencia reportados para esos valores en esta especie. El promedio de proteína plasmática en 61 aves fue de 2.7 g/dl, con un rango de 2.0 - 3.9 g/dl. El promedio de albúmina en 41 aves fue de 1.1 g/dl, variando de 0.7 hasta 1.6 g/dl. El promedio de calcio en el plasma de 61 aves fue de 7.7 mg/dl, oscilando desde 5.5 hasta 10.5 mg/dl. El promedio de fósforo en 41 aves fue de 2.5 mg/dl, con un rango de 0.8 a 5.4 mg/dl.

Muestras fecales examinadas directamente, por flotación y con tinción tricrónica de 35 aves resultaron negativas para huevecillos y endoparásitos. La frecuencia y tipo de microorganismos aislados de cultivos aerobios de cloaca y coana fueron resumidos (tabla 1). *Staphylococos/Micrococos* spp., *Streptococos* spp., y *Corynebacterium* spp. fueron los organismos más frecuentemente cultivados de muestras tomadas de las coanas. *Escherichia coli*, *Enterococos* spp., y *Streptococos* spp. fueron los organismos más frecuentemente aislados de las muestras cloacales. *Salmonella* spp., no fue cultivada en ninguna de las 46 muestras de coanas ni 61 muestras de cloaca. Ciencuenta y ocho aves dieron negativa la prueba para *Clamydia* y para enfermedad de las plumas y pico psitacino (síndrome PBFDS). Muestras cloacales de 56 aves fueron negativas para Polyomavirus. Los resultados serológicos de la enfermedad del virus de Pacheco (PDV) de 30 aves fueron evaluados en este estudio. Estas aves representan el 29% de la población del PSE. De las aves analizadas, 7 (23%) dieron positivo en la prueba de anticuerpos PDV.

En conclusión, el descubrimiento más significativo de los exámenes físicos y análisis de laboratorio del 61% de la población de cotorras serranas fue la presencia de anticuerpos neutralizantes para la enfermedad de virus de Pacheco (PDV). El significado clínico de resultados positivos o negativos en una sola familia es cuestionable, sin embargo, los resultados pueden tener serias implicaciones para aves manejadas como una sola población. Estos resultados pueden tener en la población cautiva un fuerte impacto y ayudar en los esfuerzos necesarios para ser considerados una luz en la seroprevalencia sérica de anticuerpos PDV en las cotorras serranas de vida silvestre, también qué efecto viral extendido puede tener en especies afines. Los esfuerzos son corrientemente encaminados a confirmar si las aves positivas con anticuerpos están infectadas en forma latente con PD. El aislamiento del virus de estas aves es necesario para estudiar sus patrones de virulencia en las cotorras serranas así como en otras especies.
Introduction

The goal of the thick-billed parrot Species Survival Plan (SSP) is to ensure the survival of the thick-billed parrot (*Rhynchopsitta pachyrhyncha*) within its historic range by (1) maintaining a captive population, (2) educating the public regarding the conservation of native endangered species, and (3) supporting the wild populations within North America.

It is possible that habitat destruction along with hunting pressures caused the extirpation of thick-billed parrots (*Rhynchopsitta pachyrhyncha*) from the coniferous montane forests of the southwestern United States by the 1930’s. These parrots currently exist only in the forest of Mexico’s Sierra Madre Occidental; however, their populations are declining due to deforestation and trapping for the pet bird trade.5,6

Attempts to reintroduce thick-billed parrots into Arizona began in 1986 and have as yet been unsuccessful in establishing a self-sustaining population. Experimental releases using both wild-caught and captive-reared birds have been disappointing, although the wild-caught adults appeared to be most suited for release. The captive-reared birds released to date seemed to be behaviorally deficient in predator evasion, feeding and foraging capabilities, and flocking behavior. However, efforts are currently underway to correct these deficiencies. Several disease problems were also encountered calling into question the feasibility of releasing captive birds. Due to financial constraints, health assessments of thick-billed parrots prior to previous releases were limited. More extensive disease screening prior to release may identify birds that should not be released due to disease exposure.5,9

Reintroductions can threaten existing wild populations by the inadvertent introduction of diseases not previously present in that population. This can be prevented by assessing the health of the birds prior to release and for screening for infectious disease that may impact the overall health of the population. The inability to adequately test for all infectious diseases and lack of knowledge of naturally occurring infectious diseases confuses this process.4,8

The captive population is a valuable resource and may be essential to establish a US population if translocations alone are not sufficient. Baseline medical information generated from this population contributes to the body of knowledge in avian medicine and can be used to aid conservation efforts.1,2

Assessing the health of an individual involves obtaining an adequate history (behavioral, reproductive, nutritional, exposure to disease), physical examination (including body weight), and laboratory testing. Blood is collected for complete blood counts, chemistry panels, and selected serologies. Swabs of the choana and cloaca are obtained for microbiologic cultures. Evaluation of this laboratory data is enhanced if reference values exist for comparison for both free-living and captive populations. Additionally, surveying the captive as well as the free-living population for infectious diseases will assist in assessing the prevalence of disease conditions.

**Historical Information (U.S. Captive Population)**

From 1952 until 1994, 49 deaths of captive thick-billed parrots were reported to the SSP. Necropsies
were performed on 37 (75%) of these birds. Sixteen (43%) of these birds died from confirmed infectious etiologies. Five more birds were suspected of dying from infectious causes increasing to 57% the number of birds with suspected or confirmed infectious diseases. Of these cases, 19/21 (90%) were bacterial in origin. Two of twenty-one birds (<10%) died from parasitic diseases (sarcocystosis) and 1/21 (<5%) had a viral (adenovirus suspect) and bacterial infection. There have been no confirmed reports of Pacheco’s disease virus or Proventricular Dilatation Syndrome from necropsy results reported to the SSP; however, these diseases have been a concern in birds involved in the release program. There is a concern that these birds may have been exposed to these infectious diseases while held in captive situations.

The following is a historical list of diseases which have potentially impacted the population and/or release efforts:

- Proventricular Dilatation Syndrome / Wasting Disease
- Pacheco’s Disease Virus / Herpesvirus
- Adenovirus
- Chlamydiosis
- Pasteurellosis
- *E. coli* Septicemia

**Health Advisory Team**

A Health Advisory Team has been assembled to deal with disease issues. The goals of this cooperative are to:

- review current quarantine, preventive medicine, and necropsy protocols and disseminate this information to holding institutions,
- review historical and current disease issues and make recommendations in reference to the diagnosis and control of infectious diseases,
- establish baseline medical information for the SSP population to include establishing reference values for complete blood counts, chemistry panels, and choanal and cloacal microbial flora as well as surveying this population for infectious diseases,
- establish the same information as above for the Mexican captive and free-living populations, and
- establish a nutrition advisory team.

The Health Advisory Team includes the following veterinarians:

<table>
<thead>
<tr>
<th>Name</th>
<th>Title/Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kathy Ingram</td>
<td>Arizona Representative (Phoenix Zoo)</td>
</tr>
<tr>
<td>Mike Richard</td>
<td>New Mexico Representative (Rio Grande Zoo)</td>
</tr>
<tr>
<td>Jim Koschman</td>
<td>Release Project Veterinarian (Crossroads Animal Hospital, El Paso)</td>
</tr>
<tr>
<td>Kim Joyner</td>
<td>Field Research Consultant (North Carolina State University)</td>
</tr>
<tr>
<td>Keven Flammer</td>
<td>Protocol Reviewer (North Carolina State University)</td>
</tr>
<tr>
<td>Bran Ritchie</td>
<td>Virology Consultant (University of Georgia)</td>
</tr>
<tr>
<td>Roberto Aguilar</td>
<td>Mexican Zoo Liaison (Audubon Park Zoo)</td>
</tr>
<tr>
<td>Linda Lowenstine</td>
<td>Pathology Consultant (San Diego Zoological Society)</td>
</tr>
<tr>
<td>Alberto Paras</td>
<td>Mexico Representative (Africam Safari, Puebla Mexico)</td>
</tr>
</tbody>
</table>
Nadine Lamberski  SSP Veterinary Advisor (Riverbanks Zoo)

Preliminary Study

A preliminary study was initiated in late 1995 in which SSP holding institutions were asked to evaluate their collections. The primary objective was to assess the health of the captive thick-billed parrot population and screen for the presence of infectious diseases.

Institutions were asked to evaluate as many thick-billed parrots in their collections as possible and to complete as much of the recommended health assessment as feasible for that institution. Unless otherwise specified, diagnostic samples from each institution were submitted to the laboratories of their choice. The health assessment was as follows:

1. physical examination, including assessment of body condition, body weight, and evaluation of plumage;
2. blood collection for a complete blood count (CBC), chemistry panel, chlamydia (elementary body agglutination) and Pacheco’s disease virus serology, psittacine beak and feather disease polymerase chain reaction (PCR), and plasma banking;
3. choanal and cloacal swabs for aerobic cultures;
4. cloacal swab for Salmonella spp. culture;
5. cloacal swab for polyomavirus PCR; and
6. feces for direct smear, flotation, and trichrome stain.

Results and Discussion

Of the 24 institutions holding thick-billed parrots in the United States at that time, 15 institutions (62%) participated in the health assessment. 64 birds from a total population of 105 (61%) were evaluated between 31 August 1995 and 20 March 1996.

Thirty-five of the 64 birds evaluated were males (55%) and 29 birds were females (45%). Thirty-two males ranged from 2-27 yr of age with an average age of 9 yr. Eighteen of these birds hatched in captivity, three were wild-caught, and two were of unknown origin. Twenty-six females ranged in age from 2-29 yr with an average age also of 9 yr. Nineteen of these birds hatched in captivity, four were wild-caught, and three were of unknown origin. The age and origin of the remaining birds were not available.

Thirty-two male birds ranged in weight from 275.0-381.3 g with an average weight of 323.0 g. Twenty-seven female birds ranged in weight from 265.0-354.0 g with an average weight of 306.6 g. Six male birds had evidence of self-inflicted feather damage as did five female birds; therefore, feather picking was noted as a problem in 11 out of 59 birds or nearly 20% of this population. Weights and physical exam findings were not available from the remaining five birds.

CBC’s and selected chemistries from 61 birds were compared and were found to be very similar to the reported reference intervals for these values in this species. The average plasma protein in 61 birds was 2.7 g/dl ranging from 2.0-3.9 g/dl. The average albumin in 41 birds was 1.1 g/dl ranging from 0.7-1.6 g/dl.
Plasma calcium was measured in 61 birds. The average calcium was 7.7 mg/dl ranging from 5.5-10.5 mg/dl. The average calcium in 32 males was 7.8 mg/dl ranging from 6.3-9.2 mg/dl, and the average calcium in 29 females was 7.6 mg/dl ranging from 5.5-10.5 mg/dl. Plasma inorganic phosphorus was measured in 49 birds. The average phosphorus was 2.5 mg/dl ranging from 0.8-5.4 mg/dl. The average phosphorus in 25 males was 2.6 mg/dl ranging from 0.9-4.9 mg/dl, and the average phosphorus in 24 females was 2.3 mg/dl ranging from 0.8-5.4 mg/dl.

The low plasma calcium values may be a reflection of the low albumin levels, since only the calcium bound to albumin is measured. The albumin levels reported in this study are lower than typically seen in other psittacine species. This may be a reflection of species differences, laboratory error, or may be suggestive of inadequate dietary protein. Standardization of laboratory methods as well as comparison to values in free-living thick-billed parrots would aid in the interpretation of these findings.

Intestinal parasites were not a problem for the captive population. The fecal direct smears and flotations from 35 birds were reported and all were negative. Additionally, 25 fecal samples were evaluated for protozoa by trichrome stain and all were negative.

The frequency and types of microbial isolates from choanal and cloacal aerobic cultures are summarized in Table 1. *Staphylococcus/Micrococcus* spp., *Streptococcus* spp., and *Corynebacterium* spp. were the organisms most frequently cultured from choanal swabs. Although *Pasteurella* spp. was cultured from eight out of 46 swabs, *Pasteurella multocida* was isolated only once. *Escherichia coli*, *Enterococcus* spp., and *Streptococcus* spp. were the organisms most frequently isolated from cloacal swabs. *Salmonella* spp. was not cultured from 46 choanal or 61 cloacal swabs. Gram stains and anaerobic cultures of the choana and cloaca were part of the initial health assessment request. However, these results are not reported, because the number of samples cultured anaerobically was too few to be significant, and there was a lack of consistency in the reporting of Gram stain results.

Whole, anticoagulated blood was submitted to the Avian Research Associates, Inc. Laboratory (Milford, Ohio) from 58 birds for psittacine beak and feather disease (PBFD) testing. Viral DNA probes have been designed to detect a portion of the PBFD viral nucleic acid. All 58 of these birds tested negative for psittacine beak and feather disease.

Cloacal swabs from 56 birds were submitted to this same lab for the detection of polyomavirus. Polyomaviral DNA probes were used to detect polyomavirus nucleic acid in the submitted samples. All 56 birds tested negative.

Plasma samples from 58 birds were submitted to the Texas Veterinary Medical Diagnostic Laboratory (College Station, Texas) for chlamydial serologic testing. Elementary body agglutination was the serologic method used to detect chlamydial antibody activity, and all 58 samples tested negative.

Neutralizing antibodies to Pacheco’s disease virus (PDV) were evaluated also at the Texas Veterinary Medical Diagnostic Laboratory (College Station, Texas). Serologic results were reported from 30 birds from eight institutions. Seven birds showed evidence of neutralizing antibodies to the Pacheco’s disease virus from 5 of these institutions.
The first reported outbreak of acute, fatal hepatitis in psittacine birds was described in Brazil by an investigator named Pacheco in 1929. This disease (Pacheco’s disease) was found to be caused by an avian herpesvirus in 1975 and has since been known to occur worldwide. Both virulent and avirulent strains of Pacheco’s disease virus (PDV) are thought to occur. Herpesviruses have been recovered from clinically affected as well as asymptomatic psittacine birds and there is increasing evidence that host-adapted herpesvirus strains exist. Birds that recover from the virus may develop low levels of virus-neutralizing antibodies as well as long lasting immunity to the same strain of virus.\(^7\)

PDV antibodies are frequently detected in clinically normal psittacine birds. Virus-neutralizing antibodies were detected in 34% of Amazon and African grey parrots maintained in single-bird households in one survey.\(^7\)

Since antibodies to the virus can be difficult to detect, finding them may indicate active or recent (within months) infection. These birds should be considered latently infected. Latently infected birds may shed the virus during periods of stress. This viral shedding occurs most commonly without clinical signs of disease. Antibodies that develop early in an infection can decrease within months. An increase in antibodies may occur in some latently infected birds when they are actively shedding the virus. However, a decrease in antibodies with no detectable viral shedding has also occurred.\(^7\)

Latency can be confirmed by vaccinating a bird with an inactivated vaccine followed by collection of serum 7 days later. Birds previously infected with PDV would have an anamnestic response and would develop a rapid, high titer (\(>32\)) within a short period of time. Naive birds may not develop a detectable antibody titer for 28 days (Dr. Angulo, personal communication).\(^7\)

In conclusion, the most significant finding from the physical examination and laboratory analysis of 61% of SSP thick-billed parrots was the presence of neutralizing antibodies to Pacheco’s disease virus. PDV serology results from 30 birds were evaluated in this study. These birds represent 29% of the SSP population. Of the birds tested, seven (23%) tested positive for PDV antibodies. The clinical significance of a positive or negative result is questionable in a single-bird household; however, the results can have serious implications for birds managed as a single population. The overall impact these results may have on the captive population and on release efforts needs to be considered in light of the seroprevalence of PDV antibodies in free-living thick-billed parrots as well as the effect viral shedding may have on sympatric species. Efforts are currently underway to confirm if antibody positive birds are truly latently infected with PDV. Isolating the virus from these birds is necessary in order to study its virulence patterns in thick-billed parrots as well as other species.

**ACKNOWLEDGMENTS**

The author gratefully acknowledges the assistance of the participants in the 1995 Thick-billed Parrot SSP Masterplan Meeting as well as the participation of the following institutions (and their veterinarians, bird curators, technicians, and keepers) in this health assessment: Arizona-Sonora Desert Museum, Audubon Zoo, Bush Gardens, Cincinnati Zoo, Dallas Zoo, Discovery Island, El Paso Zoo, Fort Worth Zoo, Reid Park Zoo, Riverbanks Zoo, Sacramento Zoo, San Diego Wild Animal Park, San Diego Zoo, Tulsa Zoo, and ZOOAMERICA Wildlife Park.
LITERATURE CITED


### Table 1. Frequency and types of microbial isolates from aerobic cultures of the choana and cloaca of captive thick-billed parrots.

<table>
<thead>
<tr>
<th>Microbial isolate</th>
<th>Choana (n = 46)</th>
<th>Cloaca (n = 61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter spp.</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Actinobacillus ureae</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>diphtheroids</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Enterococcus spp. (group D)</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Pasteurella gallinarum-like sp.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pasteurella hemolytica</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pasteurella spp.</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus/micrococcus spp.</td>
<td>28</td>
<td>8</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>yeast</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
CRANE SURVEY: PRELIMINARY REPORT OF THE CRANE TAXON ADVISORY GROUP (TAG) SURVEY

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Zoo Montana, P.O. Box 80905, Billings, MT 59108-0905, USA

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Abstract

A crane species survey was developed and distributed to 122 institutions in the Americas known by ISIS records to house and or display cranes. Questions were asked on the following topics: housing/husbandry, nutrition, reproduction, preventive medicine, morbidity/mortality and feedback/follow-up needs. Fifty-three (43%) of the institutions responded with returned questionnaires. This paper presents the initial findings of this survey using the information received. A more in depth study of the data with analysis will be reported elsewhere.

Institutions responding are apparently maintaining ISIS records. Most cranes are housed on exhibit outdoors in groups or pairs with other species both birds and mammals. Facilities are considered “OK” or “near optimal” in over 80% of responses. No responder considered their facilities poor. There was some variation between exhibit and off season (winter housing). Adding water sources and prevention of predation were the most often expressed concerns of responders. Netting exhibits and increasing space for holding were also significant concerns. Exposure to predatory and non predatory wild species was reported by nearly all respondents. Wing (feather) clipping and pinioning were the most common methods of flight restraint. Leg banding was the most used method of identification.

Commercially prepared diets are the most used dietary formulations by those responding to the questionnaire. The majority of respondents indicated that they use starter feed for growing birds. There was great variation in age when birds are converted to a maintenance diet (2 wk-1 yr). The majority of respondents reported using a different feed for breeding birds. These generally were commercial brands of breeder diets with supplementation.

The majority of respondents reported reproductive success in one or more species. Parent incubated eggs were the most common and desired method of incubation. Artificial incubation was a significant method however. Incubators varied as did the humidity settings whether by “wet bulb” or percent humidity; 99.5°F was the most used temperature setting. Insufficient data was gathered for individual species recommendations. A variety of foster parent species were reported as successful. Data gathering on these subjects appears to be consistent with the use of redundancy.

The majority of respondents do not regularly examine cranes. Of those who do, annual examination is the most frequent response. A variety of procedures are performed with fecal exams for parasites the most frequent test. It seems prudent to do a variety of procedures during regular examinations.
Most respondents who regularly examine their birds do perform a number of tests. Treatment of eggs to prevent infection is not done in the majority of cases presumably because of the desire to not disturb the adults and an otherwise low incidence of egg related diseases. Neonatal examinations are inconsistently done. While the majority of respondents do them regularly, a significant number (40%) never do them or they are not routinely done. This likely relates to the previous success rates and experience with neonatal problems that the different respondents have had. Candling and/or weighing are most often used to monitor eggs while daily keeper observation and/or regular weight measurements are most often used to monitor neonates. The great majority of respondents (94%) do not vaccinate the birds. Inclusion body disease is not routinely screened for generally. It is likely that the low level of vaccination and preventive screening relates to the apparent low incidence of disease in captive cranes as cold be measured by respondents to this questionnaire. Quarantine of new arrivals is an accepted reality among respondents. In general the quarantine lasts for 30 days and, although a variety of tests were mentioned, parasitology exams on feces with appropriate treatment and IBDC serology were most often listed.

Mortality and morbidity is significantly affected by trauma with predation and exhibit interspecies aggression the most important contributing variables. In addition to not being predator proof in almost all cases, facilities themselves were, in some cases, dangerous to the birds. Chick problems represented 12% of all mentioned problems. Disease outbreaks are not seen in this population. A variety of apparently isolated cases of infectious diseases are seen, however.

Data on physical injuries is consistent with responses given in an earlier section on the most common problems seen. Trauma consistently is seen and predation and interspecies aggression along with cage design appear to be significant variables. Developmental problems are significant in this population with significant numbers of angular limb deformities, curled toes and rare crossed beaks seen in a variety of species. Nutritional problems (over nutrition) are attributed as being a primary cause of most angular limb deformities by many respondents.

Neoplastic disease does not, at the present time, appear to be a significant cause of morbidity or mortality in this population of cranes. Perhaps this population is not reaching the age levels at which such maladies would become significant.

**Resumen**

Un censo sobre grullas fue desarrollado y distribuido a 122 instituciones en América conocidas a través de ISIS por albergar y/o exhibir grullas. Las preguntas fueron hechas acerca de los siguientes tópicos: alojamiento/manejo, nutrición, reproducción, medicina preventiva, morbilidad/mortalidad, retroalimentación/seguimiento sobre necesidades. Cuarenta y tres (43%) de las instituciones respondieron contestando y regresando sus cuestionarios. Este trabajo presenta los hallazgos iniciales del estudio usando la información recibida. Un estudio mas profundo de dichos datos ya analizados será reportado posteriormente.

Las instituciones que respondieron están llevando aparentemente registros ISIS. La mayor parte de las grullas son alojadas en exhibidores al aire libre en grupos o parejas con otras especies de aves y mamíferos. Las instalaciones fueron consideradas cerca de lo óptimo en más del 80% de las
respuestas. No hubieron respondieron que consideraran pobres sus instalaciones. Hubo alguna variación entre exhibidor y alojamiento de invierno. La adición de fuentes de agua y las medidas para la prevención de predadores, fueron las preocupaciones más frecuentes en las respuestas. Exhibidores con malla y el incremento de espacio para alojamiento, fueron también inquietudes significativas. La exposición a especies silvestres predadoras y no predadoras, fue también reportado en casi todas las respuestas. El recorte de plumas y la sujeción fueron los métodos más comunes de la restricción del vuelo. El bandeado (anillado) en las patas fue el método más usado para identificación.

Dietas preparadas comercialmente son las formulaciones más usadas por los encuestados. La mayoría de respuestas indica que ellos usan alimento de iniciación para aves en crianza. Aquí hubo gran variación en relación a en que edad se les cambia a una dieta de mantenimiento (de dos semanas a un año). La mayoría reportó alimento diferente para aves en reproducción. Estas generalmente fueron dietas comerciales para reproducción con suplementación.

La mayoría reportó sucesos reproductivos en una o más especies. La incubación natural por los padres fue el método más común y deseado. Sin embargo el método de incubación artificial fue muy significativo. En cuanto al porcentaje de humedad fue variado, y la temperatura de 99.5°F fue la más usada. Recomendaciones insuficientes fueron reunidas para especies individuales. Una variedad de especies como padres adoptivos fue reportado como exitoso.

La mayoría no examina regularmente sus grullas, quienes si lo hacen lo realizan anualmente. Una variedad de procedimientos son ejecutados, siendo exámenes fecales para parásitos son los más frecuentes. El hacer una variedad de procedimientos durante el examen regular parece ser prudente. El tratamiento de huevos para prevenir infecciones no es verificado en la mayoría de los casos presumiblemente por el deseo de no molestar a los adultos, además de que existe una baja incidencia de enfermedades relacionadas con el huevo. Exámenes neonatales son hechos inconsistente; de las respuestas el 40% no lo hace rutinariamente. El pesado es el monitoreo más frecuente del huevo, mientras que el animalero observa y regularmente pesa al pollo siendo esto el monitoreo usado a menudo para el neonato. El 94% no vacuna sus aves. Aparentemente el bajo nivel de vacunación y medicina preventiva se relaciona con la mínima incidencia de enfermedades de grullas en el cautiverio. La cuarentena de nuevos individuos es realizada por los que contestaron. En general la cuarentena consta de treinta días, y una gran variedad de pruebas fueron mencionadas, exámenes de parasitología con tratamiento adecuado y serología IBDC fue lo mas a menudo enlistado.

Mortalidad y morbilidad son significativamente afectadas por traumatismos, con predación y agresión interespecies en exhibidores mixtos como los más importantes contribuyentes variables. En adición al no ser a prueba de predadores, en casi todos los casos, los exhibidores por sí mismos fueron peligrosos para las aves. Problemas en pollos representó el 12% de todos los problemas mencionados. Brotes de enfermedades no son observados en esta población. Sin embargo una variedad de casos aislados de enfermedades infecciosas son apreciados.

Los daños físicos es la respuesta mas recibida en la sección de los problemas más comúnmente vistos. Los traumatismos son vistos con frecuencia; sobre todo predación y agresión interespecies derivadas del diseño del albergue. Un significativo número de problemas se presenta en relación a miembros
deformados, dedos desviados y pico cruzado y son vistos en una variedad de especies. Para varias
respuestas la causa primaria de las deformidades son problemas nutricionales.

Enfermedades neoplásicas no se han presentado hasta el momento y no es causa de morbilidad o
mortalidad en esta población de grullas. Tal vez esta población no ha alcanzado los niveles de edad
en que dichas enfermedades pueden ser significativas.

Introduction

A crane species survey was developed and distributed to 122 institutions in the Americas known by
ISIS records to house and or display cranes. Questions were asked on the following topics:
housing/husbandry, nutrition, reproduction, preventive medicine, morbidity/mortality and
feedback/follow-up needs. Fifty-three (43%) of the institutions responded with returned
questionnaires. This paper presents the initial findings of this survey using the information received.
A more in depth study of the data with analysis will be reported elsewhere.

Materials and Methods

The questionnaire was designed to allow participants to answer questions and supply additional
information where appropriate and available in a simple and quick way. It was difficult to account
for all of the possible combinations of housing, husbandry and medical practices and include enough
questions to adequately discover the occurrence of various medical problems. Nonetheless, the
attempt was made. A list of the questions included in the survey follows on the subsequent pages.
Crane Species Survey

Housing / Husbandry

A. Do you house crane species currently as listed in the ISIS database?
   - yes
   - no
   - do not know
   If no what changes have occurred?

B. How do you house the birds?
   - outside
   - inside
   - combination
   - groups
   - individually
   - in pairs
   Multiple species exhibits:
   - with mammals
   - with other birds species
   on exhibit/ off exhibit

Do you consider your facilities:
   - optimal
   - near optimal
   - OK
   - substandard
   - poor

How would you improve your facilities?

Is there exposure to free living animals?
   - yes
   - no
   - do not know

If yes, what species?

C. What method(s) of flight restraint do you employ?
   - wing clipping
   - pinioning
   - tenotomy
   - tenectomy
   - other

D. What methods(s) do you use to identify the birds?
   - transponders
   - leg bands
   - wing bands
   - tattoos
   - other

Nutrition

A. Do you use a commercially prepared diet?
   - yes
   - no
   If no, describe ingredients and analysis if available
   If yes, give product names

Which species are fed this diet?

Do you use a starter feed for growing birds?
   - yes
   - no
   If yes, at what age do you change to maintenance?

Do you supplement or use a different feed for breeding birds?
   - yes
   - no

Product name, starter:
Product name, breeder:

Reproduction

A. Have you had successful reproduction?
   - yes
   - no
   - do not know

Have you observed reproductive behavior?
   - pair bonding
   - nest building
   - copulatory behavior
   - territorial aggression
   - other

B. Do you incubate eggs?
   - yes
   - no

Methods:
   - parents
   - surrogate parents
   - surrogate species

If yes, which species of chicks?

With which species as foster parent?

Artificial:
   - yes
   - no

If yes, what kind of incubator?

Humidity:

Temperature:

C. Do you assist with hatching?
   - yes
   - no

D. How are the chicks reared?
   - parents
   - foster species
   - hand-reared

Preventive Medicine

A. Do you regularly examine the cranes?
   - yes
   - no

If yes, how often?
   - annually
   - semiannually
   - etc.

What tests are routinely done?
   - complete physical
   - CBC
   - serology
   - bacteriology
   - virology
   - biopsy
   - parasitology
   - banking of DNA
   - banking of serum
   - other

Describe or attach screening protocols.

Do you treat eggs or clean them?
   - yes
   - no

If yes, what method do you use?
   - dip
   - fumigation
   - other

With what

Do you perform neonatal exams?
   - yes
   - no

How do you monitor development prior to and after hatching?

Do you do any routine vaccinations?
   - yes
   - no

With what products?

What is the protocol?

Do you screen for Inclusion Body Disease antibodies?
   - yes
   - no

Routinely on arrival

Do you quarantine new arrivals?
   - yes
   - no

Are there differences in arrival screening protocols?
from routine physical exams and testing?

yes no

Describe the differences.

**Morbidity and Mortality**

**A. Have you, during the last five years, experienced morbidity or mortality in the cranes?**

yes no don’t know

How many cranes have died?

Adults: Chicks:

What are the most common problems you see?

Have there been disease outbreaks with significant morbidity/mortality?

yes no

If yes, did you obtain a definitive diagnosis?

yes no

Description:

**B. Infectious Diseases**

* Have you experienced cases of infectious disease? yes no

If no, skip to Physical Injuries/Disorders

1. Viral (examples EEE, arborvirus, herpes, pox) yes no unknown suspected deaths?

   crane species adult/chick etiology

2. Bacterial (examples enterobactereace, salmonellosis, mycobacteria) yes no unknown deaths?

   crane species adult/chick etiology

   Have you identified non-clinical salmonella carriers?

   yes no

   If yes, which serotypes?

3. Fungal (examples aspergillosis, mycotoxicosis) yes no unknown deaths?

   crane species adult/chick etiology

4. Protozoal (examples coccidiosis, hexamitiasis) yes no unknown deaths?

   crane species adult/chick etiology

**C. Physical Injuries/Disorders**

* Were there cases with physical etiology? yes no

1. Trauma yes no unknown

2. Chick developmental problems yes no unknown

   species describe

   legs/ wings/ toes/ beaks/ other

**D. Neoplastic Diseases**

Were there cases of neoplasia?

yes no unknown

If yes, please list giving type, location and species.

**E. Nutritional/ Toxic Diseases/Miscellaneous**

* Were there cases in this category? yes no unknown

1. Nutritional yes no unknown deaths?

   species adult/chick etiology

2. Toxicity yes no unknown deaths?

   species adult/chick etiology

3. Miscellaneous yes no unknown deaths?

   species adult/chick etiology

   Gout Anesthesia Related Other

4. Other: species adult/chick etiology

**Feedback / Follow-up**

* Would you be willing to provide additional information? yes no

On compilation of this preliminary data additional specific information may be requested. At no time will the information be published without permission.

Specific reference to an individual institution will not occur during presentation of information. A listing of cooperating zoos will be presented.

A summary of the results of this survey will be reported to the AZA Crane TAG, distributed to all participating institutions and reported to the AAZV membership.

* Would you be willing to send tissues to a centralized serum/tissue bank?
C. What types of information would you like from the Crane TAG Veterinary Advisory Group?

- Crane pathology protocol
- Crane diseases diagnostic lab information
- List of crane specialists
- Husbandry manual
- Disease updates: IBDC, TB, Disseminated Visceral Coccidiosis, leg/beak trauma
- Wild crane diseases
- Quarantine protocol
- Clinical pathology reference ranges
- Other

D. Additional materials requested at this time.

1. Please send copies of all pathology reports for cases during the last five years.

2. Please send information on specific diagnostic techniques, laboratories and individuals you have found useful for interpretation of findings in crane cases.

3. Please add any information on areas of crane husbandry or medical management that, in your opinion, need investigation or you have found useful in the care or management of cranes.

Results

Housing and Husbandry

Nearly all reporting institutions (87%) reported that they currently house crane species as listed in the ISIS database. Five reported changes related to additions or shipments of birds. Two reported that they are not current in the ISIS database but did not describe the changes.

The majority of institutions reported housing the birds outside versus inside 39/42 (93%) or having a combination of outside and inside housing 21/63 (33%). In general, most birds are found in pairs 41/68 responses (60%) while 17/68 (25%) are housed in groups and only 10/68 (15%) responses indicated housing individual birds. The great majority of the institutions report that the birds are housed in multiple species type exhibits 48/53 (91%) with nearly an equal number of responses reporting mammals (32) and/or other bird species (30). Thirty-five of 42 responses (83%) indicated that the cranes were housed on exhibit.

Facilities were considered “OK” by 31/62 (50%) of the responses, “near optimal” by 20/62 (32%) of the responses, “optimal” by 9/62 (15%) and “substandard” by 2/62 (3%) of responses. No responses indicated that facilities are “poor”. Some responses indicated conditional circumstances such as optimal “off exhibit holding” or single species holding is optimal. One respondent reported substandard winter holding and another reported OK holding for summer.
Many written responses (60) were received suggesting need for improvement in housing. Nine (15%) responses indicated the need for additional ponds or other water access. There were eight (13%) responses indicating the need to make exhibits predator proof and the same number of responses indicated the need for larger spaces to house the birds. Four responses (6.7%) recommended netting, three suggestions were to cover pens eliminating need to restrict flight and/or the birds display ability and one response was to loosely line the barriers to soften the contact with hard fencing material and, thus, reduce the incidence of injury. Winter holding space was a concern of five respondents (8.3%). Adding shelters and having separate pens for pairs was indicated as a need with three (5%) responses each. Having more varied terrain, increasing plant diversity, reducing plant maintenance activities, increasing the distance between birds and visitors, eliminating or reducing wild birds and mammals because of their consumption of food, having more off exhibit space and introduction pens, and having juvenile holding areas were responses numbering less than three each.

No respondents reported that there is freedom from exposure to free living animals. Categories were established as follows: predatory wild birds, predatory mammals, non-predatory wild birds, non-predatory wild animals, and reptiles. With a total of 171 responses 51 (30%) reported that there was exposure to non-predatory wild mammals. The same number of responses were recorded for exposure to non-predatory wild birds. Thirty-five (20%) reported possible exposure to predatory mammals and 27 (16%) reported possible exposure to predatory birds. Six respondents indicated (3.5%) possible exposure to reptiles.

Methods of flight restraint were largely wing (feather) clipping or pinioning with 28/68 (42%) and 32/68 (47%) of the responses respectively. Tenectomy or tenotomy (non distinguished) was reported in 8/68 (12%) of responses.

Bird identification was reported to be primarily by leg band 54/75 (72%). Twenty percent (15/75) of respondents reported identification of birds using transponders. Two (0.3%) reported identification via wing bands. Also two reported identification via identifying marks or sight differences. One respondent reported using tattoos and one reported to not identify individual birds.

Nutrition

Commercially prepared diets are being used by 43/56 responses (77%). A variety of individually prepared (non-commercial) diets are also being used. Ingredients often contain chicken scratch, dog food, Bird of Prey, trout chow and supplemented with insects, fish or fish meal and chopped vegetables. Commercial diets reported by respondents include Mazuri Crane Diet with or without added supplements 15/31 (48%), Zeigler Crane Maintenance with or without supplements 9/31 (29%), Purina Maintenance with or without supplementation 2/31 (6.5%). Local Milling, HMS Zoo Fowl Maintenance and Agway Game and Turkey Grower represented 3/31 (9.7%), 1/31 (3.2%) and 1/31 (3.2%) of responses respectively. One respondent replied that the cranes have a natural diet as they are housed on 450 acres.

Twenty-seven of 43 (63%) respondents stated that they use a starter feed for growing birds. Age when changing to maintenance diet varied from 2 wk to 1 yr with one respondent stating that the birds are kept on the starter diet. Zeigler Starter is reported as used by 7/20 (35%), Purina Game Bird in 6/20 (30%) and Purina Crane Starter in 3/20 (15%) respondents. Mazuri Starter, or duck starter
or a low protein game bird chow were reported by the remaining respondents (2/20, 1/20 and 1/20 respectively).

Eighteen of 34 (53%) respondents reported using a different feed for breeding birds. Only 13 respondents completed the feed information section. Of these 6/16 (37.5%) reported using Zeigler Breeder (one of these supplementing with dog kibble), 7/16 (44%) reported using Mazuri Breeder (one supplements with corn, insects and Bird of Prey and one supplements with live food). Purina laying crumbles supplemented with vitamin D, calcium and phosphorus was reported by 1/16 (6%). International Crane Foundation recommended formulation and supplementation of maintenance diet with mice and horsemeat were reported by one respondent each.

Reproduction

Thirty-two of 54 (59%) respondents reported that their institution were successful in reproducing cranes. Pair bonding, nest building, copulatory behavior and territorial aggression were all seen in most of the cases reported.

Other behaviors mentioned included unison calling in crowned, red and sarus cranes. “Dancing” was reported in demoiselle, red crowned, wattle and sarus cranes.

Twenty-six of 42 (62%) respondents reported that eggs are incubated. Parent incubation was reported to be the most frequently used method 34/66 (51.5%). Artificial incubation was the second most used method 23/66 (35%). Surrogate species were reported as being used by 5/66 (7.6%) of the respondents. Foster parent species mentioned include white naped, sandhill, red and blue crowned, demoiselle, and bantam and domestic hens.

Incubators used include Grumbach 9/25 (36%), Humidaire 7/25 (28%), Roll X 4/25 (16%), Petersime 3/25 (12%), and GQF 2/25 (8%). The most frequently reported temperature was 99.5°F 13/18 (72%) with a range of 98.1-99.5. Humidity as measured by wet bulb temperature ranges were reported as a range of 80-88 with uniform distribution of answers through this range. Humidity as measured by percent humidity ranged from 54-70% with 3/4 reported as 54-56%.

Twenty-five of 49 respondents (51%) reported parent reared chicks in this second similar question. Hand rearing occurred in 19/49 (39%) of responses. Three of these stated that hand rearing occurred only in the past, as a last resort only and only if artificially incubated. Four of 49 reported using foster species (8.2%). The use of puppets and or costumes was mentioned as comments in four cases.

Preventive Medicine

Twenty-four of 53 respondents (45%) stated that they regularly examine their cranes. Annual examinations were the most reported 15/32 (47%) followed by opportunistic exams 8/32 (25%). Semiannual exams amounted to 4/32 (12.5%) of the respondents. Exams only for emergency cases was noted in 2/32 (6.3%) respondents. Other answers were biannually, semiannually and 4-6 fecals per yr. The distribution of responses to the question on which tests are routinely done was as follows: parasitology 28/115 (24%); complete physical 25/115 (22%); CBC 20/115 (17%); serum banking 12/115 (10%); bacteriology 11/115 (9.5%); serology 9/115 (7.8%); chemistries 7/115 (6%); weight
Virological testing was the only category without response. Serological comments included testing for antibodies to EEE (three notations), Newcastle Disease and Inclusion Body Disease (one comment each). TB testing was reported once using avian old tuberculin. Fecal parasitology was the most frequent and variable testing procedure with as many as six exams per yr to as infrequent as once annually.

Out of 37 respondents 28 (76%) did not treat or clean eggs. Those that did treat eggs used a variety of agents. Ten percent betadine solution was most frequently reported 5/12 (42%). Nolvasan solution was reported in 4/12 (33%). Potassium permanganate and formaldehyde (37% formalin) was reported 2/12 (16.7%). Gentamycin and water and Virkon spray each were reported 1/12 (8.3%).

Neonatal exams are performed routinely by 19/35 (54%) respondents. Two respondents (6%) reported that neonatal exams are performed only when clinically needed. Neonatal exams are not routinely or never done in 14/35 (40%) of respondent institutions.

Development monitoring prior to hatching consists of candling 7/13 (54%), weighing 5/13 (38%), and movement 1/13 (7.7%) of respondents. Post hatching monitoring was reported to be by daily keeper observations 12/30 (40%) and regular weighings 14/30 (46.7%). Weekly observations, regular veterinary exams, toe shape and direction, and leg form each were reported 1/30 (3.3%).

Routine vaccinations were reported to not be performed in 48/52 (94%) with two respondents reporting that they are considering using EEE vaccine. Of those who are vaccinating two use human killed EEE (two doses at 4-6 wk then annually), one uses WEE Encephaloid (Fort Dodge; at 3-4 wk then annually) and one uses *Clostridium botulinum* Type C (bacterin and toxoid; United Vaccines, Inc.; beginning at 3 wk, every 3 wk times three, then annually).

More respondents do not screen 34/52 (65.4%) for Inclusion Body Disease than those who do. Of those respondents who do screen 10/18 (55.5%) do so on arrival of the birds while 5/18 (27.8%) do so routinely (4 annually and 1 biannually). Single responses 1/18 (5.5%) each were reported for United States Fish and Wildlife Service requests, for Species Survival Program requirements and as required by Dr. Dein.

Most respondents quarantine new arrivals 55/59 (93%). Three respondents stated that they do not quarantine and one reported semi-quarantine with isolation on grounds separated from other birds. Twenty-seven out of 43 (63%) respondents reported no differences between arrival screening and routine exam and testing protocols.

Parasitology 10/36 (28%), Inclusion Body Disease serology 6/36 (18%), CBC 5/36 (14%), chemistry profile 5/36 (14%), bacteriology of cloacal swabs 5/36 (14%), and chlamydial testing of feces 5/36 (14%) topped the list of differences between arrival screening and routine exams. Radiographs, acid-fast stain of fecal smears, psittacosis antigen test of fecal smears, banking serum and prophylactic deworming (no product mentioned) all received one response each 1/36 (2.8%).

Morbidity and Mortality

Morbidity or mortality was experienced during the last 5 yr by 35/52 respondents or (67%). A total
of 111 deaths were reported in adults and 76 in chicks with two deaths reported in juvenile birds. By far the most common problem seen was trauma with predators and exhibit animals being the major factors. Five of six deaths in adult birds in one institution were from trauma. Twenty-three respondents sighted general trauma. Within the trauma category predation was the most often response with 22/70 (31%) of trauma reports and 22/115 (19%) of all common problems mentioned. Interactions/aggression between exhibit species was the next highest response at 11/70 (15.7%). This was followed by listing of structural problems 5/70 (7%) such as rough walls, fence problems and escapes. Trauma to specific anatomical sights was noted in some cases (wings, beak and legs) but these specific cases will be addressed in a later section.

Chick problems such as infection (presumably bacterial) 8/115 (7%), failure to thrive 2/115 (2%), pneumonia and yolk sacculitis 2/115 (2%), neonatal deaths (no etiology noted) 2/115 (2%) totaled 14/115 or 12%.

Frostbite 3/115 and environmental conditions 1/115 accounted for 3.5% of total responses. Parasites 3/115 and Syngamus spp. infections 1/115 also accounted for 3.5% of the total responses. Bacterial septicemia 2/115, pododermitis (bumblefoot) 3/115, joint infection (presumable bacteria?) 1/115, aspergillosis 1/115 account for 7/115 or 6% of the total. Old age, seizure disorder, impacted ventriculus, myocardial degeneration, cardiac (heart failure?) and an aortic aneurysm each received one mention as common problems.

Disease outbreaks with significant morbidity or mortality were not seen with 52/53 respondents answering no to this question. The only definitive diagnosis was for IBDC in a 1978 outbreak. Experience with infectious disease was greater with 13/43 (30%) of responses answering yes to this question. Viral disease was reported by five respondents with IBDC (1978 outbreak), EEE suspected in a Stanley crane chick and an adult (unspecified) and viral myeloproliferative disease suspected in an East African crowned crane. Bacterial disease was reported by eleven respondents with a yolk sacculitis (C. perfringens), pneumonia and epicarditis of multiple bacterial nature (chick) and pneumonia and septicemia in an adult (Staphylococcus aureus) in three East African crowned cranes. Also reported in this section were mycobacteriosis in one adult and possibly in three chick Belearica varaganus. A sandhill crane adult with Staphylococcus, red crane adult with pododermitis, a grey necked crane adult with E. coli and streptococcal airsacculitis and a crowned crane adult with possible salmonellosis were reported here as well. Four out of 16 respondents reported the identification of non-clinical Salmonella. One institution reported many non-clinical Salmonella spp. Fungal disease was reported by 3/18 respondents or 16.7%. These results include aspergillosis in a chick and an adult and candida plaques during antibiotic therapy in a juvenile (no species identified). Protozoal disease was reported by 4/17 (23%) of respondents. Coccidia spp. were reported in a greater sandhill adult, a crowned adult, a demoiselle chick and a newly imported black necked adult. Leukocytotozan species was reported in a white naped chick.

Parasites were reported by respondents as follows: a greater sandhill adult with Capillaria, a grey necked crowned chick with Acuaria spp. Syngamus, Capillaria, Heterakis and rhabditiform larvae (Syngamous suspected) were reported by respondents.

Physical Injuries / Disorders
Thirty-four of 46 respondents reported that they experienced cases with a physical etiology. Trauma was the most prevalent with 32/34 (94%) of the respondents indicating this as a problem. Trauma from predators was the most reported problem by respondents 15/34 (44%). Leg fractures, tendon damage, abrasions to legs and wings, and beak lesions in adult cranes resulting from trauma either as aggression between cage mates 11/34 (32%) or contact with exhibit walls or fences 4/34 (11.8%) were the next most common reported cases from respondents. Predation, exhibit mate aggression and exhibit structure played the prominent roles in the traumas reported (see previous section).

Developmental problems were reported in 9/26 responses or 34.6%. Those mentioned include 11 cases of angular limb deformities in West African crowned, Siberian, Stanley, white naped, sandhill, blue, grey and demoiselle cranes, three cases of angel wing in whooping and East African crowned cranes, one idiopathic elbow laxity in a Siberian crane, 11 cases of twisted or curled toes in whooping, Florida sandhill and West African crowned and two cases of crossed beak in Siberian and sandhill cranes.

Neoplastic Disease

Three of 48 respondents (6.25%) reported cases of neoplasia. Eight of these respondents reported that they did not know. The cases reported were as follows: myeloproliferative disease in a East African crowned crane (viral etiology suspected); leiomyosarcoma of the foot in a sarus crane and a mass in lung (species and tumor type unidentified).

Nutritional / Toxic Diseases / Miscellaneous

Eleven of 47 (23.5%) respondents reported that they had experienced cases in these categories. Thirty-two reported no (68%) and five (12%) responded with unknown.

Nutrition

Four of 19 (21%) responses indicated nutritional problems. Angular limb deformities, low weights due to competition for food with wild species or exhibit mates or parasite load were cited.

Toxicity

Five of 23 (21.7%) responses indicated cases of toxicity in cranes. Four cases of Flunixin meglumine (Banamine) toxicity when administered at published therapeutic dosages (3 Siberian and 1 whooping crane). Toxic hepatopathy (1 case adult East African crowned crane) and hepatic necrosis with fibrosis (one case, unknown species) were reported by respondents. Other reports by respondents include lead poisoning in an adult West African crowned crane, two suspected bacterial toxicities from food or water in a Balearica pavonina (West African crowned crane) and a sarus crane and a single report of a blue crane adult eating seeds of the plant Sophora secundiflora causing death.

Miscellaneous Problems

Nine of 22 (41%) respondents answered yes to having cases in this category. Gout was reported in
an aged crowned crane female with renal disease and also stated as a complication of Flunixin meglumine administration. Anesthesia related deaths were reported in two whooping, one East African crowned, one wattled juvenile and one demoiselle juvenile. Specific etiologies were not mentioned save for two reports of Halothane toxicity. Neurological disease of unknown etiology was mentioned in one case (no species name given). Severe cardiomyopathy with epicarditis and hepatic congestion was reported in a crowned crane.

Feedback / Follow-up

Fifty-five of fifty-five (95%) respondents reported that their institutions would be willing to provide additional information. Forty-four of 45 (98%) respondents reported that they would be willing to send tissues to a centralized serum/tissue bank.

Information From the Crane TAG

All of the suggested types of information were identified by most respondents as needed. Based upon the number of responses for each category, the order of priority for the information is as follows: (1) husbandry manual, (2) disease updates, (3) crane disease diagnostic lab information, (4) clinical pathology reference ranges, (5) list of crane specialists, (6) pathology and quarantine protocols (tied), (7) wild crane diseases.

Comments and Conclusions

Institutions responding are apparently maintaining ISIS records. Most cranes are housed on exhibit outdoors in groups or pairs with other species both birds and mammals. Facilities are considered “OK” or “near optimal” in over 80% of responses. No responder considered their facilities poor. There was some variation between exhibit and off season (winter housing). Adding water sources and prevention of predation were the most often expressed concerns of respondents. Netting exhibits and increasing space for holding were also significant concerns. Exposure to predatory and non predatory wild species was reported by nearly all respondents. Wing (feather) clipping and pinioning were the most common methods of flight restraint. Leg banding was the most used method of identification.

Commercially prepared diets are the most used dietary formulations by those responding to the questionnaire. The majority of respondents indicated that they use starter feed for growing birds. There was great variation in age when birds are converted to a maintenance diet (2 wk-1 yr). The majority of respondents reported using a different feed for breeding birds. These generally were commercial brands of breeder diets with supplementation.

The majority of respondents reported reproductive success in one or more species. Parent incubated eggs were the most common and desired method of incubation. Artificial incubation was a significant method however. Incubators varied as did the humidity settings whether by “wet bulb” or percent humidity; 99.5°F was the most used temperature setting. Insufficient data was gathered for individual species recommendations. A variety of foster parent species were reported as successful. Data gathering on these subjects appears to be consistent with the use of redundancy.
The majority of respondents do not regularly examine cranes. Of those who do, annual examination is the most frequent response. A variety of procedures are performed with fecal exams for parasites the most frequent test. It seems prudent to do a variety of procedures during regular examinations. Most respondents who regularly examine their birds do perform a number of tests. Treatment of eggs to prevent infection is not done in the majority of cases presumably because of the desire to not disturb the adults and an otherwise low incidence of egg related diseases. Neonatal examinations are inconsistently done. While the majority of respondents do them regularly, a significant number (40%) never do them or they are not routinely done. This likely relates to the previous success rates and experience with neonatal problems that the different respondents have had. Candling and/or weighing are most often used to monitor eggs while daily keeper observation and/or regular weight measurements are most often used to monitor neonates. The great majority of respondents (94%) do not vaccinate the birds. Inclusion body disease is not routinely screened for generally. It is likely that the low level of vaccination and preventive screening relates to the apparent low incidence of disease in captive cranes as cold be measured by respondents to this questionnaire. Quarantine of new arrivals is an accepted reality among respondents. In general the quarantine lasts for 30 days and, although a variety of tests were mentioned, parasitology exams on feces with appropriate treatment and IBDC serology were most often listed.

Mortality and morbidity is significantly affected by trauma with predation and exhibit interspecies aggression the most important contributing variables. In addition to not being predator proof in almost all cases, facilities themselves were, in some cases, dangerous to the birds. Chick problems represented 12% of all mentioned problems. Disease outbreaks are not seen in this population. A variety of apparently isolated cases of Infectious diseases are seen, however.

Data on physical injuries is consistent with responses given in an earlier section on the most common problems seen. Trauma consistently is seen and predation and interspecies aggression along with cage design appear to be significant variables. Developmental problems are significant in this population with significant numbers of angular limb deformities, curled toes and rare crossed beaks seen in a variety of species. Nutritional problems (over nutrition) are attributed as being a primary cause of most angular limb deformities by many respondents.

Neoplastic disease does not, at the present time, appear to be a significant cause of morbidity or mortality in this population of cranes. Perhaps this population is not reaching the age levels at which such maladies would become significant.
BEAK MEASUREMENT AS A METHOD FOR SEXING KEEL-BILLED TOUCANS (Ramphastos sulfuratus) AT CHAPULTEPEC ZOO, MEXICO CITY

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Abstract

Some of the techniques developed for determining sex in monomorphic birds are: karyotyping, observation of Barr bodies, fecal steroid analysis, feather pattern and various techniques for laparoscopy. Toucans belong to the Ramphastidae family, included in the order Piciformes. Sexual dimorphism occurs in some genera of the Ramphastidae family, such as Selenidera and Pteroglossus, but keel-billed toucans (Ramphastos sulfuratus) are monomorphic. They inhabit lowland forests and forest borders from the southern part of Mexico (from Oaxaca, Puebla and Veracruz south and east) to northern Colombia and the northwest part of Venezuela. Seventeen adult keel-billed toucans were anesthetized to measure their beaks and sex was determined by means of the laparoscopic technique using an otoscope. All of the birds were at least 3 yr (sexually mature). To analyze five different characteristics including weight (g) and four different measurements of the beak (cm), the following methods were used: t-student method and simple linear regression. For weight, there was no significant difference between males and females, with a mean value of 417 g (p>0.05). Four different beak measurements showed a highly significant relationship (p<0.01) with sex. In all these cases, the observed correlation was over 85 %. According to these calculations, upper beak A (UBA) was 2.4 cm smaller in females than in males; upper beak B (UBB) in females was 1.99 cm smaller compared with males. Lower beak A (LBA) and lower beak B (LBB) showed the same characteristics being 1.82 cm (LBA) and 2.1 cm (LBB) smaller in females than in males. Males have larger beaks than females.

Resumen

Algunas de las técnicas utilizadas para el sexado de aves monomórficas son: observación del cariotipo, observación de los corpúsculos de Barr, analisis de los esteroides fecales, observación de la disposición de las plumas y varias técnicas de laparoscopía. Los tucanes pertenecen a la familia Ramphastidae incluida en el orden de los Piciformes. El dimorfismo sexual se observa en algunos géneros de esta familia como son el Selenidera y el Pteroglossus, pero los Tucanes de pecho azufrado (Ramphastos sulfuratus) son monomórficos. Estos habitan los bosques bajos y sus bordes desde el sur de México (incluyendo Oaxaca, Puebla y Veracruz hasta el sureste) hasta el norte de Colombia y el noroeste de Venezuela. Un grupo de 17 tucanes de pecho azufrado adultos fueron anestesiados para llevar a cabo la medición de sus picos y el sexo se determinó a través de la técnica de la laparoscopía utilizando un otoscopio. Todas las aves tenían al menos 3 años de edad (sexualmente maduras). Para analizar 5 características diferentes incluyendo el peso (g) y 4 diferentes medidas del pico (cm) se utilizaron los métodos de análisis estadístico t-Student y regresión lineal simple. Para
la característica de peso no hubo una diferencia significativa entre machos y hembras, encontrando un valor medio de 417 g (p>0.05). Las 4 medidas del pico mostraron una relación altamente significativa con el sexo (p<0.01). En todos los casos, la correlación observada fue arriba del 85%. De acuerdo con estos cálculos, la valva superior A (VSA) fue 2.4 cm más pequeña en hembras que en machos, la valva superior B (VSB) fue 1.99 cm más pequeña en hembras que en machos. La valva inferior A (VIA) y la valva inferior B (VIB) mostraron las mismas características siendo 1.82 cm (VIA) y 2.1 cm (VIB) más pequeña en hembras que en machos. Los machos poseen picos más grandes que las hembras.

**Introduction**

One of the main aspects for conservation of birds is reproduction in captivity. Sexing birds becomes essential to achieve reproduction but it can be a difficult task, especially when species lack physical sexual dimorphism. Some of the techniques developed for determining sex in birds are: karyotyping, observation of Barr bodies, fecal steroid analysis, feather pattern and various techniques for laparoscopy.

Toucans belong to the Ramphastidae family, included in the order Piciformes. These birds are indigenous to Central and South America, including part of North America (ranging from southern Mexico to northern Argentina).

Keel-billed toucans (*Ramphastos sulfuratus*) inhabit lowland forests and forest borders from the southern part of Mexico (from Oaxaca, Puebla and Veracruz south and east) to northern Colombia and the northwest part of Venezuela. Individuals of this species are commonly kept in Mexico as pets because of their colorful beak and feathers, and also because they become tame enough to handle if they are kept since they are young. Zoological institutions receive a large number of these “pets” donated by people that kept them in captivity for a variable period of time.

Sexual dimorphism occurs in some genera of the Ramphastidae family like *Selenidera* and *Pteroglossus*, but keel-billed toucans are monomorphic.

**Material and Methods**

Seventeen adult keel-billed toucans were anesthetized to measure their beaks and sex was determined by means of the laparoscopic technique using an otoscope. All of the birds were at least 3 yr (sexually mature). Keel-billed toucans have a large, lightweight, highly vascular and sensitive bill composed of spongy bone. Four different measurements were taken of the toucans’ beak and at the same time weight was recorded.

All birds were captured inside their cages by means of a net or a towel. Once the beak was controlled and the body and wings safely wrapped in a towel, they were anesthetized using a plastic bag as a face mask and halothane was delivered at a concentration of 4% for 40 sec for induction and 2% for maintenance. Oxygen was administered at a flow of 0.5 L/min.

All animals were placed in right lateral recumbency. Only a few feathers had to be plucked because
toucans lack of feathers in this region. The left wing was extended craniodorsally, and the left leg was fully extended caudally. The skin was prepared for aseptic technique. A skin incision was performed dorsoventrally over the last intercostal space, parallel to the ribs. Intercostal muscles were incised as well as the posterior thoracic air sac. Sex was determined by directly observing the gonads. All incisions were closed with absorbable suture material, a dose of long acting amoxicillin was used in every bird after the surgery. All animals recovered uneventfully.

Weight was recorded from 16 toucans at this time and measurements from the beak of 17 toucans were taken.

To determine beak length, measurements were taken as follows (Fig. 1):

**Upper Beak**
- UBA- the dorsal margin of the upper beak was measured from the beginning to the tip of the beak.
- UBB- the lower margin of the upper beak was measured from the beginning of the beak outward toward the tip.

**Lower Beak**
- LBA- the ventral margin of the mandible was measured in the middle of the beak from the beginning to the tip, not including any of the branches.
- LBB- the ventral margin of the mandible was measured from the beginning to the tip of the beak, including one of the branches.

All data were analyzed using the Statistical Analysis System from the Biostatistics Department from the Veterinary College, National Autonomous University of Mexico. To analyze five different characteristics including weight (g) and four different measurements of the beak (cm) the following methods were used: t-student method and simple linear regression.

**Results**

Each bird’s measurements are presented in Table 1. According to the measurements obtained from all beaks, male toucans have larger beaks than females. Sex was confirmed by laparoscopy as described earlier.

Table 2 shows mean values for five different characteristics: weight, upper beak A (UBA), upper beak B (UBB), lower beak A (LBA), and lower beak B (LBB) each compared with sex.

For weight, there was no significant difference between males and females, with a mean value of 417 g (p>0.05). In the rest of the characteristics, we found highly significant differences (p<0.01) between sexes, observing that beaks are bigger in males than in females (Fig. 2).

Table 3 shows the least squares of characteristics such as weight against sex and beak measurements against sex, finding no significant relationship between sex and weight variables. A highly significant relation (p<0.01) between sex and 4 different variables: upper beak A (UBA), upper beak B (UBB), lower beak A (LBA), and lower beak B (LBB) was found. In all these cases, the observed correlation was over 85%. According to these calculations, upper beak A (UBA) was 2.4 cm smaller
in females than in males; upper beak B (UBB) in females was 1.99 cm smaller compared with males. Lower beak A (LBA) and lower beak B (LBB) showed the same characteristics being 1.82 cm (LBA) and 2.1 cm (LBB) smaller in females than in males.

Discussion

According to literature, keel-billed toucans weigh 386 g on average; weight ranges between 317-420 g. These weights were taken from adult birds. In this case, weight from 16 birds had a mean value of 417 g (p>0.05), ranging from 275-510 g. Males’ weight mean value was 439±14.25 g (ranging from 395-510 g) and females’ weight mean value was 396±25.75 g (ranging from 275-485 g). According to our results, weight is not related significantly to sex.

In general, male birds from the Ramphastidae family have a larger beak than females.

In one report, it was found that in toco toucans (Ramphastos toco), the beak of the male is generally greater than 16 cm in length, while in the female the measurement is less than 15.5 cm. To determine beak’s length, the lower margin of the upper beak was measured from the edge of the facial skin outward toward the tip.

In this study, four different beak measurements taken from adult keel-billed toucans (Ramphastos sulfuratus), showed a highly significant relation with sex. Males have larger beaks than females. Beak measurement could be used to sex keel-billed toucans.

ACKNOWLEDGMENTS

The authors thank Chapultepec Zoo for allowing the utilization of all birds mentioned in this report; also Jose Pulido R., David Berrón H., Jose Luis Gonzalez M., Alfonso Delgado O., Carlos Sanchez R., Adriana Gallegos T., Miguel Peña R., Gustavo Ramírez Z., Jorge Guzmán R., all members of the Veterinary Staff of Chapultepec Zoo, for their assistance with this study. Special thanks to Ma. Claudia Suárez de Gual for all her assistance in translation and correction of this manuscript.

LITERATURE CITED

Table 1. Beak measurements and weight obtained from 17 keel-billed toucans (*Ramphastos sulfuratus*).

<table>
<thead>
<tr>
<th>Ring</th>
<th>Sex</th>
<th>Weight (g)</th>
<th>UBA (cm)</th>
<th>UBB (cm)</th>
<th>LBA (cm)</th>
<th>LBB (cm)</th>
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<tbody>
<tr>
<td>J-62</td>
<td>MALE</td>
<td>410</td>
<td>16.6</td>
<td>15.0</td>
<td>9.7</td>
<td>13.5</td>
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<tr>
<td>J-106</td>
<td>MALE</td>
<td>430</td>
<td>17.5</td>
<td>14.3</td>
<td>9.9</td>
<td>14.5</td>
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<td>J-111</td>
<td>MALE</td>
<td>485</td>
<td>18.3</td>
<td>15.3</td>
<td>10.5</td>
<td>14.5</td>
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<tr>
<td>J-113</td>
<td>MALE</td>
<td>450</td>
<td>16.4</td>
<td>13.6</td>
<td>10.1</td>
<td>12.9</td>
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<tr>
<td>J-166</td>
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<td>510</td>
<td>17.9</td>
<td>14.7</td>
<td>10.1</td>
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<td>J-193</td>
<td>MALE</td>
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<tr>
<td>K-182</td>
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<td>405</td>
<td>17.3</td>
<td>15.1</td>
<td>10.9</td>
<td>14.4</td>
</tr>
<tr>
<td>NO RING</td>
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<td>-</td>
<td>17.9</td>
<td>14.7</td>
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<td>14.0</td>
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<tr>
<td>J-31</td>
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<td>275</td>
<td>14.4</td>
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<tr>
<td>J-72</td>
<td>FEMALE</td>
<td>320</td>
<td>15.2</td>
<td>12.5</td>
<td>8.5</td>
<td>12.2</td>
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<tr>
<td>J-80</td>
<td>FEMALE</td>
<td>445</td>
<td>16.1</td>
<td>13.6</td>
<td>9.3</td>
<td>12.8</td>
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<tr>
<td>J-145</td>
<td>FEMALE</td>
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<tr>
<td>J-181</td>
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<td>14.4</td>
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<td>J-184</td>
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<td>K-40</td>
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</tr>
<tr>
<td>K-181</td>
<td>FEMALE</td>
<td>350</td>
<td>14.2</td>
<td>12.5</td>
<td>8.6</td>
<td>11.4</td>
</tr>
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Table 2. Mean values and standard errors for weight (g), upper beak A (UBA), upper beak B (UBB), lower beak A (LBA) and lower beak B (LBB) (cm), in comparison between males and females keel-billed toucans (*Ramphastos sulfuratus*).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>439 ± 14.25</td>
<td>396 ± 25.75</td>
</tr>
<tr>
<td>Upper beak A (UBA) (cm)</td>
<td>17.54 ± 0.22*</td>
<td>15.1 ± 0.27**</td>
</tr>
<tr>
<td>Upper beak B (UBB) (cm)</td>
<td>14.7 ± 0.17*</td>
<td>12.71 ± 0.18**</td>
</tr>
<tr>
<td>Lower beak A (LBA) (cm)</td>
<td>10.2 ± 0.14*</td>
<td>8.4 ± 0.22**</td>
</tr>
<tr>
<td>Lower beak B (LBB) (cm)</td>
<td>14.0 ± 0.18*</td>
<td>11.9 ± 0.21**</td>
</tr>
</tbody>
</table>

*, ** Statistically different values (p<0.01).

Table 3. Analysis of variance of regression for weight (g), upper beak A (UBA), upper beak B (UBB), lower beak A (LBA) and lower beak B (LBB) (cm), in keel-billed toucans (*Ramphastos sulfuratus*).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Weight (g)</th>
<th>UBA (cm)</th>
<th>UBB (cm)</th>
<th>LBA (cm)</th>
<th>LBB (cm)</th>
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<tbody>
<tr>
<td>REGRESSION</td>
<td>1</td>
<td>7656.25</td>
<td>25.30719**</td>
<td>16.73007**</td>
<td>14.06327**</td>
<td>8.67765**</td>
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<tr>
<td>ERROR</td>
<td>15</td>
<td>3463.84</td>
<td>0.51481</td>
<td>0.24992</td>
<td>0.26370</td>
<td>0.32533</td>
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<tr>
<td>TOTAL</td>
<td>16</td>
<td>11120.09</td>
<td>25.822</td>
<td>16.97999</td>
<td>14.32697</td>
<td>19.00298</td>
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<tr>
<td>R²</td>
<td></td>
<td>0.1364</td>
<td>0.7662</td>
<td>0.8169</td>
<td>0.7805</td>
<td>0.7928</td>
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** (p<0.01)
Figure 2. Mean values for upper beak A (UBA), upper beak B (UBB), lower beak A (LBA) and lower beak B (LBB) (cm), of males and females keel-billed toucans (*Ramphastos sulfuratus*).
THE CARDIOPULMONARY EFFECTS OF PROPOFOL IN MALLARD DUCKS

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Abstract

Studies of waterfowl frequently rely on radiotelemetry because it provides excellent information on movements, habitat use, behavior and survival. Inhalational anesthetics such as methoxyflurane and isoflurane, are used commonly for surgical implantation of intra-abdominal radio transmitters, but require vaporizers and oxygen delivery systems. Propofol is a rapidly metabolized intravenous agent that requires continuous administration to produce light anesthesia. Twelve healthy adult female mallard ducks (Anas platyrhynchos) were anesthetized with isoflurane for placement of catheters in the brachial artery for arterial blood pressure monitoring and blood sampling and in the medial tarsal vein for propofol delivery. After the duck recovered completely, baseline measurements were recorded. Anesthesia was induced by injecting 10 mg of propofol over 1 min. Subsequent boluses (1-4 mg) of propofol were given to effect when the duck responded to a toe pinch or feather plucking over the ventral abdomen. ECG leads were attached and a temperature probe was placed into the esophagus. Mean blood pressure, heart rate, respiratory rate and esophageal temperature were recorded before administration of anesthetic (time 0), 5, 10, 15, 20, 25, and 30 min after the initial bolus and 5 min following the last bolus. Arterial blood samples (n = 8) were collected at time 0 (baseline), 5, 10, 15, and 30 min after the initial bolus and 5 min following the last bolus for PaCO₂, PaO₂ and pH. Results of the anesthetic period (0-30 min) were analyzed using an analysis of variance (ANOVA) for repeated measures. Where significant differences (p<0.05) were found, a least significant difference (LSD) pairwise comparison was done to compare the results with the baseline values (time 0). The results from 5 min after the last bolus were compared with baseline values by using paired t-tests. During the anesthetic period (0-30 min) there was no change over time in base excess, heart rate or mean blood pressure. There was a significant decrease in PaO₂, pH and esophageal temperature and a significant increase in PaCO₂ and respiratory rate over time. Five minutes after the last bolus, PaO₂ was no longer significantly different from the baseline value. PaCO₂, pH and respiratory rate remained significantly different but improved. Propofol provides light anesthesia and muscle relaxation may be used for intra-abdominal transmitter placement with the use of supplemental analgesia. Propofol causes dose dependent respiratory depression resulting in hypventilation, apnea, respiratory acidosis and hypoxic changes in the ECG. Ducks can be anesthetized safely with propofol but they should be monitored and ventilated artificially to reduce the chance of complications.

Resumen

Los estudios de aves acuáticas frecuentemente confían en la radiotelemetría porque les proporciona excelente información sobre movimientos, comportamiento, uso de hábitat y supervivencia. Los
anestésicos inhalables, como son el methoxiflurano e isoflurano, son usados comúnmente para la implantación quirúrgica de radio-transmisores intra-abdominales, pero requieren sistemas de vaporizadores y distribución de oxígeno. El propofol es un agente rápidamente metabolizado por vía intravenosa que requiere una administración continua para producir anestesia ligera. Doce hembras adultas y saludables de pato mallard (*Anas platyrhynchos*) fueron anestesiadas con isoflurano para la colocación de catéteres en la arteria braquial para monitoreo de la presión de sangre arterial y muestreo de sangre, así como la administración de propofol a través de la vena tarsal media. Una vez que se ha recuperado el pato completamente, los parámetros basales son registrados. La anestesia es inducida por inyección de 10 mg de propofol durante un minuto. Se administraron dosis subsecuentes de 1-4 mg de propofol cuando el pato respondió a pellizcos en los dedos de las patas o al arrancar las plumas del vientre. El ECG fue colocado y una sonda de temperatura fue introducida al esófago. Presión sanguínea media, frecuencia cardíaca, frecuencia respiratoria y temperatura esofágica fueron registradas antes de la administración del anestésico (tiempo 0), y a los 5, 10, 15 y 30 min después de la dosis inicial, así como 5 minutos después de la última dosis. Se tomaron muestras de sangre arterial (n=8) al tiempo 0 (línea base), 5, 10, 15, 25 y 30 min. después de la dosis inicial y a los 5 min. siguientes de la última dosis para medir PaCO₂, PaO₂ y pH. Los resultados del periodo de anestesia (0-30 min.) se analizaron mediante un análisis de varianza (ANOVA) para medidas repetidas encontrándose diferencias significativas (p<0.05). Una diferencia significativamente menor (DSM) por comparación por pares fue hecha para comparar los resultados con los valores de la línea base (tiempo 0). Los resultados desde 5 min. después de la última dosis fueron comparados con los valores de la línea base por pruebas-t por pares. Durante el periodo de anestesia (0-30 min.) no hubo cambio en base a exceso, frecuencia cardíaca o presión media sanguínea. Hubo un decremento significativo en el PaO₂ y pH y temperatura esofágica, y un incremento significativo en PaCO₂ y frecuencia respiratoria sobre el tiempo 5 min. después de la última dosis, el PaO₂ no fue significativamente diferente del valor de la línea base. PaCO₂, pH y frecuencia respiratoria permanecieron con diferencia significativa pero mejorada. El propofol proporciona una anestesia ligera y relajación muscular, puede usarse para la colocación del transmisor intra-abdominal con el uso de analgesia suplementaria. El propofol, dependiendo de la dosis, puede causar depresión respiratoria, resultando en hipoventilación, apnea, acidosis respiratoria y cambios hipóxicos en el ECG. Los patos pueden ser anestesiados con seguridad con propofol pero deberá apoyarse con monitoreo y ventilación artificial para reducir la posibilidad de complicaciones.

**Introduction**

Studies of waterfowl frequently rely on radiotelemetry because it provides excellent information on movements, habitat use, behavior and survival. Intra-abdominal transmitters are used preferentially because harness transmitters appear to affect behavior and survival of birds adversely. Inhalational anesthetics such as methoxyflurane and isoflurane are used commonly, but require vaporizers and oxygen delivery systems. Methoxyflurane has also been administered by placing the anesthetic on gauze but overdose and other complications may occur. Injectable drugs, alone or in combination have produced variable results and are not suitable for an invasive surgical procedure.

Propofol (Rapinovet®, Mallinckrodt Groups Inc. Company) is a rapidly metabolized intravenous
agent that requires continuous administration to produce light anesthesia. Supplemental analgesia is required to ensure intra-operative pain control. The purpose of this study was to examine the cardiopulmonary effects of propofol in mallard ducks over a 30 min period and 5 min following the last bolus injection.

**Methods and Materials**

Twelve healthy adult female mallard ducks (*Anas platyrhynchos*), weighing 0.96-1.3 kg were used. Instrumentation, under isoflurane anesthesia, involved a sterile cut down to expose the brachial artery for catheterization. A sterile length of noncompliant tubing was used to connect the arterial catheter to a pressure transducer and Propaq 400® monitor. A 24-ga, ¾ inch catheter was placed in the medial tarsal vein and taped into place for propofol delivery. The duck was allowed to recover and after the duck was awake and alert for at least 5 min, baseline measurements were recorded. Anesthesia was induced by injecting 10 mg of propofol over 1 min. Subsequent boluses (1-4 mg) of propofol were given to effect throughout the procedure (30 min) when the duck moved in response to a toe pinch or feather plucking over the ventral abdomen. After induction, the birds were placed in dorsal recumbency to mimic the position required for intra-abdominal transmitter placement. ECG leads were attached using alligator clips and a temperature probe was placed at least 10 cm into the esophagus.

Mean blood pressure, heart rate, respiratory rate and esophageal temperature were recorded before administration of anesthetic (time 0), 5, 10, 15, 20, 25, and 30 min after the initial bolus and 5 min following the last bolus. Arterial blood samples (n = 8) were collected at time 0 (baseline), 5, 10, 15, and 30 min after the initial bolus and 5 min following the last bolus. Blood samples were kept on ice and were analyzed within 3 hr for PaO<sub>2</sub>, PaCO<sub>2</sub> and pH. Base excess was calculated using a standard human nomogram. Results of the anesthetic period (0-30 min) were analyzed using ANOVA for repeated measures. Where significant differences were found, a least significant difference (LSD) pairwise comparison was done to compare the results with the baseline values (time 0). The results from 5 min after the last bolus were compared with baseline values by using paired t-tests. A difference was considered significant if P was less than 0.05.

**Results**

All ducks survived the study and there were no complications after recovery. After the initial bolus of 10 mg, all ducks could be placed in dorsal recumbency. An additional bolus of 4 ± 1.6 was required to attain a plane of light anesthesia with minimal response to the toe pinch. Two ducks had profound bradycardia immediately following induction and one duck had large T-waves at 5 min post-induction which lasted approximately 4 min. Another duck developed apnea and premature ventricular contractions after a bolus of 4 mg at 28 min. All ducks had a period of apnea following the induction bolus.

During the anesthetic period (0-30 min) there was no change over time in base excess, heart rate or mean blood pressure (Table 1). There was a significant decrease in PaO<sub>2</sub>, pH and esophageal temperature and a significant increase in PaCO<sub>2</sub> and respiratory rate over time (Table 1). Five minutes after the last bolus, PaO<sub>2</sub> was no longer significantly different from the baseline value. PaCO<sub>2</sub>, pH and respiratory rate remained significantly different but improved. All birds shivered.
during the recovery period and the esophageal temperature remained significantly lower than the 30 min value.

Discussion

Propofol has been shown to depress cardiac function by slowing atrial rate and depressing AV nodal conduction.\textsuperscript{1} The change in T-wave morphology and premature ventricular contractions seen on the ECG tracing may be related to the hypoxia since abnormalities followed bolusing and a period of apnea. Propofol depressed ventilation in the mallard ducks as demonstrated by increased PaCO\textsubscript{2} and decreased PaO\textsubscript{2}. The increase in PaCO\textsubscript{2} indicates hypventilation, despite an increased respiratory rate. A reduction in minute volume may be a dose dependent function of propofol. The weight of the large pectoral muscles may also restrict ventilation in a bird placed in dorsal recumbency. The decrease in pH represents a respiratory acidosis due to increased PaCO\textsubscript{2} with no significant change in base excess.

In mammals, propofol reduces arterial blood pressure, cardiac output and total peripheral resistance.\textsuperscript{8} Peripheral resistance changes are thought to reflect a combination of decreased sympathetic tone and direct actions on vasculature.\textsuperscript{3} In this study, propofol did not affect mean blood pressure or heart rate but this may be attributed to the fact that the birds were at a light plane of anesthesia. Rapid metabolism of propofol makes the duration of action and the cardiopulmonary effects of propofol relatively short.\textsuperscript{2,3,8,9}

Conclusions

Propofol produces a smooth induction and recovery with few side effects. It provides light anesthesia and muscle relaxation that may be used for intra-abdominal transmitter placement with the use of supplemental analgesia. Propofol produces dose dependent respiratory depression resulting in hypoventilation, decreased PaO\textsubscript{2} and respiratory acidosis. In addition, administration of a large bolus of propofol will result in apnea. Ducks can be anesthetized safely with propofol but they should be monitored and ventilated artificially to reduce the chance of complications.

ACKNOWLEDGMENTS

Funding for this project was provided by Delta Waterfowl and Wetlands Research Station, Ducks Unlimited Institute for Wetland and Waterfowl Research, and the Western College of Veterinary Medicine Wildlife Health Fund (University of Saskatchewan). Support in kind was provided by the Canadian Wildlife Service. The authors gratefully acknowledge the help of Robert Brua, Robert Clark, Michelle Moroz and Colette Wheler for their help and advice.

LITERATURE CITED

Table 1. Cardiopulmonary effects of intravenous propofol in adult female mallard ducks.

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<thead>
<tr>
<th>Measurements</th>
<th>Baseline value</th>
<th>Time After Induction of Propofol Anesthesia</th>
<th>5 min following last bolus</th>
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<tr>
<td></td>
<td>(time 0)</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>203.5±36.4 (12)</td>
<td>207.1±34.8 (12)</td>
<td>198.8±32.1 (12)</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>165.7±18.3 (12)</td>
<td>170.6±37.8 (12)</td>
<td>168.2±33.5 (12)</td>
</tr>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>21.7±5.5 (12)</td>
<td>31.3±8.7* (12)</td>
<td>28.7±7.0* (12)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>39.4±1.0 (12)</td>
<td>39.3±0.9* (12)</td>
<td>39.2±0.9* (12)</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>87.5±9.2 (8)</td>
<td>60.0±8.9* (8)</td>
<td>63.1±4.2* (8)</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>31.6±4.3 (8)</td>
<td>46.6±5.4* (8)</td>
<td>47.0±4.4* (8)</td>
</tr>
<tr>
<td>pH</td>
<td>7.46±0.03 (8)</td>
<td>7.35±0.04* (8)</td>
<td>7.35±0.04* (8)</td>
</tr>
<tr>
<td>Calculated Base Excess</td>
<td>-0.71±2.36 (8)</td>
<td>-0.85±1.95 (8)</td>
<td>-0.29±2.29 (8)</td>
</tr>
</tbody>
</table>

*Significantly (p<0.05) different, compared with baseline value.
Data are expressed as: mean±standard deviation (number of observations).
EVALUATION OF HEPATOBILIARY FUNCTION IN THE COCKATIEL. (*Nymphicus hollandicus*)

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Abstract

Hepatic disease has many infectious, nutritional, metabolic, and toxic causes in companion birds, and the diagnosis of liver disease in birds is difficult primarily because of the lack of any liver specific enzyme.4,8 Tests used in diagnosis of hepatic disease in mammals vary in their usefulness in birds. Aspartate aminotransferase (AST) elevations may be caused by liver disease in psittacine birds, however, AST activity is also found in heart, skeletal muscle, brain, and kidney.1,8 Levels of AST may rise with insult to any of these tissues and amounts of AST activity vary among species. Alanine aminotransferase (ALT) analysis is useful for diagnosis of liver disease in carnivores. Serum ALT concentrations vary in avian tissues and do not increase in all cases of hepatic disease. Lactase dehydrogenase (LDH), another enzyme found in the avian hepatocyte, has isoenzymes present in many other avian tissues. Elevations of LDH may be caused by a variety of tissue damage and hemolysis. Evaluation of serum bilirubin concentrations is not applicable to avian medicine because birds lack the biliverdin reductase enzyme to produce bilirubin from biliverdin.

Liver function tests have been poorly researched in companion bird species, but have been shown to be effective indicators of hepatic disease in man and other mammals. In mammals, bile acid analysis is a sensitive indicator of hepatic function.2,6 Bile acids are synthesized by the liver from conjugates of cholesterol and secreted in the bile. Bile released into the small intestine plays a vital role in the saponification and absorption of dietary fats. In the lower small intestine, bile acids are actively reabsorbed and reused via the enterohepatic circulation. During fasting, bile acids are at low levels in normal animals but after a meal they appear in increased concentrations in the blood. In hepatocellular injury, the hepatocytes ability to extract bile acids returning in the portal blood is reduced and increased concentrations appear in blood.

Preliminary data on bile acids has been investigated in some avian species. Fasting and postprandial bile acids concentrations have been recently described in the racing pigeon, duck, and several species of raptors.2,9,10,11 Variability in normal bile acid concentrations between avian species have been documented for some large psittacine species.3,5,9 Fasting, postprandial, and diurnal variations of serum bile acids levels are not known for the cockatiel (*Nymphicus hollandicus*).
The objectives of the present research were to: 1) compare the radioimmunoassay (RIA) and colorimetric (enzymatic) methods for measuring bile acids in the cockatiel; 2) determine normal serum bile acid concentrations in fasting and postprandial cockatiels; 3) determine levels of bile acids during a diurnal cycle; and 4) determine changes in bile acid levels relative to liver enzyme values following experimentally induced hepatocellular damage resulting from the administration of aflatoxin B₁.

Complete blood counts were performed by the Clinical Pathology Laboratory, Kansas State University, Manhattan, Kansas. For chemistry analysis, including bile acids, frozen plasma samples were shipped on dry ice to the Comparative Pathology Laboratory, University of Miami School of Medicine, Miami, Florida. The following tests were included in the chemistry panel: AST, ALT, cholesterol, GGT, LDH, GLDH, uric acid, total protein, protein electrophoresis, and bile acids (using the RIA method). For comparative bile acids studies (using the colorimetric method), frozen plasma samples were also sent to Avian Technologies, Inc., California Avian Laboratory, Citrus Heights, California. At the conclusion of the study, all birds were euthanatized, and tissues from all major organs were fixed in 10% formalin and examined histologically to characterize any lesions found. Liver lesions were categorized as mild, mild to moderate, moderate to severe, or severe hepatocellular damage based on distribution and types of changes seen. Findings of the study include:

1. Both the RIA and colorimetric were good predictors of liver damage: by evaluating the bile acid levels in replicate samples, it was concluded that the colorimetric method provided more reproducible results; the colorimetric method had less variability in results and thus may be a preferred technique; because of the inherent variability in a bird’s ability to respond to a hepatic insult, false negative bile acid levels may occur in birds in early-mild stages of liver disease.

2. Difficulties in comparing the RIA and colorimetric methods included the fact that it was impossible to determine if the differences between the results were due to the method used or to the laboratory technique used. In addition, the RIA and colorimetric values were not exactly in the same scale, so direct statistical comparison of the two methods was imprecise.

3. Following are the mean pre- and post-treatment bile acid levels for the 21 treated birds.

<table>
<thead>
<tr>
<th>Method</th>
<th>Pre-treatment Values (µmol/L)</th>
<th>Post-treatment Values (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIA</td>
<td>60 ± 9</td>
<td>409 ± 123</td>
</tr>
<tr>
<td>Colorimetric</td>
<td>235 ± 30</td>
<td>395 ± 854</td>
</tr>
</tbody>
</table>

4. Of the other biochemical tests studied (AST, ALT, LDH, cholesterol, GLDH, uric acid, total protein, prealbumin, albumin, alpha [α₁ and α₂], beta [β], gamma [γ], and A/G ratio), only AST was statistically significant in evaluating liver damage. However, AST was not highly correlated with liver damage, thereby being of limited biological value in some cases.
En aves de compañía las enfermedades hepáticas tienen muchas causas: infecciosas, nutricionales, metabólicas y tóxicas, y el diagnóstico de enfermedades del hígado en aves es primariamente difícil por la ausencia de algunas enzimas específicas del hígado; pruebas usadas en diagnóstico de enfermedades hepáticas en mamíferos varían en su utilidad en aves. La elevación de la aspartato aminotransferasa (AST) puede ser causada por enfermedades del hígado en aves psitacidas, sin embargo, la actividad de AST es también localizada en corazón, musculo esquelético, cerebro y riñón. Los niveles de AST pueden elevarse con un ataque a algunos de estos tejidos y la cantidad de actividad de AST varía según la especie. El análisis de alaninotransferasa (ALT) es útil en el diagnóstico de enfermedades del hígado en carnívoros. Las concentraciones de ALT en suero varían en tejidos de aves y no se incrementa en todos los casos de enfermedad hepática. La dehidrogenasa láctica (LDH) es otra enzima localizada en el hepatocito aviar presenta isoenzimas en muchos otros tejidos aviares. La elevación de LDH puede ser causada por una gran variedad de daño al tejido y hemólisis. La evaluación de concentraciones de bilirrubina sérica no es aplicable en medicina aviar porque las aves no presentan la enzima reductasa viliverdina que produce bilirrueva a partir de viliverdina.

Las pruebas de función del hígado han sido pobremente investigadas en especies de aves de compañía, pero han demostrado ser efectivos indicadores de enfermedades hepáticas en el hombre y otros mamíferos. En mamíferos el análisis del ácido biliar es un sensible indicador de la función hepática. Los ácidos biliares son sintetizados por el hígado a partir de colesterol conjugado y secretados en la bilis. La bilis liberada en el intestino delgado juega un rol vital en la saponificación y absorción de grasa de la dieta. En el intestino delgado bajo, los ácidos biliares son activamente reabsorbidos y reusados vía la circulación enterohepática. Durante el ayuno, los ácidos biliares se encuentran a niveles bajos en animales normales, pero después de comer aparecen en concentraciones remontadas en la sangre.

Datos preliminares sobre ácidos biliares en ayuno y postprandiales han sido recientemente descritos en palomas mensajeras, patos y varias especies de rapaces. Variaciones de concentración normal de ácidos biliares entre especies de aves han sido documentada para algunas especies de grandes psitácidos. Las variaciones en ayuno, postprandial y en el ciclo diurno de los niveles de ácidos biliares en ninfas (Nimphicus hollanicus) eran desconocidas.

Los objetivos de la presente investigación fueron: 1) comparar los métodos de radioinmunoensayo (RIA) y colorimétrico (enzimático) para la medición de ácidos biliares en ninfas, 2) determinar en ninfas la concentración normal de ácidos biliares séricos en ayuno y postprandial, 3) determinar niveles de ácidos biliares durante el ciclo diurno, y 4) determinar cambios en niveles de ácidos biliares relativos a los valores de enzimas hepáticas, después de un daño hepatocelular inducido experimentalmente a través de la administración de aflatoxina B1.

La biometría hemática completa fue realizada por el laboratorio de patología clínica de la Universidad Estatal de Kansas, Manhattan, Kansas. Para el análisis químico, incluyendo ácidos biliares, se remitieron muestras de plasma congelado en hielo seco al laboratorio de patología competitiva de la Escuela de medicina de la Universidad de Miami, Miami, Florida. Las siguientes pruebas fueron incluidas en el panel de química: AST, ALT, Colesterol, GGT, LDH, GLDH, Acido úrico, Proteína total, Electroforesis de proteína y ácidos biliares (usando el método RIA). Para
estudios comparativos de ácidos biliares (usando el método colorimétrico), se enviaron muestras de plasma congelado a Tecnologías Aviares, Inc., Laboratorio Aviar de California, Citrus Heights, California. Al concluir el estudio todas las aves fueron eutanasiadas, y los tejidos de todos los órganos mayores fueron fijados con formol al 10% y examinados histológicamente para localizar alguna lesión. Las lesiones en hígado fueron categorizadas como leves, leve a moderado, moderado a severo o severo daño hepatocelular basándose en el tipo y distribución de los cambios vistos. Los hallazgos del estudio incluyen:

1. Ambos métodos (RIA y colorimetría) fueron útiles para detectar daño en el hígado: por una evaluación de niveles de ácidos biliares en muestras duplicadas, de esto se concluyó que el método colorimétrico proveyó más resultados confiables; el método colorimétrico tuvo menor variabilidad en resultados y así puede ser una técnica preferida; porque de la variabilidad inherente en la capacidad del ave a responder a un ataque hepático puede darse un falso negativo de los niveles de ácido biliar en aves en una etapa temprana de enfermedad del hígado.

2. Las dificultades en la comparación del método RIA y el colorimétrico incluyeron el hecho de que fue imposible determinar si las diferencias entre los resultados fueron debido al método usado o a la técnica de laboratorio usada. En adición los valores en los dos métodos no fueron exactamente en la misma escala, por lo que la comparación estadística de los dos métodos fue imprecisa.

3. Los siguientes son los niveles medios de ácidos biliares pre y post tratamiento en las 21 aves tratadas.

<table>
<thead>
<tr>
<th></th>
<th>valores pre-tratamiento</th>
<th>valores post-tratamiento</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(µmol/l)</td>
<td>(µmol/l)</td>
</tr>
<tr>
<td>RIA</td>
<td>60±9</td>
<td>409±123</td>
</tr>
<tr>
<td>Colorimetría</td>
<td>235±30</td>
<td>395±854</td>
</tr>
</tbody>
</table>

4. De las otras pruebas bioquímicas estudiadas (AST, ALT, LDH, colesterol, GDLH, ácido úrico, proteína total, prealbumina, álbúmina, alpha \([\alpha_1, \alpha_2, \alpha_3]\), beta \([\beta]\), gamma \([\gamma]\), y relación A/G), solamente AST fue estadísticamente significativo en la evaluación de daños hepáticos. Sin embargo, el AST no fue correlacionado fuertemente con problemas hepáticos, de tal modo que resulta de limitado valor biológico en algunos casos.

LITERATURE CITED

GLOBULIN LEVELS CORRELATED WITH TITERS OF Chlamydia psittacosis IN RED-TAILED AMAZON (Amazona brasiliensis), SOUTHEASTERN BRAZIL

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Abstract

From February 1995 to February 1996, eight red-tailed Amazon parrots (Amazona brasiliensis) were researched for globulin levels, LDH values (lactate dehydrogenase), AST values (aspartate aminotransferase), CPK values (creatine phosphokinase), indirect ELISA titers (enzyme linked immunosorbent assay) and latex agglutination titers. Globulin levels were measured three times using the Gornal-Bardawil David modified method. Chlamydiosis titers were determined by the ELISA method and compared to globulins and some enzymes. This was done to establish a reasonable, economically viable, and secure method to monitor the health status of this endangered species. The relationship between these parameters was discussed and compared to the literature values. All studied birds showed high titers in both ELISA and latex agglutination tests. Generally, AST did not differ significantly during the entire period but LDH decreased, suggesting an infection in the beginning of the study. All animals showed hyperproteinemia in the first two measurements. Such a high level is correlated with chronic inflammatory stimulation. High levels of alpha (α1) were detected on February 1995 showing a wave in the beginning of psittacosis infection. During α1 decreases between February 1995 and July 1995 and its maintenance, an increase of gamma (γ) occurred which reinforces the idea of a period of infection. The results of this preliminary study suggest that some parameters such as protein electrophoresis can be used together with an immunologic test (ELISA) to determine chlamydiosis infection and its stage, especially, when other sophisticated methods are not available and there are no clinical signs of infection. The present work points out some alternatives towards the better understanding of chlamydiosis (ornithosis) and protocols for its diagnosis in our country.

Resumen

De febrero de 1995 a febrero de 1996 ocho loros Amazona cola roja (Amazona brasiliensis) fueron investigados para establecer niveles de globulina, dehidrogenasa láctica (LDH), aspartato aminotransferasa (AST), creatinina fosfoquinasa (CPK), títulos de ELISA indirecta (Enzyme linked immunosorbent assay), y títulos de aglutinación en latex. Los niveles de globulina fueron medidos tres veces usando el método modificado de Gornal-Bardawil David. Los títulos de Chlamydiosis fueron determinados por el método ELISA y comparado con las globulinas y algunas enzimas. Esto fue hecho para establecer un razonable, económicamente viable y seguro método para monitorear el estado de salud de estas especies en peligro. La relación entre esos parámetros fue discutido y comprarado con los valores en la literatura. Todas las aves estudiadas presentaron altos niveles de títulos en las dos pruebas de ELISA y aglutinación de latex. AST no difirió significativamente durante el periodo entero, pero LDH disminuyó sugiriendo una infección en el inicio del estudio.
Todos los animales presentaron hiperproteinemia en las dos primeras mediciones. Esto sugiere que los altos niveles están correlacionados con estimulación inflamatoria crónica. Niveles altos de alpha1 fueron detectados en febrero de 1995 presentando una curva en el inicio de la infección de psitacosis. Durante el decremento de α1 entre febrero de 1995 y julio de 1995, ocurre un incremento de gamma el cual refuerza la idea de un periodo de infección. Los resultados de este estudio preliminar sugieren que algunos parámetros semejantes como electroforesis de proteínas puede ser usado junto con una prueba inmunológica (como ELISA), para determinar infección por Chlamidiosis y en esta etapa, especialmente, cuando otros métodos sofisticados no son posibles y no existen signos clínicos de la infección. El presente trabajo señala algunas alternativas hacia la mejor comprensión de la Chlamidiosis (ornitosis) y protocolos para su diagnosis en nuestro país.

Introduction

Nowadays, several countries share problems like illegal pet trade and habitat destruction as their main threat for many species, including parrots. Because of the fast forest fragmentation and rapid decline and fragmentation of wild populations, our ability to ensure their survival have often been outpaced.

The red-tailed Amazon is an endangered species (Threatened birds of the Americas, red data book, 1992) endemic to a narrow coastal stretch of Brazil between the Atlantic and the coastal mountains in the States of São Paulo, Paraná, and Santa Catarina. It is a habitat specialist species and lives in an area characterized by lowland forest on sandy soils and mangrove swamps. Beyond severe habitat fragmentation it has been suffering losses in its population through the trade of chicks and adults. Wild populations of endangered animals are more vulnerable to diseases if their habitat has been constantly modified or invaded by exotic species. Population management becomes vital in these situations.

Unfortunately, the harmful role that diseases play throughout endangered wild populations, has not received enough attention. Probably, because of difficulty in recording field data and the rarity of them.8,10,11,12

In field work conditions where enough financial support does not exist and in countries where chlamydiosis is not a recognized problem, it becomes essential to find alternative ways to monitor populations and to control animal health status.

In Brazil, at the moment, there are no test protocols nor established treatments for chlamydiosis. At zoological parks, there are no procedures to diagnose or to treat this disease in the quarantine areas. Improvement of this situation requires studies in captive and field conditions, including veterinary programs for the secure implementation of the different proposals for species protection, such as reintroduction programs, reproductive studies or breeding programs. The present work is a previous study to establish an optional protocol to facilitate diagnosis.

Subject

In this study we used one bird that came from the illegal pet trade at Bahia State (it was together
with a partner that had died before coming to us) and seven birds that were caught in the island (ilha Comprida - São Paulo) by inhabitants. These animals were placed in two cages at Estação Ecológica Juréia - Itatins (Peruíbe - São Paulo). This study is part of a project on field biology research, educational programs and veterinary care supported by Fundação o Boticário de Proteção à Natureza, World Wildlife Fund, Wildlife International, and Dresden Zoo.

The parrots were individually identified with leg bands and were sexed by karyotypic methods. Complete blood counts (CBC), parasitological fecal exams and microbiological exams to monitor the gut bacterial flora have been done together with the biochemical exams. Treatment of abnormal gut bacterial flora or intestinal parasites was done whenever necessary. They were fed twice per day but unfortunately nutritional studies have not been done.

Methods

Blood samples were taken three times during the study period, on February 1995, July 1995, and February 1996. Only one bird (3278) had its first blood sample taken in December 1994. Biochemical analyses were carried out for lactate dehydrogenase, aspartate aminotransferase, creatine phosphokinase, albumin, α₁, α₂, β, γ globulins, and total proteins. Indirect ELISA test to determine chlamydiosis titers, and latex agglutination test were done for one bird (3278) in December 1994. Latex agglutination was measured for all the other parrots on February 1995 and all of them were tested by ELISA in February 1996.

Independent t-test statistical analysis was performed to detect differences between values of globulins, total proteins and enzymes for all three measurements. Correlation between these values and chlamydiosis titers was done according to their variation in different levels of infection.

Results

No clinical signs or substantial variation in behavior, feeding, or droppings were observed during the study period. Table 1 shows average and standard deviation for total protein, albumin, globulins, total globulins, and enzymes per month. T-test significance with percent of decrease or increase, between the studied months is shown as well. T-test results with P = 0.50 are shown in Table 2.

ELISA testing was done in December 1994 on individual 3278 and it had a titer of 1:256. In February 1996 the test was performed on all birds. The titers were: 1:64, 1:32, 1:64, 1:64, 1:128, 1:64, 1:32, and 1:128.

Latex agglutination was measured in December 1994 for individual 3278 which showed a value of 800 Ua/ml. On February 1995 the same test was done for the other individuals with the following values: 520 Ua/ml, 640 Ua/ml, 490 Ua/ml, 550 Ua/ml, 500 Ua/ml, 460 Ua/ml, and 620 Ua/ml.

Discussion

Chlamydia is a zoonotic disease that can be hard to identify since clinical and laboratory findings change depending on to the organ system involved and pathogenicity of the chlamydial strain. It may vary from several to no clinical, hematological or biochemical signs.
The course of this disease in birds varies from mild, unapparent infections to septicemia. Common clinical signs are anorexia, weight loss, and diarrhea or yellowish droppings, sinusitis, air sacculitis and pneumonitis.\(^9\) However clinical recognition of chlamydiosis in an individual bird is not always precise.\(^3\)

All eight birds studied are probably unapparent carriers of chlamydiosis because of ELISA titers and latex agglutination results found. The adult individual 3278 that was introduced in the group in January 1995, had its ELISA and latex agglutination results ready, only in February 1995 (1:256 and 800 Ua/ml respectively). When latex agglutination measures were taken from the other seven birds in February 1995, all of them showed titers ranging between 460-640 Ua/ml. Later on, when an ELISA test was done on February 1996, all birds showed titers between 1:32 to 1:128.

Common situations such as transportation, purchasing, and the pet trade affects birds promoting stress. This also appears to be an important factor in inducing disease in birds with latent infection.\(^9\) It is known that, because of the large movement of birds in the pet trade, the risks of introducing highly virulent viruses into wild populations of endangered psittacine species are substantial. High titers can be stable and decrease slowly over a period of months after infection but a static titer may be considered to be indicative of cessation of an active systemic infection. However, the possibility of an intestinal carrier state infection must be considered in these cases. Such an infection could presumably result in continual antigenic stimulation, causing a titer to remain elevated.\(^4\)

Serologic methods such as latex agglutination, ELISA, and direct complement fixation have been used. However many birds do not fix complement. If this test is compared with ELISA, it appears to be less sensitive because complement fixation will react only with IgG gamma globulins and will not with IgM gamma globulins. During an experimental infection negative ELISA titers of 1:5 were found. Seven days after inoculation the titers were 1:125 (2). When direct complement fixation and latex agglutination are compared, latex agglutination test shows a sensitivity of 89.7% and a specificity of 92.8%. This test classifies a bird as probably infected if its titer is equal to or superior than 1:64 (or 256 Ua/ml).\(^3\)

Studies demonstrated the usefulness of adapting ELISA procedures made for humans for the diagnosis of chlamydial infections in birds.\(^2\) This was a large study comparing cell culture, serology and a human antigen ELISA procedure for *Chlamydia trachomatis* which found this ELISA test to be highly specific for avian *Chlamydia*. This procedure is especially helpful in identifying shedding carriers that otherwise show no clinical evidence of infection.\(^2\) Despite this, ELISA can be used but, its sensitivity is not effective or efficient unless the indirect method is used.\(^3\)

Many birds may never produce antibody at detectable levels, even though they are known to be infected, by isolation of the organism.\(^4\) Such knowledge indicates the necessity of research to establish biological parameters that can be helpful in this diagnosis.

Serum biochemistries are most often used to detect hepatic disease, kidney disorders, malnutrition and inflammatory processes. AST, LDH and ALT are often used as indicators of hepatic disorders. All three of these enzymes are found in a variety of tissues other than the liver.

AST enzyme did not show significant differences during this study. It was above the average level
(100-400 U/L) just in one bird in February 1995. This enzyme can also serve as a more general indicator of overall body condition. Despite the normal levels of CPK, there was a decrease in LDH levels during the period. Hepatic disease can be determined by simultaneous elevation of AST and LDH, with normal levels of CPK. Such a condition suggests that an infection involving the liver at the beginning of the study could have occurred. If CPK enzyme had been above the normal levels at that time, it could suggest an involvement of muscle or other tissues. Many authors have reported hepatic lesions in live animals as well as hepatomegaly in post mortem examination followed by enteritis and pericarditis in chlamydiosis confirmed cases.1,9

The hyperproteinemia measured on February 1995 and July 1995 with averages of 7.23 and 7.13 gm/dl suggested an association with chronic inflammatory stimulation with psittacosis development.5,6 Both albumin and globulins showed high levels in the two first measurements. Albumin is the largest protein fraction and in some diseases an increase of globulins with decrease of albumin may occur while total protein remains at the normal levels. Total globulin electrophoresis can be useful in monitoring birds with chronic inflammatory processes and malnutrition. However its price limits its use by the general avian practitioner, except for albumin and globulin measurements.5

Hyperproteinemia can be a consequence of dehydration, shock or infection. Extremely high serum protein values (11-15 gm/dl) have been seen in cases of chronic lymphoproliferative diseases that resemble leukosis of chickens.6 Between July 1995 and February 1996, the protein level went back to normal values (3-5 gm/dl), but this does not mean that the birds became free of any possible disease. Nutritional status can also be judged by the values of total protein, albumin, uric acid, glucose, cholesterol and alkaline phosphatase. An inadequate diet deficient in protein, reduces the levels of total protein, uric acid and glucose. Inflammatory processes or egg laying increases the levels of total protein and globulin.

Generally, an increase in globulins can indicate an infection. Alpha-1 globulin consists of glycoproteins, haptoglobin, and ceruloplasmin while alpha-2 consists of macroglobulin. An increase of alpha-2 can be related to inflammation, infection, trauma, or surgery. Its decrease can mean liver disease, malabsorption or malnutrition. The gamma fraction consists primarily of circulating antibodies and it always increases in chronic inflammation or infection. Antibodies can migrate in the beta (IgM) or gamma range in birds.6 Beta and/or gamma fractions normally increase with infectious diseases while alpha may not change.

A significant decrease of alpha-1 had occurred between February 1995 and July 1995. The values of alpha-1 on February 1996 were the same as those on July 1995, which suggests a wave at the beginning of the psittacosis infection. Such a high level in February 1995 pointed out an acute infection or infection.6 At the same time of alpha-1 reduction, a significant increase of gamma globulin occurred which reinforces the hypothesis of a period of infection with the increase of circulating antibodies.

Conclusions

Although not confirmed through diagnostic tests, it is possible that bird 3278 introduced into the group in January 1995 had infected the other ones.
The globulin electrophoresis showed variations in the period with no apparent chlamydial infection in red-tailed Amazons, with a hyperproteinemia which is recognized as one of the signs of chlamydia.

A month after the introduction of bird 3278, a significant increase of $\alpha_1$ was observed. This value decreased in July 1995 and maintained constant in February 1996. A significant increase of $\gamma$ globulin was observed between the first and the second measurements and a decrease by the third measurement. However it still remained high when compared with the February 1995 value.

The other fractions had no significant changes during the study period, but it is convenient to mention that a mean decrease of $\alpha_2$ occurred following an $\alpha_1$ bend, and a constant mean decrease of $\beta$ during all periods.

Some of the results suggest distinct phases for red-tailed Amazon’s infections as following:
- first phase—no symptomatic infection with hyperproteinemia and prevalent high values of $\alpha$ and $\beta$ globulins;
- second phase—hyperproteinemia and increase of $\gamma$ globulin (no symptomatic infection);
- third phase—decrease of total proteins and $\gamma$ globulin, although titers of the immunologic tests remain positive (no symptomatic infection).

The possibility of the occurrence of chlamydial infection without any demonstrable antibody activity also must be kept in mind when immunologic methods are used. The best diagnostic test for chlamydia infection diagnosis is still considered to be propagation, in either cell culture or embryo inoculation. However these methods may have high costs and long turn-over time since organism shedding is often intermittent. Confirming chlamydiosis, mainly in cases where there are no clinical clues, seems to be more secure by adding biochemical parameters with serological methods. It can be done through ELISA test and globulin electrophoresis. Levels of LDH, AST and total protein can also be useful. All of these measurements can be done with a single blood sample.

This preliminary study concludes that simultaneous use of globulin electrophoresis and immunologic tests can be adequate in determining the period of infection in ill birds with no clinical signs. Those exams can not confirm diagnosis always, but they have been shown to be very useful when done together.

Improving our knowledge of chlamydial infection with more studies in captivity and wild birds is necessary towards the better understanding of the pathogenicity of chlamydiosis and the mechanisms of response developed by the host.

ACKNOWLEDGMENTS

We are most grateful for the financial support of the Fundação Boticário de Proteção à Natureza. Once again, our sincere thanks to them, whose trust in us made this work possible. To the Comprida Island municipality and Estação Ecológica Juréia -Itatins for their cooperation during the role study period. To Carlos Firkowski who helped us since the first drafts of this paper.

LITERATURE CITED


Table 1. Mean and standard deviation for proteins, enzymes, and globulins per studied month and independent T-test significance between studied months.

<table>
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<th>Research for</th>
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<th>Between months</th>
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<tr>
<td>CPK (U/l)</td>
<td>mean</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
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</table>

* There were no data in Feb/95
(+) T-test significance (P = 0.50) with increase.
(-) T-test significance (P = 0.50) with decrease.
Table 2. Independent T-test results.

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<td>T. Protein (g/%)</td>
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<td>Albumin (g/%)</td>
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<td>14.734</td>
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<td>Alpha1 (g/%)</td>
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<td>Alpha2 (g/%)</td>
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<td>Beta (g/%)</td>
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* There were no data in Feb/95.

INVESTIGATION OF A HERPES VIRAL INFECTION IN THREE SPECIES OF PHEASANTS
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Abstract

Three species of exotic pheasants including Temmincks tragopans (Tragopan temminckii), mountain peacock pheasants (Polyplectron inopinatum) and Malayan peacock pheasants (Polyplectron malacense) died due to a disseminated herpes viral infection. Gross lesions consisted of hepatosplenomegaly, and enteric and serosal hemorrhages. Light microscopic lesions included diffuse, severe necrosis of splenic and gastrointestinal submucosal lymphoid tissue and multifocal hepatic and enteric mucosal necrosis. Electron microscopic evaluation revealed budding virions with capsids and variably dense cores consistent with a herpes virus within nuclei and cytoplasm of hepatocytes and splenic macrophages. Virus was isolated from pheasant tissues and recovered from experimentally inoculated specific pathogen free chickens. Chickens developed the same gross, microscopic, and ultrastructural lesions as seen in the naturally infected pheasants.

Resumen

Tres especies de faisanes exóticos incluyendo Tragopán de Temminck (Tragopan temminckii), Faisán pavo de montaña (Polyplectron inopinatum) y Faisán pavo Malayo (Polyplectron malacense) murieron debido a una infección viral por herpes. Las principales lesiones macroscópicas consistieron en hepato-esplenomegalía y hemorragias en serosa intestinal. Las lesiones más claras microscópicalemente incluyeron necrosis severa difusa de bazo y tejido linfóide de submucosa intestinal, y necrosis multifocal hepática y de mucosa intestinal. Una evaluación con microscopio electrónico reveló viriones brotando con cápsides y núcleos de densidad variable compatible con un virus herpes dentro del núcleo y citoplasma de hepatocitos y macrófagos esplénicos. El virus fue aislado de tejidos de faisán y recuperado de pollos libres de patógenos específicos inoculados experimentalmente. Los pollos desarrollaron las mismas lesiones macroscópicas, microscópicas y ultraestructurales observadas en los faisanes infectados naturalmente.

Introduction

Various orders of birds have been found to be susceptible to experimental or natural herpesvirus infections. These include Anseriformes, Psittaciformes, Falconiformes, Strigiformes, Columbiformes, Gruiformes, Ciconiiformes, Passeriformes and Galliformes. In the order Galliformes, infections have been described in chickens, turkeys, and quail.¹ However, to our knowledge, no herpes infections have yet been described in exotic pheasants.

¹ 1996 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS
Since 1987, six gallinaceous birds, all belonging to the family Phasianidae, have succumbed to a disseminated herpes viral infection at the Wildlife Conservation Society. In this paper, we present the gross, histologic and ultrastructural features of this infection. Viral isolation and transmission studies are also described.

**Case Report**

A herpes viral infection resulted in the loss of three Temmincks tragopans (*Tragopan temminckii*), two mountain peacock pheasants (*Polyplectron inopinatum*), and one Malayan peacock pheasant (*Polyplectron malacense*). All six birds died with little to no antecedent clinical history. Three birds had one day histories of being depressed to moribund but three others were found dead without premonitory signs.

All six birds presented with varying degrees of hepatosplenomegaly and enteric hemorrhage. Livers and spleens were variably swollen, congested, mottled and often contained miliary pinpoint white foci. There was disseminated serosal petechiation. Multifocally severe hemorrhage was found throughout the gastrointestinal tract extending from the crop to the cloaca.

Histologically, there was diffuse, severe necrosis of the splenic periarteriolar lymphoid and gastrointestinal submucosal lymphoid tissue. Random foci of necrosis were seen in all livers. Intranuclear inclusion bodies were readily identified in the periphery of necrotic foci in the liver and spleen. Inclusions were also found in lesser numbers in intestinal epithelial cells. The inclusions were eosinophilic to basophilic and variable in size. In some instances, they filled the nucleus resulting in marked margination of chromatin. These resembled the signet-ring like inclusions as described in the Type II adenoviral infection (AAVII) of pheasants known as Marble Spleen Disease.¹ This diagnosis was, however, subsequently ruled out with serologic and ultrastructural studies.

Tissues from four of the six cases were routinely processed for electron microscopy, and thin sections were examined with a JEOL 1000 X Electron Microscope. Virions were readily identified in hepatic and splenic tissue. Clear, complete virions with capsids and variably dense cores were seen within nuclei as well as focally within the cytoplasmic compartment. Virions measured 125 nm to 167 nm in diameter. The size, location, and morphology of the viral particles were consistent with a herpes virus.

Frozen liver and spleen (maintained at -70°C) were submitted for viral isolation and transmission studies. Pheasant liver tissue was homogenized using a TenBroeck tissue grinder. The homogenized tissue was diluted 1:5 in phosphate buffered saline containing 1 ml/10 ml Gibco tissue culture antibiotic (Life Technologies Inc., Grand Island, NY, 14072, USA). The homogenized tissues were centrifuged in a clinical centrifuge at 1500 RPM for 10 min. The supernatant fluid was then inoculated onto 48-hr-old primary chicken kidney cells (CKCs) obtained from 2-3 wk-old chickens from a specific pathogen (SPF) flock, CKCs were cultured in Gibco M-199 + 5% bovine fetal serum. (Life Technologies Inc., Grand Island, NY, 14072, USA). The CKCs were kept at 37°C with 5% CO₂ and 85% relative humidity. Syncytia were observed in the CKCs starting at 5 days postinoculation (DPI). At 7 DPI most of the CKC monolayer was destroyed, and the cells and supernatant fluids were harvested and frozen. This material was then used to inoculate CKCs for
One and 2-day-old SPF chickens were inoculated intraabdominally with $10^0$, $10^1$, $10^2$, $10^3$ dilutions of the passaged pheasant isolate. Chicks developed gross and histologic lesions as described in the pheasants. Virus was then recovered from tissues of inoculated chickens in a manner similar to that described with the pheasant tissue. Electron microscopic evaluation of the splenic and hepatic tissues of the infected chickens revealed viral particles as seen in the naturally infected pheasants.

**Discussion**

The gross, light microscopic, ultrastructural and viral transmission studies support the diagnosis of a herpes viral infection in these pheasants. This is further supported by the finding that activity of the pheasant isolate as well as a known herpes virus (Infectious Laryngotracheitis) were both inhibited on CKCs by fluoroaminoarabinosylpyrimidine.

Additional transmission studies were performed with turkeys, quail, chukar partridge, and ring neck pheasants. The exotic pheasant isolate did not cause disease in these species. Further serologic studies will be required to determine if these species are susceptible to infection and to define the nature of this pheasant isolate.

**ACKNOWLEDGMENTS**

We would like to thank Mr. Rodman Gepchell for technical help with viral isolation and Dr. Stetter and Dr. Trupkiewicz for performing several necropsies.

**LITERATURE CITED**

USE OF PARASITE-SPECIFIC MONOCLONAL ANTIBODIES TO STUDY INVASION AND EARLY DEVELOPMENT OF Eimeria gruis IN THE FLORIDA SANDHILL CRANE

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Abstract

Eimeria gruis and E. reichenowi are common parasites of a number of species of cranes including the sandhill cranes (Grus canadensis) and the whooping crane (Grus americana). The pathology of the acute stage of the infection has been described in detail.2,4 However, neither the site for invasion by the sporozoites nor the dynamics of their early development have been defined, primarily because the stages are difficult to identify in tissues by conventional staining methods. The aims of the present study were to determine if monoclonal antibodies (McAb), elicited against Eimeria spp. of chickens and turkeys,3 would cross-react with sporozoites and developmental stages of E. gruis, and to use the cross-reacting McAb, along with a fluorescein-conjugated antibody, as probes to identify sporozoites and developmental stages of E. gruis in the intestines and visceral organs of Florida sandhill cranes (FSHC).1

Ten day-old FSHC chicks were inoculated by oral gavage with a suspension of either 1.5 X 10^7 or 2.5 X 10^4 oocysts of E. gruis to examine invasion and development, respectively. A separate group of chicks served as the control by receiving an oral gavage of an equal volume of sterile water. Chicks which received the concentrated inoculum were humanely euthanatized at 6 hr post-inoculation (PI) to evaluate intestinal invasion of the sporozoites. Chicks which received the less concentrated inoculum were euthanatized at 14 day PI to assess the developmental stages of infection. Necropsies were performed and tissues were collected in 10% buffered formalin for histopathology and in Carnoy’s solution for fluorescence microscopy.

E. gruis sporozoites were found to invade primarily from just proximal to Meckle’s diverticulum in the jejunum to the ileocecal juncture (average of 169 sporozoites per cross-section of intestine), and, to a lesser extent, in the ceca and colon (average of 5 sporozoites per cross-section). No sporozoites were found in the duodenum. Within the tissues, invasion by the sporozoites occurred in the middle third of the intestinal villi in the lamina propria. At 14 day PI, life cycle stages were observed in the intestine (ceca and jejunum), liver, and lungs. Development in the intestine was more abundant than in the other organs. In the ceca, the stages consisted of small schizonts or macrogamonts and were located in the crypts, either in the epithelial cells or lamina propria. No stages were seen at the villus tips. In the jejunum, the stages were more mature, and development occurred along the entire villus, with the heaviest concentration of stages at mid-villus. The livers of 3 chicks at 14 day PI contained 91, 175, and 191 small schizonts or macrogamonts per cross-section. In 2 of these 3 chicks, there were 200 and 246 small schizonts or macrogamonts per cross-section of lung; in the third chick, their numbers were far fewer. No stages were found in the heart, kidney, or brain of any of the chicks. These experiments reveal the value of McAb for the
quantification of invasion and, potentially, for the elucidation of the early development by *Eimeria* spp. in the crane.

**Resumen**

*Eimeria gruis* y *E. reichenowi* son parásitos comunes de numerosas especies de grullas incluyendo las grullas canadienses (*Grus canadensis*) y la grulla gritona (*Grus americana*). La patología de la etapa aguda de la infección ha sido descrita en detalle. Sin embargo, ni la vía de entrada de los esporozoitos ni la dinámica de este desarrollo temprano han sido definidos, principalmente porque las etapas son difíciles de identificar en los tejidos por métodos convencionales de tinción. Las intenciones del presente estudio son determinar si los anticuerpos monoclonales (AcMc) contra *Eimeria* spp. extraídos de pollos y pavos, tienen una reacción cruzada con esporozoitos y etapas evolutivas de *E. gruis* y si el uso de este AcMc de reacción cruzada, junto con un anticuerpo conjugado con fluoresceína es una prueba útil para identificar esporozoitos y etapas de desarrollo de *E. gruis* en intestinos y órganos viscerales de las grullas canadienses de Florida (GSHF).

Pollos GSHF de diez días de edad fueron inoculados por vía oral con una suspensión de $1.5 \times 10^7$ o bien de $2.5 \times 10^4$ oocistos de *E. gruis* para estudiar su invasión y desarrollo respectivamente. Un grupo separado de pollos sirvió como control, recibiendo una dosis de igual volumen de agua esterilizada. Los pollos que recibieron el concentrado inoculado fueron humanitariamente sacrificados a las 6 horas post-inoculación (PI) para evaluar la invasión intestinal de los esporozoitos. Los pollos que recibieron el inóculo de menor concentración se eutanasiaron al día 14 PI para estimar las etapas de desarrollo de la infección. Las necropsias se realizaron y fueron colectados tejidos en formol al 10% para histopatología y en solución de Carnoy para microscopía fluorescente.

Se encontraron esporozoitos de *E. gruis* en una invasión primaria justo desde el divertículo de Meckle en el yeyuno hasta la unión ileocecal (un promedio de 169 esporozoitos por sección cruzada de intestino) y a la más pequeña extensión, en el ciego y colon (promedio de 5 esporozoitos por sección cruzada). No fueron encontrados esporozoitos en el duodeno. Dentro de los tejidos, la invasión por esporozoitos ocurrió en el tercio medio de la vellosidad intestinal en la lámina propia. Al día 14 después de la inoculación fueron observadas las etapas del ciclo de vida en el intestino (ciego y yeyuno), higado y pulmones. El desarrollo en el intestino fue más abundante que en los otros órganos. En el ciego las etapas consistieron en pequeños esquizontes y fueron localizados en las criptas, en las células epiteliales o lámina propia. No fueron vistos en las puntas de las vellosidades. En el yeyuno las fases fueron más maduras y el desarrollo ocurrió a lo largo de todas las vellosidades con la mayor concentración de etapas en las vellosidades medias. Los higados de tres pollos al día 14 PI, contenían 91, 175, y 191 pequeños esquizontes por sección cruzada. En dos de estos tres pollos se encontraron 200 y 246 pequeños esquizontes por sección cruzada de pulmón, en el tercer pollo estas cifras fueron bastante bajas. No se encontraron huellas de etapas en el corazón, riñón o cerebro de ninguno de los pollos. Estos experimentos revelaron el valor de los anticuerpos monoclonales (AcMc) para la cuantificación de la invasión y potencialmente para la dilucidación del desarrollo temprano de *Eimeria* spp. en la grulla.
LITERATURE CITED


GRANULOMATOUS DERMATITIS CAUSED BY Mycobacterium genavense IN TWO PSITTACINE BIRDS

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Abstract

Mycobacterial infections are quite common in psittacine birds. The most frequent being those caused by Mycobacterium avium-intracellulare (MAI complex), which shows high affinity with the digestive tract. However, in most cases the involved bacterial species cannot be identified. In such infections, classic tubercles rarely develop, so they are named non-tuberculous or atypical mycobacteriosis. Mycobacterium genavense is a recently described Mycobacterium that causes disseminated infections in adult human AIDS patients and displays a pathogenicity similar to that of the MAI complex. The present communication describes two cases of granulomatous dermatitis in psittacine birds, in which M. genavense was identified by means of PCR.

Case 1

A 5-yr-old, apparently healthy, rainbow lory (Trichoglossus haematodus) was presented to the Veterinary Medicine School of Barcelona with the complaint of having a 3 cm diameter, featherless, non-painful, non-pruritic nodule. The nodule was surgically excised and samples were taken for histopathologic and bacteriological studies. Microscopically, a diffuse granulomatous dermatitis with necrotic areas surrounded by macrophages and multinucleated giant cells which showed numerous acid-fast organisms in their cytoplasm was observed. However, in bacteriological studies onto Coletosos and Löwenstein-Jensen media at 37°C and 42°C respectively, during a 3 mo period, no growth was observed. A cutaneous mycobacteriosis caused by an undetermined mycobacterial species was diagnosed.

Later on, PCR studies to detect mycobacterial DNA were performed on a portion of nodule which had been preserved at -80°C. Using primers 246 and 264, the relevant fragment of the 16S rRNA gene, typical of mycobacteria, was amplified. Lastly, the sequence of the amplified DNA was analyzed and it was characteristic of M. genavense.

A year and a half later, the bird has not shown any relapse.

Case 2

A 3-yr-old, blue fronted Amazon (Amazona aestiva) belonging to the psittacine collection of the
Zoological Gardens of Barcelona showed a diffuse cutaneous swelling of the tibiotarsal region of both rear extremities and subcutaneous nodules of variable size in different areas. Nodules were similar to those described in Case 1. A presumptive diagnosis of neoplasia was done and a skin biopsy was taken to perform histopathologic study. Microscopically, a diffuse granulomatous reaction similar to that of Case 1 was seen and a cutaneous mycobacteriosis was also diagnosed. Endoscopic examination of the intestinal tract of the bird revealed the presence of numerous yellowish nodules in intestinal serosa and liver.

Due to the generalized nature of the process, the bird was euthanatized. At necropsy, samples of cutaneous and internal nodules were taken to perform cultures and detection of mycobacterial DNA. No growth was observed in cultures, but PCR identified DNA characteristic of *M. genavense*.

The description of these two cases leads to the following conclusions:

- *M. genavense* is a pathogenic *Mycobacterium* that affects psittacine birds. It can produce an exclusively cutaneous form and a disseminated form in which cutaneous involvement is also present.
- As distinguished from human infections, psittacine birds infected by *M. genavense* are not immunosuppressed.
- A transcutaneous transmission route should be considered in some mycobacterioses.
- Cutaneous mycobacteriosis should be considered in the differential diagnosis of cutaneous nodules in psittacine birds.

**Resumen**

Las infecciones por mycobacterias son comunes en aves psitáceas. Las más frecuentes son las causadas por *Mycobacterium avium-intracelular* (MAI complejo) el cual muestra gran afinidad con el tracto digestivo. Sin embargo, en muchos casos las especies bacterianas involucradas no son identificadas. En tales infecciones los tubérculos clásicos raramente se desarrollan, siendo entonces denominadas no tuberculosas o mycobacteriosis atípica. *Mycobacterium genavense* ha sido recientemente descrita como la mycobacteria causante de infecciones diseminadas en humanos adultos con SIDA (HIV) y muestra una patogenicidad similar al complejo MIA. El presente estudio describe dos casos de dermatitis granulomatosa en aves psitáceas, en los cuales *M. genavense* fue identificado por medio de PCR.

**Caso 1**

Loro arco iris (*Trichoglossus haematodus*) de 5 años de edad, aparentemente sano, que fue presentado en la Escuela de Medicina Veterinaria de Barcelona con la molestia de tener un nódulo de 3 cm de diámetro, con poco plumaje, indoloro, no prurítico. El nódulo fue estirpado quirúrgicamente y se tomaron muestras para estudios histopatológicos y bacteriológicos. Microscópicamente se observó dermatitis granulomatosa difusa con áreas necróticas rodeadas por macrófagos y células gigantes multinucleadas, las cuales mostraron numerosos organismos acidófilos en su citoplasma. Sin embargo, en medio de cultivo Coletsos y Löwenstein-Jensen a 37 ºC y 42 ºC respectivamente, durante un periodo de tres meses, no se observó crecimiento del microorganismo. Fue diagnosticada una mycobacteriosis cutánea causada por una mycobacteria
indeterminada.

Posteriormente por medio de PCR se detectó ADN mycobacteriano representado en una porción del nódulo el cual fue preservado a -80°C. Utilizando los primers 246 y 264 se amplificó el fragmento pertinente de ARN del gen 16S, típico de mycobacteria. Finalmente la secuencia amplificada de ADN fue analizada y resultó la característica de *M. genavense*.

Un año y medio después, el ave no mostró ninguna recaída.

**Caso 2**

Loro frente azul (*Amazona aestiva*) de tres años de edad, perteneciente a la colección de psitácidas de los jardines zoológicos de Barcelona, mostraba una inflamación cutanea difusa de la región tibio-tarsal de ambas extremidades posteriores y nódulos subcutáneos de tamaño variable en diferentes áreas. Los nódulos eran similares al descrito en el caso 1. Un diagnóstico probable de neoplasia fue hecho y se tomó una biopsia de piel para llevar a cabo el estudio histopatológico. Microscópicamente se detectó reacción granulomatosa difusa similar a lo observado en el caso 1. En este caso también se diagnosticó una mycobacteriosis cutanea. El examen endoscópico del tracto intestinal del ave reveló la presencia de numerosos nódulos amarillentos en mucosa intestinal e higado.

Debido al estado generalizado del proceso, el ave fue sometido a la eutanasia. En la necropsia, muestras de nódulos cutáneos e internos fueron tomadas para cultivos y detección de ADN mycobacteriano. No se observó desarrollo en los cultivos, pero el PCR identificó características de *M. genavense*.

La descripción de estos dos casos nos conduce a las siguientes conclusiones:

- *M. genavense* es una mycobacteria patógena que afecta aves psitácidas. Lo que puede producir una forma exclusivamente cutanea y una forma diseminada en la que la afectación cutanea también se presenta.
- Se distingue de las infecciones humanas, en que las aves psitácidas infectadas por *M. genavense* no están inmunodeprimidas.
- La ruta de transmisión transcutanea podría ser considerada en algunas mycobacteriosis.
- La mycobacteriosis bacteriana podría ser considerada en el diagnóstico diferencial de nódulos cutaneos en aves psitácidas.
AMIKACIN SULFATE PHARMACOKINETICS IN RING-NECKED PHEASANTS (Phasianus colchicus): AGE AND ROUTE DEPENDENT EFFECTS

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Abstract

The highly polar aminoglycoside antibiotic amikacin sulfate has had long standing use in avian clinical practice. Although very effective in treating a variety of gram-negative bacterial infections in birds, this drug is not devoid of potentially serious nephrotoxic side effects. This is in part due to the small number of species-targeted pharmacokinetic studies published. Additionally these studies, to a large extent, have only addressed a single dose, route, and age of bird, providing only part of the overall information needed for efficacious and safe clinical application.

Our study involved the Ring-necked pheasant (Phasianus colchicus), a semi-domesticated representative of the Superfamily Phasianidae containing 45 genera and 177 species, many of which are maintained and bred in captivity for exhibition, wild population restoration, and for food. The birds used were captive reared, of mixed sexes, and in five age groups (2, 4, 6, 10, and greater than 24 wk). Dosing regimes included single dose (10 mg/kg) by different routes (i.v., i.m. [pectoral], s.c. [right subaxillary area]), and multiple (10 mg/kg) i.m. and s.c. route studies. Pheasant weights ranged from 75 g (2-wk-old) to 1800 g (adults). For pheasants 4 wk and older, each study group consisted of 5 birds (for each route, single 10 mg/kg dose, and multiple 10 mg/kg doses). For 2-wk-old pheasants, a group consisted of 15 individuals in 5 groups (totaling 45) for each single dose 10 mg/kg route (i.v., i.m., s.c.). Multiple dose studies (10 mg/kg amikacin sulfate i.m. or s.c. t.i.d. for 14 days) were performed in pheasants 24 wk of age or older. These studies were performed following tabulation of single dose pharmacokinetic data. State of hydration (assessed by PCV and total solids) and renal function/dysfunction were monitored using plasma creatinine and uric acid levels initially, at 48 hr intervals over the 14 treatment days, and at 72 hr intervals for 14 days post treatment. Blood was drawn for trough amikacin plasma levels at 48 hr intervals prior to each third daily injection. All birds in the single and multiple dose and multiple route studies were offered water ad libitum and a complete grower or maintenance pheasant diet (crumbles). The birds used for the single dose studies were segregated by age and group housed indoors. For the multiple dose studies, pheasants were housed indoors in standard stainless steel tiered laboratory animal (rabbit) cages, weighed daily, and their food and water intake determined quantitatively each morning. For all studies, blood (0.2 ml) was collected from the brachial or jugular vein and immediately placed in tubes containing lithium heparin. All plasma samples were frozen at -20 C until analyses for amikacin were performed using a fluorescence polarization immunoassay-based system (Abbott TDX Fluorescence Polarization Analyzer, Abbott Laboratories, North Chicago, IL). Pharmacokinetic data were derived using a standard polyexponential parameter estimate software package (ESTRIP BASIC®).
Data from this study reinforced and refuted some previously published conclusions concerning amikacin sulfate use in birds. The single dose (multi-age and route) studies reinforced the importance of allometry as applied to the clinical use of antimicrobial agents in birds. Pheasants in the 75-350 g weight range eliminated amikacin very rapidly following all routes of administration compared to pheasants in the older age groups. These data help to substantiate the importance of body surface area/weight ratios, given normal renal function and hydration. Additionally, it was determined that amikacin sulfate was absorbed well via the s.c. route in all age groups, and resulted in plasma concentrations comparable to or greater than those produced via the i.m. route. This has definite clinical application where repeated i.m. injections in small birds are known to cause severe myositis and/or myonecrosis.

Results from the multi-dose studies indicated that efficacious levels of amikacin sulfate could be maintained if 10 mg/kg was given i.m. or s.c. t.i.d. over 14 days. It is important to note, based on clinical laboratory data, objective assessment of the pheasants’ food and water intake, and subjective assessment of their attitudes, that renal toxicosis began to appear in 3 birds at days 11 through 14 of dosing. This reinforces the importance of serum drug level monitoring at these times (in order to adjust dosage and/or dosing interval or to discontinue use), until clinical laboratory parameters return to within normal baseline ranges. In this study the parameters (uric acid/creatinine) remained abnormal for up to 7 days following cessation of the repeated amikacin administration.

Resumen

El antibiótico aminoglicósido sulfato de amikacina se ha mantenido por largo tiempo en uso dentro de la práctica de clínica aviar. Aun cuando es muy efectivo en el tratamiento de una gran variedad de infecciones bacterianas gram negativas en aves, esta droga no está exenta producir potencialmente serios efectos nefrotóxicos. Esto es en parte debido a los escasos estudios publicados sobre farmacocinética en diferentes especies animales. Adicionalmente estos estudios en su mayoría sólo hacen referencia a una sola dosis, vía de administración y edad del ave, proporcionando sólo parte de la información necesaria para una segura y eficaz aplicación clínica.

Nuestro estudio involucra al faisán de collar (Phasianus colchicus), un representante semidomesticado de la superfamilia Phasianidae que contiene 45 géneros y 177 especies, muchos de los cuales son mantenidos y reproducidos en cautiverio para exhibición, restablecimiento de poblaciones silvestres y para alimentación. Las aves usadas fueron criadas en cautiverio, de sexos mixtos y en cinco grupos de edades diferentes (2 semanas, 4, 6, 10 y mayores de 24 semanas). El régimen de dosificación incluyó dosis únicas (10 mg/kg) por diferentes vías (i.v., i.m. (pectoral), s.c. (área subaxilar derecha)), y una dosificación múltiple (10 mg/kg) vía i.m. y s.c. El peso de los faisanes presentó un rango de 75 g (2 semanas de edad) a 1800 g (adultos). Para faisanes de 4 semanas o más, cada grupo de estudio consistió en 5 aves (para cada vía, dosis única de 10 mg/kg y dosis múltiple de 10 mg/kg). Para faisanes de 2 semanas de edad, un grupo consistió de 5 subgrupos con 15 individuos cada uno (totalizando 45) para cada dosis única de 10 mg/kg vía (i.v., i.m., s.c.). Las dosis múltiples estudiadas (10 mg/kg sulfato de amikacina i.m. ó s.c. t.i.d. por 14 días) fueron administradas en faisanes de 24 semanas de edad o mayores. Estos estudios fueron realizados siguiendo la tabulación de datos de farmacocinética/dosis única. El estado de hidratación
(calculado por P.C.V. y sólidos totales) y función/disfunción renal, fueron monitoreados usando niveles de creatinina plasmática y ácido úrico, inicialmente a intervalos de 48 hrs durante los 14 días de tratamiento, y a intervalos de 72 hrs para los 14 días posteriores al tratamiento. La sangre fue trabajada para revisar niveles de amikacina en plasma a intervalos de 48 hrs antes de cada tercera inyección diaria. A todas las aves se les ofreció agua ad libitum y una dieta completa para faisanes en mantenimiento. Las aves usadas para dosis únicas fueron separadas por edad y grupos, alojadas en interiores (jaulas). Para el estudio de dosis múltiple, los faisanes fueron alojados en el interior de jaulas standard de acero inoxidable usadas normalmente para animales de laboratorio como conejos. Los faisanes se pesaron diariamente, y su comida y agua fue medida cuantitativamente cada mañana. Para los estudios sanguíneos (0.2 ml.) fueron colectados de la vena yugular o braquial y colocada inmediatamente en tubos con heparina. Todas las muestras de plasma fueron refrigeradas a -20 ºC. hasta que los análisis para amikacina fueron realizados usando un sistema de polarización basado en inmunoensayo fluorescente (Abbott tDX Fluorescence Polarization Analyser, Abbott Laboratories North Chicago, IL). Los datos de farmacocinética fueron obtenidos usando un paquete informático para la estimación de parámetros poliexponenciales estándar (Estrip Basic).

Los datos en este estudio refuerzan y refutan algunas conclusiones publicadas previamente en relación al uso de sulfato de amikacina en aves. La dosis única (multi edad y ruta) estudiada, señala la importancia de la alometría aplicada al uso clínico de agentes antimicrobianos en aves. Faisanes en el rango de peso de 75-350 g. eliminaron la amikacina rápidamente siguiendo todas las rutas de administración comparado a los faisanes en los grupos de mayor edad. Estos datos apoyan la importancia del área de superficie corporal/cociente de peso basados en una función renal e hidratación normales. Adicionalmente fue determinado que el sulfato de amikacina fue absorbido bien vía s.c. en todos los grupos de edad, y resultó en unos niveles de concentración plasmática iguales o mayores a los obtenidos vía i.m. Esto tiene aplicación clínica, ya que las dosis repetidas en pequeñas aves son causa de severas miositis y/o mionecrosis.

Los resultados de estudios multidosis indicaron que niveles eficaces de sulfato de amikacina puede ser mantenido si 10mg/ kg son aplicados i.m. o s.c. t.i.d. durante 14 días. Es importante notar que basado en datos de laboratorio clínico, apreciación objetiva del consumo de alimento y agua y subjetivamente por las actitudes de los faisanes, se apreció una toxicosis renal que empezó a aparecer en 3 aves entre los 11 y 14 días de dosificación. Esto refuerza la importancia de monitorear los niveles de droga en suero a este tiempo (y en base a ello ajustar o interrumpir la administración) hasta regresar a parámetros de laboratorio clínico dentro de los rangos basales normales. En este estudio los parámetros (ácido-úrico/creatinina) permanecieron anormales por más de 7 días siguientes a la terminación de la administración repetida de amikacina.
ITRACONAZOLE-IMPREGNATED SYNTHETIC GRIT FOR SUSTAINED RELEASE DOsing IN AVIAN SPECIES

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Abstract

Itraconazole is an antifungal agent which has been successfully used to treat avian aspergillosis. Traditional treatment protocols include twice daily oral medication for extended periods of time. In this study, the use of medicated synthetic grit was investigated as a potential delivery method which would provide sustained serum levels following a single oral treatment. Itraconazole-impregnated polymethyl methacrylate was formulated and used as synthetic grit. Following a single oral dose, serum samples were collected from birds over a nine day period. Birds medicated with synthetic grit had higher and more sustained serum itraconazole levels as compared to birds medicated with a standard avian dose.

Resumen

El itraconazol es un agente antifúngico que ha sido utilizado con éxito en el tratamiento de aspergilosis aviar. Los protocolos de tratamiento tradicional incluyen medicación oral dos veces al día por periodos prolongados de tiempo. En este estudio, el uso de arena (gravilla) sintética medicada fue investigado como un método de administración potencial, el cual puede proporcionar niveles sostenidos en suero siguiendo un solo tratamiento oral. El polimetil metacrilato impregnado con itraconazol fue formulado y usado como arena sintética. Seguido de una sola toma oral, fueron tomadas muestras de suero de aves durante un periodo de nueve días. Las aves medicadas con gravilla sintética tuvieron niveles más altos y sostenidos de itraconazol en suero que las aves medicadas con una dosificación aviar estándar.

Introduction

Morbidity and mortality from aspergillosis is not only one of the most common disease problems facing avian species, but is also one of the most difficult to treat. In a zoological setting, treatment often requires removal of the individual from its’ environment and long term treatment with repeated handling. An alternative therapy which includes a single oral dosage of a sustained-release antifungal medication would greatly reduce many of the problems encountered in medicating these birds.

Antibiotic impregnated polymethyl methacrylate (PMMA) has been used as bone cement for orthopedic procedures. This technique is used to treat osteomyelitis by providing a long term, high local concentration of antibiotics.

Itraconazole is a new antifungal drug which is less toxic and more efficacious than previously used antifungal agents. It has been successfully used in human and veterinary medicine for the treatment
of aspergillosis.

The unique digestive tract of many avian species allows large firm material (grit) to remain in the gizzard for long periods of time. Preliminary in-vitro work by the authors demonstrated that itraconazole-impregnated synthetic grit (IISG) placed in a solution resembling a gizzard’s chemical and mechanical environment had a sustained release of itraconazole over a 7-day period. This manuscript describes the initial in-vivo use of IISG in the Indian peafowl (Pavo cristatus).

Materials and Methods

Indian peafowl were divided into three groups. The first group, (n=5) was used for an initial non-grit, single oral dose pharmacokinetics trial. Birds were given an oral bolus of 15 mg/kg itraconazole via capsule. Blood samples were collected at 0, 0.5, 3, 5, 8, 13, 22 and 24 hr. The second group of birds (n=6) were given an oral bolus of 7 g of IISG consisting of 16% itraconazole by weight. The third group (n=6) was given an oral bolus of 7 g of IISG consisting of 21% itraconazole by weight. Blood samples from birds in group two and three were collected on days, 0, 1, 2, 3, 4, 5, 6, 7 & 9.

Synthetic grit was prepared using PMMA bone cement and 100 mg itraconazole capsules. The PMMA powder, liquid polymer and the contents from itraconazole capsules were uniformly mixed and allowed to harden. Bone cutters were utilized to cut grit into approximately 1 g size pieces.

Serum samples were stored at -30°C. for shipment. Serum itraconazole levels were determined via biological assay at the Fungus Testing Laboratory, University of Texas, Health Science Center, San Antonio, Texas.

Results

Group 1 birds demonstrated peak levels (1.3 - 2.3 µg/ml) at 8 hr. By 24 hr, serum levels had fallen to ≤1 µg/ml. One bird demonstrated a biphasic peak at 8 and at 22 hr. This biphasic peak may have resulted from partial crop emptying. All Group 1 birds had relatively low serum levels with only two of the five demonstrating levels which are commonly considered therapeutic in humans (≥2 µg/ml).

The birds in Groups 2 and 3 demonstrated high serum itraconazole levels which were maintained for 3-6 days. In group 2 (16% itraconazole grit) all birds reached high therapeutic levels (≥5 µg/ml) by day 2. At day 7, serum levels had declined to zero in most birds. The peak levels ranged from 5-17 µg/ml and were achieved in 1-3 days.

In Group 3 (21% itraconazole grit) birds reached higher levels more rapidly than the birds in group two. Five of the six birds in Group 3 had levels in excess of 10 µg/ml at 24-48 hr. These levels declined more rapidly compared to group two birds. Most birds in Group 3 had less than therapeutic levels by day three and negligible amounts at 4-5 days.

Discussion
Birds receiving oral medications have been reported to have inconsistent peak serum levels. Variability in crop emptying times is thought to be primarily responsible. The crop may empty within an hour or remain full for up to 24 hr. Emptying times are influenced by a number of variables including food composition, quantity, physiologic state and disease. Ventriculus anatomy and physiology is highly species specific. Some species lack a true muscular ventriculus while others have a highly developed muscular organ. Ventriculus contractions are dependent upon quantity and texture of ingesta. Material may remain within the ventriculus for extended periods of time. In the authors experience, Indian peafowl were capable of retaining the same pieces of grit in the gizzard for more than a month. Grit leaves the gizzard either by degradation or via passage of the intact material into the duodenum. Size, shape, texture and amount of grit may all influence rate of passage. Softer material is more rapidly broken down and passed as compared to harder material. The higher the percentage of medication incorporated within the PMMA, the softer and more rapidly it may be degraded. Group 3 birds had been given 21% itraconazole grit, which is softer and more rapidly broken down than the 16% itraconazole grit. These differences in grit consistency and degradation may have resulted in the Group 2 birds having a more sustained release than the birds in group three.

The interval from oral dosing to peak serum levels is probably most influenced my crop emptying. Delayed crop emptying will slow drug passage into the ventriculus. Itraconazole is optimally absorbed in an acidic environment with fatty foods. In humans, peak serum concentrations are reached after 7 days of treatment. A higher loading dose may be used to reach therapeutic levels more rapidly. In humans, serum itraconazole levels consistently below 2 mg/ml are associated with treatment failure.

Conclusion

This use of IISG provided much higher and more sustained serum levels as compared to routine oral dosing at published avian doses. The incorporation of a medication into a synthetic grit has many potential therapeutic uses in avian species. At the Wildlife Conservation Society, birds which are highly susceptible to aspergillosis are often prophylactically medicated with antifungal agents during potentially stressful times (shipment, introductions, disease conditions). During these periods, it is often counterproductive to manually gavage these birds. Medications are commonly placed in food items however diseased and stressed birds may become hypophagic or discard tainted food items. Incorporating medicated synthetic grit into daily feeding regimes or its use as a single oral bolus may help provide sustained therapeutic levels during periods of increased susceptibility to aspergillosis. IISG could also be used for the treatment of aspergillus providing higher and more sustained levels than conventional therapy. Although the concept of medicated grit is intriguing, the unique anatomy and physiology of each avian species coupled with the different chemical properties and release rates of each drug, leave a great deal of investigation to be done prior to routine clinical use.

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LITERATURE CITED


THE SUCCESSFUL TREATMENT OF SARCOCYSTOSIS IN TWO KEAS (*Nestor notabilis*)
AT THE FRANKLIN PARK ZOO

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Abstract

Two keas (*Nestor notabilis*) were diagnosed as having sarcocystosis at the Franklin Park Zoo, Boston, Massachusetts in the late summer of 1995. The birds appeared fluffed and had a slow onset of lethargy, anorexia, and periodic weakness. Sarcocystosis was suspected following a finding of extremely elevated creatine phosphokinase (CPK) and aspartate amino transferase (AST) levels on serum biochemistry profiles. The diagnosis was confirmed by identifying *Sarcocystis* cysts in pectoral and quadriceps muscle biopsies. The birds were treated with a combination of Amprolium (CORID Merck and Co. Inc., Ag. Vet Division, Whitehouse Station, NJ, USA) at a dosage of 0.025% in drinking water for seven days, Fansidar (500 mg sulfadoxine / 25 mg pyrimethamine) (Roche branch of Hoffman / LaRoche Inc., 340 Kingsland St., Nutley, NJ, USA 07110) at a dosage of 0.5 mg/kg p.o. b.i.d. for 45 days and Primaquine phosphate (Sanofi / Winthrop Pharmaceutical, N.Y., N.Y. USA) at a dosage of 1 mg/kg p.o. s.i.d. for 45 days. Regular serum biochemistries and complete blood counts were drawn throughout the course of the treatment regime. Body weights were taken once a week during the treatment time and for 7 mo post treatment.

Resumen

Dos keas (*Nestor notabilis*) fueron diagnosticadas positivas a sarcocistosis en el Franklin Park Zoo, Boston, Massachusetts en el pasado verano de 1995. Las aves aparecieron con la pluma erizada y tuvieron un lento principio de letargia, anorexia y debilidad periódica. Se sospechó de sarcocistosis al detectarse niveles extremadamente elevados de creatinina fosfoquinasa (CPK) y aspartato aminotransferasa (AST) en suero. El diagnóstico fue confirmado al identificar quistes sarcocísticos en biopsias musculares de cuadriceps y área pectoral. Las aves fueron tratadas con una combinación de amprolium (Corid, Merck and Co. Inc., Agro Vet Division, Whitehouse Station, NJ, USA) a una dosis de 0.025% en agua de bebida por siete días, Fansidar (sulfadoxina 500 mg/pirimetamina 25 mg) (Roche branch of Hoffman-LaRoche Inc., 340 Kingsland St., Nutley, NJ USA 07110) a una dosis de 0.5 mg/kg oral dos veces por día durante 45 días y fosfato de primaquina (Sanofi/Winthrop Pharmaceutical, N.Y., N.Y., USA) a una dosis de 1 mg/kg oral una vez por día durante 45 días. Se realizaron bioquímica de suero y biometrías hemáticas durante el curso del tratamiento. El peso corporal fue tomado y registrado una vez a la semana durante el tratamiento y por siete meses posteriores al tratamiento.

Introduction

*Sarcocystis* species are apicomplexan coccidian parasites that undergo an obligatory two host life
cycle that alternates between predator and prey hosts. Sexual multiplication occurs in the intestines of the definitive host (predator) and infectious, sporulated oocysts are passed in the feces. The intermediate host, (prey), serves as a place where asexual reproduction, including schizogony and sarcocyst formation, occurs. This may result in the formation of muscular cysts. Mammalian species of *Sarcocystis* have been demonstrated to be host specific, while avian species of *Sarcocystis* have differing levels of infectivity based on the taxonomic order that is infected.

Keas (*Nestor notabilis*) are old world psittacines in the order psittaciformes and as such are considered highly susceptible to the acute, fatal form of sarcocystosis. This paper will discuss the presentation, diagnosis, and treatment of a pathologic muscular form of sarcocystosis seen in two keas.

**Case Report**

One male and one female, juvenile keas (*Nestor notabilis*) were acquired from the San Diego Zoo in the fall of 1994. The birds were placed in an isolated, outdoor cage that had a dirt floor, wire on three sides and the roof, and a solid wall on the fourth. Except for a mild illness in the fall of 1994 in the female bird, both birds remained healthy until the late summer of 1995. At that time, both birds appeared to be slightly puffed, lethargic, weak, and had poor appetites. The male appeared to be sicker than the female. Both birds had lost approximately 12-15% of their body weight.

Diagnostic procedures done included venipuncture for a complete blood count, a serum biochemistry, lead levels, cloacal swabs were sent out for culture and sensitivity, and radiographs were taken. Supportive care was initiated and consisted of subcutaneous fluids, antibiotic therapy with injectable enrofloxacin (Baytril Miles Inc., Agricultural Division, Animal Health Products, Shawnee Mission, KS, USA 66201) given i.m. at a dosage of 7.5 mg/kg s.i.d. and force fed a mixture of multivitamins, a caloric supplement, baby food and yogurt.

Radiographs indicated hepatomegaly in the male and the female appeared normal on survey films. Significant findings of the complete blood count in both birds indicated a leukocytosis, anemia, and a lymphocytosis. The serum biochemistries indicated extremely elevated creatine phosphokinase (CPK) levels in both birds and elevations in their aspartate amino transferase (AST) levels. Pertinent laboratory values are documented in Table 1.

Cloacal swabs were negative for pathogenic bacteria. Blood lead levels also were within normal limits on both birds (2.5 µg/dl and 2.7 µg/dl).

Twelve days after the initial signs of illness, the birds were continuing to appear ill and based on the elevated CPK and AST levels, abnormal radiographs, and lack of response to treatment, muscle biopsies were taken and laparoscopy was performed.

The birds were anesthetically induced via face mask with isoflurane and oxygen and maintained via mask. Laparoscopy confirmed the sexes of the birds and nothing else abnormal was detected. Skeletal muscle biopsies were taken from the pectoral and quadriceps muscle of each bird and placed in 10% buffered formalin for histopathology. Both birds had a moderate chronic multifocal lymphohistiocytic myositis but only the female had intraliesional *Sarcocystis* spp. organisms seen.
At this time it was assumed that the male bird also had sarcocystosis due to the similarity in clinical signs, blood chemistries and histopathology results.

**Treatment Protocol**

Initial treatment given consisted of enrofloxacin (Baytril) at a dosage of 7.5 mg/kg i.m. b.i.d., s.c. fluid therapy once daily, and force feeding a multivitamin, baby food combination once per day.

Once the diagnosis of sarcocystosis was made, the treatment regime was changed as follows. The birds were given Amprolium at a dilution of 0.025% as their sole water source for seven days. They were started on Fansidar (500 mg Sulfadoxine/25 mg Pyrimethamine) and primaquine phosphate.

The Fansidar was given at a dosage of 0.5 mg/kg Pyrimethamine p.o. b.i.d. for 45 days. A Fansidar solution was prepared weekly by mixing one tablet of Fansidar with 21 ml water and 4 ml K-Y Jelly (Johnson & Johnson, Inc., Skillman, NJ 08558-9418) to make a 1 mg/ml solution of Pyrimethamine. The Primaquine was started at a dosage of 1 mg/kg at 6 hr, 18 hr (post Fansidar) then s.i.d. p.o. for 45 days. A Primaquine solution was prepared weekly by adding one tablet of Primaquine to 11 ml of water and 4 ml of K-Y Jelly to make a 1 mg/ml solution.

The medications were mixed with a multivitamin, high calorie supplement and baby food to be delivered p.o. via syringe. This method both acted as a positive reinforcement for accepting treatment, and as a nutritional supplement for the birds.

Approximately 2 wk into the course of treatment, the birds started acting lethargic and weak again. Blood work indicated an increase in AST, and a leukocytosis. The birds were again started on Baytril s.c. s.i.d. for 7 days and s.c. fluids were given s.i.d. if the birds appeared to be off feed.

Renovations, including a solid roof and a soil change in the exhibit, were completed and the birds were returned to the exhibit after treatment. Weekly weights indicated that the male kea was doing fine and gaining weight at a steady rate. The female kea started to drop weight and blood work indicated an elevation in CPK levels. Both birds were started on a multivitamin, high calorie supplement and appeared to be clinically normal. Because the female kea appeared clinically normal, the elevated CPK levels were attributed to muscle scarring and treatment was not reinstituted. Her weight loss eventually stabilized and she continued to appear clinically normal.

**Discussion**

There are at least six species of *Sarcocystis* that affect birds. The opossum (*Didelphis virginiana*) is the definitive host for the coccidian parasite *Sarcocystis falcatula*, which has been shown to affect several orders of birds, including passerines, columbiformes, and psittaciformes. Cowbirds (*Molothrus ater*) and grackles (*Cassidix mexicanus, Quiscalus quiscula*) serve as the intermediate hosts. Cockroaches and flies can also act as transport vectors.

Exposure to either infective sporocyst in opossum feces or to transport vectors is most probably the route of infection in the exhibit. The possibility of opossum feces getting into the kea enclosure existed. The roof of the enclosure consisted of a wire mesh with trees overhanging it. The building
whose wall made up the fourth side of the enclosure also has been known to have opossums nesting in the eaves.

There are five clinical manifestations of sarcocystosis in avian species. These include an incidental muscular form, an acute pulmonary disease, an encephalitic disease, a clinical muscular disease, and a cardiac form. It is believed that survival of the birds may be related to the number of sporocyst that infects them. Psittacines usually suffer from a peracute form of the disease with a high rate of mortality due to severe pulmonary lesions. The keas in this report suffered from the clinical muscular form of the disease.

Diagnosis of avian sarcocystosis is based on clinical signs, elevated muscular enzymes on blood chemistries, and histopathological evidence of Sarcocystis spp. in muscular biopsies.

Historically, the treatment of sarcocystosis has consisted of a combination therapy consisting of pyrimethamine and trimethoprim-sulfadiazine. The treatment used in this report was based on therapy for malaria in humans.

Fansidar is an antimalarial agent which acts by reciprocal potentiation of its two components, achieved by a sequential blockade of two enzymes involved in the biosynthesis of folinic acid within the parasites. Fansidar has been shown to be effective against certain strains of Plasmodium falciparum that are resistant to chloroquine. Fansidar is supplied as scored tablets, each containing 500 mg sulfadoxine and 25 mg pyrimethamine. The dosage used was 0.5 mg/kg pyrimethamine p.o. b.i.d. for 45 days.

Primaquine phosphate is a synthetic antimalarial agent which is an 8-amino quinoline derivative. The exact mechanism of antimalarial activity of primaquine has not been determined, but the drug appears to interfere with the function of plasmodial DNA. Primaquine is a tissue schizonticidal agent and is active against the preerythrocytic and exoerythrocytic forms of Plasmodium falciparum, P. malariae, P. ovale, and P. vivax. Primaquine is also gametocyticidal against plasmodia. Primaquine has been used, in conjunction with blood schizonticidal agents, such as chloroquine, to decrease the risk of delayed primary attacks, and relapse of P. ovale and P. vivax malaria. The dosage of Primaquine used was 1 mg/kg starting 6 hr, and 18 hr post Fansidar, then s.i.d. for 45 days.

Amprolium (Corid) is a coccidiostat that inhibits the utilization of thiamine causing the organism to starve. It also impairs the development of first-generation schizonts within the intestines. The dosage was 0.025% given as the birds sole water source for seven days.

Originally, the treatment course was going to only last 30 days, but when drugs were discontinued on day 30, the birds appeared puffed again, developed a partial anorexia and appeared lethargic. Medications were resumed and the birds improved within a few days. At the end of the 45 day treatment regime, the birds did not relapse and medications were not continued.

The therapy used in these cases requires additional investigation in order to establish the efficacy. The severity of clinical signs seen may have been due to a light infection; therefore, it is hard to determine how effective the drugs were in these cases. The fact that the birds had a recurrence of
clinical signs when treatments were discontinued, that then responded to resumed treatment, seems to support the benefit of the therapy.

ACKNOWLEDGMENTS

The author would like to thank Dr. Mary Duncan, DVM, for her assistance.

LITERATURE CITED

Table 1. Selected laboratory values from the male and female keas (*Nestor notabilis*) infected with a clinical muscular form of *Sarcocystis* at the Franklin Park Zoo.

<table>
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<th>AST (U/L)</th>
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CONSERVATION OF MEXICAN PRIMATES: PRESENT PROJECTS AND EXPECTATIONS

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Abstract

The septentrional limit for the distribution of the neotropical primates is located in the South of Mexico. Within this area, the presence of populations of spider monkeys (Ateles geoffroyi vellerosus and A. g. yucatanensis), mantled howler monkeys (Alouatta palliata mexicana) and black howler monkeys (Alouatta pigra) have suffered a dramatic reduction. Workshops were held with the purpose of evaluation of the state of these taxa, and recommendations for steps towards their conservation were made. The workshops highlighted the participation of specialized, experienced scientists that have worked with animals in captivity and in the wild using evaluation techniques for habitat and populations. From these discussions it was established that A. g. vellerosus, A. g. yucatanensis and A. palliata mexicana should be considered as vulnerable species, while A. pigra could still be considered at a lesser risk, according to the new criteria of the IUCN red list.

Of all the factors that affect Mexican primates, the most severe is the fragmentation and loss of habitat, followed by illegal traffic and poaching for human consumption.

Several conservation actions were proposed to reduce the process of extermination of Mexican primates. It was first indicated that conservation in situ needs to be strengthened and increased, along with the implementation of various ex situ conservation tactics; primarily those involving monkey translocation and rehabilitation. Emphasis was made on the need of counting on strong legal backing, and of effective enforcement and control of animal extraction from their habitat. The development of an educational program has also been proposed to secure public participation in the custody of this natural resource of Mexico.

Following these lines of action, there are several projects, with interesting preliminary results. Nevertheless, there are still numerous activities to continue the research of these taxa, in the wild and in captivity. For these purpose there are priority outlines of research being developed.

Resumen

En el sur de México se encuentra el límite septentrional para la distribución de los primates Neotropicales. Dentro de esta zona, la presencia de poblaciones de mono araña (Ateles geoffroyi vellerosus y A. g. yucatanensis), de mono aullador de manto (Alouatta palliata mexicana) y de aullador negro (Alouatta pigra) ha sufrido una dramática reducción. Con el propósito de evaluar el estado de estos taxa y hacer recomendaciones para su conservación, se han realizado talleres con la participación de especialistas, con experiencia en el campo y en cautiverio, utilizando técnicas de evaluación de hábitat y de poblaciones. De estos análisis se estableció que A. g. vellerosus, A. g. yucatanensis y A. palliata mexicana deberían ser consideradas como especies Vulnerables, mientras
que A. pigra tod avía podría ser considerada en Menor Riesgo, de acuerdo a los nuevos criterios de la Lista Roja de la UICN.

De todos los factores que afectan a los primates mexicanos, el más severo es la fragmentación y pérdida de hábitat, seguido del tráfico ilegal y la cacería de animales para consumo humano.

Para reducir el proceso de exterminio de los primates mexicanos, se propusieron diversas acciones conservacionistas; en primer lugar se propuso fortalecer y ampliar la conservación in situ, acompañada de varias tácticas de conservación ex situ, destacando la translocación y rehabilitación de monos. Se enfatizó la necesidad de contar con un sistema legal y de vigilancia efectiva para controlar la extracción de animales de su hábitat. También se ha propuesto el desarrollo de un programa educativo que asegure la incorporación de la sociedad civil, en la custodia de este patrimonio natural.

Siguiendo estas líneas de acción, hay varios proyectos en curso, cuyos resultados preliminares parecen ser promisorios. Sin embargo, aún hay numerosas tareas por realizar para asegurar la permanencia de los primates mexicanos. Una de estas tareas es incrementar la investigación sobre estos taxa, tanto en estado silvestre como en cautiverio. Para tal fin, se han planteado líneas de investigación a ser desarrolladas de manera prioritaria.
REPRODUCTIVE CONSEQUENCES OF SOCIAL SUBORDINATION IN FEMALE CALLITRICHID PRIMATES

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Abstract

Marmosets and tamarins of the family Callitrichidae are small-bodied New World primates. They share the distinction of being the only non-human primates to develop a cooperative breeding reproductive strategy. Offspring remain within their natal families into adulthood and all members of a group participate in infant care. Such a kin-selected breeding strategy may have arisen because of (1) competition for resources that limited breeding opportunities, (2) predation and (3) the high risks involved in dispersal.1

Callitrichid primates provide a unique specialized commitment to cooperative breeding that is unrivaled by any other mammalian family. They provide excellent opportunities to study (1) the neural mechanisms translating social subordination into sexual inactivity and infertility and (2) phylogenetic and ecological constraints on the proximate regulation of female reproductive success.

Resumen

Las Marmosetas y los tamarines de la familia Callitrichidae, son primates de pequeño tamaño del nuevo mundo, que muestran la distinción de que son los únicos primates no humanos en desarrollar una estrategia reproductiva de cooperación en la crianza. Los hijos permanecen con sus familias natales en la edad adulta y todos los miembros del grupo participan en el cuidado de los infantes. La estrategia de selección para la reproducción se origina debido a: (1) competencia por los recursos que son limitados (2) predación, y (3) el alto riesgo que involucra su dispersión.

Los primates Callitrichidos poseen un cometido especializado de cooperación en la crianza que no tiene comparación con ninguna otra familia de mamíferos. Ello proporciona una excelente oportunidad para estudiar dos factores: (1) los mecanismos neurales de traslación social en la subordinación dentro de la inactividad sexual y la infertilidad, y la (2) restricción filogenética y ecológica en la regulación proximal del éxito reproductivo de las hembras.

Introduction

Marmosets and tamarins of the family Callitrichidae are small-bodied New World primates. They...
share the distinction of being the only non-human primates to develop a cooperative breeding reproductive strategy. Offspring remain within their natal families into adulthood and all members of a group participate in infant care. Such a kin-selected breeding strategy may have arisen because of (1) competition for resources that limited breeding opportunities, (2) predation and (3) the high risks involved in dispersal.\(^1\)

Both field\(^1\) and laboratory\(^2\) studies of marmosets and tamarins typically report that only a single, dominant female breeds in each social group. While this restriction of female reproduction is a characteristic of cooperatively breeding mammals, from naked mole-rats \(^3\) to dwarf mongooses, \(^4\) it is unusually extreme for female primates.\(^5\) Socially subordinate female primates usually have poorer birth rates or fail to rear as many offspring as dominant females, but still manifest continued attempts to breed.\(^6\) Harassment-induced stress is traditionally invoked to explain impaired reproductive function in subordinate animals.\(^7\) However, such a mechanism does not appear to be causally involved in maintaining reproductive suppression among subordinates in cooperatively breeding species.\(^8\) In the two callitrichid species in which adrenocortical function has been studied (the common marmoset, Callithrix jacchus, and cotton top tamarin, Saguinus oedipus), cortisol levels in nonbreeding subordinate females not only failed to exceed those of the breeding dominant females, but they also were significantly lower than those in dominant females.\(^9,10\) Moreover, other endocrine and physical disturbances typically associated with chronic stress, such as low body weight, elevated plasma prolactin levels and altered melatonin secretion, are not found in subordinate female common marmosets.\(^8\) In callitrichid primates, therefore, specialized neuroendocrine and behavioral mechanisms not involving generalized stress may mediate the inhibition of sexual behavior and/or ovulation in female subordinates.

**Saguinus**

This genus may represent the most primitive of the Callitrichidae.\(^11\) Field studies of Saguinus tamarins have associated these callitrichids with secondary growth, successional forest and edge habitat.\(^1\) Such disturbed forest habitat provides the tamarins with seasonal supplies of fruit and small animal prey. Reproductive suppression in subordinate females is displayed at its most extreme in this genus. In captive studies of intact natal families, ovulation and sexual behavior were completely suppressed in all subordinate daughters remaining in their families (cotton top tamarin\(^12\); saddleback tamarin, S. fuscicollis\(^13\); red-bellied tamarin, S. labiatus\(^14\)). Hypogonadotropism may be the underlying physiological cause of the ovarian inhibition, but urinary luteinizing hormone (LH) levels were not always found to be low in subordinate female cotton top tamarins\(^15\) and ovarian morphology in subordinates indicated the presence of numerous luteinized follicles, suggestive of some gonadotropic activity. Ovulation and sexual activity were readily activated in subordinate females by removing them from their families and pairing them with unfamiliar males. Onset of ovulation was delayed if the male partner was familiar (a brother) or if scent contact was maintained with the natal family.\(^16\)

**Callithrix**

Marmosets are found generally eastwards of Saguinus, in successional forest and edge habitat which also includes gallery forest in savannas. This genus has specialized in tree exudate feeding to a much greater extent than Saguinus, so that during temporary or permanent fruit shortages, Callithrix
species can switch to gum feeding, whereas *Saguinus* cannot. Such adaptations may have allowed *Callithrix* to produce two litters per year, in comparison to the single annual litter usually produced by *Saguinus*, and to permit the two most gumivorous species, the common marmoset and the black-tufted-ear marmoset, *C. penicillata*, to colonize more changeable and extremely seasonal habitats. Such adaptations to changeable and less predictable environments may be reflected in the more varied degrees of reproductive suppression found among subordinate female common marmosets. While sexual behavior was clearly suppressed in subordinate daughters remaining within their captive natal families, ovulation could spontaneously occur in 0-67% of eldest daughters (common marmoset, Wied’s marmoset, *C. kuhlii*). The presence of an unrelated male, group composition and the fecundity of the dominant breeding female all influenced the degree of ovarian suppression in female subordinates.

The physiological mechanisms mediating suppression of ovulation in subordinate female marmosets have been studied most extensively in mixed-sex social groups of unrelated adult common marmosets. Hypogonadotropism was responsible for the anovulatory condition of subordinates. The ovaries of subordinate female marmosets were filled with many small antral follicles and presented a morphology consistent with a hypogonadotropic condition. A combination of enhanced sensitivity to estradiol negative feedback and inhibitory endogenous opioid peptides contributed to reduced LH release from the anterior pituitary and insufficient gonadotropic stimulation of the ovary. Hypothalamic release of gonadotropin-releasing hormone (GnRH), however, was not dramatically altered in anovulatory subordinate female common marmosets. Such minimal perturbations in hypothalamic function in anovulatory subordinate females would be consistent with the rapid ability of female common marmosets to generate an ovulatory LH surge and to conceive following removal from subordinate status. Olfactory and visual cues from the familiar dominant female play an important role in maintaining the anovulatory condition in subordinates and implicate associative learning in the neural mechanism of reproductive suppression.

*Cebuella*

The pygmy marmoset, *Cebuella pygmaea*, is the smallest anthropoid primate (weighing approximately 120 g). It has developed an extreme specialization in tree exudate feeding and inhabits seasonally inundated riverine forest. While sexual behavior appears limited to the breeding (presumed dominant) female in a social group, ovulatory function may not always be suppressed in subordinate daughters in captive natal families. Given their adaptation to a changeable habitat, pygmy marmosets may resemble *Callithrix* species most closely in their flexible reproductive responses to cues from their social environment.

*Leontopithecus*

The lion tamarins are the largest callitrichids. They occupy mature forest and may have specialized in insect foraging to exploit the abundance of large insects in such habitat. The physiological and behavioral mechanisms involved in reproductive suppression have only been investigated in one species, the golden lion tamarin, *Leontopithecus rosalia*. In contrast to *Saguinus* and *Callithrix*, there was no evidence of suppressed ovulation in subordinate daughters remaining in their captive natal families. All mature subordinate females ovulated. Furthermore, the ovarian cycles of
subordinate daughters were synchronized with those of their mothers. Nevertheless, sexual behavior remained inhibited in daughters. Leontopithecus, unlike Saguinus and Callithrix, appears to rely only on behavioral mechanisms to regulate female reproduction within social groups. The lion tamarins may have secondarily lost the ability to physiologically regulate female reproduction within social groups because the increased carrying capacity of their habitat may readily support more than one female breeding in a social group.

Summary

Callitrichid primates provide a unique specialized commitment to cooperative breeding that is unrivaled by any other mammalian family. They provide excellent opportunities to study (1) the neural mechanisms translating social subordination into sexual inactivity and infertility and (2) phylogenetic and ecological constraints on the proximate regulation of female reproductive success.

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LITERATURE CITED


STATUS AND CONSERVATION OF NEOTROPICAL PRIMATES AND THEIR HABITATS

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Abstract

The mammalian Order Primates is one of the most important and interesting in the Animal Kingdom, including as it does some 250 living species of apes, monkeys, lemurs, lorises, galagos and tarsiers, and of course, our own species, Homo sapiens. Our nonhuman primate relatives are valuable to us in many ways, and the rapid growth of the science of primatology over the past 30 yr has reflected this. Studies of these animals have taught us a great deal about the intricacies of our own behavior, they have clarified questions about our evolution and our origins, and they have played a significant role in biomedical research. Nonhuman primates are also one of the most conspicuous groups of animals in the world’s tropical forests and are often the best symbols for tropical forest conservation.

Unfortunately, wild populations of most nonhuman primates are decreasing all over the world, with many spectacular species like the mountain gorilla, the golden lion tamarin, the muriqui, and the indri are already on the verge of extinction, and numerous others headed in the same direction. The major reason for the decline of primates is destruction of their tropical forest habitat, but hunting, live capture and other factors have also come into play, bringing about a worldwide decline in primate populations. Approximately half of the world’s primate species are already in some danger, and one in five could be extinct by the turn of the century or even sooner if something isn’t done quickly.

The Neotropical Real is the most important area on the planet for its overall biodiversity, and this is reflected in the primate fauna as well. The status of Neotropical primates will be discussed, as well as some of the more important efforts underway to conserve primates and primate habitat. The comparative global importance of the Neotropical primate fauna will be underscored, and a summary of the long-term trends in Neotropical primatology and biodiversity conservation in general will be presented.

Resumen

De los mamíferos, el orden de los primates es uno de los más importantes e interesantes del reino animal, incluyendo en éste algunos de los 250 spp. vivas de sámulos, monos, lemuris, lorises galagos y társidos, y por supuesto a nuestra especie, Homo sapiens. Nuestros parientes primates no humanos son muy valiosos para nosotros en muchos sentidos, y el rápido crecimiento de la ciencia de primatología en los pasados 30 años ha reflejado esto. Estudios de estos animales nos han enseñado mucho sobre la complejidad de nuestra conducta, ellos nos han aclarado preguntas sobre nuestra evolución y nuestros orígenes y han jugado un papel muy importante en la investigación biomédica. Los primates no humanos son también unos de los grupos más conspicuos de animales en el mundo de los bosques tropicales y son los mejores símbolos para la conservación de estos bosques.
Desafortunadamente, las poblaciones silvestres de primates no humanos están declinando alrededor de todo el mundo, junto con muchas especies espectaculares como el gorila de montaña, el tamarín dorado, el maquiqui y el indri, que están a punto de la extinción, y muchos otros más que van en la misma dirección.

La mayor razón por la que están declinando los primates es la destrucción del hábitat del bosque tropical, pero la cacería, la captura en vivo y otros factores vienen a contribuir a que las poblaciones de primates declinen en estado silvestres. Aproximadamente la mitad de las especies de primates del mundo se encuentra en algún grado de peligro, y una de cada cinco estará extinta al final del siglo o más rápido si no hacemos algo para evitarlo.

El Neotrópico es el área más importante en el planeta por su biodiversidad, y esto está reflejado en los primates también. El estatus de los primates neotropicales será discutido, como también los esfuerzos más importantes que están en camino para la conservación de los primates y su hábitat. La importancia global comparativa de los primates neotropicales será discutida y será presentado un resumen de sus tendencias a largo plazo. La observación general de la biodiversidad en estos primates será presentada.
SURGICAL TREATMENT OF VITAMIN C DEFICIENCY INDUCED CEPHALHEMATOMAS IN A BREEDING COLONY OF SQUIRREL MONKEYS (Saimiri sciureus)

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Abstract

A vitamin C deficient diet was accidentally fed to a large breeding colony (691 animals) of squirrel monkeys (Saimiri sciureus) at the Pasteur Institute of French Guiana. Twenty-nine animals (4.2%) developed subperiosteal cranial hematomas, a condition well-known to occur as the first sign of vitamin C deficiency in this species. 16.8% of the juveniles (6-18 mo of age) and 1.9% of the adults (over 3 yr of age) were affected. Additional clinical findings included various degrees of normocytic, normochromic anemia. Hemoglobin ranged from 4.9 to 14.4 gm/dl. The deficiency was initially corrected by intramuscular injection of ascorbic acid. Treatment of 17 animals presenting a cephalhematoma consisted of aspiration of the lesion. Two ml to 100 ml (x=23 ml) of serosanguinous fluid was collected. In five cases, aspiration was repeated. Cephalhematomas reoccurred in 9 of the 17 cases. A surgical procedure was undertaken in the remaining 8 cases and in six additional severe cases. The surgery consisted of a large midline scalp incision followed by a resection of hyperostoses and a heat cautery of resected edges. The distended scalp skin was then removed and the skin was closed in a manner that resulted in pressure over the underlying tissues. To our knowledge this is the first report of the use of a surgical approach to reduce severe cephalhematomas in the squirrel monkey. All animals recovered from this outbreak and were in excellent condition 6 mo later. Serum vitamin C levels were not determined but the specificity of the clinical signs, the improvement seen after vitamin supplementation and return to normal diet argued in favor of a vitamin C deficiency.

Resumen

Una dieta deficiente de vitamina C fue accidentalmente proporcionada a una gran colonia (691 animales) de monos ardilla (Saimiri sciureus) en el Instituto Pasteur de la Guinea francesa. Veintinueve animales (4.2%) desarrollaron hematomas craneales subperiosteo, una condición bien conocida que ocurre como el primer signo de deficiencia de vitamina C en estas especies. Fueron afectados 16.8% de los juveniles (6-18 meses) y 1.9% de los adultos (superior a los 3 años de edad). Hallazgos clínicos adicionales incluyeron varios grados de anemia normocítica-normocrómica. El rango de hemoglobina fue de 4.9-14.4 gm/dl. La deficiencia fue corregida inicialmente por inyecciones intramusculares de ácido ascórbico. El tratamiento de 17 animales que presentaban cefalohematomas consistió en la aspiración de la lesión. Entre 2 y 100 ml. (media-23 ml.) de líquido serosanguíneo fue colectado de cada animal afectado. En 5 casos, la aspiración fue repetida. Los cefalohematomas reaparecieron en 9 de los 17 casos. Un procedimiento quirúrgico fue emprendido en los 8 casos restantes y en 6 casos severos adicionales. La cirugía consistió en una larga incisión con un escalpelo seguido de una resección de la hiperostosis y una cauterización de bordes. La piel distendida fue removida y entonces la piel fue cerrada de tal forma que resultó en presión sobre el tejido superpuesto. A nuestro saber, este es el primer reporte del uso de un recurso
quirúrgico para reducir cefalohematomas severos en el mono ardilla. Todos los animales se recuperaron y estaban en excelente condición 6 meses después. Los niveles de suero de vitamina C no fueron determinados, pero la especificidad de los signos clínicos, la mejoría observada después de los suplementos vitamínicos y el regreso a una dieta normal apuntan en favor de una deficiencia de vitamina C.
DISEASES OF OWL MONKEYS (Aotus spp.)

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Abstract

The owl monkey, Aotus spp., is a small nocturnal neotropical primate. The genera is genetically diverse; composed of two phenotypes and ten karyotypes with a diploid chromosome number ranging from 46-58. Red-necked owl monkeys (karyotypes I, VI, VII, XII) are located south of the Amazon river and grey-necked owl monkeys (karyotypes II, III, IV, V, VIII, IX) north of the river. Correct identification of karyotype is important in that certain karyotypes are predisposed to the development of some diseases. This presentation will cover the more frequently diagnosed diseases of owl monkeys.

Bacterial infections in owl monkeys generally cause pneumonia or air sacculitis, enteritis, or septicemia. Cellulitis and abscess formation occur less frequently. Pasteurella multocida, Klebsiella pneumoniae and to a lesser extent Bordetella bronchiseptica are the most frequent pathogens of the respiratory tract. Clinical signs of pneumonia and air sac infection include anorexia, listlessness, depression, mucopurulent nasal discharge, conjunctivitis, sneezing, coughing and dyspnea. Meningitis can develop secondarily. Several bacteria have been isolated from owl monkeys with enteric disease: Campylobacter jejuni, E. coli, Klebsiella, Proteus, Shigella and Salmonella. Colibacillosis characterized by dehydration, depression and profuse foul-smelling bloody diarrhea has been reported. Owl monkeys with Yersinia enterocolitica infection may present with diarrhea, hepatomegaly, splenomegaly, and abdominal distension. Pseudomonas, Streptococcus, and Staphylococcus have been associated with septicemia in owl monkeys.

The most frequent fungal infection in owl monkeys is moniliasis caused by an overgrowth of Candida albicans. Moniliasis may occur in debilitated or immunosuppressed monkeys or in those monkeys that have had prolonged antibiotic therapy. Infection with Histoplasma capsulatum resulting in clinical hypercalcemia and granulomatous hepatitis has been described in one owl monkey. A disseminated fungal infection characterized by 7-8 un oval, singly budding yeast cells with a large capsule occurs in karyotype I and V owl monkeys. Affected monkeys present with weight loss and marked splenomegaly. The yeast does not appear to be any of the commonly described pathogenic fungi. Dermatophilosis or cutaneous streptothricosis, caused by Dermatophilus congolensis, is characterized by exudative dermatitis resulting in papillomatous lesions of the extremities, head, and trunk.

Viral infections in owl monkeys usually result from contact with man or other nonhuman primates. Herpesvirus hominis and Herpesvirus tamarinus cause pantropic ulcerative, usually fatal disease in owl monkeys. Measles virus infection results in giant cell pneumonia and diarrhea. Encephalomyocarditis virus infection has been associated with acute death with pulmonary edema and hemorrhage. With the exception of toxoplasmosis most parasitic infestations in owl monkeys are not clinically significant.

Non-infectious diseases of owl monkeys are among the most clinically important. Owl monkeys
that have been in captivity have a high incidence of cardiovascular disease including cardiomegaly, left ventricular hypertrophy, congestive heart failure, dissecting aortic aneurysms, atrial and aortic thrombosis, and cerebrovascular accidents. The etiology of these cardiovascular diseases is unknown. Congestive heart failure is responsive to treatment with furosemide at 2 mg/kg body weight; but eventually becomes refractory to treatment. Renal disease including glomerulonephropathy, interstitial renal disease and nephrotic syndrome is a common cause of morbidity and mortality in owl monkeys. Feeding a protein restricted diet and treatment with furosemide may ameliorate clinical signs. Sub-adult, gray-necked owl monkeys are susceptible to vitamin E-responsive hemolytic anemia. Affected monkeys are weak, pale, icteric, and hypothermic with hematocrit <15. Treatment with vitamin E and selenium (10 IU α-tocopherol, 0.22 mg sodium selenite/kg body weight/wk), provision of oxygenated environment, and transfusion for severely anemic monkeys is effective. Less common non-infectious diseases include cholelithiasis, hepatic lipidosis and idiopathic eosinophilia (karyotypes II, III, IV and VI).

Resumen

El Mono Nocturno (Aotus spp.) es un primate neotropical pequeño y nocturno. El género es genéticamente diverso, compuesto por dos fenotipos y diez cariotipos con un número cromosomal diploide en un rango de 48-58 cromosomas. El mico de noche cuello rojo (cariotipo I,VI,VII.XII) localizados al sur del río Amazonas, y los de cuello gris (cariotipos II, III, IV, V VIII, IX) situados al norte de el río. La identificación correcta de los cariotipos es importante, ya que ciertos cariotipos pueden revelar la predisposición a algunas enfermedades. Este trabajo pretende presentar las enfermedades más frecuentes de micos de noche.

Las infecciones bacterianas en mono nocturno generalmente causan neumonía o aerosaculitis, enteritis o septicemia. La celulitis y la formación de abscesos ocurre menos frecuentemente. Pasteurella multocida, Klepsiella pneumonae y menos frecuentemente Bordetella bronchiseptica son los patógenos más frecuentes del tracto respiratorio. Los signos clínicos de la neumonía e infección de los sacos aéreos incluyen anorexia, indiferencia, depresión, descarga nasal mucopurulenta, conjuntivitis, estornudos tos y disnea. Se puede desarrollar una meningitis secundaria. Muchas bacterias han sido aisladas de monos con enfermedad entérica: Campylobacter jejuni, E. coli, Klebsiella, Proteus, Shigella y Salmonella. Ha sido reportada una colibacilosis caracterizada por deshidratación, depresión y diarrea mucosanguinolenta severa. Los micos con una infección por Yersinia enterocolica pueden presentar diarrea, hepatomegalia, esplenomegalia y distensión abdominal. Pseudomonas, Estreptococos y Estafilococos han sido asociados con septicemia en micos de noche.

La infección fúngica más frecuente en micos de noche es la moniliasis causada por el desarrollo de Candida albicans. La moniliasis puede ocurrir en monos debilitados o inmunodeprimidos o en aquellos monos que han tenido una terapia prolongada con antibióticos. Ha sido descrita en un mono la infección con Histoplasma capsulatum, dando como resultado hipercalemia y hepatitis granulomatosa. Una infección fúngica diseminada caracterizada por un botón oval de 7-8 un. con células de levaduras, y con una cápsula grande ocurrió en los monos con los cariotipos I y V. Los monos afectados presentaron pérdida de peso y esplenomegalia marcada. La levadura no parece ser ninguno de los hongos patógenos comúnmente descritos. La dermatofitosis o estreptotrichosis
La infección cutánea, causada por *Dermatophilus congolensis*, es caracterizada por dermatitis exudativa que da lugar a lesiones papilomatosas en las extremidades, cabeza y tronco.

Las infecciones virales en los micos usualmente resultan por el contacto con el humano u otro primate no humano. El *Herpesvirus hominis* y el *Herpesvirus tamarinus* causa una enfermedad pantrópica ulcerativa usualmente fatal. La infección por el virus del sarampión provoca una neumonía con células gigantes y diarrea. Una infección por encefalomiocarditis vírica ha sido asociada con una muerte aguda presentando edema pulmonar y hemorrágico. Con excepción del *Toxoplasma* la mayoría de las infestaciones parasitarias no son clínicamente significativas.

Las enfermedades no infecciosas están entre las más importantes clínicamente. Monos que han estado en cautiverio tienen una alta incidencia de enfermedades cardiovasculares incluyendo cardiomegalia, hipertrofia ventricular izquierda, falla cardíaca congestiva, ruptura de aneurisma aórtico, trombosis atrial y aórtica y accidentes cerebrovasculares. La etiología de estas enfermedades cardiovasculares es desconocida. La falla cardíaca congestiva responde al tratamiento con Furosemida a una dosis 2 mg/kg; pero eventualmente este tratamiento deja de ser efectivo. Las enfermedades renales incluyendo glomerulonefropatía, enfermedad intersticial renal y síndrome nefrótico comúnmente causan morbilidad y mortalidad en monos. Proporcionando una dieta restringida en proteínas y un tratamiento con furosemida pueden aminorar los signos clínicos. Los micos cuello gris subadultos son susceptibles a la anemia hemolítica que responde a la vitamina E. Los monos afectados están pálidos, ictericos, débiles e hipotérmicos con un hematocrito < 15. Es efectivo el tratamiento con vitamina E y Selenio (10 UI Alfatocoferol, 0.22 mg de Selenito de sodio/kg/semana), la provisión de un ambiente oxigenado y la transfusión para monos severamente anémicos. Enfermedades no infecciosas menos comunes incluyen colelitiasis, lipidosis hepática y eosinofilia idiopática (cariotipos II, III, IV, VI).
FATAL LYMPHOPROLIFERATIVE DISEASE/LYMPHOSARCOMA IN A CAPTIVE POPULATION OF COMMON MARMOSETS (*Callithrix jacchus*)

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Abstract

Eleven cases of fatal lymphoproliferative disease/lymphosarcoma have been diagnosed at necropsy in a laboratory housed population of 230 common marmosets (*Callithrix jacchus*) at the Wisconsin Regional Primate Research Center since May of 1992. Six of these cases occurred in 1995. Animals typically presented with a history of weight loss, inappetence, +/- diarrhea. In several cases there were palpable abdominal masses. The mesenteric lymph nodes and intestinal mucosa were consistently infiltrated by a mixed population of lymphocytes that often obliterated the normal architecture. Peripheral lymph nodes and other organs were rarely affected. Two animals had a lymphocytic leukemia. Affected animals range in age from 16 mo-9 yr and there is no gender prevalence. Of the 11 cases there are 2 sets of twins who died 2 yr apart (1993 and 1995) and a set of half siblings who died in the same year (1995). The other 5 cases are unrelated. While familial lymphoma is an etiological consideration, the cluster of unrelated cases in 1995 and lack of other connecting factors lead us to the hypothesis that there is a viral etiology.

Marmosets are known to be susceptible to tumor induction by gamma herpes viruses (*Herpes saimiri, Herpes ateles* and Epstein-Barr Virus). The common marmoset colony at the Wisconsin Regional Primate Research Center is currently being screened for EBV and *H. saimiri* titers. All animals tested to date have been EBV positive and *H. saimiri* negative. Virus isolation was attempted on lymph node and splenic tissue from 1 of the 11 confirmed cases, but no virus was isolated. Virus isolation, PCR and southern blots are currently in progress on cells from 2 confirmed cases. Cell typing is being investigated.

Resumen

Las marmosetas son conocidas por ser susceptibles a los tumores por los gamma herpes virus (*Herpes saimiri, Herpes ateles* y virus Epstein-Barr). La colonia de marmosetas comunes en el Centro Regional de Investigación de Primates en Wisconsin fue actualmente analizada para la detección de títulos contra EBV y *H. saimiri*. Todos los animales estudiados hasta la fecha han sido EBV positivos y *H. saimiri* negativos. Se trato de aislar el virus de un nódulo linfático y del bazo de uno de los 11 casos confirmados, sin ningún éxito. Está en progreso el aislamiento vírico, PCR y southern blots de células de dos casos confirmados. La tipificación celular esta siendo investigada.
ANESTHESIA OF WILD RED HOWLER MONKEYS (Alouatta seniculus) WITH MEDETOMIDINE-KETAMINE AND REVERSAL BY ATIPAMEZOLE

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Abstract

Wild red howler monkeys (Alouatta seniculus) were translocated during the flooding of the forest at a hydroelectric dam site in French Guiana. For a variety of minor clinical procedures, 95 monkeys were anesthetized with various i.m. combinations of medetomidine and ketamine. The howler population was composed of healthy animals (41 males and 54 females) of various ages. Medetomidine (0.15 mg/kg) associated with ketamine (4 mg/kg) was used on 62 animals and gave the best results. The injection rapidly resulted in complete immobilization with good to excellent myorelaxation. The induction stage was quiet with absence of both corneal and pedal withdrawal reflexes in fifty seven animals after 2.9 ± 1.4 min. Five animals required an additional injection. Rectal temperature, respiratory and heart rates decreased during anesthesia whereas relative oxyhemoglobin saturation increased. One death occurred but no adverse effects were noted in the other animals. Atipamezole given i.m. at a dose of five times the medetomidine, 38.3 ± 7.9 min after the anesthetic injection, led to standing recovery in 7.1 ± 4.5 min. Spontaneous recovery occurred in seventeen animals before the atipamezole injection, after an average of 30.6 ± 9.6 min, and earlier in juveniles. We recommend this association of medetomidine (0.15 mg/kg) with ketamine (4 mg/kg) for short procedures, including minor surgery, in red howler monkeys.

Resumen

Varios monos aulladores rojos (Alouatta seniculus) silvestres fueron traslocados durante la inundación de la selva por la construcción de una hidroeléctrica en la Guyana francesa. Para una variedad de procedimientos clínicos menores, 95 monos fueron anestesiados con varias combinaciones intramusculares de medetomidina y ketamina. La población de aulladores estaba compuesta de animales sanos (41 machos y 54 hembras) de varias edades. Los mejores resultados se obtuvieron con 0.15 mg/kg de medetomidina asociado con 4mg/kg de ketamina, mismas que fueron utilizadas en 62 animales. La rápida inyección dio como resultado una inmovilización completa con una buena a excelente miorelajación. El estado de inducción fue calmado, con la ausencia de reflejos retractor pedal y corneal en 57 individuos después de 2.9 ± 1.4 min. Cinco animales requirieron una inyección adicional. La temperatura rectal, frecuencia cardíaca y respiratoria disminuyeron durante la anestesia, presentando un relativo incremento en la saturación de la oxihemoglobina. Ocurrió una muerte, pero no fueron encontrados efectos adversos en otros animales. El Atipamezole administrado i.m. a una dosis de 5 veces la dosis de medetomidina 38.3 ± 7.9 min. después de la inyección del anestésico condujo a la recuperación en 7.1 ± 4.5 min. Una recuperación espontánea ocurrió en 17 animales antes de la inyección de atipamizole y después de un promedio 30.6 ± 9.6 min. y más tempranamente en juveniles. Nosotros recomendamos esta asociación anestésica para procedimientos cortos incluyendo cirugía menor en mono aullador rojo.

ENDOCRINE NEOPLASIA IN NEW WORLD PRIMATES

José L. Catão Dias, DVM, PhD*
Abstract

From 1975 to 1994, 1106 New World nonhuman primates were necropsied at the Department of Pathology, National Zoological Park (871), Washington, D.C., and at the Department of Comparative Pathology, Johns Hopkins University School of Medicine (235), Baltimore. Twenty-two (1.9%) animals were identified with 27 neoplasms. Of this group, nine (two females; seven males) animals had a total of 13 endocrine tumors. All individuals were adults, with an age range of 2.7-25 yr (average, 12.1 yr). Seven were Callitrichidae and two were Cebidae. The individual cases and corresponding neoplasms are identified in Table 1.

The adrenal gland was the most affected organ with seven (53.8%) neoplasms, followed by the pituitary and thyroid gland with two (15.4%) cases each, and pancreas and parathyroid gland each with one (7.7%) tumor. All neoplastic disorders were benign. Immunocytochemistry assays for growth hormone, adrenocorticotropic hormone, prolactin, follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone and chromogranin A were performed on two pituitary neoplasms. Pheochromocytoma was the most frequent neoplasm, representing five (38.4%) of the 13 neoplasms. The remaining were thyroid cystadenoma (2-15.4%), corticotrophic cell pituitary adenoma (2-15.4%), adrenal ganglioneuroma (1-7.7%), actively secreting adrenal cortical adenoma (1-7.7%), parathyroid chief-cell adenoma (1-7.7%) and pancreatic islet-cell adenoma (1-7.7%).

Resumen

Desde 1975 a 1994, 1106 primates del nuevo mundo fueron sometidos a necropsia en los Departamentos de Patología del National Zoological Park (871), Washington D.C. y el Departamento de Patología Comparativa de Johns Hopkins University School of Medicine (235) en Baltimore. 22 animales (1.9%) fueron identificados con 27 neoplasias. De este grupo, 9 (2 hembras, 7 machos) tenían un total de 13 tumores endocrinos. Todos los individuos eran adultos, con un rango de edad de 2.7 a 25 años (promedio 12.1 años). Siete de ellos eran Callitrichidos y 2 Cébidos. Los casos individuales y neoplasias correspondientes son identificados en la tabla 1.

La glándula adrenal fue el órgano más afectado con 7 (53.8%) neoplasias, seguidos por las glándulas
pituitaria y tiroides con dos casos cada una (15.4%), y el páncreas y la glándula paratiroides cada una con un tumor (7.7%). Todos los desórdenes neoplásicos fueron benignos. La inmunocitoquímica para hormonas de crecimiento, hormona adrenocorticotrópica, prolactina, hormonafoliculo estimulante, hormona luteinizante, hormona estimulante de tiroides y cromogranina A fueron llevadas a cabo en 2 neoplasias pituitarias. Los pheochromocytomas fueron las neoplasias más frecuentes, representado cinco de las 13 neoplasias (38.4%). El resto eran Cystadenoma tiroidal (2-15.4%) adenoma corticotrópico de la células pituitarias (2-15.4%), ganglioneuroma adrenal (1-7.7%), adenoma secretor cortical adrenal activo (1-7.7%), adenoma de células parenquimatosas en paratiroides (1-7.7%) y adenoma de células del islote pancreatico (1-7.7%).

Introduction

The information available on neoplasms arising from the endocrine system in New World nonhuman primates (NWP) is remarkably scant, and few spontaneous endocrine tumors have been described.\(^1,3,4\) The aim of this report is to present 13 endocrine neoplasms in nine NWP, belonging to the primate colonies of the National Zoological Park (NZP) and Johns Hopkins University School of Medicine (JHU).

Materials and Methods

A total of 1,106 complete necropsies of NWP were evaluated (871 from the NZP, and 235 from the JHU). Specimens from all organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4-6 µm and stained with hematoxylin and eosin (HE). Special stains were performed selectively in some cases, including periodic acid Schiff (PAS), PAS - orange G and Gomori’s chrome alum-hematoxylin-phloxine. Immunocytochemistry assays for growth hormone, adrenocorticotropic hormone, prolactin, follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone and chromogranin A were performed on two pituitary neoplasms.

Results and Discussion

Of 1,106 NWP, 22 individuals had 27 neoplasms affecting most organ-systems. Of this group, nine were identified with 13 endocrine neoplasm. All nine animals were adults, ranging from 2.7-25 yr, and the average age was 12.1 yr. The individual cases and corresponding neoplasms are identified in Table 1.

The adrenal was the most affected gland with seven (53.8%) neoplasms, followed by the hypophysis and thyroid gland with two (15.4%) each. The pancreas and parathyroid gland each had one tumor (7.7%). All tumors were classified as benign, according to theirs gross and microscopic characteristics, primary sites, absence of metastasis and/or invasive ability.\(^2\)

Pheochromocytoma was the most frequent neoplasm, representing five (38.4%) of the 13 neoplasms. The remaining were thyroid cystadenoma (2-15.4%), pituitary adenoma (2-15.4%), adrenal ganglioneuroma (1-7.7%), actively secreting cortical adrenal adenoma (1-7.7%), parathyroid chief-cell adenoma (1-7.7%) and pancreatic islet-cell adenoma (1-7.7%). Both pituitary adenomas were positive for ACTH and negative for all other immunocytochemistry assays.
The frequency of spontaneous endocrine neoplasia among the NWP is controversial. In the present study, endocrine tumors dominated and accounted for 48.1% (13/27) of all cases. The reasons for this high incidence is unknown, but one point might be considered. Due to better management conditions, animals were able to have a long life span, with an average age of 12.1 yr. It has been proposed that prolonged stimulation of endocrine glands would predispose to a higher incidence of neoplasia. Therefore, it is reasonable to propose that stress which often attends captivity, associated with a longer life span, could have triggered the high endocrine tumor frequency observed.

Similarly, the causes of such high pheochromocytoma (cases 4, 5, 7, 8, 9) frequency are also unclear. Pheochromocytomas are tumors of chromaffin cells, located mainly in the adrenal medulla. In NWP, a pheochromocytoma has only been described in a 14-yr-old male mantled howler monkey (A. villosa), in association with thyroid C-cell adenoma and an islet cell adenoma. The relationship between pheochromocytoma and calcitonin-secreting C-cell adenoma appears to be well-established, representing a neoplastic change of neuroectoderm in the same individual. This hypothesis also could explain the endocrine neoplastic syndromes observed in Cases 8 and 9. Yet, although measurements of catecholamines were not performed, it is probable that the brown-headed spider monkey (Case 9) had a actively-secreting pheochromocytoma, based on the clinical and pathological evidence of cardiovascular failure.

The occurrence of ganglioneuroma and pheochromocytoma (Case 7) is well-defined in the literature and represents an example of bipolar neoplastic cell differentiation. Hyperadrenocorticism was clinically confirmed in the black-tailed marmoset (Case 2), based on basal plasma cortisol measurement and ACTH stimulation test. Osteoporosis was a significant clinico-pathological aspect of this case, as seen progressively on serial radiographs and pathologic bone fractures and deformities.

The remaining neoplasms reported in this study (Cases 1, 3, 4, 5) appeared to be inactive tumors, and were considered incidental necropsy findings.

ACKNOWLEDGMENTS

The authors thanks Mr. GL Bratthauer, B.A., MT(ASCP), Ms. Vera Bonshock, and Ms. Robin Anne Ferris for their helpful suggestions and assistance. JLC Dias was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant No. 92/4717-9), and by the Smithsonian Institution Visiting Scientist Award. This work was supported by the Friends of the National Zoo (FONZ) grant No. 94-014.

LITERATURE CITED

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EPIDEMIC OF ENTERITIS AND COLITIS IN A PYGMY MARMOSET (Cebuella pygmaea) COLONY

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Abstract

A breeding colony of pygmy marmosets (Cebuella pygmaea) (n=35-40) is maintained by the University of Wisconsin Department of Psychology. The colony was initiated in 1960 and animals were imported into the colony until 1980. Additional animals were added from an NIH colony in 1990-1991. Animals are housed in family groups or pairs for purposes of studying vocal communication and reproductive endocrinology. In 1992, diarrhea disease became a significant cause of morbidity and mortality in the colony. Enteritis related health problems have continued through 1996. Treatment procedures were instituted in 1993 which decreased mortality, but the disease remains a significant cause of morbidity.

Histological lesions of enteritis and colitis have been identified in 18 animals from a necropsy total of 31 animals (58%) in the period 8/23/92 - 8/30/95. Seven of the affected animals were infants and animals less than 5 mo of age, one animal was a juvenile (defined as 5 mo - adult, not paired and still in a family group), and 10 animals were adults. Five of the ten adults identified with enteritis lesions died due to causes other than enteritis or colitis.

The clinical presentation included diarrhea and dehydration. The stools were soft to watery, sometimes mucoid but rarely bloody. The infants tended to be depressed and severely dehydrated, estimated by skin turgor at 12-15%. Gross necropsy included the following findings: thin body condition (often an absence of intraabdominal adipose tissue in the young animals), and rectal prolapse and peritonitis due to colonic perforation. Histological diagnoses often included a description of diffuse, necrotizing, suppurative colitis, subacute gastritis, suppurative enteritis with villar atrophy, and necrosis of the mesenteric lymph nodes if peritonitis was present. Amyloidosis of the liver and kidneys was present in several chronic cases.

The epidemiology and necropsy findings directed the differential diagnosis towards an infectious disease problem. Bacteriological cultures for enteric pathogens, including Campylobacter, have been negative. Negative fecal floatations, direct fecal smears and IFA for Giardia and Cryptosporidium ruled out enteric parasites. Viral etiologies under consideration are listed in Table 1.

At present, tests have been negative for paramyxovirus, rotavirus, coronavirus, simian immunodeficiency virus, and lymphocytic choriomeningitis virus. Virus isolation studies and PCR for parvovirus B19 are pending. Histologically, intranuclear inclusions were identified in one marmoset and electron-microscopically these are adenovirus-like particles. Thirteen serum samples were opportunistically collected in 1995 from living colony animals and are all seropositive for
Resumen


Lesiones histológicas de enteritis y colitis han sido identificados en 18 animales a partir de necropsias de un total de 31 marmosetas (58%) en el periodo 8/23/92 - 8/30/95. Siete de los animales afectados fueron infantes y animales menores de 5 meses de edad. Un animal era juvenil (definido como adulto de 5 meses, sin aparearse y aún en un grupo familiar), y 10 animales eran adultos. Cinco de los 10 adultos identificados con lesiones de enteritis murieron debido a causas diferentes de enteritis o colitis.

La presentación crónica incluyó diarrea y deshidratación. Las heces eran de suaves a acuosas, algunas veces mucoides pero rara vez con sangre. Los infantes tendieron a estar deprimidos y severamente deshidratados, estimado por el grosor de la piel del 12-15% de deshidratación. Los hallazgos macroscópicos en la necropsia incluyeron lo siguiente: condición corporal delgada (frecuentemente ausencia de tejido adiposo intra abdominal en los animales jóvenes) y prolapso rectal y peritonitis debido a la perforación del colon. El diagnóstico histológico también incluyó a menudo, una descripción de una colitis difusa, necrótica y supurativa, gastritis subaguda, enteritis supurativa con atrofia biliar y necrosis de los nódulos linfáticos mesentéricos si la peritonitis estaba presente. Amiloidosis del hígado y riñones estaba presente en muchos casos crónicos.

La epidemiología y los resultados de la necropsia dirigieron el diagnóstico diferencial hacia el problema de una enfermedad infecciosa. Cultivos bacteriológicos de patógenos entéricos, incluidos *Campylobacter*, han sido negativos. Flotaciones fecales negativas, frotis fecales directos e IFA para *Giardia* y *Cryptosporidium* descartan parásitos entéricos. Etiologías virales bajo consideración son listados en la Tabla 1.

Hasta el momento, las pruebas han sido negativas para Paramyxovirus, Rotavirus, Coronavirus, virus de inmunodeficiencia en simios y virus de la coriomeningitis linfocítica. Estudios de aislamiento de virus y PCR para parvovirus B19 siguen pendientes. Histológicamente, se identificaron inclusiones intranucleares en una marmoseta, las cuales semejan, bajo el microscopio electrónico, partículas similares a adenovirus. Trece muestras de suero fueron colectadas oportunisticamente en 1995 a partir de colonias de animales vivos y todos fueron seropositivos al adenovirus. En muchas ocasiones, el aislamiento del virus no ha sido posible.
Table 1. Differential of virus pathogens, laboratories used for analysis and type of sample used for analysis.

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*Special arrangements for testing. Not a routine procedure for the laboratory.
Salmonella arizona OSTEOMYELITIS IN A COLONY OF ARIZONA RIDGE-NOSED RATTLESNAKES (Crotalus w. willardi)

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Abstract

Osteomyelitis was diagnosed in 6 of 14 Arizona ridge-nosed rattlesnakes (Crotalus w. willardi) evaluated at the Knoxville Zoo in 1995. Clinical signs ranged from asymptomatic to focal, firm nodules over the ribs and less movement by the animal due to a decreased range of motion of the spinal vertebrae. Diagnosis was based upon radiographic lesions including expansile bony proliferation associated with multiple, adjacent vertebrae and similar appearing, focal rib lesions. Salmonella induced osteomyelitis was confirmed by rib biopsy in one snake. Salmonella arizona serotype 56:Z4,Z23 was the predominant bacterium cultured from the biopsy. This snake developed septicemia following the biopsy procedure and died. Necropsy confirmed a marked, chronic, locally extensive osteomyelitis, with osteonecrosis, of the vertebrae and ribs. Mild to moderate pneumonia, celomitis, hepatitis, and oophoritis were also present. Salmonella arizona was grown in pure culture from an ovum.

Review of reptile collection inventory and necropsy records documented two additional cases of Salmonella arizona spinal osteomyelitis in Arizona ridge-nosed rattlesnakes during the past 5 yr. Necropsy records also identified two other Arizona ridge-nosed rattlesnakes which died of enteritis and had Salmonella arizona cultured from the intestine. Only one Salmonella arizona isolate has been serotyped to date. All of the isolates have been susceptible to the antibiotics most commonly tested. No long-term therapies directed at the osteomyelitis have been attempted to date.

Salmonella spp. and Salmonella arizona, in particular, are common pathogens in snakes in our collection. This is in contrast to observations from previous reports that have suggested Salmonella spp. to be infrequently pathogenic in reptile collections. Salmonella arizona osteomyelitis has also been diagnosed in a colony of montane rattlesnakes (B. Raphael, personal communication) and appears to be a pathogen with bone as a target tissue. Research efforts directed toward understanding pathogenesis, early detection, and medical therapy are needed.

Resumen

La osteomelitis fue diagnosticada en 6 de 14 serpientes de cascabel de Arizona (Crotalus w. willardi) evaluadas en el Zoológico de Knoxville en 1995. Los signos clínicos variaron de asintomáticos a firmes nódulos focales sobre las costillas y menor movimiento del animal debido a la disminución del movimiento de la columna vertebral. El diagnóstico fue basado en lesiones radiográficas que incluyen proliferación expansiva múltiple asociada con vértebras adyacentes y
lesiones similares en las costillas. La osteomielitis inducida por *Salmonella* fue confirmada por la biopsia de una costilla de una serpiente. La *Salmonella arizona*, serotipo 56:Z4, Z23 fue el cultivo bacteriano predominante de la biopsia. Esta serpiente desarrolló septicemia después del procedimiento de biopsia, y luego murió. La necropsia confirmó una marcada osteomielitis extensiva focal y crónica con osteonecrosis de vértebras y costillas. Una neumonía leve a moderada, celomitis, hepatitis y oforitis también estuvieron presentes. La *Salmonella arizona* fue desarrollada en cultivo puro a partir de un óvulo.

Una revisión de la colección de reptiles y archivos de necropsia documentaron otros dos casos de osteomielitis espinal por *Salmonella arizona* en las serpientes de cascabel durante los últimos cinco años. Los registros de necropsia también identificaron otras dos serpientes de cascabel de Arizona que murieron de enteritis y tenían *Salmonella arizona* cultivada en el intestino. Solo una *Salmonella arizona* ha sido serotipificada hasta ahora. Todas las Salmonellas aisladas han sido susceptibles a los antibióticos más comúnmente utilizados. No se han intentado terapias directas en la osteomielitis hasta la fecha.

*Salmonella* spp. y *S. arizona* son patógenos comunes en serpientes de nuestra colección. Esto en contraste a observaciones de reportes previos que sugieren que la *Salmonella* spp. es un patógeno poco frecuente en las colecciones de reptiles. La osteomielitis causada por la *Salmonella arizona* ha sido diagnosticada también en una colonia de serpientes de cascabel de montaña (B. Raphael comunicación personal) y parece ser un patógeno con el tejido óseo como blanco principal. Son necesarios esfuerzos en la investigación dirigidos a entender la patogénesis, detección temprana y terapia médica.
DERMATITIS OF ANURANS CAUSED BY FUNGAL-LIKE PROTISTS

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Abstract

During a 3 mo period in 1991, high mortality occurred in a captive colony of arroyo toads (Bufo microscaphus californicus). Affected toads usually displayed signs of lethargy, anorexia, pupillary miosis, and muscle incoordination for 2-5 wk prior to death. Gross necropsy findings and results of bacterial and fungal cultures of livers, kidneys, and coelomic fluid were unremarkable. Histologically, significant lesions were limited to the skin where multifocal epidermal hyperplasia, hyperkeratosis, and degeneration were often accompanied by edema and inflammation of the underlying dermis. These lesions were associated with the presence of round 15-30 µm single-celled organisms within the epidermis. Death of these toads was attributed to the cutaneous lesions with resultant disruption of respiration and maintenance of osmotic and electrolyte homeostasis. The organisms present in the toads’ skins were originally thought to be a protozoa and therefore, treatment of the surviving toads with trimethaprim-sulfadiazine (TMS) soaks was initiated. The soaking solution consisted of 1 part injectable TMS, 240 mg/ml (Di-Trim, Syntex Animals Health, Inc., West Des Moines, Iowa 50265) added to 250 parts of 0.6% saline solution. Toads were soaked in a volume of solution sufficient to cover the lower one-half of their bodies for 24 hr every other day of 2 wk. Although this was successful in halting mortality, it did not completely clear the toads of the organisms. The primary effect of this therapy was to dramatically increase the rate of skin shedding by the toads and thereby apparently decrease the numbers of organisms present in and on the toads’ skins.

Three stages of the causative organisms were identified by light microscopy: an uninucleate vegetative form, a multinucleate endosporulating stage, and a thick-walled cyst containing multiple 2-3 µm round spores. Special histologic stains revealed that cell walls of all forms of the organism were periodic acid-Schiff (PAS) positive and stained with Gomori’s methenamine silver (GMS). They were not acid-fast and did not stain with Gridley’s fungal stain. The small spore forms within the larger cystic structures were gram positive and stained weakly with Giemsa. Transmission electron microscopy also revealed that the spores had flagella. These results indicated that the organisms are aquatic protists taxonomically related to the diverse group of “water molds” which were formerly classified as primitive fungi.

In a retrospective study of the pathology records at the National Zoological Park from 1975 - 1995, three additional cases of fatal dermatitis caused by morphologically similar organisms were discovered. These cases occurred in two White’s tree frogs (Litoria caerulea) and an ornate horned frog (Ceratophrys ornata). Subsequently, other cases have been identified during post mortem histologic examination of captive amargosa toads (Bufo nelsoni) and a woodhouse toad (Bufo w.
The organisms present in the cutaneous lesions of these cases have not apparently been previously reported to be amphibian pathogens. However, their occurrence in several anuran species from different facilities suggest that these are widespread and may be relatively common. The source of the infections was undetermined. More than one species of aquatic protist may have been present in the various anuran species; definitive speciation of these protists requires special cultures and/or morphologic studies of viable organisms. These cases illustrate the importance of careful examination and sampling of skin when investigating disease in amphibians.

Resumen

Durante un periodo de tres meses en 1991, ocurrió una gran mortandad de una colonia de sapos de arroyo (*Bufo microscaphus californicus*) en cautiverio. Los sapos afectados generalmente presentaban signos de letargo, anorexia, miosis en pupilas e incoordinación muscular de 2 a 5 semanas antes de morir. Los hallazgos a la necropsia y los resultados de los cultivos bacterianos y micóticos de riñón, hígado y líquido celómico fueron irrelevantes. Histológicamente las lesiones significativas fueron limitadas a la piel donde la hiperplasia epidermal multifocal, hiperqueratosis y degeneración fueron acompañadas varias veces con edema e inflamación de la dermis inferior. Estas lesiones fueron asociadas con la presencia de aproximadamente 15-30 µm. de organismos unicelulares dentro de la epidermis. La muerte de estos sapos fue atribuida a las lesiones cutáneas con la resultante interrupción de la respiración y mantenimiento de una homeostasis osmótica y electrólítica. Los organismos presentes en la piel de los sapos sobrevivientes fueron identificados originalmente como protozoarios y por lo tanto el tratamiento se inició mojándolos con trimetoprin-sulfadiazina (TMS). La solución consistía en una parte inyectable de TMS, 240 mg/ml (Di-Trim, Syntex Animals Health, Inc., West Des Moines, Iowa 50265) además de 250 partes de solución salina al 0.6%. Los sapos fueron empapados en un volumen de solución suficiente para cubrir la mitad de sus cuerpos durante 24 hrs diariamente por 2 semanas. Pese a que fue exitoso al disminuir la mortalidad, no limpió a los sapos por completo de los organismos. El efecto primario de esta terapia fue el dramático incremento de la muda de la piel de los sapos y aparentemente eso disminuyó el numero de organismos en la piel.

Tres estados del organismo causal fueron identificados mediante el uso del microscopio óptico: una forma vegetativa unicelular, un estado endoesporulado multinuclear y un quiste de pared delgada conteniendo esporas de 2 a 3 µm. de circunferencia. Cortes histológicos especiales revelaron que las paredes celulares de todas las formas del organismo fueron positivas al ácido-Schiff periódico (PAS) y teñidas con metenamina de plata Gomori (GMS). Ellos no fueron ácido alcohol resistente y no se tiñeron con la tinción para hongos de Gridley. Las pequeñas formas de esporas dentro de las grandes estructuras císticas fueron Gram positivas y se tiñeron levemente con Giemsa. El microscopio de transmisión electrónica reveló que las esporas poseían flagelo. Esos resultados indicaron que los organismos eran protistas acuáticos taxonómicamente relacionados con el diverso grupo de “mohos acuáticos” que estaban antiguamente clasificados como hongos primitivos.

En un estudio retrospectivo de los reportes patológicos del National Zoological Park de 1975-1995, tres casos adicionales de dermatitis fatal causada por organismos morfológicamente similares fueron
descubiertos. Estos casos ocurrieron en dos ranas arborícolas blancas (*Litoria caerulea*) y en una rana cornuda (*Ceratophrys ornata*). Subsecuentemente, otros casos habían sido identificados durante el examen postmortem de sapos amargosa cautivos (*Bufo nelsoni*) y un sapo de woodhouse (*Bufo w. woodhousei*).

El organismo presente en las lesiones cutáneas en estos casos no habían sido aparentemente reportados como patógenos en anfibios. Sin embargo, estos ocurrieron en varias especies de anuros en diferentes grados sugiere que estos están diseminados y pueden ser relativamente comunes. La ruta de la infección no fue determinada. Más de una especie de protistas acuáticos puede estar presente en varias especies de anuros, la especificación definitiva de esos protistas requieren cultivos especiales y/o estudios morfológicos de organismos viables. Esos casos ilustran la importancia del cuidadoso examen y un muestreo de piel cuando se investigan enfermedades en anfibios.
HIGH PREVALENCE OF GOUT AT NECROPSY IN GIANT DAY GECKOES (Phelsuma madagascariensis) AT THE NATIONAL ZOOLOGICAL PARK

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Abstract

The National Zoological Park (NZP) in Washington, DC has maintained two breeding colonies of giant day geckoes: Phelsuma madagascariensis grandis and P. m. martensi. The population of P. m. grandis, founded in October 1972 and still present as of November 1995, has included 175 geckoes consisting of 35 males, 73 females, and 67 animals of unknown sex (35.73.67). The population of P. m. martensi, founded in October 1990 and removed from the collection in January 1995, included 46 geckoes (10.25.11). It was noted recently that these animals were frequently diagnosed with gout at necropsy. A retrospective study was designed to characterize the problem and identify possible causative factors. All giant day gecko necropsy reports from November 1975 to November 1995 were reviewed. Extremely autolyzed animals and animals less than 6 mo of age were excluded from the study, yielding two study populations of 55 (15.36.4) P. m. grandis and 25 (7.18.0) P. m. martensi. The prevalence of gout at necropsy was calculated for each gecko subspecies and for various cohorts within each subspecies (Table 1). Cases of gout were classified by the distribution of lesions (articular and/or renal) and by etiology (primary or secondary) (Table 2). Primary gout was diagnosed if no underlying conditions were detected at necropsy. Secondary gout was diagnosed if other conditions were detected which likely led to the demise of the animal. Differences in the prevalence of gout between pairs of cohorts were tested for statistical significance using the Pearson’s Chi-square test or the two-tailed Fisher exact test (Table 3).

Among animals presented for necropsy, gout was detected in 36% of P. m. grandis (20 cases out of 55 necropsies) and 70% of P. m. martensi (14 cases out of 20 necropsies). The prevalence of gout in P. m. grandis varied over time (Table 1). Between November 1975 and December 1984, the prevalence of gout in P. m. grandis was only 5% (one case out of 20 necropsies). Between January 1985 and November 1995, the prevalence of gout in P. m. grandis was 54% (19 cases out of 35 necropsies), a statistically significant difference (p < 0.001). P. m. martensi were only present at the NZP between November 1990 and January 1995. There was not a significant difference between the prevalence of gout in P. m. grandis and P. m. martensi during this period (p > 0.15). However, there was a significant difference between the two subspecies with respect to the type of gout diagnosed. Fifty-five percent of P. m. grandis cases were considered primary gout compared to 100% of P. m. martensi cases (p < 0.01). The remaining 45% of P. m. grandis cases were considered secondary gout. Underlying lesions identified in these cases included: bacterial
septicemia, infectious stomatitis with granulomatous pneumonia, granulomatous hepatitis, hepatocellular adenoma, hepatic fibrosis, chronic hepatopathy, renal carcinoma, and chronic nephropathy. The two subspecies also differed significantly with respect to the distribution of gouty lesions. Forty-five percent of *P. m. grandis* cases had articular lesions compared to 93% of *P. m. martensi* cases (p < 0.01). Eighty-five percent of *P. m. grandis* cases had renal lesions compared to 36% of *P. m. martensi* cases (p < 0.01). There was no statistical difference between the prevalence of gout in males versus females in either subspecies.

In summary, *P. m. grandis* exhibited a dramatic increase in the prevalence of gout at necropsy after January 1985. *P. m. martensi* added to the collection in November 1990 exhibited a similarly high prevalence. However, the two subspecies differed significantly in their tendencies to develop primary or secondary gout, and in their predilections for the articular and renal forms of gout. It is unclear why the two subspecies behaved differently in these respects. One factor that might have contributed to the high prevalence of gout after January 1985 is a change in the diet of the crickets offered to the geckoes. In 1985, the feeder cricket diet was changed from mouse chow to a commercial cricket diet. While the total nitrogen content of crickets on the two diets did not differ, it is possible that a relative excess of one or more amino acids occurred, thus increasing the production of uric acid. Analyses of relative amino acid concentrations were not performed. Other potential contributing factors include: a change in the housing of some of the geckoes in the early 1980’s from glass aquaria to screen cages; a possible increase in the average age of the geckoes in the NZP populations; and a potential, inherent predisposition of this species to the development of gout. Further study to investigate the high prevalence of gout at the NZP, with particular focus on the possible role of the cricket diet, is warranted.

**Resumen**

El parque Zoológico Nacional (NZP) en Washington D.C. ha mantenido en reproducción colonias de geckos gigantes: *Phelsuma medagascariencis grandis* y *P. m. martensi*. La población de *P. m. grandis* comenzó en octubre de 1972 y aún continua en noviembre del 1995, con un total de 175 geckos que consisten en 35 machos, 73 hembras y 67 indiferenciados (35.73.67). La población de *P. m. martensi* que comenzó en octubre de 1990 y que fue removida de la colección en enero de 1995, llegó a sumar 46 ejemplares (10.23.11). Recientemente fue observado que en estas especies de gecko, con frecuencia se diagnosticaba gota a la necropsia. Se designó un estudio retrospectivo para detectar el problema e identificar los posibles factores que la causan, se revisaron de nuevo todas las necropsias que se habían practicado en los geckos gigantes entre noviembre de 1975 y noviembre de 1995. Para este estudio se hizo una cuidadosa selección de animales y todos aquellos que presentaron una autolisis extrema o que tenían menos de 6 meses de edad fueron excluidos. Las dos poblaciones escogidas fueron de 55 individuos de *P.m. grandis* (15.34.4) y de 25 de *P.m. martensi* (7.18.0). La frecuencia de gota en la necropsia fue calculada por cada subespecie de gecko y por varios grupos entre cada subespecie (Tabla 1). Los casos de gota fueron clasificados por la distribución de la lesión (articular y/o renal) y por la etiología (primaria y secundaria) (Tabla 2). La gota primaria fue diagnosticada cuando las condiciones esenciales no se detectaron a la necropsia. La gota secundaria fue diagnosticada cuando otras condiciones se detectaban como posibles causas del fallecimiento del animal. La diferencia en la frecuencia de la gota en los 2 pares de grupos se examinó estadísticamente usando la prueba de Chi cuadrada de Pearson o la prueba
exacta de Fisher (Tabla 3).

En todos los animales que se efectuó la necropsia, el 36% de *P. m. grandis* presentaron gota (20 casos de 55 necropsias) y el 70% de *P. m. martensi* (14 casos de 20 necropsias). La frecuencia de gota en *P. m. grandis* varió durante todo el tiempo (Tabla 1). Entre nov. de 1975 y dic. de 1984, la gota en *P. m. grandis* fue sólo del 5% (1 caso de 20 necropsias). Entre enero de 1985 y nov. de 1995, la gota en la misma subespecie aumentó al 54% (19 casos de 35 necropsias) con diferencia estadística significativa (p<0.001). La subespecie *P. m. martensi* fue presentada en el NZP entre noviembre de 1990 y enero de 1995. No hubo diferencias significativas entre la gota de *P. m. grandis* y *P. m. martensi* durante ese periodo (p>0.15). En lo que sí hubo diferencia significativa fue en el tipo de gota existente en las 2 subespecies. El 55% de los casos en *P. m. grandis* fueron consideradas como Gota primaria comparado al 100% entre los casos del *P. m. martensi* durante ese periodo (p<0.01). El 45% restante de los casos de *P. m. grandis* fueron considerados como gota secundaria. Las lesiones principales que se identificaron en estos casos incluyeron: septicemia bacteriana, estomatitis granulomatosa infecciosa, hepatitis granulomatosa, adenoma hepatocelular, fibrosis hepática, hepatopatía crónica, carcinoma renal y nefropatía crónica. Las subespecies también difieren significativamente con respecto a la distribución de la lesión de la gota. El 45% de los casos de *P. m. grandis* tuvieron lesiones articulares, comparados con el 93% de los casos de *P. m. martensi* (p<0.001). El 85% de los casos de *P. m. grandis* tuvieron lesiones renales comparada con el 36% de los casos de *P. m. martensi* (p<0.01). No hubo diferencia estadística entre la frecuencia de gota en machos y en hembras, ni en una u otra subespecie.

En resumen, desde enero de 1985, *P. m. grandis* mostró un incremento dramático en la frecuencia de la gota en las necropsias. Un *P. m. martensi* en la colección en nov. de 1990 también mostró un incremento similar de gota. Como siempre, hubo diferencias significativas entre las dos subespecies en cuanto a tendencias para desarrollar la gota primaria o secundaria y sus predilecciones por la gota renal o la articular. Esto todavía no está muy claro, porque las dos subespecies se comportaron de diferente forma a este respecto. Un factor que pudo contribuir a la alta prevalencia de la gota después de enero de 1985 fue un cambio en la dieta de los grillos que eran ofrecidos como alimentación. Se les cambió la dieta a los grillos que normalmente se alimentaban con comida para ratón a una dieta comercial para grillos. El contenido de nitrógeno en las dos dietas no se diferenció. Es posible que un exceso relativo de uno o más aminoácidos hayan ocurrido y se haya incrementado el nivel de ácido úrico. No se hizo un análisis de la concentración relativa de aminoácidos. Otros factores potenciales que pudieron haber contribuido son: el cambio de albergue que tenían los geckos a principios de los ‘80s, que de estar en terrario de vidrio pasaron a jaulas de metal, un posible aumento en las edades promedio de los geckos del Parque Zoológico Nacional (PZN) y a una posible predisposición innata de estas especies para desarrollar la gota. Un estudio más amplio para investigar la alta frecuencia de la gota en el Parque Zoológico Nacional (PZN) con un enfoque particular en el posible papel que tenga la dieta de los grillos, está garantizado.

ACKNOWLEDGMENTS

The authors wish to thank the Friends of the National Zoo for providing a summer research traineeship grant that made this study possible.
Table 1. Prevalence of gout at necropsy in various cohorts of giant day gecko (*Phelsuma madagascariensis*) at the National Zoological Park.

<table>
<thead>
<tr>
<th>Population of giant day gecko</th>
<th>Prevalence of gout at necropsy among animals &gt; 6 mo&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. m. grandis</em></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>36% (20/55)</td>
</tr>
<tr>
<td>November 1975 to December 1984</td>
<td>5.0% (1/20)</td>
</tr>
<tr>
<td>January 1985 to November 1995</td>
<td>54% (19/35)</td>
</tr>
<tr>
<td>November 1990 to January 1995</td>
<td>47% (9/19)</td>
</tr>
<tr>
<td>Males only</td>
<td>53% (8/15)</td>
</tr>
<tr>
<td>Females only</td>
<td>33% (12/36)</td>
</tr>
<tr>
<td><em>P. m. martensi</em></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>70% (14/20)</td>
</tr>
<tr>
<td>Males only</td>
<td>57% (4/7)</td>
</tr>
<tr>
<td>Females only</td>
<td>77% (10/13)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in parentheses indicate the number of cases of gout at necropsy out of the total number of necropsies in which gout was either ruled in or out.
Table 2. Prevalence of primary, secondary, articular, and renal gout at necropsy in giant day geckos (*Phelsuma madagascariensis*) at the National Zoological Park.

<table>
<thead>
<tr>
<th>Type</th>
<th><em>P. m. grandis</em></th>
<th><em>P. m. martensi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>55% (11/20)</td>
<td>100% (14/14)</td>
</tr>
<tr>
<td>Secondary</td>
<td>45% (9/20)</td>
<td>0% (0/14)</td>
</tr>
</tbody>
</table>

Lesion Distribution

<table>
<thead>
<tr>
<th>Type</th>
<th><em>P. m. grandis</em></th>
<th><em>P. m. martensi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Articular</td>
<td>45% (9/20)</td>
<td>93% (13/14)</td>
</tr>
<tr>
<td>Renal</td>
<td>85% (17/20)</td>
<td>36% (5/14)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses represent the number of cases in that category out of the total number of cases of gout.

b Cases of gout were classified at necropsy as either primary, if no other significant lesions were discovered, or secondary, if other lesions were present which most likely led to the animal’s demise and potentially led to the development of gout.

c The sum of the prevalences of articular and renal gout in each subspecies exceeds 100% because both lesions may be present in the same animal.

Table 3. Results of statistical analysis of differences in the prevalence of gout between paired cohorts of giant day gecko (*Phelsuma madagascariensis*) at the National Zoological Park.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All <em>P. m. grandis</em></td>
<td>All <em>P. m. martensi</em></td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><em>P. m. grandis</em>-Nov 1975-Dec 1984</td>
<td><em>P. m. grandis</em>-Jan 1985-Nov 1995*</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><em>P. m. grandis</em>-Nov 1990-Jan 1995</td>
<td>All <em>P. m. martensi</em></td>
<td>&gt; 0.15</td>
</tr>
<tr>
<td>All <em>P. madagascariensis</em> males</td>
<td>All <em>P. madagascariensis</em> females</td>
<td>&gt; 0.45</td>
</tr>
<tr>
<td><em>P. m. grandis</em> males</td>
<td><em>P. m. grandis</em> females</td>
<td>&gt; 0.18</td>
</tr>
<tr>
<td><em>P. m. martensi</em> males</td>
<td><em>P. m. martensi</em> females</td>
<td>&gt; 0.60</td>
</tr>
<tr>
<td><em>P. m. grandis</em> males</td>
<td><em>P. m. martensi</em> males</td>
<td>&gt; 0.90</td>
</tr>
<tr>
<td><em>P. m. grandis</em> females</td>
<td><em>P. m. martensi</em> females*</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><em>P. m. grandis</em>-primary gout</td>
<td><em>P. m. martensi</em>-primary gout*</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><em>P. m. grandis</em>-articular gout</td>
<td><em>P. m. martensi</em>-articular gout*</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><em>P. m. grandis</em>-renal gout*</td>
<td><em>P. m. martensi</em>-renal gout</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

* *P* values represent the probability that the differences between the two groups are due to random error.

* Indicates the group with the higher prevalence when a statistically significant difference was found.
AN UPDATE ON INCLUSION BODY DISEASE OF BOID SNAKES

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Abstract

A disease characterized by the presence of intracytoplasmic inclusions in cutaneous and visceral epithelial cells, and neurons, has been recognized in members of the families Boidae and Pythonidae for over 20 yr. This disease has been named inclusion body disease (IBD) of boid snakes.1 Starting in the late 1970s and extending into the mid 1980s, Burmese pythons (Python molurus bivittatus) were the most common boid snake diagnosed with this disease. In affected Burmese pythons, signs of central nervous system disease are typically seen. While also seen in boa constrictors (Boa constrictor) during this period of time, within the last 5 yr more cases have been seen in boa constrictors compared to Burmese pythons. The exact explanation for this shift is not known but may have to do with increased numbers of boa constrictors been bred for the pet trade and the ease in which these snakes are transported for breeding and sale. Initially, clinical signs in boa constrictors included regurgitation and signs of central nervous system disease. However, over the last few years a number of snakes with atypical clinical manifestations have been seen including stomatitis, pneumonia, and lymphoproliferative disorders. While a retro-like virus was identified in tissues of affected snakes and Koch’s postulates were fulfilled using virus-positive tissue culture supernatant derived from kidney of an infected snake and injected into Burmese pythons,1 the original isolate was lost and never recovered. Recently, a virus has been isolated from an IBD-positive boa constrictor in our laboratory and the electron microscopic features and presence of reverse transcriptase activity in infected cell cultures support this virus as being a member of the family Retroviridae. This isolate will be injected into clinically healthy captive-bred Burmese pythons to determine its pathogenicity. We are in the process of purifying this virus for the production of specific antibodies in rabbits and the development of a virus-specific immunofluorescence assay. Currently, antemortem diagnosis is based upon the presence of intracytoplasmic inclusions in esophageal, stomach, and liver biopsies, and the presence of elevated peripheral white blood cell counts (> 30,000 cells/µl). The exact route of transmission is unknown. The snake mite, (Ophionyssus natricis), has been seen in multiple epizootics of IBD and may be involved in transmission. Direct contact and venereal spread may be involved. Some infected snakes may act as silent carriers, not exhibiting clinical signs for many years. Control centers around identification and elimination of known positive animals.

Resumen

Una enfermedad caracterizada por la presencia de inclusiones intracitoplasmáticas en células del epitelio cutáneo y visceral y neuronas ha sido reconocida en miembros de la familia Boidae y Pythonidae por más de 20 años. Esta enfermedad ha sido llamada Enfermedad de cuerpos de inclusión (IBD) en boas. Empezando al final de los 1970’s y extendiéndose a la mitad de los 1980’s, pitones de Burma (Phyton molurus bivittatus) fueron las pitónidos más comúnmente diagnosticadas con esta enfermedad. En pitones de Burma, signos de una enfermedad del sistema nervioso central son vistos...
típicamente. También se ha visto en boa constrictor durante este periodo de tiempo. En los últimos cinco años se han visto más casos en boa constrictor en comparación con los de pitones de Burma. La explicación exacta de esto no es bien conocida pero puede tener relación con el incremento de boas que son criadas para el mercado de mascotas y la facilidad con que estos animales son transportados para su crianza y venta. Inicialmente los signos clínicos en la boa constrictor incluyen regurgitación y señales de una enfermedad del sistema nervioso. Hace pocos años, un número de serpientes con síntomas irregulares han aparecido, incluyendo estomatitis, neumonía y desórdenes linfoproliferativos. Mientras que un virus similar a un retrovirus fue identificado en tejidos de serpientes afectadas y los postulados de Koch fueron realizados usando el sobrenadante del cultivo de tejidos con virus positivo derivados del riñón de una serpiente infectada e inyectado en pitones de Burma, el aislamiento original fue perdido y nunca recuperado. Recientemente un virus ha sido aislado de una boa constrictor IBD positivo en nuestro laboratorio y su presencia en el microscopio electrónico así como la presencia de actividad de la transcriptasa inversa en cultivos celulares infectados apoya a este virus como miembro de la familia Retroviridae. Este virus aislado será inyectado a pitones de Burma clínicamente sanos y criados artificialmente en cautiverio para determinar su patogenicidad. Estamos en el proceso de purificar este virus para la producción de anticuerpos específicos en conejos y el desarrollo de un virus inmunofluorescente de prueba. Actualmente el diagnóstico antemortem está basado en la presencia de inclusiones intracitoplasmáticas en biopsias de esófago, estómago e hígado, y la presencia de recuentos elevados de glóbulos blancos periféricos (>30,000 células/µl). La ruta exacta de transmisión es desconocida. El ácaro de la serpiente (Ophionyssus natricis) ha sido vista en múltiples epizootias de IBD y puede estar involucrado en la transmisión. El contacto directo y venéreo puede estar relacionado. Algunas serpientes infectadas pueden actuar como portadoras sanas sin exhibir signos clínicos por muchos años. Existen centros de control que identifican y eliminan a los animales positivos ya conocidos.

ACKNOWLEDGMENTS

The author thanks Ms. Sylvia Tucker, Ms. Betty Hall, Dr. Bruce Homer, and Dr. Ayalew Mergia for their technical assistance.

LITERATURE CITED

MYCOPLASMA EPIZOOTIC IN A HERD OF BULL ALLIGATORS (*Alligator mississippiensis*)

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Abstract

Nine bull American alligators (*Alligator mississippiensis*) from a herd of 74 conspecifics died within a 10 day period. This herd was composed entirely of 3-4 m long, 200-300 kg, adult male alligators that were older than 30 yr. The herd was housed in a 20 x 50 m outdoor exhibit with a centrally located spring-fed pool. Management was not aware of any recent exposure to other reptiles, infectious agents, nor toxic insult.

Clinical signs shown by the affected alligators were vague but included anorexia, lethargy, muscle weakness, paraparesis, bilateral white ocular discharge, and varying degrees of periocular, facial, cervical, and limb edema. Necropsy evaluation of four alligators that died with these signs and five that were euthanatized showed pneumonia in all cases (Table 1). Aerobic and anaerobic cultures of lung tissue yielded mixed populations of bacteria, including *Morganella morganii*, *Proteus vulgaris*, β-hemolytic *Streptococcus* spp., *Salmonella* spp., *Aeromonas* spp., *Enterobacter* spp., *Enterococcus* spp., and *Clostridium bifermentans*. Different bacteria were isolated from different individuals and there was no pattern to aid in pinpointing the disease etiology (Table 1). Virus isolation attempts on lung samples were negative.

Blood cultures yielded *Clostridium* spp. in two of four affected and two of two apparently healthy alligators. Complete blood counts and plasma biochemistry analyses from several individuals did not demonstrate consistent abnormalities among affected alligators. White blood cell distribution patterns showed a trend toward a relative lymphocytosis and hyperfibrinogenemia. Tissue toxicant, heavy metal and mineral analyses of liver and kidney did not support acute toxicity. No contributory deficiencies in vitamin A, vitamin E, nor selenium were detected in serum or tissue analyses.

Culture and polymerase chain reaction (PCR) screening of fresh lung and synovial fluid of affected alligator #14 revealed the presence of a rapidly growing mycoplasma. Subsequent culture of banked frozen lung and fresh lung and fresh synovial fluid on newly-affected alligators confirmed the presence of a *Mycoplasma* spp. in seven of eight individuals tested.

Efforts to control the spread of disease included water chlorination (1 ppm chlorine) and reduction of herd density. Intramuscular treatment with oxytetracycline at approximately 10 mg/kg (based upon estimated body weight) every 7 days was instituted for 4 wk. After 4 wk of treatment, the administration interval was increased to every 14 days.

Five months after the index case fourteen of the initial 74 alligators remain. Five animals were euthanatized for diagnostic necropsy. Thirty-three animals died within the first month before the
Eight additional animals died over the 4-mo treatment period. Thirteen animals were euthanatized for humane reasons. The onset of the winter season, a generally quiescent time for alligators in the southeastern climate, made clinical evaluation of improvement or response to treatment difficult. Permission was not granted for complete necropsies on euthanatized animals from the treatment group. Lung and trachea samples from two euthanatized treatment animals were evaluated for mycoplasma via culture. Mycoplasma was not recovered from these specimens; however, these individuals were never evaluated for the presence of the organism prior to treatment. The source of the implicated Mycoplasma organism has not been identified.

Occurring worldwide, mycoplasmas are the smallest free-living bacteria and generally produce clinically silent, chronic infections. In animals, articular surfaces, mucous membranes of the upper respiratory tract, intestinal, and genital tract, as well as, the mammary gland may harbor both pathogenic and non-pathogenic mycoplasmas. Stress, environmental factors, and synergism with other infectious agents predisposes to recrudescence of latent or low grade infections. Vertical, venereal, and aerosol transmission has been shown to occur with various species of Mycoplasma. In general, mycoplasmas are host specific and do not survive well outside their host.

Five crocodile farms in Zimbabwe reported outbreaks of polyarthritis that was attributed to a Mycoplasma spp. in 1-3 yr-old rearing stock. The Zimbabwe isolates resembled Mycoplasma capricolum, which causes clinical syndromes in goats; however, the isolate differed serologically. Our reported isolate appears to be a new species in the mycoplasma family as its nucleotide sequence does not match that of any characterized mycoplasma (over 75 species), including the chelonian mycoplasma, Mycoplasma agassizii. Along with the Zimbabwe isolates, our isolate grows rapidly, producing colonies within 24 hr, while most Mycoplasma spp. colonies require a 2-21 day growth period.

Animal density, geriatric clinical state, and mycoplasma virulence likely contributed to the course of disease in this population of American alligators.

**Resumen**

Nueve caimanes toro de una manada de 74 murieron en un periodo de 10 días. Esta manada estaba compuesta totalmente por machos adultos de 3-4 mts. de largo y con un peso de 200-300 kg que tenían una edad de más de 30 años. La manada fue albergada en un exhibidor de 20 x 50 mts. al aire libre con un estanque central. En el manejo no se tuvo conocimiento de ninguna exposición con otros reptiles, agentes infecciosos o tóxicos.

Los signos clínicos mostrados por los caimanes afectados fueron vagos pero incluyeron anorexia, letargia, debilidad muscular, paraparesis, descarga ocular blanca bilateral y varios grados de edema periorcular, facial, cervical y de las extremidades. La evaluación de la necropsia de 4 caimanes que murieron con esos signos y de 5 que fueron eutanasiados, mostró neumonía en todos los casos (Tabla 1). Los cultivos aeróbicos y anaeróbicos de tejido pulmonar mostraron poblaciones mixtas de bacterias, incluyendo Morganella morgani, Proteus vulgaris, Estreptococos hemolíticos spp, Salmonella spp., Enterobacter spp., Enterococos spp., y Clostridium bifermentans. Diferentes bacterias fueron aisladas de diferentes individuos y no había un patrón para precisar la etiología de
la enfermedad (Tabla 1). Los intentos por aislar virus en las muestras de pulmón fueron negativos.

Los cultivos sanguíneos fueron positivos a *Clostridium* spp. en 2 de 4 afectados y en 2 de 4 aparentemente sanos. Los recuentos sanguíneos completos y análisis bioquímicos del plasma de varios individuos no demostraron anormalidades consistentes en los caimanes afectados. Los patrones de distribución de las células blancas sanguíneas mostraron una tendencia a una linfocitosis relativa e hiperfibrinogenemia. Análisis tóxicos en tejidos, metales pesados y minerales en el hígado y en el riñón no mostraron una toxicidad aguda. No fue detectada la deficiencia de vitamina A, E o selenio en el análisis del suero o tejidos.

El cultivo y la reacción en cadena de la polimerasa (PCR) en muestras frescas de pulmón y líquido sinovial del caimán afectado #14 reveló la presencia de un mycoplasma de rápido crecimiento. Subsecuentes cultivos de pulmón congelado y fresco y de líquido sinovial fresco de un caimán recientemente afectado confirmó la presencia de un *Mycoplasma* spp., en 7 de 8 individuos muestreados.

Los esfuerzos para controlar la propagación de la enfermedad incluyeron la cloración del agua (1ppm de cloro) y la reducción de la densidad del hato. Fue instituido un tratamiento intramuscular con oxitetraciclina a una dosis aproximada de 10 mg/kg (basado en el peso corporal estimado) cada 7 días por 4 semanas. Después de 4 semanas de tratamiento el intervalo de administración fue aumentado a cada 14 días.

Cinco meses después del inicio del caso, de los 74 lagartos sólo quedan 14. Cinco animales fueron eutanasiados para realizar la necropsia para el diagnóstico. Treinta y tres animales murieron dentro del primer mes después de iniciar la terapia antibiótica. Ocho animales murieron durante los 4 meses del periodo de tratamiento. A 13 animales se les practicó la eutanasia por razones humanitarias. Al llegar el invierno, que es una temporada de quietud para los caimanes en el sudeste, la evaluación clínica, la implementación y la respuesta del tratamiento fue difícil. No fue otorgado el permiso para realizar una necropsia completa en los animales eutanasiados del grupo del tratamiento. Muestras de pulmón y traquea de dos individuos del grupo de tratamiento a los que se les realizó la necropsia fueron evaluadas contra *Mycoplasma* por cultivo. El *Mycoplasma* no fue identificado en esos especímenes; sin embargo, esos individuos nunca fueron evaluados para demostrar la presencia del organismo antes del tratamiento. La ruta del *Mycoplasma* no ha sido identificada.

Presente en todo el mundo, el *Mycoplasma* es la bacteria más pequeña de vida libre y generalmente produce una infección clínica silenciosa y crónica. En animales, las articulaciones, la membrana mucosa del tracto respiratorio superior, intestinal y genital, así como la glándula mamaria pueden albergar Mycoplasmas patógenos o no patógenos. El estrés, factores ambientales y el sinergismo con otros agentes infecciosos, predispone a un agravamiento de infecciones latentes o de menor grado. Se ha visto que ocurre transmisión vertical, venérea y por aerosoles en varias especies de *Mycoplasma*. En general, los Mycoplasmas tienen un huésped específico fuera del cual no logran sobrevivir por largo tiempo.

Cinco cocodrilos de una granja en Zimbabwe reportaron un brote de poliartritis que fue atribuido al *Mycoplasma* spp., en un grupo de 1-3 años de crianza. En Zimbabwe aislaron un organismo parecido al *Mycoplasma*, que causa síndromes clínicos en cabras. Sin embargo el organismo aislado
difirió serológicamente. Nuestro aislamiento reportado parece ser una nueva especie en la familia del mycoplasma, debido a que su secuencia de nucleótidos no se tiñe como ningún otro *Mycoplasma* identificado (alrededor de 75 especies) incluyendo el micoplasma de las tortugas, *Mycoplasma agassizii*. Junto con el microorganismo aislado en Zimbabwe, nuestro organismo aislado crece rápidamente, produciendo colonias a las 24 hrs., mientras que la mayoría de las colonias de *Mycoplasma* spp., requieren de un periodo de crecimiento de 2 a 21 días.

La densidad animal, el estado geriátrico de los caimanes y la virulencia del *Mycoplasma* posiblemente contribuyó al curso de la enfermedad en esta población de caimanes americanos.

**LITERATURE CITED**

Table 1. Summary of gross and histopathologic findings on necropsied alligators from affected enclosure.

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Condition</th>
<th>Lung lesions</th>
<th>Lung culture</th>
<th>Other lesions</th>
<th>Other culture</th>
<th>Mycoplasma status</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 (N95-684)</td>
<td>Autolyzed carcass</td>
<td>Fibronecrotic pneumonia</td>
<td><em>Morganella morganii</em> (60%) and <em>Proteus vulgaris</em> (30%)</td>
<td>Lead pellets in stomach</td>
<td>Not done</td>
<td>Not done</td>
<td>Elevated lead levels in hepatic, renal, and gastric luminal tissue</td>
</tr>
<tr>
<td>02 (N95-690)</td>
<td>Autolyzed carcass</td>
<td>Mild acute pneumonia</td>
<td><em>β</em>-hemolytic <em>Streptococcus</em> sp.</td>
<td>Necrosis of abdominal fat body</td>
<td><em>Edwardsiella tarda</em> (fat body)</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td>08 (N95-701)</td>
<td>Fresh carcass</td>
<td>Granulomatous pneumonia</td>
<td>Mixed growth with subgroup 3 <em>Salmonella</em> sp (95%)</td>
<td>Necrotizing myositis and edema, pectoral muscles</td>
<td>Not done</td>
<td>Frozen lung <em>Mycoplasma</em> culture positive</td>
<td></td>
</tr>
<tr>
<td>09 (N95-713)</td>
<td>Autolyzed carcass</td>
<td>Fibrinous pneumonia</td>
<td><em>Proteus vulgaris</em> (60%); <em>Aeromonas</em> sp (40%)</td>
<td>Myocarditis and epicarditis; multifocal arthritis</td>
<td>Not done</td>
<td>Frozen lung <em>Mycoplasma</em> culture positive</td>
<td></td>
</tr>
<tr>
<td>10 (N95-714)</td>
<td>Fresh euthanasia</td>
<td>Mild interstitial pneumonia</td>
<td>Aerobic negative; <em>Clostridium bifermantans</em>; <em>Penicillum</em> sp (1 colony)</td>
<td>Fibrinous pericarditis; fibrinous arthritis; splenomegaly; cholangiohepatitis; nephritis; tongue abscess; portal hepatitis</td>
<td><em>Edwardsiella tarda</em> (pericardial fluid); no growth (joint fluid)</td>
<td>Frozen lung <em>Mycoplasma</em> culture positive</td>
<td></td>
</tr>
<tr>
<td>12 (N95-723)</td>
<td>Fresh euthanasia</td>
<td>Mild peribronchiolar pneumonia</td>
<td>Aerobic negative</td>
<td>Endocarditis; endocardiosis; portal hepatitis; submandibular abscess</td>
<td><em>Clostridium sporogenes</em> (blood culture); no growth (liver, submandibular abscesses)</td>
<td>Not done</td>
<td>Virus isolation negative; Electron microscopy of lung, liver, kidney, and fat unremarkable</td>
</tr>
<tr>
<td>14 (N95-740)</td>
<td>Fresh euthanasia</td>
<td>Interstitial pneumonia</td>
<td>Aerobic negative; fungal negative</td>
<td>Portal hepatitis; granulomatous myocarditis</td>
<td>3 types <em>Clostridium</em> sp (blood)</td>
<td>Fresh lung positive on <em>Mycoplasma</em> culture and PCR</td>
<td>Joint fluid positive on <em>Mycoplasma</em> culture and PCR</td>
</tr>
<tr>
<td>45 (N95-792)</td>
<td>Fresh euthanasia</td>
<td>Pyogranulomatous pneumonia</td>
<td><em>Enterobacter</em> sp (70%) and <em>Enterococcus</em> sp (30%); fungal negative</td>
<td>Hemosiderosis; nephritis; edema; periocular abscess; cholelith</td>
<td>Aerobic negative (liver)</td>
<td>Fresh lung negative culture</td>
<td>Elevated mercury and selenium levels in hepatic and renal tissue</td>
</tr>
<tr>
<td>46 (N95-793)</td>
<td>Fresh euthanasia</td>
<td>Mild Interstitial pneumonia</td>
<td>Not done</td>
<td>Hemosiderosis; synovitis</td>
<td>Aerobic negative (liver)</td>
<td>Fresh lung <em>Mycoplasma</em> culture positive</td>
<td>Joint fluid <em>Mycoplasma</em> culture positive</td>
</tr>
</tbody>
</table>
CYTOGENETIC TECHNIQUE FOR KARYOTYPING IN *Heloderma horridum horridum* IN THE GUADALAJARA ZOO

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Abstract

Karyotyping was carried out on cultured lymphocytes from 6 individuals of the *Heloderma horridum horridum* species. Fourteen macrochromosomes (8 metacentric and 6 submetacentric) and 18-22 microchromosomes were observed. It was not possible to differentiate a sexual pair. Further evaluation with radiography and ultrasonography demonstrated that all 6 individuals were males.

Resumen

Fué realizado el cariotipo de cultivo de linfocitos de 6 individuos de la especie *Heloderma horridum horridum* fueron observados 14 macrocromosomas (8 metacentricos y 6 submetacentricos) y 18-22 microcromosomas. No se pudieron diferenciar cromosomas sexuales. Evaluaciones con radiología y ultrasonografía demostraron que los 6 individuos eran machos.

Introduction

One of the most important missions of institutions which maintain wild animals is the reproduction of endangered species. This study investigates the lizard species *Heloderma horridum horridum*. Captive breeding of these reptile has been complicated by the lack of sexual dimorphism. Several published reports have described possible sexing methods of Heloderma species, but no research has focused on cytogenetic studies. Chromosomal studies of reptiles have previously been limited due to the difficulty in obtaining samples, low cellular cultivation, and difficulties in chromosomal slide preparation. Karyologic studies of reptiles show that 215 of the 1,074 species evaluated have distinguishable sex chromosomes with the highest incidence of sex chromosomes being found in snakes. Of the 3,307 living Saurian species, about 607 species have been karyotyped and 85 of these species show sex chromosomes. Three gonosomal types have been identified: 1) Simple male heteromorphism (XY), most frequently in Iguanidae; 2) Simple female heteromorphism (ZW); multiple sex chromosomes (X₁, X₂Y and Z₁,Z₂W) have been described.

Materials and Methods

Six *Heloderma horridum horridum* from the Guadalajara Zoo were used in the study. Animals were individually identified by dermal markings. Age and sex were unknown on all animals. Heparinized blood samples were collected from the caudal tail vein (1.5-3 ml). Lymphocytes were obtained via a cell suspension of autologous plasma and buffy coat (ap/bc). Cultures were grown on RPMI-60 medium fortified with 20% Fetal Bovine Serum, 2% L-glutamine (45 mg/ml) and protected with 2% antibiotics in a total volume of 10 ml.
Lymphocytes were stimulated with 0.06 ml phytohemagglutinin (PHA-P) and 0.06 ml pokeweed. Cultures were incubated at 27-29 C. Metaphase lymphocyte stages were obtained by adding 0.06 ml of demicolchicine (0.05 mg/ml) for 5 hr. A hypotonic solution of KCl (0.075 M) was used for 30 min at 27-29 C to swell the cells and separate the chromosomes. The sample was fixed with a methanol:acetic acid solution (3:1).

Fixed preparation of chromosomes were made and stained with the Giemsa technique. Due to difficulties matching chromosomes, the C banding technique was utilized.

Results

Chromosomes were obtained from all six animals that were sampled. Fixed preparations demonstrated 15-70 metaphyses. This karyotyping demonstrates the diploid number (2n) to be 34, some metaphase variation between 32-36 was noted and attributed to the microchromosomes (Table 1). The samples were unable to establish morphology of a sex chromosomes. Using the C Banding technique, no bands were observed and chromosomes could not be matched due to blurring. Karyotyping was based upon the size of chromosome and position of centromere (Figs. 1 and 2).

Discussion

The karyotype of all six Heloderma horridum demonstrated four pairs of metacentric chromosomes (pairs 1,3,4,5) and three pairs of submetacentric chromosomes (pairs 2,6,7). These results indicate either that there is no difference in the sex chromosomes in Heloderma lizards or that all animals were of the same sex. Karyotyping of reptiles has not been commonly performed and most of the saurian species do not show distinguishable sex chromosomes.

The C Banding technique is widely used in avian species and stains the W chromosome an intense color. Samples from the lizards in this study did not demonstrate the intense band.

In a separate, but related study, bone marrow was collected from a female Heloderma suspectum at necropsy. The karyotype obtained from this sample demonstrated three pairs of metacentric chromosomes (pairs 1, 4, 5) and three pairs of submetacentric chromosomes (pairs, 2,6,7). In pair number 3 a difference was noted in the position of the centromere. One chromosome was metacentric while the other was submetacentric, and ranged from 18-22 microchromosomes (Fig. 2). Further work is presently underway to determine if this difference in pair number 3 is consistent and could be used for genetic sexing of animals.

Other methods of sexing monomorphic lizards include radiography, ultrasonography, laparoscopy and hemipene eversion. Radiographs of sexually mature, similar sized Heloderma suspectum demonstrates a variation in the ischium bone length between male and females. (In the present study, radiographs of similar sized animals showed no differences in the ischium bones.) However, two different forms of pelvis were noted (Fig. 3). Structural variations occur in pelvic anatomy of certain mammals, but have not been well-documented in other groups of vertebrates. Studies are underway to further investigate these anatomical variations. Ultrasonography of mature female Helodermated lizards has demonstrated ovarian follicles. Ultrasonography of the six lizards in this study did not allow visualization of gonads or associated reproductive tissues. Neither laparoscopy
or hemipene eversion was performed on these animals.

ACKNOWLEDGMENTS

To Dr. J. Genaro Santoscoy Gomez and to Dr. José Antonio Ornelas Sanchez for Unidad de Patologia Clinica for their collaboration with the radiology techniques and the ultrasonic techniques. To Mrs. Barbara Keenan for the translation of the manuscript. To Francisco Rodriguez Herrejón, D.V.M., Director of the Guadalajara Zoo and the staff of Herpetarium of the Guadalajara Zoo.

LITERATURE CITED

Table 1. Characteristic of the karyotype of Heloderma.

<table>
<thead>
<tr>
<th>Specimen number</th>
<th>No. of microchromosomes</th>
<th>No. of microchromosomes</th>
<th>Centromere position in the pairs</th>
<th>Sex chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-032-01</td>
<td>14</td>
<td>18-.20</td>
<td>M S M M M S S S</td>
<td>?</td>
</tr>
<tr>
<td>R-032-02</td>
<td>14</td>
<td>18-22</td>
<td>M S M M M S S S</td>
<td>?</td>
</tr>
<tr>
<td>R-032-03</td>
<td>14</td>
<td>20-22</td>
<td>M S M M M S S S</td>
<td>?</td>
</tr>
<tr>
<td>R-032-04</td>
<td>14</td>
<td>20</td>
<td>M S M M M S S S</td>
<td>?</td>
</tr>
<tr>
<td>R-032-05</td>
<td>14</td>
<td>18-20</td>
<td>M S M M M S S S</td>
<td>?</td>
</tr>
<tr>
<td>R-032-06</td>
<td>14</td>
<td>20</td>
<td>M S M M M S S S</td>
<td>?</td>
</tr>
<tr>
<td><em>Heloderma suspectum</em></td>
<td>14</td>
<td>18-22</td>
<td>M S M/S M M S S</td>
<td>?</td>
</tr>
</tbody>
</table>

M= Metacentric
S = Submetacentric
Figure 1. Karyotype of *Heloderma horridum horridum*, showing seven pairs of macrochromosomes.
Figure 2. Karyotype of *Heloderma suspectum* showing a metacentric chromosome and a submetacentric chromosome in the third pair of macrochromosomes.

*Heloderma suspectum* No.145

Zoológico Guadalajara

R. 23.30
Figure 3. Radiographs of *Heloderma horridum horridum*, two different forms of pelvis, A: rhomboidal, and B: Oval.
SPECTACLE WOUND HEALING OF THE BALL PYTHON (Python regius)

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Abstract

The spectacle of snakes forms from the embryonic fusion of the upper and lower lids. It has a well-developed superficial keratinized layer and a more deeply located germinal layer that contains a vascular supply. Immediately posterior to the spectacle is the subspectacular space which contains tears. The spectacle provides protection for the cornea, must be transparent for normal vision, and its keratinized layer is shed during the normal process of ecdysis. The most common ophthalmic problems seen in snakes involve the spectacle either directly (retained spectacle) or indirectly (subspectacular abscess). The spectacle can develop defects as a result of disease processes (spectaculitis or severe subspectacular abscesses), can be mistakenly avulsed in the treatment of retained spectacles or a surgical defect may be intentionally created during the treatment of subspectacular abscesses. Any defect in the spectacle exposes the underlying cornea and spectacle wound healing must occur in order to reestablish ocular integrity. We initiated this study to evaluate normal spectacle wound healing in the ball python.

Twenty five ball pythons ranging in weight from 0.26-1.3 kg were purchased from a commercial vendor, treated for parasites (Ivermectin s.c. 0.2 mg/kg), and allowed to acclimate at the University of Wisconsin SVM Laboratory Animal Facility for 60 days prior to surgery. A complete ophthalmic examination was performed on all snakes. All snakes were intubated, anesthesia induced and maintained with isoflurane. A 30-ga needle was introduced into the subspectacular space and the space distended by injection of 2.5% methylcellulose. A Vannas scissors was then used to resect 25% of the spectacle in a “pie shaped” wedge from the inferior-temporal portion of one eye. The fellow eye was not operated in 20 snakes, and these served as the controls for comparison. Five snakes had surgery done on both eyes separated by 2.5 mo. A single drop of topical triple antibiotic solution (bacitracin, polymyxin, neomycin) was applied to the surgical wound at the end of surgery. No other medications were employed in the remainder of the study.

Sequential slit lamp exams were performed and observations recorded. Groups of five snakes were euthanatized at differing time points after spectacle wounding; from 24 hr postoperatively up to 3 mo. In each group, two animals were injected with silicone to visualize the effect of wounding on the vascular supply to the spectacle. After euthanasia, the eyes were fixed in either formalin or Bouin’s solution and sectioned through the wound for histological evaluation.

Engorgement of the spectacle vessels and edema adjacent to the wound edges was observed immediately postoperatively and subsided over several weeks. An amorphous plaque of homogeneous material completely filled the defect within 24 hr and allowed reestablishment of the subspectacular space within 7 days. A variable heterophil infiltrate of the spectacle occurred immediately post operatively and subsided over 30 days. The epithelium of the spectacle was seen to migrate under the amorphous plaque by 3 wk postop, reestablishing a germinal center for
production of new spectacle material. By 3 mo many animals evidenced substantial progress towards reestablishment of normal spectacle morphology.

Resumen

El lente de las serpientes se forma de la fusión embrionaria de los párpados inferiores y superiores. Tienen una capa superficial queratinizada bien desarrollada y una capa germinal localizada más profundamente que contiene el abastecimiento vascular. Inmediatamente después del lente está el espacio sublenticular el cual contiene las lágrimas. El lente provee protección a la córnea, debe ser transparente para que exista una visión normal y su capa queratinizada se desprende durante el proceso normal de ecdisis. El problema oftálmico más común que se ha visto en serpientes involucra a un lente o a otro directa (retención de lente) o indirectamente (absceso sublenticular). El lente puede desarrollar defectos como resultado de una enfermedad (lenticulitis o absceso severo sublenticular) y puede desprenderse incorrectamente en el tratamiento de retención de lentes o un defecto quirúrgico puede ser causado intencionalmente durante el tratamiento del absceso sublenticular. Cualquier defecto del lente expone principalmente a la córnea y la curación de la lesión del lente debe ocurrir para restablecer la integridad ocular. Nosotros iniciamos este estudio para evaluar la curación normal de las lesiones del lente en el Pitón bola.

Se adquirieron 25 pitones bola que variaban en peso entre 0.26-1.3 kg, de un vendedor comercial, se desparasitaron (Ivermectina s.c. 0.2 mg/kg) y su aclimatación fue en el laboratorio animal SVM de la Universidad de Wisconsin durante 60 días antes de la cirugía. A todas las serpientes se les realizó un examen oftálmico completo. Todas fueron intubadas, se indujo la anestesia y se mantuvo con isoflurano. Una aguja del 30 fue introducida al espacio sublenticular y el lente fue dilatado inyectándole metilcelulosa al 2.5%. El 25% del lente fue seccionado con una tijeras Vannas en forma triangular, en la porción infero-temporal del ojo. El otro ojo no fue operado en 20 serpientes, mismas que sirvieron como control de comparación. A 5 serpientes se les realizó cirugía en ambos ojos con un intervalo de 2.5 meses. Una sola gota de solución tópica de antibiótico triple (Bacitracina, polimixina, neomicina) fue aplicada a la lesión quirúrgica al final de la cirugía. Ningún otro medicamento fue aplicado durante el resto del estudio.

Se hicieron exámenes secuenciales con una lámpara y las observaciones fueron registradas. Se aplicó eutanasia a grupos de 5 serpientes a diferentes tiempos una vez que se efectuó la lesión del lente, desde 24 horas post intervención hasta 3 meses después. En cada grupo, dos animales fueron inyectados con silicón para visualizar el efecto de las lesiones en la irrigación vascular del lente. Después de la eutanasia, los ojos fueron fijados con solución de formalina o de Bourns, y seccionados a través de la lesión para efectuar una evaluación histológica.

Inmediatamente después de la operación se observó una dilatación del vaso sanguíneo del lente y un edema adyacente en los bordes de la lesión, que desapareció después de varias semanas. Una placa amorfa de material homogéneo, llenó completamente el defecto en 24 hr y permitió el restablecimiento del espacio sublenticular en 7 días. Una infiltración variable de heterófilos ocurrió inmediatamente después de la operación y se mantuvo por 30 días. Se observó migración del epitelio del lente hacia abajo de la placa amorfa por 3 semanas después de la operación restableciendo un centro germinal para la producción del material para el nuevo lente. En tres meses varios animales
evidenciaron un progreso substancial hacia un restablecimiento de la morfología normal del lente.
PLASMA ITRACONAZOLE PHARMACOKINETICS IN SPINY LIZARDS (Sceloporus spp.) FROM ONCE-DAILY DOSING

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Abstract

Fungal infections in reptiles are not uncommon and can present as a primary disease problem or be associated with a secondary infection in already compromised individuals. Antifungal drugs may provide appropriate treatment options but treatment regimens have not been established for most reptiles. Itraconazole (Sporanox, Janssen Pharmaceutica, Inc., Titusville, New Jersey 08560, USA) is an oral human antifungal drug which is efficacious for many fungal pathogens of man. The pharmacokinetics of itraconazole in mammals and avian species is under investigation but there have not been any reports of its use in reptiles. This report investigates the pharmacokinetics of itraconazole in spiny lizards (Sceloporus spp.)

Thirty-five lizards were divided into 10 groups (3-4 individuals per group). The small size of individual animals precluded individual sample analysis and thus plasma was collected and pooled from each group following itraconazole administration. Animals were orally gavaged with itraconazole and a protein based baby food at 23.5 mg/kg (mean), s.i.d. for three consecutive days. This dose was based on itraconazole trials in peacocks (20-30 mg/kg). Blood was collected into heparinized syringes by cardiopuncture on days 0,1,2,3,4,6,9,12, and 18.

Plasma itraconazole levels were determined by microbiological assay (Fungal Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas 78284, USA). Untreated Spiny lizard plasma was provided as a specific control. Time-plasma concentration data were plotted semilogarithmically to identify pharmacokinetic values.

Drug elimination was evaluated as a loading dose administration. The area under the curve (AUC) for itraconazole was 377.21 µg/hr/ml and terminal elimination half-life was 48.3 hr. Itraconazole reached a peak concentration of 2.48 µg/ml in two half-lives. Commonly five half-lives are required to reach steady state plasma concentrations. Itraconazole would be expected to achieve steady state in 10 days with a concentration of at least 3.1 µg/ml. Using the above dosing regimen, itraconazole concentrations would be above the literature minimum inhibitory concentration for many fungal pathogens for 6-8 days beyond the peak concentration.

Resumen

Las infecciones fungales en reptiles son comunes y pueden presentarse como un problema primario o estar asociadas a una infección secundaria en individuos comprometidos. Las drogas antimicóticas pueden proveer un tratamiento adecuado pero los regímenes no han sido establecidos para la mayor parte de los reptiles. El Itraconazole (Sporanox, Janssen Pharmaceutica, Inc., Titusville, New Jersey 08560, USA) es un antifúngico oral que es eficaz para varios hongos patógenos en el hombre. La farmacocinética del Itraconazol en mamíferos y aves está bajo investigación pero no ha habido
ningún reporte de su uso en reptiles. Este reporte investiga la farmacocinética del Itraconazole en lagartos espinosos.

Treinta y cinco lagartos fueron divididos en tres grupos (tres a cuatro individuos por grupo). El pequeño tamaño de los animales impidió el análisis de muestras individuales, así que el plasma fue colocado y combinado por cada grupo después de la administración de Itraconazole. Los animales fueron medicados oralmente con Itraconazole a una dosis de 23.5 mg/kg (promedio) y con un alimento proteínico para bebés, una vez al día por tres días consecutivos. Esta dosis estuvo basada en el conocimiento de las dosis de Itraconazole en pavos (20 a 30 mg/kg). Se recolectó sangre en jeringas heparinizadas por medio de punción cardíaca en los días 0, 1, 2, 3, 4, 6, 9, 12 y 18.

Los niveles plasmáticos de itraconazole fueron determinados por ensayos microbiológicos (Fungal Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas 78284, USA). El plasma de los lagartos no tratados sirvió de control. La curva de concentración-tiempo en el plasma fue trazada semilogarítmicamente para identificar los valores farmacocinéticos.

La eliminación de la droga fue evaluada de acuerdo a la dosis administrada. El área bajo la curva (AUC) para el Itraconazole fue 377.21 µg/hr/ml y la vida media hasta la terminación total fue de 48.3 hr. El Itraconazole alcanzó un máximo de concentración de 2.48 µg/ml en dos vidas medias. Comunmente cinco vidas medias son requeridas para que la concentración en plasma alcance su máxima estabilidad. Se puede esperar que el Itraconazole tenga un nivel continuo en 10 días con una concentración de 3.1 µg/ml. Utilizando el régimen anteriormente descrito, las concentraciones del Itraconazole estarían arriba de lo que la literatura reporta como concentración mínima para inhibir la mayoría de los hongos patógenos por 6 a 8 días más allá del pico de concentración.
ANESTHETIC TECHNIQUES IN KOMODO DRAGONS (*Varanus komodoensis*)

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Abstract

Anesthetic protocols were developed for routine diagnostic procedures in Komodo dragons (*Varanus komodoensis*). Young komodo dragons (1.5-3.5 yr) weighing 1-7 kg were manually restrained for anesthetic induction with isoflurane/oxygen via facemask. Anesthesia was induced in adult Komodo dragons weighing 11-55 kg with either tiletamine-zolazepam (5.5 mg/kg combined) or ketamine (10-12 mg/kg) combined with midazolam (0.2-0.5 mg/kg) administered intramuscularly in the forelimb via dart or hand syringe. Anesthesia was maintained using isoflurane/oxygen delivered via facemask or endotracheal tube. The anesthetic techniques described were suitable for physical examination, blood collection, radiography, transcutaneous and transintestinal ultrasonography.

Resumen

Los protocolos de anestesia fueron desarrollados para procedimientos rutinarios de diagnóstico en el dragón de Komodo (*Varanus komodoensis*). Dragones de Komodo jóvenes pesando de 1-7 kg fueron retenidos manualmente para la aplicación anestésica con isoflurano/oxígeno mediante una máscara. La anestesia inducida en dragones de Komodo adultos, pesando de 11-55 kg fue con tielatamina/zolazepam (5.5 mg/kg combinado) o con ketamina (10-12 mg/kg) combinado con midazolam (0.2-0.5 mg/kg) administrado intramuscularmente en el miembro anterior mediante dardo o manualmente con jeringa. La anestesia fue mantenida usando isoflurano/oxígeno mediante una máscara o sonda endotraqueal. Las técnicas de anestesia descritas fueron apropiadas para el examen físico, toma de muestras de sangre, radiografías y ultrasonido transcutáneo o transintestinal.

Introduction

Anesthetic techniques for large varanid lizards have not been well-described. Historically at the National Zoological Park, manual restraint has been adequate for routine procedures (physical examination, blood collection) in juvenile Komodo dragons (*Varanus komodoensis*). However, in order to fully evaluate an adult 55-kg male dragon for a chronic forelimb lameness, it was necessary to develop a protocol for chemical restraint. In addition, anesthesia was required for a collaborative study on transintestinal ultrasonography in young Komodo dragons for sex determination.4

Materials and Methods

Juvenile Komodo dragons (1.5-3.5 yr) ranging in weight from 1-7 kg were manually restrained for mask induction using 3% isoflurane (Aerrane, Ohmeda PPD Inc., Liberty Corner, New Jersey 07938, USA) delivered in oxygen at 1 L/min via a small canine facemask. For maintenance, isoflurane was delivered via mask or endotracheal tube (animals greater than 3 kg were intubated)
and concentrations were reduced incrementally (2 to 1%) to maintain a light plane of anesthesia for 20-30 min.

Anesthesia was induced in adult Komodo dragons (15-25 yr) ranging in weight from 11 to 55 kg with either 5.5 mg/kg (combined) tiletamine-zolazepam (Telazol, Fort Dodge Laboratories Inc., Fort Dodge, Iowa, USA) or 10-12 mg/kg ketamine (Ketaset, Aveco Co., Fort Dodge, Iowa 50501, USA) mixed with 0.2-0.5 mg/kg midazolam (5 mg/ml, Versed, Hoffman-LaRoche, Nutley, New Jersey 07110, USA). The induction agent was delivered intramuscularly via dart (Telinject USA, Saugus, California 91350, USA) or hand syringe in the forelimb. Following induction, the snout and limbs were secured with heavy tape and the animal was transported to the hospital. Supplemental isoflurane (2-3%) and oxygen (1-2 L/min) were delivered initially via a large canine facemask and then via endotracheal tube. Isoflurane concentrations were maintained as low as possible (1-2%) to prevent spontaneous movement during physical examination, radiography, transcutaneous and transintestinal ultrasonography (40-90 min).

For animals maintained on isoflurane via mask, heart rate was monitored using doppler (Ultrasound Doppler Flow Detector 811-BTS, Parks Medical Electronics, Inc., Aloha, Oregon, USA) with the probe placed on the ventral thorax or within the cloaca. For intubated animals, heart rate and relative oxyhemoglobin saturation was monitored using a Nellcor 180 pulse oximeter (Nellcor Inc., Hayward, California 94545, USA). Consistent SPO2 readings were obtained using the RS-10 reflectance transducer in the oral cavity. Respiratory rate was monitored by direct observation of thoracic excursions. Body temperature was maintained during anesthesia using heating pads. The juvenile Komodo dragons were placed in plastic holding containers for recovery and the adults were returned to their exhibit (ambient temperature 32°C). Anesthesia times and physiologic data were recorded and entered into MedARKS.

Results and Discussion

Mask induction with 3% isoflurane in young Komodo dragons was easily accomplished and rapid (2-4 min). Unlike many other lizard species which tend to hold their breath during isoflurane induction, the dragons breathed regularly. High concentrations of isoflurane (4-5%) were intentionally avoided in order to minimize respiratory depression. Throughout the anesthetic period, respiratory rate (8-12 breaths/min), relative oxyhemoglobin saturation (100%), and heart rate (30-50 beats/min) remained stable. Muscle relaxation was excellent during anesthesia, and complete recovery was rapid (5-7 min).

Two options were considered for anesthesia in adult Komodo dragons to ensure the safety of both staff and animals. Either a reversible injectable anesthetic protocol, or a relatively low dose of injectable anesthetic for induction followed by maintenance with isoflurane. The second approach was chosen for several reasons. Current information on the use of reversible α2-agonists or narcotic anesthetics in reptiles is limited, and these agents have not been evaluated in varanid lizards. By comparison, dissociative anesthetics have been used successfully to induce anesthesia in a variety of reptile species and generally have a wide margin of safety, particularly at low dosages.1,3,5,6,8

Tiletamine-zolazepam (5.5 mg/kg combined) was initially selected for anesthetic induction in the
55-kg adult male based upon ease of administration (small volume) and reported efficacy in large monitor lizards (D. Mader and P. Morris, personal communication, 1996). Anesthetic administration via dart was routine, although pole syringe delivery would also have been possible given the docile nature of this individual. Initial effects were observed within 4 min (ataxia, sedation) followed by a period of repetitive head and limb movements (paddling) which lasted from 10-25 min. The animal was easily handled at 30 min and supplemental isoflurane was required at 45 min to maintain light anesthesia for radiography. Heart rate (24 beats/min) and respiratory rate (1-3 breaths/min) were slow and regular throughout anesthesia and recovery. Eye and limb movements returned 1 hr after discontinuing isoflurane but heavy sedation persisted for the remainder of the day. The lizard appeared mildly sedated the following morning but was ambulatory.

Due to the prolonged recovery following tiletamine-zolazepam, ketamine combined with midazolam was used for the next induction in the adult male. Although limited studies have been conducted with ketamine and midazolam in reptiles, this combination in mammals typically produces more rapid recovery than tiletamine-zolazepam due to shorter half-lives of each component. The primary disadvantages of ketamine mixed with midazolam include larger drug volume and expense.

Anesthetic induction in the adult male with 11.6 mg/kg ketamine and 0.2 mg/kg midazolam was very similar to tiletamine-zolazepam, although muscle relaxation was not as complete after 30 min. Once restrained, the animal was handled as before and supplemented with isoflurane at 54 min. In comparison to tiletamine-zolazepam, both heart rate (40-50 beats/min) and respiratory rate (6-10 breaths/min) were higher during induction and maintenance on isoflurane. Recovery was less prolonged, with eye and limb movements returning 30 min after discontinuing isoflurane. Mild sedation persisted for several hours but the lizard was fully recovered the next day. A higher dosage of midazolam (0.34-0.45 mg/kg) combined with the same dosage of ketamine was subsequently used in two adult female Komodo dragons in an effort to smooth induction. Muscle relaxation was excellent in both females within 20 min of administration of ketamine and midazolam. Heart and respiratory rates were similar to those of the male, and recovery time was further reduced (4 hr).

Pulse oximetry provided useful information in anesthetized Komodo dragons. Despite intubation and regular respiratory rates on isoflurane, initial relative oxyhemoglobin saturation in adults induced with ketamine and midazolam or tiletamine-zolazepam were extremely low (less than 70%). Following a single mechanical ventilation, SPO$_2$ values increased markedly (95-100%). Intermittent positive pressure ventilation was subsequently administered in order to maintain adequate oxyhemoglobin saturation (90-95%) without depressing spontaneous respiration. By contrast, juvenile Komodo dragons masked down with isoflurane exhibited high relative oxyhemoglobin saturation levels (100%) throughout anesthesia.

**Conclusions**

Although the sample size was limited, several anesthetic techniques can be recommended for Komodo dragons. Both tiletamine-zolazepam and ketamine combined with midazolam were suitable induction agents in adults, followed by maintenance anesthesia using isoflurane. In subadult Komodo dragons, mask induction and maintenance with isoflurane was effective and easily
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LITERATURE CITED

SONOMORPHOLOGICAL SEX DETERMINATION IN SUBADULT KOMODO DRAGONS

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Abstract

Transintestinal sonography (TIS) is a non-invasive, practical method for early sex determination in Komodo dragons (Varanus komodensis) aged 19-36 mo and weighing 0.97-6.10 kg. Additionally, TIS can provide information on general physical condition and gonadal status of subadults and adults, which is useful for effective breeding management in this endangered species.

Resumen

El ultrasonido transintestinal (TIS), es un nuevo método no invasivo práctico para la determinación sexual temprana en dragones de Komodo (Varanus komodensis) de 19-36 meses de edad y pesando de 0.97-6.10 kg. Adicionalmente el TIS provee información general de la condición física y estado gonadal de subadultos y adultos, lo cual es útil para el manejo efectivo de la crianza de esta especie en peligro.

Introduction

The Komodo dragon (Varanus komodensis) is the largest extant lizard species, living in lowland areas of several islands, including Komodo, Flores and Rintja, of the Lesser Sunda archipelago in Indonesia. It is one of the most endangered lizard species and the existing population of 3,000-5,000 individuals range over a total of only 1,000 sq km. Since the first description of the Komodo dragon by Ouwens in 1912, it has been a very popular exhibit species in zoos because of its large size and fearsome reputation. However, captive breeding success outside of Indonesia has been limited to only two zoos (National Zoological Park and Cincinnati Zoo).

Intraspecific aggression in this species makes it preferable to house animals individually or in breeding pairs. The protracted juvenile phase (~5-10 yr) and absence of morphological and behavioral sexual dimorphism in subadults makes it necessary to develop early sexing techniques. Laparoscopy is the most widely used method of sex determination in varanid lizards5,6,7 because the use of sex probes is not effective in this group of reptiles. Although relatively simple, laparoscopy is still invasive and presents some degree of risk, especially problematic in such a rare, high-profile species. Cloacal manipulation has not been effective for sex determination in subadults. Female Komodo dragons have musk glands that can be mistaken for hemipenes when everted. Radiographic identification of the hemipenis structure in subadults has not yet been successful. Although transcutaneous ultrasonography is effective for sex and sexual cycle determination in other reptilian
species,\textsuperscript{3,4,8,10,11} its use in Komodo dragons is limited because of the hyperechoic skin plates which interfere with resolution of the fine detail of gonadal structures. Although effective for identifying females with large follicles, transcutaneous ultrasonography cannot positively identify males or reproductively-inactive females. Transintestinal sonography (TIS) has been used in avian species for imaging the internal urogenital organs.\textsuperscript{1,2} In the present study, the practicality of TIS was compared to that of transcutaneous sonography.

Methods

Eight subadult Komodo dragons of undetermined sex from two North American zoos, aged 19-36 mo, with a body mass of 0.97-6.10 kg were examined. Additionally, both members of an adult breeding pair (male=55 kg; female=29 kg) were also examined during the non-breeding season for verification of gonadal imaging. All examinations were performed with a computer sonograph (Hitachi CS9100) equipped with a 3.5 MHz transcutaneous convex transducer and a 7.5 MHz endosonographic convex transducer. Additionally, a 7.5 MHz transcutaneous linear transducer was used on the smallest lizards. The subadults were anesthetized with isoflurane and the adults with i.m. injection of telazol or ketamine, with midazolam followed by isoflurane.\textsuperscript{9} All animals were examined in dorsal recumbency. For transcutaneous ultrasonography, ultrasound gel was massaged into the abdominal and inguinal skin and allowed to penetrate for about 5 min. The 3.5 MHz transducer was then applied to the skin. For TIS, the rectum of each individual was lavaged extensively with warm water. This step was critical to secure undisturbed image quality and to avoid mechanical irritation by any intestinal contents. Ultrasound gel was placed in the cloaca as an acoustic coupling medium and for lubrication. The 7.5 MHz convex transducer was placed carefully into the cloaca and then gently moved cranially. Each examination was video-recorded.

Results

There were no indications of injury or infection resulting from the procedures. All animals recovered quickly without incident. The transcutaneous ultrasound procedure required about 10-15 min and the TIS procedure took about 8-10 min to complete. The transcutaneous method took longer because of the difficulty in identification of internal organs. A large number of small (10 mm) follicles were visualized by the transcutaneous method (3.5 MHz probe) in the adult female breeder. In the adult male, no gonadal structures could be identified transcutaneously. Both female and male gonads of the breeding pair were visualized in high resolution with TIS at a rectal distance of 280 mm from the cloaca. In the female, both inactive ovaries were elongated (~70x16 mm) and contained a total of ~30-40 follicles of uniform size (9-11 mm). However, the internal structure of the follicles varied. The ovarian parenchyma was hyperechoic without a distinct border. In the adult male, the inactive testes were cigar-shaped (55x17.5 mm) and positioned adjacent to the cranial renal poles. The testicular parenchyma appeared homogeneous and more echoic than the kidneys, and was surrounded by a distinct, thin, hyperechoic border. No vessel system or rete testis were internally detectable.

Transcutaneous ultrasonography was not useful for sex determination in the group of subadults. Gonadal visualization was only possible in one 3-yr-old female during the breeding season. Five follicles (~25 mm) were detected. Visualization of the internal organs such as kidney and testis was ambiguous and allowed no diagnosis of their condition. The TIS technique facilitated sex
determination in all eight subadults. The sex ratio was 5:3 (male:female). The eight subadults showed remarkable differences in gonadal development. Among the female subadults, ovarian size ranged from 8x10 mm to 45x28 mm and the size of visible follicles ranged from 2-25 mm. In subadult males, the testes were cigar-shaped like in adults, but without such distinct borders. The testes ranged in size from 14x6 mm to 20x8 mm.

The heart, liver and gall bladder were well-visualized transcutaneously in all animals. Additionally, the kidneys, adrenal glands, fat bodies, intestinal loops and caudal parts of the liver were visualized with the TIS method. The kidneys appeared very homogeneous, as in avian species, and were characterized by a longitudinally-running central blood vessel system. In both adults and three of the subadults, the urinary bladder was so full of hypoechoic urine that it extended into the area between the rectal wall and the ventral border of the gonad sometimes all the way to the caudal part of the liver.

Conclusions

Transintestinal ultrasonography is an effective, non-invasive method for sex determination of subadult Komodo dragons and affords more accurate visualization of the internal organs than transcutaneous ultrasonography.

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LITERATURE CITED

ISOFLURANE ANESTHESIA IN AMPHIBIANS: COMPARISON OF FIVE APPLICATION METHODS

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Abstract

Amphibians have a unique skin which allows absorption of various substances. Application of isoflurane to the skin of anurans results in percutaneous absorption and provides sedation and/or anesthesia. This study investigated five methods of isoflurane administration with two different anuran species.

Resumen

Los anfibios tienen una piel única, que permite la absorción de varias sustancias. La aplicación de isoflurano en la piel de los anuros resulta en la absorción percutánea y proveyó sedación y/o anestesia. En este estudio se investigaron cinco métodos de administración de isoflurano con dos diferentes especies de anuros.

Introduction

Increased interest in amphibian medicine and surgery has spurred important advancements in the health care of these animals. Although a variety of medical and surgical procedures are being conducted, amphibian anesthetic techniques have not kept pace with the many other advances in zoological medication. Isoflurane (Aerrane, Ohmeda PPD Inc. Liberty Corner, New Jersey 07938, USA) is one of the safest, most versatile anesthetics in human and veterinary medicine. It is an inhalant anesthetic which when vaporized with oxygen is carried to the pulmonary alveoli and rapidly absorbed into the generalized circulation. Amphibians are, to various degrees, aquatic animals which can respire and absorb substances through the skin. Preliminary work by the authors has demonstrated that cutaneous application of isoflurane in amphibians can produce sedation or anesthesia.

This study investigated and compared five methods of isoflurane administration for the induction of anesthesia in amphibians. Two ecologically different species of anurans were selected as model species to compare anesthetic induction of a primarily aquatic (*Xenopus*) and terrestrial (*Bufo*) species. The methods of isoflurane application include: direct application of liquid isoflurane to the skin, application of a liquid isoflurane and water mixture to the skin, application of an isoflurane, KY-jelly and water mixture to the skin, vaporized isoflurane in a water bath and vaporized isoflurane in an air chamber.

Materials and Methods

Each animal was examined, weighed, identified and maintained in a group tank for 3 mo prior to the
initiation of the study. *Xenopus* were identified via transponder chip and the toads via color patterns and anatomical characteristics. Animals were used multiple times in different application trials and randomly selected.

An anesthesia form was completed for every animal each time it participated in an anesthetic event. Data collection included body weight, method of isoflurane application, isoflurane concentration and volume, air temperature, water temperature, time until first effect, induction and recovery times. Sedation was classified as any one or combination of the following events: decreased movement, decreased gular movement, slow withdrawal reflex and/or slow righting reflex. Anesthesia was classified as any one or combination of the following events: loss of withdrawal reflex, loss of response to physical stimuli and/or loss of righting reflex. After induction of anesthesia, the amphibian was removed from the isoflurane and recovery was monitored.

In the following four trials, induction occurred in vented plastic containers beneath an air scavenger system. The direct application study was performed by dripping 100% liquid isoflurane onto the animal’s dorsum. *Xenopus* were administered 0.007 cc/g body weight isoflurane and *Bufo* toads were 0.015 cc/g of body weight. The liquid isoflurane and water application method was performed by placing the animals in a shallow water bath. The bath contained 0.28% isoflurane (0.35 cc of isoflurane liquid in 125 cc of water). After induction, animals were removed from the water bath for recovery in another plastic container. The isoflurane, KY-jelly and water mixture application method was performed by topical application of the thick solution upon the animal’s dorsum. A stock solution was made daily and consisted of 3 cc of liquid isoflurane, 3.5 cc KY-Jelly, and 1.5 cc water. This mixture was vigorously shaken in a closed glass container until becoming a uniform, viscous solution. *Xenopus* received 0.025 cc/g and *Bufo* toads 0.035 cc/g. Once anesthetized, the solution was removed from the skin with a damp gauze and the animals were recovered in another plastic container. The vaporized isoflurane in water method was performed by placing the animals in a shallow water bath. Isoflurane was vaporized at 5% with oxygen and bubbled into the water. The vaporized isoflurane induction chamber method was performed by placing the animals in an empty plastic container with an air tight lid. The container was attached to an inhalant anesthetic unit and 5% isoflurane was passed into the chamber via a precision vaporizer. Animals were induced in a closed chamber and removed to another isoflurane free container for recovery.

**Results and Discussion**

All five isoflurane application methods provided adequate sedation and/or anesthesia. There were no anesthesia complications or mortalities. Rates of induction, levels of anesthesia and recovery times were dependent upon the method of application, dosage, and the species evaluated.

Isoflurane is supplied as a liquid inhalant anesthetic which rapidly vaporizes when exposed to air. Isoflurane applied directly to the amphibians skin vaporizes at a very rapid rate. Higher ambient temperatures and increased local air flow (i.e., an open container with a scavenger unit versus a sealed container) will increase the rate of vaporization. In this study, the direct application method provided lighter sedation and poorer anesthetic levels than the other methods of application. This may be due to the vaporization of isoflurane from the skin prior to adequate percutaneous absorption.
The rate of isoflurane vaporization can be decreased by mixing liquid isoflurane with water or a water based soluble jelly. Isoflurane is poorly miscible in water and is denser than water. Small droplets form at the bottom of the container when liquid isoflurane is added to water. Combining liquid isoflurane with a water soluble jelly also decreases the rate of vaporization and allows increased dermal contact and absorption. In general, both the liquid isoflurane/water and the liquid isoflurane/KY-jelly/water application methods provided consistent levels of surgical anesthesia. The animals should be removed from the isoflurane mixture immediately after induction (loss of righting reflex) to prevent deep anesthesia and prolonged recoveries.

Both of the more traditional methods of isoflurane application (vaporized isoflurane into a water bath or gas chamber) provided adequate sedation and anesthesia. In general, these methods have longer induction times and more rapid recovery rates. These two application methods can be cumbersome for use with surgical procedures. The relatively rapid recovery, the use of air tight containers and difficulty in supplementing isoflurane while working on the patient make these methods better suited for short procedures.

The percutaneous absorption of isoflurane appears to be species dependent. In this study the thinner, more absorptive skin of frogs allowed a lower dose to be used than the thicker dermis of toads. Besides the two species evaluated in this project, dermal application of isoflurane has been effectively used by the authors for medical and surgical procedures in poison dart frogs (Dendrobates spp.), marine toads (Bufo marinus), smokey jungle frogs (Leptodactylus pentadactylus), horned toads (Ceratophrys spp.), and Colorado river toads (Bufo alverius).

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ALL STAR LINEUP OF MARINE INVERTEBRATES FOR PUBLIC DISPLAY

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Abstract

In spite of the fact that the number of invertebrate species in the world outnumbers those of fish species twenty-five to one, public aquariums and zoos have predominantly exhibited fish in the past. This fact is supported by Chart 1.

![Distribution of exhibits](image)

Fortunately, this trend has been changing and invertebrates are becoming more and more prevalent. This paper will look at the most popular marine invertebrates, other than corals, exhibited by these aquariums and an overview of their basic husbandry needs. It will also examine the advantages and disadvantages of these groups and examples of graphics.

Cnidarians make attractive and colorful displays such as the carpet anemones and their symbiotic clownfish. These invertebrates require good water quality, water current and lighting of the correct spectrum, intensity and duration. The pelagic moon jellyfish, in comparison, require a special tank known as a kreisel which creates water movement in a circular pattern. Diet consists of newly hatched *Artemia*.

Among the arthropods, the class Crustacea offers numerous advantages making it an almost perfect display animal. Most species are very hardy requiring simple dietary and filtration needs. For this reason they make excellent candidates for educational purposes. The major disadvantage is their aggressive and sometimes predacious nature.

Among the molluscs, the cephalopods are the most popular in public aquariums. This includes the octopus, nautilus and cuttlefish. Life support requirements are not complicated, but water quality and diet are important factors. Among octopus and cuttlefish, disadvantages include a short life span
and compatibility problems of varying degrees.

Among the echinoderms, public aquariums are now displaying exhibits of just starfish, including tropical and cold water. These animals have relatively simple dietary and life support needs.

**Resumen**

A pesar del hecho de que el número de especies de invertebrados en el mundo supera al de especies de peces en 25 a 1, los acuarios públicos y zoológicos han exhibido predominantemente peces en el pasado. Este hecho queda demostrado en la tabla 1.

Afortunadamente, esta tendencia ha estado cambiando y los invertebrados se han hecho más y más prevalentes. Este documento se enfocará en los invertebrados marinos más populares, diferentes a los corales, exhibidos por estos acuarios con una revisión general de sus necesidades de crianza. También examinara las ventajas y desventajas de estos grupos y los explicará en gráficas.

Los Cnidarios hacen exhibidores atractivos y coloridos, tales como las de las anémonas alfombra y su simbólico pez payaso. Estos invertebrados requieren una alta calidad de agua, que el agua este en circulación y una iluminación de intensidad y de espectro correcto. La medusa luna pelágica, en comparación, requiere un tanque especial conocido como kreisel que crea un movimiento de agua de forma circular. Su dieta consiste en *Artemia* recién nacida.

Dentro de los artrópodos, la clase crustácea ofrece numerosas ventajas, haciéndolos animales casi perfectos para la exhibición. La mayoría de las especies son muy resistentes, y tienen requisitos dietéticos y de filtración muy simples. Por esta razón son excelentes candidatos para propósitos educativos. Su mayor desventaja es su agresividad y algunas veces su naturaleza predatoria.

Dentro de los moluscos, los cefalópodos son los más populares en los acuarios públicos. Estos incluyen los pulpos, Nautilus y calamares. Sus requerimientos no son complicados pero la calidad del agua y la dieta son factores importantes. Dentro de los pulpos y los calamares las desventajas incluyen un periodo de vida corto y problemas de compatibilidad en grados variables.

Dentro de los equinodermos, los acuarios públicos están montando exhibiciones solo de estrellas de mar, incluyendo de aguas tropicales y frías. Estos animales tienen necesidades dietéticas y fisiológicas relativamente simples.
IDENTITY AND HUSBANDRY OF SOFT AND STONY CORALS

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Abstract

The husbandry of corals in captivity involves an ecosystem management approach combined with knowledge of the requirements of the individual species. Management of the ecosystem via different methods of water purification have been tried. Some of the methods include several types of biological filtration, algal turf scrubbing, protein skimming (foam fractionation), and frequent partial water change. The use of living substrates (rock and sand from the reef) as a filter has proven very beneficial to the stability of the system and its ability to support high biodiversity. In addition to filtration, other important concerns for the husbandry of corals include lighting, water motion, methods for securing the corals in position, trace and minor element supplements and the maintenance of calcium and alkalinity. Finally, with certain fast growing species good husbandry requires frequent pruning to avoid overshadowing and competitive interactions.

Resumen

La crianza de los corales en cautiverio involucra el manejo y el aprovechamiento de los ecosistemas, combinados con el conocimiento de los requisitos individuales de las especies. El manejo del ecosistema se ha probado a través de diferentes métodos de purificación del agua. Algunos de los métodos incluyen diferentes tipos de filtración biológica, raspado de las algas que cubren el tanque, separación de proteínas (por fraccionamiento espumoso), y un frecuente cambio parcial de agua. El uso de substratos vivos (piedras y arena de arrecifes) como filtro ha sido muy beneficioso para la estabilización de los sistemas y para su capacidad de soportar una alta biodiversidad. Además de la filtración, otros factores importantes para la crianza de los corales incluyen la iluminación, movimiento del agua, métodos para asegurar la posición de los corales, suplemento de elementos menores y elementos traza, y el mantenimiento del calcio y la alcalinidad adecuada. Finalmente, con ciertas especies de crecimiento rápido una buena crianza requiere frecuentes podas para evitar las sombras y la competencia.

Overview

The husbandry of corals in captivity involves an ecosystem management approach combined with knowledge of the requirements of the individual species. Management of the ecosystem via different methods of water purification have been tried. Some of the methods include several types of biological filtration, algal turf scrubbing, protein skimming (foam fractionation), and frequent partial water change.

Biological filtration for aquarium systems typically concentrates on the conversion of ammonia to nitrate. So-called “wet/dry” trickle filters that efficiently make this conversion have been employed for many years on closed recirculating systems. While their use is well-accepted for fish displays,
The results achieved with trickle filters are not good, unless other forms of biological filtration and protein skimming are also employed. In the process of converting ammonia to nitrate, hydrogen ions are released which acidify the water, depleting alkalinity. Therefore the trickle filter tends to make an environment unsuitable for calcification by the corals. In addition, the high efficiency of these filters also quickly converts available ammonia, which is food for photosynthetic corals, to nitrate, which is less easily utilized by corals.

The process of denitrification sometimes used in combination with trickle filters tends to reverse the loss of alkalinity by generating carbonates while converting the accumulated nitrate to nitrogen gas and nitrous oxide, which easily escape from the water. Such denitrification filters are tricky to manage as they employ regulated water flow and the dosage of an organic food source such as lactose for the denitrifying bacteria. Without the proper adjustment these filters may feed hydrogen sulfide or nitrite back to the aquarium, though most systems pass the water exiting the denitrification filter back through the trickle filter which would oxidize any hydrogen sulfide or nitrite. In any case, the use of trickle filters and associated denitrification filters is overly complicated for reef aquarium systems.

Some aquariums employ algal turf filters to remove nitrate created by the trickle filter. This combination can also be complicated, and while the algae do remove nitrate and phosphate and elevate the pH, they may further deplete alkalinity if they are illuminated during the day when the water has little available carbon dioxide, because photosynthesis by the plants and photosynthetic corals in the aquarium removes carbon dioxide and carbonates from the water. Therefore, algal turf filters are best employed on a light schedule opposite of the display aquarium. The results achieved with them are very good for fish displays but not so good for live corals. The detriment of algal turf filters used without any other means of filtration is that they remove inorganic nitrogen (i.e., ammonia), which is food for the corals, while leaching back organic nitrogen, which is toxic to corals and which stains the water. Algal turf filters also remove trace elements from the water, but this is easily remedied with supplemental additions to replenish them. The yellow water stain from organic substances leached by the algae prevents UV wavelengths from penetrating the water and reduces total light penetration. The use of activated carbon effectively removes such organic compounds from any aquarium. Trace element removal by activated carbon is also easily replenished. A benefit of algal turf filters is that they are refugia for many species of algae and associated organisms.

Protein skimming or foam fractionation is a very beneficial method of maintaining water quality in any closed system aquarium. This method employs tiny bubbles to filter the water in a column. Organic molecules are attracted to the bubbles, and a foamy froth collects at the top of the column where it exits via a spout. This filtration method reduces biological oxygen demand and removes many compounds that would otherwise be broken down biologically in the system at the expense of water quality.

Frequent partial water change has been used as a water quality maintenance method alone or in combination with other methods. For public aquaria located next to the sea this is a natural option. For inland aquariums the cost of using this method is very high. In open system aquariums the quality of the feed water may vary, and the introduction of pathogens is possible. Furthermore, high silicate availability combined with strong illumination may create a perpetual problem with diatoms,
which rapidly coat the display window with a brown film that must be removed daily. Well-designed, stable reef aquariums require very little water change.

The use of living substrates (rock and sand from the reef) as a filter has proven very beneficial to the stability of the system and its ability to support high biodiversity. The sand and rock combined provide complete biological filtration (nitrification and denitrification). Therefore, these substrates provide the basic filtration without the need for external devices. Protein skimming, however, does improve the condition of the water over what is achieved by the natural biological filtration.

In addition to filtration, there are other important concerns for the husbandry of corals. These include, for example, lighting, for which high color temperature daylight metal halide lamps and blue fluorescents are typical, among newer options. Water motion is also critical, and there are special pumps and devices available to effect the high energy water flow characteristic of many reef environments. Methods for securing the corals in position are essential to prevent falls that can be fatal. Underwater epoxies and other devices now make this task simple. Trace and minor element supplements and the maintenance of calcium and alkalinity are important water quality issues, and several schools of thought exist regarding the recipes and techniques. Calcium and alkalinity can be maintained via calcium hydroxide enriched make-up water, via calcium chloride and sodium bicarbonate solutions, often improved by the addition of other important ions, and with calcium reactors that use CO₂ to dissolve coral gravel or limestone. Trace element supplements are commercially available and also can be made based on published recipes. Finally, with certain fast growing species good husbandry requires frequent pruning to avoid overshadowing and competitive interactions.
ARTHROPOD PUBLIC DISPLAY, REARING, AND CONTAINMENT AT CINCINNATI ZOO'S WORLD OF THE INSECT EXHIBIT

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Abstract

The World of the Insect exhibit, informally called the Insectarium, is part of the Cincinnati Zoo’s Animal Department. The Insectarium publicly opened August 1978, was the first North American exhibit of its size and scope, and received the 1979 National Exhibit Award for best new display in any zoo or aquarium.

Reflecting modern zoo philosophy, the Insectarium strives to serve four broad social needs: public education, entertainment, research and conservation. Our visitors pass through 12 major theme areas (i.e., What is an Arthropod?, What eats Insects?, Defense & Escape), each offering a fascinating mixture of live animal displays, interpretative graphics, prepared museum specimens and interactive teaching tools. A non-public rearing area supports biological investigations, husbandry technique development and standardized rearing programs. The exhibit received Edward H. Bean Awards for the first zoo captive breeding of the Royal Goliath Beetle (1987), Giant Asian Walkingstick (1979), Hercules Beetle (1985) and Harlequin Beetle (1986), and Significant Achievement Award for the endangered American Burying Beetle (1993).

The Insectarium is staffed by two graduate entomologists, two skilled entomology technicians, and two part-time public area security guards; all work staggered schedules to ensure daily coverage. The entomology staff is responsible for animal acquisition, husbandry and breeding program development, display design and repair, and related duties.

We maintain over 100 live species: mostly insects, arachnids and other arthropods, and a few vertebrates. The arthropods are diverse and include native and exotic predators, scavengers and herbivores endemic to varied habitats like deserts, ponds and rainforests. Effective live display animals generally are colorful (i.e., butterflies and beetles), behaviorally active (bees and ants), large and bizarre (stick and leaf insects), or have notorious reputations (black widow spiders and scorpions). We emphasize captive propagation for continuous livestock production and to limit field collection need; some species serve secondarily as prey animals. Vertebrates featured are insectivorous to demonstrate arthropod importance in food chains and represent all five major classes (i.e., archer fish, poison dart frogs, whiptail lizards, hummingbirds, and tree shrews).

Since we import, hold and breed exotic herbivores, the Insectarium is accountable to the U.S. Department of Agriculture and designed to function and operate as an arthropod containment facility. The building has numerous physical features preventing escape including self-closing doorways with perimeter gaskets and sill sweeps, and fine mesh over floor drains and air vents. A separate interior quarantine room holds incoming wild caught animals for necessary disease or parasite screening, or for isolating sick animals away from the main rearing area.
Resumen

La exhibición del Mundo de los Insectos, informalmente llamado “El Insectario”, es parte del departamento animal del Zoológico de Cincinnati. El Insectario se abrió al público en agosto de 1978 y fue la primera exhibición de este tamaño y finalidad, recibiendo en 1979 el premio “National Exhibit” por el mejor exhibidor en todos los zoológicos o acuarios.

Reflexionando sobre la filosofía moderna de los zoológicos, el Insectario se esfuerza en servir cuatro necesidades sociales: educación al público, entretenimiento, investigación, y conservación. Nuestros visitantes pasan a través de los 12 grandes temas (p.ej. ¿Qué es un Artrópodo?, ¿Qué come un insecto?, Defensa y Escape). Cada uno ofrece una fascinante mezcla de vitrinas con animales vivos, gráficas interpretativas, exposición de individuos preparados por el museo y herramientas de enseñanza interactivas. Existe un área de crianza que no se exhibe y se utiliza para realizar investigaciones biológicas, desarrollo y estandarización de técnicas de crianza. La exhibición recibió el premio Edward H. Bean por ser el primer zoológico que crió en cautiverio el escarabajo Goliath real (1987), el insecto palo gigante asiático (1979), el escarabajo Hércules (1985) y el escarabajo Harlequín (1986), así como el premio “Al Logro Significativo” por la cría del amenazado escarabajo enterrador americano (1993).

El equipo de trabajo del insectario está integrado por 2 entomólogos graduados, 2 técnicos en entomología, y 2 guardias de seguridad de tiempo parcial; el calendario de trabajo es rotativo para asegurar la atención diaria. El equipo de entomólogos es responsable de la adquisición de los animales, del desarrollo de programas de reproducción y crianza, diseño y reparación de terrarios, y tareas relacionadas.

Mantenemos alrededor de 100 especies vivas: en su mayoría insectos, arácnidos y otros artrópodos, y un pequeño número de vertebrados. Los artrópodos son diversos e incluyen predadores exóticos y nativos, carroñeros y herbívoros endémicos de una variedad de hábitats como desertos, pantanos y bosques tropical. Las exposiciones de animales vivos suelen ser coloridas (mariposas y escarabajos), de comportamiento activo (abejas y hormigas), de animales grandes y raros (insectos palo e insectos hoja), o que tienen una reputación notoria (araña viuda negra y escorpión). Enfatizamos la propagación en cautiverio para una continua producción de animales y así limitar las necesidades de colecta del campo; algunas especies sirven en forma secundaria como animales de presa. Los vertebrados insectívoros demuestran la importancia de los artrópodos en la cadena alimenticia y representan a las cinco clases mayores (pez arco, rana venenosa, lagarto cola de látigo, colibríes y musarañas).

Desde que nosotros importamos, mantenemos y reproducimos herbívoros exóticos, el insectario es tomado en cuenta por el Departamento de Agricultura de los Estado Unidos y designado para funcionar y operar como centro de acopio para artrópodos. El edificio tiene numerosas barricadas fí­sicas para prevenir el escape, incluyendo puertas de cerrado automático selladas en su perímetro y con una aleta de hule en la parte inferior, y tela de mosquitero en drenajes y ventanas de aire. Un cuarto interior para cuarentena mantiene animales silvestres recién llegados para su análisis de enfermedades y parásitos, o para el aislamiento de animales enfermos lejos del área principal de crianza.
THE HUSBANDRY OF DESERT ARTHROPODS

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Abstract

The desire by zoological parks and aquariums to maintain terrestrial arthropods as part of their collections is steadily increasing. In some cases, taxa are chosen to illustrate a particular theme or behavior, or they may be chosen as representatives in a replicated biotic community. Due to its biological diversity and the relative ease of specimen acquisition from the area, many zoos find the Sonoran Desert region, particularly southeastern Arizona, rich in material to meet their arthropod exhibit requirements. The area’s non-insect arthropods are of special interest due to their long lives and relative ease in providing proper captive care.

Resumen

El deseo de parques zoológicos y acuarios de mantener artrópodos terrestres como una parte de su colección está aumentando firmemente. En algunos casos el taxón es escogido para ilustrar un tema particular o el comportamiento, o bien, puede ser escogido como representante en una réplica de una comunidad biótica. Debido a su diversidad biológica y a la relativa facilidad para la adquisición de especímenes del área, muchos zoológicos encuentran la región del Desierto de Sonora, específicamente el suroeste de Arizona, rico en material para satisfacer los requerimientos de sus exhibiciones de artrópodos. Los artrópodos del área que no son insectos son de especial interés debido a su longevidad y relativa facilidad de cuidado en cautiverio.

Introduction

The diversity of this area is due to the influence of several biogeographic provinces which meet in southeastern Arizona. With plant communities representing the Madrean evergreen woodlands and Sinaloan deciduous thorn forests from the south, the Chihuahuan Desertscrub from the east and the Sonoran Desertscrub from the west, species diversity is quite high. Mountain islands punctuate the landscape and elevations range from 600-3000 m. Most commonly exhibited arthropods occur at the lower elevations where annual precipitation ranges from 25-60 cm. Desirable taxa are most readily available during the brief but heavy rainy season in July and August. The area’s non-insect arthropods are of special interest due to their long lives and relative ease in providing proper captive care.

The husbandry of the most frequently displayed arthropods from this region is fairly straightforward. Sonoran Arthropod Studies Institute maintains a variety of local arthropods for its workshops and outreach programs in a building that was originally a small residence. Environmental conditions are maintained with a central air conditioning unit and a humidifier. A near constant temperature of 27°C (80°F) is maintained. The relative humidity fluctuates due to the conflict between the refrigeration unit and humidifier but an average of 50% is achieved. The basics
of clean caging and substrates, adequate nutrition and fresh water are necessary in maintaining desert arthropods. Details of their husbandry are here presented.

**Centipedes**

The giant desert centipede, *Scolopendra heros*, with its striking black and orange aposematic coloration is a popular display animal. Although frequently attaining a length of more than 20 cm they are extremely agile and quick moving. Care in handling should be exercised as their bite is very painful. They, like most Sonoran Desert arthropods commonly exhibited, are most active during the summer rainy season. With proper care, giant desert centipedes can live several years in captivity.

Centipedes may be kept in a variety of containers including wide mouth gallon jars, plastic sweater boxes and standard aquaria. A soil substrate of at least 6 cm facilitates the centipede’s burrowing behavior and minimal ventilation helps to maintain desirable humidity within the cage. Exhibit situations where visibility is important may call for less substrate and a shelter carefully devised to provide for visitor viewing while providing a sense of security to the nocturnal animal. Centipedes should always be housed individually.

A dish of fresh water will help maintain higher humidity in the enclosure but may frequently be spilled or covered during burrowing activities and result in a soggy substrate. Giant desert centipedes will also drink water droplets off the sides of a glass aquarium or jar. They feed upon a variety of insects and crickets are most commonly offered. Larger centipedes also prey upon small rodents and lizards and some keepers have found them to relish chicken livers. Such food items, however, tend to foul caging rapidly. One cricket, three times weekly satisfies most appetites. After molting, the desert centipede eats its exuvia, presumably to recover valuable nutrients.

**Millipedes**

The desert millipede, *Orthoporus ornatus*, requires high humidity and prefers a moist porous soil substrate. While maintaining a high humidity is important, open ventilation has led to greater longevity. A flat rock or piece of bark will provide shelter for the animal during the daylight hours and when cage conditions become too dry. A daily misting provides a reasonably stable humidity and the millipedes regularly drink moisture from off the glass.

Millipedes eat both fresh and decaying vegetation. In captivity they can be fed leaf lettuce, broccoli, grapes (cut open), bananas, spinach and sweet potato. SASI has kept desert millipedes successfully on a diet of only primate chow. Several millipedes can be housed in a single container. Due to soil moisture requirements and the frequent problem of food stuffs becoming moldy, daily servicing is generally required.

**Arachnids**

*Vinegaroons or Whip scorpions (Uropygida)*
The vinegaroon, *Mastigoproctus giganteus*, is common in southeastern Arizona where it is found crossing the roads at night during the summer rainy season. They are generally impossible to find except during the summer months even under rocks or in burrows where they spend the summer daylight hours. Vinegaroons are harmless and easy to handle but like many arachnids, are quite fragile. When threatened by predators, they spray acetic acid from the base of the telson.

Vinegaroons may be kept in plastic shoe boxes or aquaria and must be housed individually. Vinegaroons will readily burrow under rocks and leaf litter to make a small cavern, particularly if the cage is well-ventilated. A dish of water should be available at all times although burrowing activities may result in soggy substrate. A periodic heavy misting seems to be enjoyed by vinegaroons. They will eat a variety of insects including crickets, small grasshoppers and beetles. One or two crickets per week are quickly eaten and vinegaroons have been maintained in captivity for more than 6 yr.

**Tailless whip scorpions (Amblypygida)**

The tailless whip scorpion, *Paraphrynus mexicana*, is found in the drier south/central portion of Arizona. They are most active in the spring and summer and spend much of their lives secluded in rodent burrows, and beneath bark or rocks. Because they are fragile and very responsive to vibrations and movements, it is recommended they be handled as little as possible.

Tailless whip scorpions prefer vertical surfaces to rest on and to hide behind during the daylight hours. Multiple specimens may be housed together if adequate food and shelter is available. A regular misting of the enclosure provides water as amblypygids readily take droplets from surfaces. Crickets are the standard captive fare but other live prey may be taken. Prey items should not be too large. The author has maintained three adult tailless whip scorpions for more than 3 yr.

**Scorpions (Scorpionida)**

Scorpions are abundant throughout southeastern Arizona. They are most active during the warmer months, hunting at night and resting during the day. Scorpions may be found readily with the aid of an ultraviolet light while walking through the desert at night. Bark scorpions, *Centruroides* sp. and devil scorpions, *Vaejovis* sp. can be found under rocks, loose bark and old debris throughout the year. Giant hairy scorpions, *Hadrurus arizonensis*, the largest and most desirable exhibit species, construct burrows in the ground and are not easily found during the cooler months.

Scorpions are long lived and will survive several years in captivity with good care.

Only the bark scorpion is potentially life threatening but all species commonly displayed from the region have painful stings and care should be exercised when handling. Use forceps to grasp the scorpion’s telson (tail).

Plastic shoe boxes, aquariums or gallon jars may be used to house scorpions. Provided with sufficient shelter and food, many specimens of the same species can be housed together. Different species of scorpions should not be mixed. One will feed upon the other as will individuals of the same species not provided enough food.
All scorpions are predatory and feed primarily upon soft-bodied insects. Small prey items are grasped and readily eaten; larger insects are grasped and subdued with a sting. Due to commercial availability, mealworms or crickets are standard offerings. Live roaches, grasshoppers, flies and moths may also be taken. Long term captive scorpions may, on occasion, decline eating for several weeks. Removal of uneaten prey after a day or so is recommended to minimize stress. Although they hardly seem to drink, a shallow dish of water will help maintain higher humidity levels.

Scorpions give birth to living young which ride upon their mother’s back until their first molt; 1-3 wk depending on the species. Baby scorpions should be removed from the mother’s cage after they crawl off her back as they will be considered prey by larger scorpions including the mother. Baby scorpions can be fed fruit flies or pinhead crickets. If you plan to rear young scorpions, it is easier to reduce the number of the original brood to a manageable size by allowing the young scorpions to prey upon one another.

**Solpugids (Solifugi)**

Solpugids can be found wandering in search of insect prey during late spring and summer evenings. They are fast runners with a high metabolism and are easily agitated. As such, these harmless arachnids are difficult to keep for any length of time and do not lend themselves to long-term exhibition. They seem to do best with a deep silty substrate which should be kept slightly damp. They are persistent burrowers and construct intricate systems of tunnels, some of which may be visible against the glass.

Solpugids are efficient predators with voracious appetites. Their pedipalps have sticky tips to allow them to catch insects and pull them in with great ease. In captivity they may eat several crickets every day. The smaller species do well on fruit flies and can consume up to a dozen twice per day.

The author has had only moderate success in keeping solpugids. The smaller species have been kept for longer periods, but the larger species rarely survive a month in captivity. Further experimentation in solpugid husbandry is warranted.

**Tarantulas (Theraphosidae)**

Tarantulas, *Aphonopelma* sp. are the most commonly kept desert arthropod. During the 5-8 yr tarantulas require to reach sexual maturity, they remain close to their burrows and are rarely seen. Mature males seeking females, which remain in their burrows, are commonly seen crossing roads in late summer and early fall. Male tarantulas generally die within the year of achieving sexual maturity while females may live for another 10 yr or more. Immatures and females are considered superior exhibit animals for this reason.

As with all arthropods, tarantulas shed their exoskeletons in order to grow. Young tarantulas molt several times per year until they are 6 or 7-yr-old. At this age, they molt once per year, usually in April or May. A couple of weeks prior to molting, the tarantula may appear lethargic and reject food. Just before ecdysis (molting) it will turn over and lie motionless on its back. Do not disturb during this time as the spider may be easily injured. Tarantulas always die right side up, legs folded beneath the body.
A plastic shoe box provides the minimum spatial requirements for a mature tarantula. Larger sweater boxes or aquaria are preferred. The substrate and cage furniture used is a matter of aesthetics and serviceability and may include paper toweling, newspaper or soil. Many tarantula specialists are now using a vermiculite and sphagnum moss. Soil may be banked towards one side of the container to encourage burrowing or other materials can be used to provide shelter. Most tarantulas are nocturnal and like to seclude themselves during the daylight hours. Reptile hiding boxes, paper tubes and broken flower pots may be used in non-exhibit situations.

Tarantulas eat a variety of live insects but are especially fond of crickets, grasshoppers and beetles. It has been shown that beetles are necessary for tarantula reproduction in captivity and variety of prey will increase longevity. Tarantulas will also eat pink mice and small lizards. Commercially available crickets are most commonly fed to tarantulas with one or two adult crickets weekly being adequate. Although tarantulas derive some moisture from their prey, they should have a shallow dish of clean water available at all times.

Conclusion

Desert arthropods are easily maintained in zoos, museums and nature centers. Many species obtained in southeastern Arizona during the summer months are being exhibited by institutions around the country. For some, particularly the popular arachnids and myriapods, their husbandry is simple. For others, including many which offer great interpretive value, little is known about their captive care requirements and successful husbandry techniques await development.

LITERATURE CITED

BUTTERFLIES ARE NOT FREE: LIVE BUTTERFLY “ZOOS” IN NORTH AMERICA

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Abstract

The last 15 yr or so have seen a great proliferation of live butterfly exhibits, called variously butterfly houses, butterfly centers, butterfly conservatories, etc., in North America (their tradition is much longer standing in Europe, especially England, where some date from the beginning of the century or earlier). In this paper I will discuss basic butterfly biology, the concept and operating principles behind butterfly “zoos”, where the butterflies come from and how they are handled, and some current permitting and containment concerns with these public walk-through displays.

Butterflies are excellent subjects for public displays, and offer many opportunities for education about insects in general, their importance to the natural world, and the role of conservation. Live butterfly exhibits are popular and proliferating. Although containment of non-indigenous species is a concern, government agencies should work in collaboration with large butterfly facilities rather than taking an adversarial position. These exhibits can be important in raising the public’s awareness about the importance of conservation of both species and habitats, as well as the dangers of introducing exotic species.

Resumen

Durante los últimos 15 años, aproximadamente, ha habido una gran proliferación de exhibiciones de mariposas vivas llamadas Casas de Mariposas, Mariposarios, Centro de Mariposas, Conservatorios de Mariposas, etc. (Su tradición es mucho más antigua en Europa, especialmente en Inglaterra, donde algunos reportes son anteriores al principio de este siglo). En este trabajo se trata de la biología básica de las mariposas, el concepto y principios operativos detrás de los “zoológicos de mariposas”, de donde vienen las mariposas y como son manejadas, y algunos aspectos concernientes al trámite de permisos e instalaciones de estos escenarios al aire libre.

Las mariposas son excelentes animales para exhibiciones públicas, y ofrecen muchas oportunidades para la educación acerca de insectos en general, su importancia para la naturaleza y su papel en la conservación. Las exhibiciones vivas de mariposas son populares y están proliferando. Aunque el confinamiento de especies exóticas representa una seria preocupación, los organismos gubernamentales deben de trabajar en colaboración con los grandes mariposarios, en lugar de tomar una posición adversa. Estas exhibiciones pueden ser importantes para incrementar la concientización del público acerca de la importancia de la conservación de especies y hábitat, así como el daño que puede producir el introducir especies exóticas.

Introduction

The last 15 yr or so have seen a great proliferation of live butterfly exhibits, called variously
butterfly houses, butterfly centers, butterfly conservatories, etc., in North America (their tradition is much longer standing in Europe, especially England, where some date from the beginning of the century or earlier). In this paper I will discuss basic butterfly biology, the concept and operating principles behind butterfly “zoos”, where the butterflies come from and how they are handled, and some current permitting and containment concerns with these public walk-through displays.

**Basic Butterfly Biology**

The popularity of these displays stems from the attraction of the butterflies themselves. Butterflies are almost everyone’s favorite insects. Associated with sunshine and flowers, they appear to have a life of frivolous ease and innocence. They are silent and beautiful, coming in as many colors and shapes and sizes as the flowers they visit. With their beautiful wings, delicate bodies, slender legs, and perhaps most importantly their inability to bite or sting, they are completely nonthreatening. People seem to view them more like birds than as insects, most of which are considered with loathing or fear.

Yet take the same people who wax sentimental over a butterfly and show them a caterpillar--their reactions are completely different! Most people would rather squash these “ugly worms” than look at them--even though they would not consider killing a butterfly! They forget that a caterpillar IS a butterfly, and vice versa.....

Truly the transformation from “ugly worm” to beautiful butterfly is one of the miracles of nature. Butterflies, and moths, together forming the insect order Lepidoptera or “scaly-winged” insects, are insects with complete metamorphosis. Along with beetles, flies, wasps, ants, and bees, etc., they have four life stages--egg, larva, pupa, and adult, in contrast to such insects as grasshoppers, cicadas, and cockroaches, which have no pupal stage and change much less drastically as they grow from juvenile to adult. The pupal stage, called a chrysalis in butterflies, is what separates the so-called holometabolous and hemimetabolous insects. Sometimes called the “resting stage”, the period in the pupa is when the larval body is completely transformed into that of the adult insect. In butterflies the change is particularly dramatic. Caterpillars have thick, cylindrical bodies, tiny legs, and a large head dominated by powerful chewing mandibles. Almost all caterpillars, whether butterflies or moths, eat the leaves of plants. They spend most of their time eating, and grow rapidly from the first stage or instar to the final in star, the pupa or chrysalis, shedding their skin or exoskeleton several times as they grow. With only simple eyes called ocelli, that mostly see dark and light, caterpillars are almost blind. They are relatively sedentary, rarely leaving the hostplant where their egg was laid, although some may “wander” away from the hostplant just before they pupate.

The butterfly appears to be a completely different animal. It is no longer sedentary and earth-bound, but a creature of the air. Dominated by large wings, its body is thin with long, delicate legs. The head is small and bears long antenna, large compound eyes, and the coiled proboscis or tongue. Gone are the chewing mandibles; the straw-like proboscis limits butterflies to a liquid diet. Most people are aware that many butterflies visit flowers for nectar, pollinating them in the process. Yet some butterflies never visit flowers, surviving instead on the fermenting juices of fallen, over-ripe fruit. And many butterflies will occasionally partake of other less savory liquids, including urine, stagnant water, and the juices from dung or carrion. These substances provide minerals and salts
Butterflies thus are relatively catholic in their tastes. Nectar-feeding butterflies, for example, seldom show strict loyalty to any one species of flower, but visit a variety of good nectar producers and will even feed on "artificial" nectar (sugar water or other nectar equivalents). This makes them relatively easy to maintain in displays. Caterpillars, on the other hand, are much more limited in what they can and will eat. Most butterfly species are relatively "host specific" as caterpillars; that is, they feed on only one or a few species of plants. Indeed many families or genera of butterflies are characterized by the plants they eat as caterpillars. For example, the monarch and queen butterflies (Family Danaidae) are called "milkweed butterflies" after the larval food plant; passionflower or long-wing butterflies (Heliconiidae) specialize on passion flower vines; and the so-called "poison feeders" are the group of swallowtails (Papilionidae) that eat Dutchman's pipevine or Aristolochia. The chemical composition of the hostplant is a powerful ovipositional stimulant for the female, as well as a feeding stimulant for the caterpillar, and most female butterflies will die before laying their eggs on the wrong plant for their larvae. This host specificity is also important, as will be seen below under legal considerations: because butterflies are so host specific as caterpillars, their egg-laying behavior (and caterpillar feeding damage) can be controlled or eliminated by avoiding host plants in the display.

Thus for several reasons these fascinating insects are ideal for public displays. As mentioned, butterflies are "user-friendly"--because they are completely harmless and non-threatening, they can be allowed to fly freely in the same space as human visitors. And since they are relatively oblivious to humans, visitors can watch their behaviors up close and even (although this is discouraged) touch the butterflies.

**Butterfly "Zoos" and How They Operate**

Live butterfly exhibits range in scope from small outdoor flower gardens designed to attract native species, typically open during the summer months only, to large, glass enclosed, year-round facilities that display mostly exotic tropical, i.e., non-native, butterfly species from around the world. There are approximately 10 of the latter type of butterfly exhibits currently in North America (see Table 1), and several more are in the planning or construction stages. They are housed in zoos, museums, botanical gardens or amusement parks, or are stand-alone facilities. A few are combined with exhibits of other animals, such as birds. Most have educational displays attached, including exhibitions of preserved butterflies and sometimes other insects, posters and other signage, etc. Of course associated gift shops bring in substantial revenue.

All of the large butterfly exhibits, while different in design, are similar in concept and scope. Most feature tropical vegetation and display mostly exotic tropical, i.e., non-native, butterfly species (some display only exotic butterflies). Certain tried and true nectar sources can be found in almost every display (see Table 2). Plates of ripe fruit (bananas and mangos are favorites) are provided for fruit-feeding species. Temperatures and humidity are kept high and relatively constant, typically about 75-80°F and about 80% humidity. These are ideal conditions for most butterflies, which will not fly in cool weather. Most displays have some sort of a water feature, whether a pond, stream, or waterfall, and many have other animals that hopefully are compatible with the butterflies (especially non-insectivorous birds, but also turtles, fish, etc.).
The butterflies flown in these exhibits are imported in the chrysalis stage from butterfly “farms” around the world (see below). Once received, the chrysalises are pinned or hung from boards or string to allow the butterflies to emerge naturally. Most of the large displays fly from 1000 to 2000 individual butterflies at any one time. Because the average life span of butterflies in these displays is only about 2 wk, and because mortality in the chrysalis stage averages about 20% in an average shipment (personal observation), butterfly centers must import well over twice this number every month. This represents a considerable expense, since the average cost of a chrysalis is about $3.00 US, and since shipments must be sent via costly express airmail because of the typically short period (10 days to 2 wk) spent in the pupal stage.

Several butterfly centers rear at least a few species of butterflies on site. Because large-scale rearing inside public displays is prohibited (see Legality Issues), auxiliary greenhouses or other areas are needed.

Sources and Availability of Butterflies

At present most butterfly producers are located in Central America or South America and in tropical Asia. Native North American species are mostly available from several growers in south Florida.

The foreign butterfly producers are of two types. Some are large rearing facilities that farm their butterflies on-site. Others function as brokers for a number of small-scale producers. Both represent the recent development of an unusual cottage industry using natural resources. Although the worldwide demand for butterflies is relatively limited, some of the butterfly farms represent real local success stories. For example, Bioproductores de El Salvador was developed on 20 ha of highly degraded, deforested land that formerly supported three families at a subsistence level. Today the property is in the process of vegetational succession, and the farm, presently utilizing only about three of the 20 ha, provides full-time employment for 16 heads of households. In Costa Rica, Joris Brinkerhoff, a former Peace Corps volunteer, now manages a butterfly brokerage in Costa Rica called Suministros Entomológicos. Most of his suppliers formerly subsisted as small-scale cattle ranchers, but several of these have now essentially abandoned cattle in order to concentrate on butterfly rearing, which is more profitable and reliable as a source of income.

Whether conducted in farms dedicated to butterfly rearing or in a cattle-rancher’s backyard, the process is the same. Eggs are collected on hostplants, and the caterpillars are raised until the pupal stage. The chrysalises are harvested, packed carefully, and shipped to buyers via express airmail. Almost all butterfly exhibits use an air courier service such as DHL or Federal Express, which provide door-to-door service between supplier and buyer, and take each shipment through the required inspections.

People ask if butterfly farming is depleting local butterfly populations. In fact, the opposite is true. Because the eggs are collected and caterpillars are reared in cages or protected conditions, mortality due to parasites and predators is greatly reduced. Indeed most butterfly farms produce a surplus of butterflies and release those they cannot use back into the wild. Thus butterfly farming represents a wonderful example of the sustainable use of a natural resource. It is compatible with preservation of natural habitat such as rainforest, since butterfly hostplants are typically “weeds” or non-commercial plants that grow in relatively pristine areas.
Most of the butterflies raised by the butterfly farms around the world are common, large, showy species that do well in captivity. Table 3 lists some of the most often-seen genera of butterflies used in live butterfly exhibits. Many are very colorful; the brightly patterned species tend to be distasteful to predators (their bright colors are aposematic or warning colors). The typical behavior of aposematic species (e.g., slow, languid flight, boldness, etc.) also enhances their success in displays. Several of them, especially the danaids and heliconiids, are unusually long-lived.

Legality Issues: The Permitting Process

In this day of the CITES, the Endangered Species Act, concern with uncontrolled or inadvertent introductions of non-indigenous organisms, and illegal transport of exotic species, the permitting process for live butterfly exhibits displaying exotic species is formidable. The U.S. Department of Agriculture’s Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) is the agency primarily involved in issuing permits to facilities wishing to display non-native insect species. Butterfly zoos must submit lists of species they wish to import, including country of origin and the caterpillar hostplant. Some species may be refused because they eat (as caterpillars) a local plant, such as citrus. Thus the species permitted at a given facility are partially dependent on the climatic zone in which the facility is located--presumably northern exhibits should be allowed a wider range of species than those located in the extreme south of the USA, since survival of any escapees is less likely, due to both climatic conditions and potential hostplant availability.

Before the USDA will issue butterfly import permits to a facility, they require an on-site inspection to assess the escape risk from that facility. In the past couple of years, the USDA permitting office has become increasingly active and stringent in their requirements. Federal officials are presently drafting new “containment guidelines” for facilities exhibiting live exotic insects, including butterflies. Although several existing facilities do not conform exactly to the proposed containment requirements, all new exhibits are required to have at least two sets of self-closing doors, separated by foyers or vestibules, between the exhibit and the exterior. Air curtains over all exits will probably soon be mandatory in walk-through butterfly exhibits. Minimal hostplant material is allowed in the exhibit itself to discourage uncontrolled reproduction, as caterpillars are seen as having even greater escape potential than butterflies (although this is probably not the case in reality). Containment is also a concern in any auxiliary greenhouses used as rearing facilities, even though these are typically not open to the public.

In addition to ensuring that exotic organisms are securely contained in their destination zoos or exhibits, the USDA is becoming increasingly concerned with preventing the introduction of any accompanying parasites or pathogens. Recently USDA containment officials have suggested that all waste and packing material be autoclaved, for example. Packaging must be secure, and packages must be opened in a contained area away from public access. Only trained personnel are allowed access to service and rearing areas.

Facilities importing over $90,000.00 worth of stock annually must also obtain an import permit from the US Fish and Wildlife Service. The supplier (butterfly farm or broker) is responsible for obtaining export permits and any other required certification from the country of origin. Each shipment coming from abroad must pass through USA Customs where it is inspected by officials.
from Fish and Wildlife Service and the U.S. Department of Agriculture. Fish and Wildlife makes sure the export permits are in order, and that no endangered species are being transported. The USDA ensures that only species on the buyer’s import list are included in the shipment. Questionable shipments, improperly packed shipments, or shipments without accompanying documentation are destroyed or returned to the country of origin.

Conclusion

Butterflies are excellent subjects for public displays, and offer many opportunities for education about insects in general, their importance to the natural world, and the role of conservation. Live butterfly exhibits are popular and proliferating. Although containment of non-indigenous species is a concern, government agencies should work in collaboration with large butterfly facilities rather than taking an adversarial position. These exhibits can be important in raising the public’s awareness about the importance of conservation of both species and habitats, as well as the dangers of introducing exotic species.

ACKNOWLEDGMENTS

The author thanks Mike Weissman for generously sharing his wealth of information!
Table 1. Permanent, year-round, live walk-through butterfly exhibits currently in North America. In addition to the existing butterfly centers listed here, several comparable facilities are in the planning or construction stages, including Niagara Falls, Canada; St. Louis, Missouri; Branson, Missouri; Cancun, Mexico, etc. Numerous smaller and/or seasonal butterfly gardens scattered throughout Canada and the USA are not included.

<table>
<thead>
<tr>
<th>NAME OF FACILITY</th>
<th>YEAR OPENED</th>
<th>LOCATION</th>
<th>TYPE OF FACILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butterfly World*</td>
<td>1988</td>
<td>Ft. Lauderdale, FL</td>
<td>stand-alone facility, for profit</td>
</tr>
<tr>
<td>Butterfly World</td>
<td>1988</td>
<td>Vallejo, CA</td>
<td>exhibit within Marine World Africa USA Park (zoo/marina), non-profit</td>
</tr>
<tr>
<td>Day Butterfly Center</td>
<td>1988</td>
<td>Callaway Gardens, GA</td>
<td>exhibit within a large botanical garden, non-profit (in a for-profit resort)</td>
</tr>
<tr>
<td>Wings of Wonder</td>
<td>1993</td>
<td>Cypress Gardens, FL</td>
<td>exhibit within a large botanical garden, for-profit</td>
</tr>
<tr>
<td>Moody Gardens Rainforest Pyramid</td>
<td>1993</td>
<td>Galveston, TX</td>
<td>part of convention and mental health center complex, non-profit</td>
</tr>
<tr>
<td>Hidden Jungle</td>
<td>1993</td>
<td>1993 Escondido, CA</td>
<td>exhibit within San Diego Wild Animal Park (zoo), non-profit</td>
</tr>
<tr>
<td>Cockrell Butterfly Center</td>
<td>1994</td>
<td>Houston, TX</td>
<td>exhibit (separate entry fee) within museum of natural science, non-profit</td>
</tr>
<tr>
<td>Butterflies in Flight</td>
<td>1995</td>
<td>New Orleans, LA</td>
<td>in aviary of Audubon zoo, non-profit</td>
</tr>
<tr>
<td>Butterfly Pavilion and Insect Center</td>
<td>1995</td>
<td>Westminster, CO</td>
<td>stand-alone facility; non-profit</td>
</tr>
</tbody>
</table>

*Butterfly World is housed in a large, screened enclosure. All other butterfly zoos are in glasshouses.
**Table 2.** Main nectar plants used in live butterfly exhibits.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>COMMON NAME</th>
<th>FAMILY</th>
<th>FLOWER COLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Buddleia davidii</em></td>
<td>Butterfly bush</td>
<td>Loganiaceae</td>
<td>lavender, purple, pink, white</td>
</tr>
<tr>
<td><em>Clerodendron spectabilis</em></td>
<td>Glory bower</td>
<td>Verbenaceae</td>
<td>red</td>
</tr>
<tr>
<td><em>Cuphea hyssopifolia</em></td>
<td>Mexican heather</td>
<td>Lythraceae</td>
<td>lavender</td>
</tr>
<tr>
<td><em>Hamelia patens</em></td>
<td>Flame bush</td>
<td>Rubiaceae</td>
<td>red-orange</td>
</tr>
<tr>
<td><em>Jatropha integerrima</em></td>
<td>Jatropha</td>
<td>Euphorbiaceae</td>
<td>red</td>
</tr>
<tr>
<td><em>Lantana</em> (several species and cultivars)</td>
<td>Lantana</td>
<td>Verbenaceae</td>
<td>orange, red, yellow, white, lavender</td>
</tr>
<tr>
<td><em>Pentas lanceolata</em></td>
<td>Egyptian star</td>
<td>Rubiaceae</td>
<td>red, pink, white</td>
</tr>
<tr>
<td><em>Senecio confusa</em></td>
<td>Mexican flame vine</td>
<td>Asteraceae</td>
<td>orange</td>
</tr>
<tr>
<td><em>Stachytarpheta jamaicensis</em></td>
<td>Porter weed</td>
<td>Verbenaceae</td>
<td>purple, blue, pink</td>
</tr>
<tr>
<td><em>Tithonia rotundifolia</em></td>
<td>Mexican sunflower</td>
<td>Asteraceae</td>
<td>orange</td>
</tr>
</tbody>
</table>
Table 3. Most frequently seen/widely available butterflies flown in live butterfly exhibits.

<table>
<thead>
<tr>
<th>GENUS</th>
<th>FAMILY</th>
<th>COMMON NAME</th>
<th>SOURCE</th>
<th>EDIBILITY*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caligo</td>
<td>Brassolidae</td>
<td>Owl butterfly</td>
<td>Central and South America</td>
<td>palatable</td>
</tr>
<tr>
<td>Catonephele</td>
<td>Numphalidae</td>
<td></td>
<td>Central and South America</td>
<td>palatable</td>
</tr>
<tr>
<td>Danaus</td>
<td>Danaidae</td>
<td>Milkweed butterflies</td>
<td>Old and New World</td>
<td>unpalatable</td>
</tr>
<tr>
<td>Euploea</td>
<td>Danaidae</td>
<td>Crow</td>
<td>Tropical Asia</td>
<td>unpalatable</td>
</tr>
<tr>
<td>Hamadryas</td>
<td>Nymphalidae</td>
<td>Cracker butterflies</td>
<td>Central and South America</td>
<td>various</td>
</tr>
<tr>
<td>Heliconius</td>
<td>Heliconiidae</td>
<td>Longwings or Passionflower butterflies</td>
<td>Central and South America</td>
<td>unpalatable</td>
</tr>
<tr>
<td>Hypolimnas</td>
<td>Nymphalidae</td>
<td>Egg fly</td>
<td>Tropical Asia</td>
<td>palatable</td>
</tr>
<tr>
<td>Idea, Ideopsis</td>
<td>Danaidae</td>
<td>Rice paper butterfly</td>
<td>Tropical Asia</td>
<td>unpalatable</td>
</tr>
<tr>
<td>Morpho</td>
<td>Morphidae</td>
<td>Blue morpho</td>
<td>Central and South America</td>
<td>palatable</td>
</tr>
<tr>
<td>Papilio</td>
<td>Papilionidae</td>
<td>True swallowtails</td>
<td>Old and New World</td>
<td>various</td>
</tr>
<tr>
<td>Parides</td>
<td>Papilionidae</td>
<td>Cattlehearts or Poisonfeeders</td>
<td>Central and South America</td>
<td>unpalatable</td>
</tr>
<tr>
<td>Parthenos</td>
<td>Nymphalidae</td>
<td>Clipper</td>
<td>Tropical Asia</td>
<td>palatable</td>
</tr>
<tr>
<td>Phoebis</td>
<td>Pieridae</td>
<td>Sulpher butterflies</td>
<td>New World</td>
<td>Palatable</td>
</tr>
<tr>
<td>Trogonoptera, Troides</td>
<td>Papilionidae</td>
<td>Birdwings</td>
<td>Tropical Asia</td>
<td>unpalatable</td>
</tr>
</tbody>
</table>

*i.e., to natural predators such as birds*
HOPPERS, HERMITS AND HAEMOLYMPH: A VETERINARY APPROACH TO INVERTEBRATES

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Abstract

The veterinary profession is involved with invertebrates and their care for a variety of reasons. Invertebrates are kept in captivity in zoos, in private collections and in laboratories; they are the subject of many scientific studies as well as providing interest, entertainment and educational benefit. These animals may require veterinary attention and this can include diagnosis of disease, medical and surgical treatment, and advice on management and care. There are also indications for veterinary involvement in work with free-living invertebrates, particularly when these are threatened species that are the subject of management programs or attempts at reintroduction or translocation. Under such circumstances the basic rules that are followed when working with free-living vertebrates are applicable. Health programs for invertebrates should include quarantine, monitoring and appropriate preventive measures as well as attention to infectious and non-infectious disease if it occurs. The opportunities for expansion into invertebrate medicine are great and veterinarians need to take advantage of this.

Resumen

La profesión veterinaria está involucrada con los invertebrados y su cuidado por una variedad de razones. Los invertebrados son mantenidos en cautiverio en zoológicos, colecciones privadas y en laboratorios; están sujetos a muchos estudios científicos de interés, y de igual modo aportan entretenimiento y beneficios educacionales. Estos animales pueden requerir atención veterinaria que puede incluir el diagnóstico de enfermedades, tratamiento médico y quirúrgico, y consejos sobre su cuidado y manejo. También hay algunas indicaciones para el trabajo veterinario involucrado con invertebrados en libertad, particularmente cuando son especies en peligro que son objetos de programas de manejo o en intento de reproducción o translocación. Bajo estas circunstancias las reglas básicas seguidas cuando se trabaja con vertebrados silvestres son aplicables. Los programas de salud para invertebrados deberían incluir: cuarentena, monitoreo y medidas preventivas apropiadas, así como atención de enfermedades infecciosas y no infecciosas si ocurren. La oportunidad para expandirse en medicina de invertebrados es grande, y los veterinarios necesitan tomar ventaja de esto.

Introduction

The invertebrates comprise well over ninety per cent of all living species. There are at least one million species compared with nine thousand species of bird, four thousand mammals, six thousand reptiles, four thousand amphibians and over twenty thousand fish. While the vertebrate animals belong to only one phylum (Chordata), there are approximately thirty phyla of invertebrates.
Invertebrates range from single-celled Protozoa to complex and highly organized arthropods, molluscs and helminths. Virtually all invertebrates are exothermic and thus unable to control their body temperature, other than by behavioral means, although a very small number of species (mainly Lepidoptera) can tolerate the winter in temperate climates by slightly raising their body temperature. Some invertebrates, such as the insects and crustaceans, have tough exoskeletons while others, such as sea anemones, are composed solely of soft tissue. Invertebrates vary greatly in behavior, nutrition and habitat requirements.

Interest in the diseases and pathology of invertebrates is not new. Two thousand years ago, Greek and Roman observers recognized disorders of economically important invertebrates such as honey bees (Apis mellifera). In the late Middle Ages in Europe, invertebrates were frequently the subjects of study by scientists who had an interest in comparative anatomy, physiology and pathology.

In the last century the famous French scientist, Louis Pasteur, did research on diseases of the silkworm (Bombyx mori) and in so doing possibly saved the French silk industry and the economy of France.

Involvement by veterinarians is far more recent, however. In some veterinary schools, the syllabus has, for some time, included certain invertebrates of economic importance, particularly the honey bee, but generally the veterinary profession has regarded invertebrates as parasites and pests, deleterious to the health and well-being of domesticated animals, rather than considering them as animals in their own right.

The situation began to change about 20 yr ago when a small number of veterinarians on both sides of the Atlantic realized that a veterinary input into work with invertebrates was necessary. This was prompted by a number of factors, not least the popularity of invertebrates as companion animals and their importance in laboratories and scientific study. In addition, the growing importance of biological control and the emergence of invertebrate pathology as a discipline led some vets to realize that there were many areas of common ground between those who worked with vertebrates and those who worked with invertebrates.

In this paper the growing need for a veterinary input into the health, well-being and management of invertebrates is discussed and ways in which this role might be expanded are outlined. It is also suggested that work with free-living invertebrates, particularly those that are under threat, may assume increasing importance.

In this paper the word “invertebrate” will be used mainly to describe the larger metazoan species, particularly arthropods and molluscs, but where appropriate, reference will be made to other groups.

Background

Invertebrates are kept in captivity for a variety of purposes. These can be conveniently divided as follows:
1. as companion animals (pets);
2. for educational purposes in the home, classroom or college;
3. for display in zoos, butterfly houses or similar collections;
4. for research in laboratories;
5. as a source of food or other products, e.g. honey, silk; and
6. as an aid to treating human or animal patients or diagnosing disease, e.g. medicinal leeches (*Hirudo medicinalis*) used in surgery, haemolymph of molluscs employed in certain diagnostic tests.

Sometimes there may be overlap between these categories or between invertebrates that are kept in captivity and those that are free-living. Thus, for example, studies on endangered spiders and snails have involved captive breeding as well as field research. Those who work with invertebrates, including the veterinarian, may contribute to conservation efforts as well as to high standards of captive maintenance.

**Requirements of the Veterinarian**

The veterinarian who is involved with invertebrates requires the following:

1. a sound knowledge of the basic biology and natural history of the important groups of invertebrates. Such information may be gleaned from standard textbooks on entomology, on biology and on wildlife. Ideally it should be supplemented with practical experience of the species involved. Many veterinarians kept insects or other animals when they were children, and some (including the author) continue to do so;

2. access to information on the care of invertebrates in captivity and the diseases to which they are susceptible. Over the past few years a considerable volume of literature has become available, some relating to invertebrates of economic importance and some on specific veterinary care. Many popular books provide information on the care of pet invertebrates and laboratory animal publications sometimes cover such species. More specialized publications on the pathology of, and diagnostic techniques for, invertebrates include some specifically intended for veterinarians and zoologists and others for those working with invertebrates that are pests or are of economic importance. All in all, there is a wealth of information now available on invertebrates and their diseases and with modern electronic databases and access to international expertise, the veterinarian has no excuse for pleading ignorance;

3. contact with colleagues who can assist with diagnostic tests and provide second opinions. These are accessible through the zoo veterinary world and through laboratories; and

4. appropriate equipment and facilities. These will depend upon the extent to which the veterinarian is involved with invertebrates. The practitioner who sees an occasional tarantula spider or giant land snail will need relatively little compared with the colleague who is regularly visiting invertebrate collections in a zoo or laboratory.

In addition to the above, it is important that the veterinarian who works with invertebrates acquaints
him/herself with relevant legislation, regulations and codes of practice concerning the conservation, welfare and diseases of invertebrates. Further information on this may be obtained from various textbooks or publications of organizations such as the Federation of Zoological Gardens in the UK.

Handling and Restraint

Much depends on the species involved. Some, such as molluscs, millipedes and terrestrial crustaceans, can be grasped in the hand. Others, for example large spiders, tolerate gentle handling, but may bite if restrained. Certain invertebrates require the use of nets, rubber gloves or padded forceps; examples are leeches, certain hairy caterpillars and scorpions respectively. Caution should always be exercised if there is any doubt as to the safety of handling, either to the animal or the handier. Under such circumstances, the veterinarian may find it useful to view the patient through a glass or plastic container or to anesthetize it lightly (see later). In most species, hypothermia can be employed to facilitate handling; 30 min in a refrigerator at a temperature of 4°C is usually adequate. However, under no circumstances should hypothermia be used for surgical or other procedures that may be painful.

Diseases and Clinical Care

The “higher” invertebrates are susceptible to a wide range of diseases and these are comparable in many ways to those seen in vertebrates. Control of disease is based largely upon prevention and this in turn depends on good management and hygiene.

Treatment of individual invertebrates is possible but is generally restricted to the larger species.

Changes in management will often have a beneficial effect in reducing morbidity or mortality and is particularly valuable when dealing with invertebrates that are kept in large groups or colonies. If many animals are affected, the veterinarian should separate some and keep them under different conditions from the others. Changes to temperature, relative humidity or terrain may per se prove beneficial.

Hygiene was mentioned earlier. It plays an important part in disease control, especially amongst arthropods where many microorganisms are recognized pathogens. In a few cases, there may be a risk of spread of infection to humans (see later). Regular cleaning of cages and the removal of sloughed skins and feces will go a long way towards minimizing the risk of an epizootic.

Care must be taken over some species however. Giant land snails (Achatina spp.), for example, appear to prefer dirty conditions and will often thrive in the presence of decaying vegetation. Some molluscs are dependent upon a bacterial flora in order to ensure optimum digestion and metabolism; in these cases, too over-enthusiastic use of disinfectants may prove deleterious. One must, therefore, be selective when implementing hygienic measures. While hot water is the cheapest and safest disinfectant, cetrimide, hypochlorite or dilute formalin can be used with care. It is always a wise precaution to rinse thoroughly after their use.

Signs of ill health in invertebrates include anorexia, lethargy, change of color, discharges and
dysecdylysis (difficulty in shedding the skin). Behavioral changes may also be seen; for example, mealworms (Tenebrio molitor) will assemble on the surface of their container if the carbon dioxide levels are high. However, some apparently aberrant behavior may be perfectly normal; for example, a spider which is shedding its skin may lie on its back and appear to be dead or dying.

A full investigation must always be carried out and details taken of the history and method of management. The animal must be handled and examined. Even small invertebrates can be examined using a hand lens. Clients who keep invertebrates should be encouraged to keep records and also to save such specimens as shed skins and empty pupal cases so that these can be examined for parasites or lesions.

Diagnostic samples can be taken from invertebrates - for example, swabs from lesion can be cultured or examined direct. In some cases it may be possible to take blood (haemolymph); a technique in lobsters (Homarus spp.) was described by Greenwood and in snails (Achatina spp.) by Cooper.

Post-mortem examination of invertebrates can prove useful. Even if the veterinarian is not familiar with the detailed anatomy of the species, he/she should be able to detect gross lesions, to demonstrate the presence of parasites and to take samples for microbiology and histopathology.

Anesthesia

Anesthesia may be needed to immobilize invertebrates or to permit the performance of procedures which may cause pain. It is important that veterinarians are both acquainted with, and able to use, appropriate anesthetic techniques. The higher invertebrates are not difficult to anesthetize and most of the procedures recommended appear to be relatively safe. For a detailed account, reference should be made to Cooper but the following is a list of commonly used techniques:

- Carbon dioxide, ether, halothane or isoflurane by inhalation (terrestrial species)
- Tricaine methanesulphonate (MS222) or benzocaine in acetone by absorption (aquatic species)
- Carbon dioxide by absorption - bubbled through the water (aquatic species)

The question of how best to kill captive invertebrates has been addressed by some authors including the Federation of Zoological Gardens in the UK. As a general rule, invertebrates should be killed either by physical means or by an overdose of an anesthetic agent.

Discussion

The veterinary care of invertebrates has assumed increasing importance in recent years and there is now no doubt that the veterinary profession must be prepared to deal with such animals. In addition, concern over the status in the wild of some species and the need for management programs, including attention to health and disease, means that there can be a need for a medical involvement here also. Although most invertebrates differ greatly in morphology and physiology from other animals, the basic principles of disease prevention and treatment are similar. The veterinarian must
be prepared to respond to the challenge presented by these species.

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LITERATURE CITED

USE OF RECYCLED WASTE WATER IN ANIMAL EXHIBITS

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Abstract

Wildlife Safari (WS) is a 610 acre drive-through exotic animal park located in Winston, Oregon. The drive-through is divided into three main geographical regions: Asia, Africa, and North America. In each area, animals native to the geographical region are displayed in open habitat much like what would be found in their native home. The park also includes a five acre area of walk-through exhibits and visitor amenities.

WS currently pumps water from the South Umpqua River to maintain water levels in the exhibit ponds and stream flow through the park. In the dry summer months, up to 300,000 gallons of river water may be pumped into the park each day with virtually no outflow. This water is consumed through evaporation, absorption, or animal intake.

The Winston-Green Regional Treatment Facility (WGRFT) has proposed providing up to 1.5 million gallons/day of treated waste water for the park’s use. This is a unique cooperative project that brings together a non-profit institution and local government agencies. The project also falls under the jurisdiction of the Department of Environmental Quality (DEQ), the Environmental Protection Agency, the Army Corps of Engineers and other federal government agencies.

The waste water will be treated to DEQ standards that allow limited human exposure, but will not be of drinking quality. The treated waste water will be utilized to replace the water currently being pumped from the South Umpqua River. The additional volume of water available from the WGRFT will also allow development of new water features in the exhibit areas, irrigation for a planned arboretum and the creation of a greenbelt as a perimeter firebreak at WS. In addition, the water reuse program will improve the habitat along the South Umpqua River where effluent is currently discharged, provide additional water downstream from WS for development, agriculture and serve as a model for future state water re-use plans.

Monitoring of water quality will be conducted throughout the park. Water sampling and analysis will be done from eight water test locations: one at WGRFT, and seven others throughout WS. Water evaluation includes weekly analysis for flow, temperature, pH, and suspended solids. Monthly testing will include biological oxygen demand for 5 days (BOD5), total suspended solids (TSS), ammonia, nitrates and phosphates. Because local industries do not currently produce any significant toxic by-products, we expect the recycled waste water to be low in harmful substances. Long term health concerns for the animal collection will be addressed by monitoring bioaccumulation of heavy metals and other toxins.

At this time treated waste water is not being used in animal contact situations on a widespread basis. Known examples will be discussed both from the zoological community and private industry. Given the high level of water use in zoological parks and the increasing concern for conserving water resources, we believe that recycled water will become an important part of exhibit programs. By
evaluating this project, we hope to demonstrate its potential use in the zoological community.

Resumen


El Wildlife Safari actualmente se abastece de agua del río Umpqua del Sur para mantener los niveles de agua en estanques y arroyos situados a lo largo de todo el parque. En los meses más secos, durante el verano, se bombean más de 1,500 m³ de agua del río al parque diariamente, sin que salga del parque ningún volumen importante. Esta agua se consume a través de la evaporación, la absorción, o el consumo directo por parte de los animales.

La planta regional de tratamiento Winston-Green (WGRTF) ha propuesto suministrar por encima de 6 millones de litros diarios de agua tratada para uso del parque. Este es un proyecto de cooperación que vincula a una institución no lucrativa con un organismo gubernamental local. El proyecto también recae sobre la jurisdicción del Departamento de Calidad Ambiental (DEQ), la Agencia de Protección del Medio Ambiente, el Cuerpo de Ingenieros de la Armada, y otras agencias del gobierno federal.

El agua residual será tratada hasta alcanzar estándares que permitan la exposición limitada al hombre, pero no tendrá la calidad suficiente para ser potable. El agua tratada será utilizada para remplazar el agua que actualmente se bombea del río. El volumen adicional de agua disponible de la WGRTF permitirá el desarrollo de nuevas atracciones acuáticas, servirá para irrigar un vergel que se planea a futuro, y para la creación un cinturón verde que se utilizará como barrera de fuego en el WS. Además, el programa de agua reciclada mejorará el medio ambiente a lo largo del río Umpqua del Sur, donde se descarga actualmente el efluente. El río proporcionará agua adicional para el desarrollo de la agricultura y servirá como un modelo para los planes futuros de la re-utilización del agua en el estado.

El monitorío de la calidad del agua será conducido en todo el parque. El agua será muestreada y analizada a partir de ocho localidades; uno en la WGRTF y siete más dentro del parque. La evaluación del agua incluye el análisis de flujo, temperatura, pH y sólidos suspendidos. Mensualmente se incluirán las pruebas de Demanda Biológica de Oxígeno por 5 días (DBO5), el total de sólidos suspendidos, así como niveles de amonio, nitratos y fosfatos. Debido a que las industrias locales actualmente no producen ningún sub-producto de toxicidad importante, esperamos que los desechos del agua reciclada sean bajos en substancias dañinas. Se monitorizará lo concerniente a la salud de los animales a largo plazo, sobre todo en lo que respecta a la bioacumulación demetales pesados y otras toxinas.

Por el momento las agua grises tratadas no han sido utilizadas en situaciones de contacto generalizado con animales. En esta presentación se discutirán los ejemplos conocidos en la
comunidad zoológica y en la industria privada. Dados los altos volúmenes de agua que se utilizan en los parques zoológicos, así como la creciente preocupación por la conservación de los recursos acuíferos, creemos que el agua reciclada será una parte importante en los programas de exhibición. Mediante la evaluación de este proyecto esperamos demostrar su uso potencial a la comunidad zoológica.
APPLIED PEST CONTROL AT WOODLAND PARK ZOOLOGICAL GARDENS

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Abstract

Integrated pest management (IPM) is an approach to pest control that utilizes regular monitoring to determine if and when control measures are needed. IPM employs indirect and direct suppression tactics to keep pest numbers at tolerable levels. Ongoing pest management monitoring is critical to an effective program. Contracting with professional pest control agencies can provide valuable recommendations to existing programs. Indirect suppression strategies are exemplified by educating the zoo keeper and maintenance staff in the proper sanitation, mechanical and structural needs in relation to the control of pests. Direct suppression of zoo pests by trapping is common and effective. The blood and tissue sampling of live-trapped pests can be a means of disease surveillance in zoos. All zoo employees can apply different strategies to take every aspect of a zoo’s pest control program to a higher level of effectiveness.

Resumen

El manejo integrado de plagas (IPM), es un sistema de control de plagas con monitoréo regular que se utiliza para determinar si las medidas de control son necesarias o no. El IPM emplea tácticas de eliminación directa e indirecta para mantener el número de individuos a un nivel tolerable. Actualmente el monitoréo del manejo de plagas es esencial para el éxito de del programa. La contratación de agencias profesionales de control puede proporcionar algunas recomendaciones muy valiosas para los programas ya existentes. Las estrategias de eliminación indirectas son ejemplificadas al educar a los animaleros del zoológico y al personal de mantenimiento en como llevar a cabo un saneamiento adecuado, así como en las necesidades mecánicas y estructurales en relación al control de las plagas. La eliminación de plagas con trampas en el zoológico es un método común y efectivo. La sangre y las muestras de tejido de las plagas atrapadas ayuda a determinar y vigilar enfermedades en los zoológicos. Todos los empleados de los zoológicos pueden implementar diferentes estrategias para asegurar que cada aspecto del programa de control de plagas alcance los niveles más altos de efectividad.

Introduction

Integrated pest management (IPM) is an approach to pest control that utilizes regular monitoring to determine if and when control measures are needed. IPM employs direct and indirect suppression tactics to keep pest numbers at tolerable levels. The goal of IPM is to control pests using a minimum of pesticides. Pesticide treatments are used only when and where monitoring has indicated that the pest population is causing unacceptable economic, medical or aesthetic damage. Woodland Park Zoological Gardens (WPZG) maintains an IPM approach to pest control.

An IPM program consists of the following elements: pest identification, monitoring, deciding at
what point a pest population becomes a problem, indirect suppression (modifying exhibits, changing human behaviors and educating staff), and direct suppression (mechanical, biological and/or chemical control). Indirect suppression is the most important strategy for long term pest control. Direct suppression methods will only result in a temporary reduction in pest numbers.

These programs should not be designed unilaterally. Zoo keepers should have input because they will maintain and monitor the program daily in their respective areas. The zoo keeper’s supervisor should be responsible for supervising the zoo keeper and communicating routine results to the pest control operator (PCO). The PCO designs and modifies the programs with the zoo keepers to meet changing pest patterns and pressures. The zoo veterinarian monitors and provides suggestions on the progress and direction of the overall program.

All zoo employees have the responsibility to promote the health and well-being of the animals in their care; to include the reduction of pest contamination and infestation in their areas. Applying different strategies at all staffing levels has improved the overall pest control program at WPZG.

PEST MANAGEMENT MONITORING/Pest Control Operator

WPZG experienced a potential interruption in the monitoring of pest control services during the lengthy absence of the PCO following an injury. A private pest control company was contracted to develop a plan for the zoo’s immediate needs and to critique the zoo’s existing program. The recommendations that resulted were a departure from the protocols currently in place and reflected a change to a more zoo-wide program due to a then increased problem from rodents (Addendum 1). Emphasized by the consultant company was the necessity of a person dedicated to the overall monitoring of the zoo’s pest control program.

The PCO is tested and licensed by the Washington State Department of Agriculture (WSDA). This licensing is required of individuals who professionally use or provide advice on the commercial use of pesticides, including the more hazardous restricted-use pesticides as defined by the Environmental Protection Agency (EPA). The PCO investigates reported pest problems and determines an appropriate control strategy, then reviews pesticides and application methods with the zoo veterinarian for final approval. A treatment schedule is developed with input from the zoo keeper staff. After treatment, the PCO follows up with periodic evaluations of the control measures in cooperation with zoo keeper staff, oversees the disposal of used products and empty containers and keeps accurate records of pesticide use as required by law.

INDIRECT SUPPRESSION/Education

Zoo keepers and maintenance staff are the first line of defense against pests due to their daily presence in each area. They are responsible for the cleanliness and sanitation of their respective areas. They notify the PCO if they identify signs of pest infestation, prepare areas for treatment and move animals as necessary. Within their units or areas of responsibility, they remove dead and dying pests, maintain and monitor bait stations and set traps as supplied and recommended by the PCO. The indirect suppression of pests through these measures is part of the strategy of efficient and effective long term pest control.
Zoo management has a responsibility to educate and encourage zoo keepers and maintenance workers to maintain a minimum level of sanitation and structural integrity. Sanitation standards should be maintained throughout the zoo, including concessions, administrative and education facilities. Zoo keeper assistance is needed in communicating any pest sightings to the PCO. Such communication should be made directly or in writing by way of a Daily Keeper Report or Pest Sighting Form.

Sanitation and maintenance guidelines as they pertain to the care and well-being of zoo animals have been established by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) through The Animal Welfare Act. The maintenance of an effective program for the control of insects, feral birds and feral rodents in animal areas and the main commissary storage area is the legal obligation of USDA licensed facilities. USDA inspections will identify areas of institutional noncompliance. USDA policy is to encourage compliance through the education and cooperation of zoo personnel.

Scheduled simulated USDA inspections in zoo units at WPZG has proven to be helpful in achieving compliance with pest control goals through staff education. These inspections are reviews with the zoo staff and are scheduled annually on the zoo’s preventive medicine calendar. A Zoo Pest Inspection Checklist serves as a worksheet for the veterinarian and supervisory staff doing the inspection (Addendum 2). The worksheet provides an interactive list for the zoo keeper and maintenance staff to know what is being inspected and identified as priority items by the zoo veterinarian and PCO. The report that results is useful to advise the zoo keeper and maintenance staff of appropriate sanitation, mechanical and structural needs in relation to the control of pest problems.

**DIRECT SUPPRESSION/Mechanical Control**

The direct suppression of pest species by live trapping is common in zoos. Live trapping allows for the euthanasia or translocation of the offending animal. The euthanasia of wild animals may or may not be legally permitted by federal, state or municipal laws. Exemptions may be granted by federal and state wildlife agencies on a case by case basis. Public sentiment towards some species may also preclude a more aggressive euthanasia policy. Educating the zoo staff as to the necessity of euthanasia is important and helps with compliance from all zoo staff. Domestic species such as dogs and cats may also be live-trapped. Educating the owners of such animals as to the disease risk to the zoo collection should be attempted as well as working with local Animal Control officers who should institute effective fines for the reclaiming of such animals.

Trapping and eliminating a pest species may reduce the risk of zoonotic and infectious disease. Immobilizing pest species for blood and tissue sampling may be indicated as a means of disease surveillance. A zoo policy and protocol should be established for the efficient processing of animals and samples when pest species are trapped. Pest species may also be trapped, tested and translocated if no evidence of infectious disease is obvious.

Should translocation be the established zoo policy, individual animal identification should be pursued by standard zoo animal identification methods such as ear tagging, tattooing and/or transpondering. Vaccinating against infectious disease before release may also be considered to potentially lessen the risk of contagious disease transmission to the zoo collection. This may need
to be done in cooperation with local or state wildlife officials where the handling and altering of wildlife may be regulated.

Conclusion

The control and prevention of pest infestations in a zoo requires the full cooperation of all zoo personnel. The key to the success of this partnership is effective communication. This requires all appropriate personnel to have a full understanding of the pest control program objectives. Zoo management must keep personnel informed as to the progress of the program. All zoo employees can apply different strategies and take every aspect of the zoo’s pest control program to a higher level of effectiveness.

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LITERATURE CITED

Addendum 1. Bait station recommendations.

The basic strategy of rodent control is a layered defensive strategy of property perimeter, the building exteriors and interiors. The goal is to intercept immigrating rodents before they enter the buildings.

- Exterior bait stations should be secured to the ground or a building. Stakes, barbed anchors, wire and concrete blocks are some alternatives.
- The bait in the stations should be identified with a card or label as to the type of toxin.
- Baits should be individually secured within a bait station.
- In areas of infestation, bait station should be spaced at 8 m intervals with less bait placed in each station, and not within 3 m of doors.
- Waxed based baits are preferable because of their palatability and weather resistance.
- Liquid baits are alternatives when access to free water is restricted.
- Rodents living inside buildings also forage outside for food. Increasing the number of bait stations outside may help control inside populations.
- Individuals stations should be mapped and numbered. This will help prevent lost stations.
- Date cards should be placed in the stations to record when the station was last checked.
- Schedule routine changes of bait in all stations. Keeping bait fresh will increase the acceptance by the rodents. As a general rule, the stations should be checked often enough to always have bait present. Changing the bait on a regular routine should provide information on rodent movement within the zoo and information on reservoir populations.
- Different formulations of bait rodenticides with the same active ingredient may be more palatable to the rodents. Different types of rats are known to be more discriminating in their diet.
- Rodent populations are typically “on the move” and bait stations in all units are more likely to affect rodents moving from location to location.
- Reduction of harborage areas inside and outside buildings is critical.
- Reduction of food sources is critical to the success of rodent control.
- Tracking powder as a toxicant may have limited areas for use.
- Stations should be baited with a bar of soap when rodenticides are not being used. Rodents will leave tooth marks when gnawing the bar soap which helps in pest identification.
- Gassing burrows during wet periods of the year may have some value.
- Mice only need about 5 mm to enter structures. Add weather stripping or door sweeps to the bottom of all doors. Use steel wool to semi-permanently rodent proof the bottom of doors.
- Keep bait in bait stations only.
- Keep an 45 cm wide inspection and cleaning area along all walls.
- Whenever possible, no food should be left out overnight for rodents to consume.
- Rodents shy of traps can be reduced by:
  1. Set out baited traps for 3-5 days before setting them.
  2. Use non-baited traps along runways.
  3. Use an assortment of traps if one particular type is not effective.
  4. Change and experiment with different types of bait.
Addendum 2. Zoo pest inspection checklist.
The inspected items below are to be checked ACCEPTABLE, NOT ACCEPTABLE or QUESTIONABLE. Comments that follow are meant to clarify and give direction to the Unit Keeper.

A. Building Exterior
   A NA Q 1. Rodent, bird, insect and predator proofed with perimeter pest control?
   A NA Q 2. Weeds, brush and litter harborage areas eliminated?
   A NA Q 3. Equipment and garage storage/handling areas kept clean and orderly?
   A NA Q 4. Paving and drainage maintained?

B. Building Interior in Non-Animal Areas
   A NA Q 1. Walls, floors, ceilings clean and free of cracks/holes?
   A NA Q 2. Windows kept closed and screened?
   A NA Q 3. Doors fit tightly with door sweeps where indicated?
   A NA Q 4. Ventilation and lighting adequate?

C. Food Storage and Preparation Areas
   A NA Q 1. Package and dry food storage in covered containers and on racks?
   A NA Q 2. Cold food storage in covered containers and in refrigeration/freezer?
   A NA Q 3. Food preparation counter and surface area is kept clean and uncluttered?
   A NA Q 4. Damage/moldy/infested food products stored separately?

D. Employee Areas
   A NA Q 1. Are lunch or break rooms clean and accessible for cleaning?
   A NA Q 2. Is employee food stored and/or consumed in animal areas?
   A NA Q 3. Are toilet facilities sanitary in and in good repair?
   A NA Q 4. Locker rooms free of old clothes, trash and regularly emptied and cleaned?

E. Garbage and Recycling Areas (Indoor)
   A NA Q 1. Storage area for garbage receptacles adequate and kept clean?
   A NA Q 2. Garbage containers are of proper type and regularly covered?
   A NA Q 3. Garbage areas show evidence of regular cleaning?
   A NA Q 4. Are recycle containers are regularly emptied?

F. Building Interior/Exterior in Animal Areas
   A NA Q 1. Area free of rodent, insect, bird, mammal or predator infestation?
   A NA Q 2. Area free of excessive animal food/feces?
   A NA Q 3. Area free of odors coming from animal waste or spoiled foods?
   A NA Q 4. Cleaning/disinfection schedule is acceptable?

G. Documentation
   A NA Q 1. Are pest sighting logs being used regularly and routed appropriately?
   A NA Q 2. Are bait station maps being used regularly and routed appropriately?
   A NA Q 3. Are requests for maintenance made through the Daily Keeper Report?
   A NA Q 4. Is the PCO being contacted when the evidence of pests are found?

Comments_____________________________________________________________________________________
___________________________________________D.V.M.
____________________________________________Unit Zoo Keeper
____________________________________________Zoo Keeper Supervisor
____________________________________________Pest Control Operator
SALT TOXICOSIS IN WILD BIRDS

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Abstract

Salt toxicosis has been well-documented in the literature for domestic mammals and poultry.2,3 This condition is commonly attributed to limited access to water, in addition to an increase in sodium intake. Most animals with functioning kidneys can tolerate increased dietary sodium, if adequate water is available. There is also substantial documentation of avian extrarenal salt secretion, and the structure and function of the nasal gland.5 This gland is present in many birds and allows them to consume and process saline water without ill effects. Documentation of salt toxicosis in wild bird species is uncommon, and this paper provides an overview of literature reports, and cases submitted to the National Wildlife Health Center. Reports of salt toxicosis, source and species involved are given in Table 1.

Resumen

La toxicosis salina ha sido bien documentada en la literatura sobre mamíferos domésticos y de granja.2,3 Esta condición es comúnmente atribuida a una restricción en el consumo de agua, además a un incremento en el consumo de sodio. Muchos animales con funcion renal normal pueden tolerar el incremento de sodio en su dieta si tienen una cantidad adecuada de agua disponible. También es considerable la documentación sobre la secreción extrarenal de sal en aves, y la función y estructura de la glándula nasal.5 Esta glándula está presente en muchas aves y les ayuda a consumir y procesar el agua salina sin efectos patológicos aparentes. Los reportes de toxicosis por sal en aves silvestres son poco comunes. En este trabajo se muestra una revisión bibliográfica, así como casos a presentados en el Centro Nacional de Salud de la Fauna Silvestre. En la tabla No. 1 se pueden ver reportes de toxicosis salina, fuentes y especies involucradas.

LITERATURE CITED

Table 1. Species, source and location of reports of salt toxicosis in wild birds.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
<th>Location</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pheasant (<em>Phasianus colchicus</em>)</td>
<td>Road salt</td>
<td>Wisconsin, USA</td>
<td>6</td>
</tr>
<tr>
<td>Unspecified</td>
<td>Water concentration for mining</td>
<td>New Mexico, USA</td>
<td>1</td>
</tr>
<tr>
<td>Mountain ducks (<em>Tadorna tadornoides</em>)</td>
<td>Drought</td>
<td>Western Australia</td>
<td>4</td>
</tr>
<tr>
<td>Common merganser (<em>Mergus merganser</em>)</td>
<td>Ice</td>
<td>North Dakota, USA</td>
<td>7</td>
</tr>
<tr>
<td>Lesser Canada geese (<em>Branta canadensis parvipes</em>)&lt;br&gt;Northern shoveler (<em>Anas clypeata</em>)</td>
<td>Rapid temperature change on a hypersaline lake</td>
<td>Saskatchewan, Canada</td>
<td>8</td>
</tr>
<tr>
<td>Fulvous whistling duck (<em>Dendrocygna bicolor</em>)&lt;br&gt;Gull (<em>Larus spp.</em>)&lt;br&gt;Tri-colored blackbird (<em>Agelaius tricolor</em>)</td>
<td>Manufacturing discharge pond</td>
<td>Texas, USA</td>
<td>NWHC records</td>
</tr>
<tr>
<td>Lesser scaup (<em>Aythya affinis</em>)&lt;br&gt;Pintail (<em>Anas acuta</em>)</td>
<td>Oil drilling produced waters</td>
<td>New Mexico, USA</td>
<td>NWHC records</td>
</tr>
<tr>
<td>Mallard (<em>Anas platyrhynchos</em>)&lt;br&gt;Ring-billed gull (<em>Larus delawarensis</em>)&lt;br&gt;Merganser (<em>Mergus spp.</em>)&lt;br&gt;Black duck (<em>Anas rubripes</em>)&lt;br&gt;Gadwall (<em>Anas strepera</em>)&lt;br&gt;Common goldeneye (<em>Bucephala clangula</em>)&lt;br&gt;Canada goose (<em>Branta canadensis</em>)</td>
<td>Power plant discharge; road salt</td>
<td>Utah, USA</td>
<td>NWHC records</td>
</tr>
<tr>
<td>White pelican (<em>Pelecanus erythrorhynchos</em>)&lt;br&gt;Gull (<em>Larus spp.</em>)</td>
<td>Undetermined</td>
<td>Utah, USA</td>
<td>NWHC records</td>
</tr>
</tbody>
</table>
LEAD POISONING IN CAPTIVE GENTOO PENGUINS (Pygoscelis papua papua)

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Abstract

Lead poisoning was diagnosed in an arctic exhibit in a colony of 20 gentoo (Pygoscelis papua papua) and 20 king (Aptenodytes patagonica) penguins. The diagnosis was made using radiography and fluoroscopy, measuring blood lead levels, and necropsy. The source of lead was found to be ankle weights worn by the divers responsible for cleaning the pool. The weights contained 1-2 mm lead pellets that were expelled through faulty seams. These lead pellets were consumed by the gentoo penguins. Eleven out of 20 gentoos were positive for pellets radiographically. Despite massive clean-up efforts and aggressive treatment with chelating agents, a 50% mortality was observed in the gentoo penguins. No king penguins died or showed clinical signs of lead poisoning.

Resumen

Se diagnosticó envenenamiento con plomo en el exhibidor ártico en una colonia de 20 pingüinos gentoo (Pygoscelis papua papua) y 20 pingüinos rey (Aptenodytes patagonica). El diagnóstico fue hecho utilizando radiografías y fluoroscopía, midiendo los niveles de plomo en sangre y por hallazgos a la necropsia. La fuente del plomo fueron las anclas de peso utilizadas por los buzos responsables de limpiar la alberca. Estas anclas contenían pellets de 1 a 2 mm que se salieron a través de una costura mal cerrada. Estos pellets de plomo fueron consumidos por los pingüinos. Once de los 20 pingüinos fueron diagnosticados positivos por evidencias de pellets en las radiografías. A pesar de los grandes esfuerzos de limpieza y un tratamiento agresivo con agentes quelantes, se observó un 50% de mortalidad en pingüinos gentoo. Ningún pingüino rey murió o mostró signos clínicos de envenenamiento por plomo.

Introduction

Lead poisoning is a common toxicity of birds. This report discusses a case of lead poisoning in gentoo penguins at Omaha’s Henry Doorly Zoo. The challenges of diagnosis and treatment of a
A colony of 20 gentoo penguins and 20 king penguins were housed in a new arctic exhibit. The display consisted of a pool, approximately 10-15 feet wide, 8-13 feet deep and 60 feet long, separating viewers and swimming penguins by only the thickness of the glass. The pool sides contained numerous crevices, simulating cliff-like rock work. The ambient temperature was maintained at a constant 36°F, and the water temperature was 40°F. The rock work and glass were vacuumed and cleaned regularly by divers wearing waist and ankle weights.

Within 15 wk of arrival, one male gentoo penguin developed anorexia, vomiting, depression, periocular yellow crusty exudate, and diarrhea. Hematology and serum chemistries demonstrated leukocytosis, heterophilia, elevated SGOT and CPK, and low blood glucose when compared to other avian normal values. Bacterial and fungal cultures of the crop and feces failed to demonstrate pathogens.

Ten days after the onset of clinical signs, gastroscopy revealed no abnormalities. Survey radiographs appeared normal except for a 2 mm diameter metal density in the caudal abdomen.

Fourteen days after the onset of clinical signs a blood sample was obtained, and lead content was 1.5 ppm, indicating toxicosis. Blood lead concentrations greater than 0.5 ppm are considered toxic in waterfowl; no values are available for penguins.

Fifteen days after the penguin became ill, it was discovered that the ankle weights worn by the divers had faulty seams and were expelling 1-2 mm diameter lead pellets into the pool. Keepers had not detected them due to their small size and color identical to the grey rock. At this time, the penguin was started on D-penicillamine (Depen®, Wallace Laboratories Division of Carter-Wallace, Inc. Cranbury, New Jersey 08512, USA) at 55 mg/kg p.o. b.i.d. The bird was also force fed psyllium-containing fish.

Seventeen days after the first bird became ill, another gentoo penguin became anorectic. Radiographs revealed five 2 mm diameter metal densities in the abdomen. D-penicillamine therapy was instituted at the dosage stated above. At this time, five additional birds were selected for radiographs. This procedure was considered extremely stressful on the birds because they had to be removed from their cold environment, so it was performed reluctantly. Radiographs taken with the portable unit were not of sufficient quality to be diagnostic; however, three of the five birds had metal densities in the abdomen. The entire flock of gentoo penguins was placed on D-penicillamine, in addition to injecting all fish with psyllium before feeding them to the penguins.

Eighteen days after the first bird developed clinical signs, the first and second birds died.

On day 23, a fluoroscopy unit was obtained for use on all 18 remaining gentoo penguins and five randomly selected king penguins. Fluoroscopy was positive for metal in seven of the gentoo and none of the king penguins. The positive gentoos were then started on CaEDTA (Calcium Disodium Versenate®, Manufactured for 3M Pharmaceuticals, Northbridge, CA 91324, USA by Sanofi...
Winthrop Pharmaceuticals, McPherson, KS 67460, USA) at 40 mg/kg i.m. b.i.d. along with the oral D-penicillamine. On day 23, two birds sampled had blood lead concentrations of 2.4 and 0.44, respectively. On day 25, these birds’ lead concentrations were 0.5 and 0.3 respectively. On day 25, the remaining fourteen birds were also tested for blood lead. Two penguins had blood lead concentrations of 0.3 and 0.4 ppm, indicating exposure, one was < 0.2 ppm (negative), and 11 were < 0.1 ppm (negative). Massive pellet clean-up efforts were instituted in the enclosure.

Eight birds died between days 25-27, including all the birds that had blood lead contents indicating exposure or toxic concentrations. The predominant clinical sign observed in birds that died were seizures. Two of the birds that died were negative on fluoroscopy. Treatment was modified, and CaEDTA replaced the D-penicillamine therapy in all birds. In addition, all birds were placed on ampicillin (Amp-equine®, SmithKline Beecham Animal Health, West Chester, PA 19380 USA) at 200 mg/kg, s.c. b.i.d. and fluconazole (Diflucan®, Division of Pfizer, Inc. New York, NY 10017, USA) at 5 mg/kg p.o. s.i.d. Psyllium continued to be added to all the fish fed during the entire treatment.

After discontinuing the D-penicillamine therapy, no additional birds died or demonstrated clinical signs of toxicity.

In the first two dead birds, kidney lead concentrations were 14.6 and 20.4 ppm, while liver lead concentrations were 7.35 and 7.87 ppm respectively. Surprisingly, tissue lead concentrations were negative in the remaining eight animals that died.

On necropsy, the only consistent findings were swollen kidneys, enlarged, flabby hearts, and bloody mucus in the small intestines. Secondary infections were common, but varied greatly among individual birds. They included fungal airsacculitis and epicarditis, bacterial meningitis, hepatitis, and enteritis. Two of the birds necropsied had gastrointestinal foreign body perforation. Histologically, the most consistent microscopic change was tubular nephrosis and hemosiderosis.

Discussion

Interestingly, none of the king penguins died or even demonstrated clinical signs of lead toxicity. Since the king penguins are sedate by nature and far less exploratory than the gentoos, it was speculated that they never discovered the lead pellets, and therefore, did not ingest them.

The most reliable test to detect the presence of metallic foreign bodies appeared to be fluoroscopy or radiography, although none of the diagnostic tests utilized proved to be accurate one hundred percent of the time. Surgical removal of the minute pellets was impossible, due to their wide dispersion throughout the digestive tract. Psyllium in the diet appeared to make no positive clinical difference in the speed with which the pellets passed. The low blood and tissue lead concentrations could partially be explained by the fact that all the birds tested had been on 4-7 days of chelation therapy. However, one of the treated birds did have a high blood lead content.

Despite aggressive treatment, 50% of the gentoo penguins died. Two birds that died were negative on fluoroscopy and had no detectable blood lead. Only one bird that was positive on fluoroscopy (but had blood lead content < 0.2 or lower throughout treatment) survived the ordeal. It is suspected
that the nine other surviving birds did not ingest any lead pellets. They were negative on fluoroscopy, blood lead, and showed no clinical signs. The high death rate may be explained with several possible theories:

1. Immunosuppression may have occurred from the lead toxicity and the stress of frequent handling. This may have resulted in a high incidence of secondary disease.
2. Combined nephrotoxicity and neurotoxicity of lead intoxication and the chelating agents may have occurred. Dosage regimens used for treatment were those found in the general avian literature. Specific dosages for penguins were not available.
3. Therapy may have been started too late in the progression of the disease. Theoretically, birds could have been exposed to lead for up to 15 wk since this was the age of the display.

This case was a clinical challenge because it took several days of investigation before lead toxicity was diagnosed. It reminds the clinician to remain open-minded to seemingly impossible differentials. It demonstrates the challenge and frustration of treating a toxicosis in a species for which drug dosage regimes have not been determined.
WELCOME TO THE REVOLUTION--OTHERWISE KNOWN AS, INNOVATE OR EVAPORATE: THE SEVEN CHARACTERISTICS OF INNOVATIVE PEOPLE

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Abstract

Some people constantly create new and better ways of doing things - delighting some, irritating others and often times, jolting the competitors. In pest management, the competitors are not other pest management companies, they are the pests themselves! I believe that innovation occurs in three forms: Innovation by Improvement which entails process improvements--making things run more efficiently, cost-effectively or faster, Innovation by Extension which initiates new schemes or reconfigures new product components to yield some new benefit, and Innovation by Paradigm which can bring about totally new patterns or unique innovations. Change has become the dominant concern not only in our society but also in cockroach, ant, fly and rodent societies. They have become resistant to conventional chemicals or seem to avoid the ones that are of concern around the sensitive areas of zoological parks and aquariums. In solving pest management problems, you can follow or you can lead. You can wait until someone finds a solution or you can think up original ideas that meet the needs of your specific problems.

I believe that the real challenge is to develop a different sort of program, one in which everyone knows their real function. This function is not to get things done through people. It is to make genuine use of the people: first to discover what should be done and next to discover how to get it done with the most satisfaction for all involved. When it comes to pest management, there are two strategies for beating your competitor (the pests). First you out-think them, then you out-work them. The race belongs not just to the swift, but to the indefatigable marathoners with great imaginations. This is how the new Electronic Fly Traps and Electrostatic Spraying Equipment were created. This is why the newest innovation in rodent control was discovered with the advent of The Food Energy Inhibitor which eliminates rodents but whose components are listed on the FDA, G.R.A.S. list as Generally Recognized As Safe and yet are safe enough to eat yourself.

I’m involved in synectics, which is the attempt to bring together problems or opportunities and the creative talents of people that cry to be used. Creativity is a vital component of any business because it develops alternatives, enriches possibilities and imagines the consequences. Synectics does two things: first it brings out Imaginative Speculation and then it promotes, values and encourages speculation. The probability of success is increased if more information is collected. Synectics helps to bring out more information than could expected to solve difficult pest management problems or any problems that you may encounter.

Resumen

Algunas personas constantemente crean nuevas y mejores formas de hacer las cosas - haciendo felices a unos, irritando a otros y frecuentemente, sorprendiendo a otros competidores. En el manejo
de plagas, los competidores no son sólo las otras compañías de tratamiento de plagas; son las plagas mismas! Creo que la innovación ocurre en tres diferentes formas: La innovación por perfeccionamiento el cual implica todo un proceso de mejoras--haciendo que las cosas funcionen mejor y mas eficientemente, con eficiencia o rapidez en los costos; la innovación por extensión, la cual origina nuevos proyectos o reconfigura nuevos componentes de productos que permiten algún nuevo beneficio, y la innovación mediante paradigmas, la cual brinda todo un nuevo patrón de innovaciones. El cambio se ha convertido en la preocupación primordial no solo para nuestra sociedad sino también para las sociedades de cucarachas, hormigas, insectos voladores y roedores. Ellos se han hecho más resistentes a los químicos convencionales o parecen evitar aquellos que tienen algo que ver con las áreas más sensibles de los zoológicos o acuarios. En la resolución del problema del manejo de fauna nociva, usted solo puede hacer lo que hacen los demás, o puede inventar algo nuevo. Usted puede esperar hasta que otro encuentre una solución, o puede pensar en ideas originales que satisfagan las necesidades de sus problemas específicos.

Yo creo que el verdadero reto es el desarrollar un tipo diferente de programa, uno en el cual todos sepan su verdadera función. Esta función no consiste en obtener las cosas hechas a través de otras personas. Es hacer un uso genuino de la gente: primero es descubrir lo que debería de hacerse y después descubrir como debe hacerse obteniendo la mayor satisfacción de todos aquellos involucrados. Cuando se trata del manejo de fauna nociva, existen dos estrategias para derrotar a la competencia (o sea a la plaga). Primero usted los estudia, después usted los trabaja. Esta carrera no solo la ganan los más velozes, sino los infatigables corredores de maratón que posean una gran imaginación. Esta fue la manera en que las nuevas Trampas Electrónicas para Insectos Voladores y el Equipo de Rocio Electrostático se crearon. Esta es la razón por la que la mayor innovación de control de roedores fue descubierta con la llegada de el Inhibidor de Energía Comestible el cual elimina a los roedores, pero cuyos componentes están enlistados en el FDA, y en la lista de G.R.A.S. como generalmente reconocidos como seguros, tanto así que podría comerlos usted mismo.

Yo estoy involucrado en lo que es la sinéctica, la cual intenta conjuntar problemas u oportunidades con gente talentosa y creativa que dice a gritos que quieren trabajar. La creatividad es un componente vital de cualquier negocio, pues con ella se desarrollan alternativas, se enriquecen las posibilidades, y se vislumbran las consecuencias. La sinéctica hace dos cosas: primero saca a la luz la Imaginación Especulativa y después la promueve, valora y alienta. La probabilidad de éxito es incrementada si se reúne mas información. La sinéctica ayuda a sacar mas información de la que se podría esperar para resolver problemas difíciles en el manejo de fauna nociva o de cualquier otro problema con el que usted se pueda encontrar.

Introduction

Some people constantly create new and better ways of doing things--delighting some, irritating others and often times, jolting the competitors. In pest management, the competitors are not other pest management companies, they are the pests themselves! Change has become the dominant concern not only in our society but also in cockroach, ant, fly, and rodent societies. They have become resistant to conventional chemicals or seem to avoid the ones that are of concern around the
Sensitive areas of zoological parks and aquariums.

Think for a moment about the three or four things that matter most to you. What are they? Now ask yourself, am I giving them the care, emphasis, and the time they deserve? If you are like most professionals, the answer is no because of a phrase I have heard so many people use to describe why they can’t accomplish meaningful objectives, “It’s Always Something.” Some friends one day were telling me about one of these somethings, and I told them, if the shoe doesn’t fit, try it anyway. Put yourself in someone else’s shoes. So they did or at least tried to, and by the middle of the afternoon they had gained a different perspective. While it may not have been the exact outlook, they could better understand the other view. My point? Whether you’re facing challenges and problems as the chief veterinarian, zoo director, animal specialist, pest management specialist or whatever, it truly helps to, as the American Indians believe, “walk in another person’s moccasins.”

Solving the Problems

In solving pest management problems, you can follow or you can lead. You can wait until someone finds a solution or you can think up original ideas that meet the needs of your specific problems. When it comes to pest management, there are two strategies for beating your competitor (the pests). First, you out-think them, then you out-work them. To out-think them you need to begin thinking about the seven (7) Characteristics of Innovative People and organizations and why they are all interdependent to achieve success (in any endeavor).

1. A Stated and Working Strategy of Innovation. It is not necessary to be some multi-billion dollar corporation in order to have a stated and working strategy of innovation. Innovation is not an event but a process that must have guidelines to function. This seems to be a paradox at first, but the framework for innovation is an organization that believes in taking risks.

2. Forming Teams. Forming teams in your own organization can be highly rewarding to everyone, but forming teams with your vendors, suppliers and even the end-users can be enlightening, productive, and profitable.

3. Rewarding Creativity and Innovation. Until recently, many experts believed that scientists, researchers, professional people and other innovators were best motivated by the work itself--by the technical challenge, the opportunity to create, and the autonomy. In some instances this is true, but they are now realizing that professionals are very receptive to financial and other nonintrinsic rewards. Does this mean we are greedy? No, it means that we live in a “reality” that we want to do more than just survive.

4. Allowing Mistakes. I used to say that a mistake is not a mistake unless you decide not to correct it. But not all mistakes are correctable, but we can learn from them. However, the message is to create an environment where the “fear of failure” becomes less and less. In fact, congratulate failure and you will go on to achieve many successes.

5. Education in Creativity. “Creativity; you either have it or you don’t” or “you are born with it.” How many times have you heard these statements about creativity and innovation? We often times think that creativity is genius from great painters, song writers, advertising gurus, etc. I have never
met an uncreative person, only uncreative environments. Once you educate people to create an environment that will foster, protect and nurture people, you will experience a geometric increase in creative problem solving.

6. Managing the Organizational Culture. Now that you have created it, what shall I do with it? In my experience with product development, creating the product was the fun part, innovating the product was the challenge, but managing the product and bringing it to the market place was the real toil and work. To succeed in managing the creative culture, you must follow one simple rule: “Empowerment.”

7. Creating New Opportunities Proactively. Creativity left to chance is exactly what the word chance means; “the absence of any known reason why an event should turn out one way rather than another.” You have all heard of the phrase, “if it ain’t broke don’t fix it.” I have always been a proponent of, “if it isn’t broke, fix it anyway.” Understanding the future is important, but that growth is the future is paramount to the quality of our lives and the world we live in. In other words, “creating the future is more important if we want to truly have an impact upon our lives and the lives of others”.

Where does this leave us when not just trying to beat the revolution, but when trying to create the revolution in innovation? First, always think “win-win” because it is the only option that produces productivity and success over the long term. It is as simple as mutual respect for each other. Second, create the vision together and share it with each other. To create a vision that is based on empowerment, ask each other to help form the vision (not a mission statement), the power to perceive what is actually not there, and the principles that will nurture the “win-win” environment.

As Steven Covey states, “You’ll know you’re on the right track if it’s in harmony with the universal mission: To Improve The Economic Well Being And Quality Of Life Of All Stakeholders.” Lastly, “lubricate” the working parts by creating the organizational environment that cares about each other enough to identify the common goals that transcend politics, back-biting, and ruthless corporate behavior. Doing so will improve and elevate everyone’s quality of life.
Abstract

The clinical approach to marine mammals is similar to any terrestrial species. Basic knowledge of behavior, normal clinical pathology, proper methods of handling and treatment may avoid clinical delay in possible illness.

Interpretation of CBC’s and chemistries is similar though it is common to run erythrocyte sedimentation rates, serum fibrinogen and iron levels to help gauge the presence of inflammation. Elevated alkaline phosphatase levels, commonly undesirable in terrestrials are desirable in cetaceans. Blood urea nitrogen levels are normally elevated between 30 and 55 mg/dl.

Diagnostic cultures in cetaceans may include blowhole, gastric, and colonic. Anaerobic cultures are commonly performed on the colon to monitor for Clostridium perfringens. The majority of these are type A and 70% are toxins producers. Cytologic examination of blow exudate, stomach fluid and fecal material may aid in diagnosis of respiratory inflammation, gastritis and colitis. Endoscopic evaluation can be useful in tracheobronchitis, gastritis, and cystitis. Radiographic evaluation can aid in respiratory evaluation, foreign body ingestion and kidney stones. Sonographic evaluation of the chest can help to detect pleural effusion, pleuritis, and surface related pleural abnormalities. Ultrasound of the abdomen can aid in evaluation of pregnancy, kidney stones and other anomalies.

As with all animals, recuperation and healing are greatly affected by the animal’s nutritional status. All cetaceans should be routinely weighed when healthy and weighed every 3-7 days when ill and losing weight. Nutritional supplementation is most often accomplished by the use of larger fish such as herring which are easier to force feed during periods of inappetence. Herring meals should be spaced to allow an adequate amount of digestion time such as 4-6 hr when meal sizes are 4-6 lbs of herring. Smaller sized fish can be used when voluntarily eating to increase food intake since the smaller fish break down quicker. The veterinarian or caretaker should be aware of the caloric value of the different food items since they change at different times of the year.

Resumen

El acercamiento clínico de los mamíferos marinos es similar al de cualquier especie terrestre. Basándose en el conocimiento del comportamiento, patología clínica normal, métodos apropiados de manejo, etc, se puede evitar un retraso en el tratamiento o prevención de posibles enfermedades.

La interpretación de la biometría completa y la química sanguínea es similar a la de los mamíferos terrestres, ya que es común realizar pruebas de sedimentación de eritrocitos, fibrinógeno en suero y niveles de hierro para medir la presencia de inflamación. Los niveles elevados de fosfatasa alcalina, que comúnmente no son deseables en mamíferos terrestres, son normales en cetáceos. Los
niveles de urea nitrogenada en sangre también son normalmente elevados, entre 30 y 55 mg/dl.

Los cultivos para diagnóstico en cetáceos pueden incluir exudados del opérculo, muestras gástricas, y del colon. Los cultivos anaerobeos son comúnmente practicados en el colon para monitorear la presencia de Clostridium perfringes. La mayoría de estos son del tipo A y el 70% producen toxinas. La citología del exudado respiratorio, fluidos gástricos y materia fecal pueden ayudar en el diagnóstico de una inflamación respiratoria, gastritis o colitis. La evaluación endoscópica puede ser muy útil en traqueobronquitis, gastritis y cistitis. La radiología puede ayudar en la evaluación respiratoria, diagnóstico de ingestión de cuerpos extraños y de piedras en el riñón. La evaluación sonográfica del tórax es de utilidad para el diagnóstico de efusión pleural, pleuritis y anormalidades pleurales superficiales. El ultrasonido en abdomen puede ayudar en la evaluación de gestación, piedras en los riñones y otras anormalidades.

También, como en todos los animales, una recuperación saludable está muy influenciada por el estado nutricional del animal. Todos los cetáceos se deben pesar rutinariamente cuando están sanos y cada 3 ó 7 días cuando están enfermos o están perdiendo peso. La suplementación nutricional es frecuentemente complementada con el uso de peces más grandes como el arenque, los cuales son mucho más fáciles de dar como un alimento forzado en periodos de inapetencia. La alimentación con arenques debe ser espaciada permitiendo un tiempo adecuado para la digestión, generalmente de 4-6 hrs cuando el tamaño de la ración es de 2 a 3 kg de arenque. Se pueden utilizar peces de menor tamaño cuando la alimentación es voluntaria, e incrementar el tamaño de la ración, ya que la digestión de los peces pequeños es más rápida. Los veterinarios y cuidadores deben de estar pendientes del valor calórico de los diferentes tipos de alimentos, puesto que las necesidades calóricas cambian en las diferentes épocas del año.
CLINICAL PATHOLOGY INTERPRETATION IN DELPHINIDAE WITH EMPHASIS ON INFLAMMATION

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Abstract

Early diagnosis of inflammatory disease is essential if the veterinarian is to achieve a favorable therapeutic response. In spite of this well-understood fact, on more than a few occasions a potentially fatal inflammatory disease has gone undetected. Why?

Marine mammals, especially cetaceans, present the clinician with a difficult if not unique set of diagnostic challenges. The aquatic environment compromises observation. The blubber encased, ridged body and explosive respiratory cycle limits the value of physical examination. As with most wild mammals, masking the signs of illness is the rule, not the exception. Depressed appetite or behavior is a sign of illness but can be the result of negative social interaction with humans or conspecifics. Total white cell and differential counts can be misleading due to unpredictable variability. As a result, distinguishing a normal from a stress leukogram or a localized inflammatory leukogram can be problematic. Due to these and other complications, the veterinarian is usually forced to use and depend on clinical pathology parameters to a much greater extent than in non-marine species and to use parameters not traditionally used as indicators of inflammatory disease.

The most useful of these clinical pathology parameters are alkaline phosphatase, serum iron, plasma fibrinogen, erythrocyte sedimentation rate, and serum albumin. Rapidly declining serum alkaline phosphatase has historically been a dependable indicator of inflammatory disease and prognosis in cetaceans. A fall of 25-50% is significant if not associated with chronic caloric deprivation. Serum iron will decrease drastically in the presence of inflammatory disease. Decreases of more than 50% are commonplace. Confusion may arise with hepatic cell necrosis as a noticeable elevation may occur, often more than four times normal levels. Plasma fibrinogen levels consistently rise 20% or more very early during inflammatory disease episodes and remain elevated until resolution occurs. Erythrocyte sedimentation rate (ESR), often used as an inflammatory marker in marine mammals, is reliable if rising, but is not a good prognostic indicator and does not reliably parallel serum fibrinogen levels. ESR has the advantage of being a rapid and simple on-site test for the veterinary clinician to use if rapid access to chemistries is not available. Serum albumin is an additional indicator. Liver synthesis of albumin is slowed or stopped as a result of the effects of bacterial toxins. This decreased synthesis is readily detectable by serum electrophoresis as a 20% or greater decrease. There is usually a coinciding increase in globulins. To obtain the most value from these tests, they must be considered as a group and compared with normal values from the same individual.

An equivocal leukogram associated with other parameters indicative of inflammatory disease should warrant a second look. Careful evaluation of all available clinical pathology parameters will minimize confusion and lead to early recognition of inflammatory disease in members of the dolphin family.
Resumen

El diagnóstico temprano de la enfermedad inflamatoria se considera esencial cuando el veterinario tiene que obtener una respuesta terapéutica favorable. A pesar de este factor bien comprendido, en varias ocasiones, una enfermedad inflamatoria potencialmente fatal se ha presentado sin ser detectada. Por qué?

Los mamíferos marinos, especialmente los cetáceos, representan para el clínico, cuadros únicos y difíciles que representan retos diagnósticos. El medio ambiente acuático hace difícil la observación. La capa de grasa que se encuentra por debajo de la piel, con la aleta dorsal y el ciclo respiratorio explosivo, limitan el valor del examen físico. Así como la mayoría de los mamíferos silvestres, en que como regla enmascaran los signos de enfermedad, los cetáceos no son la excepción. La disminución del apetito y los cambios de conducta pueden interpretarse como signos de enfermedad, pero también podrían ser el resultado de una interacción social negativa con los humanos o con sus congéneres.

Una cuenta diferencial total de células blancas, podría darnos pistas falsas debido a lo impredecible de sus variaciones. Como resultado, el diferenciar un leucograma normal del de uno bajo condiciones de estrés o de una serie blanca inflamatoria localizada presenta una gran dificultad. Debido a esta y otras complicaciones, el veterinario está generalmente obligado a utilizar y depender de los parámetros de patología clínica en una mucha mayor extensión que en los casos de especies no marinas, así como de utilizar parámetros que no son tradicionalmente usados como indicadores de una enfermedad inflamatoria.

Los más utilizados de estos parámetros de patología clínica son: la fosfatasa alcalina, el hierro sérico, el fibrinógeno plasmático, el volumen de sedimentación globular y la albúmina sérica. La rápida disminución de la fosfatasa alcalina sérica ha sido históricamente un indicador confiable en la enfermedad inflamatoria así como en el prognóstico clínico en los cetáceos. Una caída del 25 al 50% será importante cuando no se encuentra asociada con una privación calórica crónica (desnutrición). El hierro sérico disminuye drásticamente en presencia de una enfermedad inflamatoria. La disminución de más del 50% es común. Puede causar confusión, en caso de necrosis celular hepática, una elevación notable que podrá presentarse frecuentemente hasta en 4 veces mayor de los valores normales. Los niveles de fibrinógeno plasmático consistentemente se elevan en un 20%, o más rápidamente durante los episodios de la enfermedad inflamatoria, y permanecen elevados hasta que se presenta una mejoría. El volumen de sedimentación de eritrocitos (ESR), frecuentemente utilizado como un marcador inflamatorio en mamíferos marinos, será un valor de confianza cuando se elevan sus valores, pero no es un buen indicador del pronóstico y no paraleliza de forma fiable los valores de fibrinógeno sérico. ESR tiene la ventaja de ser una prueba rápida y simple que el clínico puede realizar cuando no tiene acceso inmediato a otras pruebas químicas más específicas. La albúmina sérica puede ser también un indicador adicional. La síntesis hepática de albúmina se enlentece o detiene como resultado de los efectos de algunas toxinas bacterianas. Esta disminución de la síntesis puede detectarse por electroforesis del suero cuando es igual o superior a un 20%. Suele haber un aumento coincidente de las globulinas. Para obtener el máximo valor de estas pruebas, deben considerarse como un grupo y compararse con los valores normales del mismo individuo.

Un leucograma alterado, asociado a otros parámetros indicativos de una enfermedad inflamatoria,
deben de obligarnos a una segunda revisión. La evaluación cuidadosa de todos los parámetros obtenidos minimizarán una posible confusión y nos conducirán al reconocimiento temprano de la enfermedad inflamatoria en los miembros de la familia de los delfines.
ADVANCES IN *Tursiops* GASTROENTEROLOGY

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Abstract

A large population of bottlenose dolphins in the Navy Marine Mammal Program are kept at NCCOSC (NRaD) in San Diego, CA. Recently NRaD veterinary clinicians have been focusing on advancing diagnostic and treatment methods concerning the cetacean (bottlenose dolphin, *Tursiops truncatus*) gastrointestinal (GI) tract. Relying on previously reported modalities and investigating new techniques, clinicians at NRaD’s Vetlab are attempting to answer some questions regarding disease, physiology, and medicine. This abstract describes our findings regarding diagnostic techniques, normal physiologic parameters, and pathology and treatments.

Diagnostic Techniques: Clinical history continues to be our initial diagnostic tool. Partial or complete anorexia, vomiting, or changes in feces character are among the signs that may lead us to a work-up of the case. Diagnostic procedures that may be employed in a case workup are listed as follows:

- Hematology and serum chemistries
- Esophageal, forestomach, and fundic stomach gastroscopy
- Abdominal ultrasound
- Radiology
- Colonoscopy

Normal Physiologic Values: There are a number of ongoing clinical trials designed to answer questions regarding what is “normal” for the gastrointestinal tract of our animals. We have demonstrated that the fasting pH of the forestomach is between 1.22 and 1.80 (n=43), and that the pH climbs to between 3.0 and 4.0 within 15 min following a fish meal and maintains this pH for approximately 3 hr postprandial. Forestomach emptying appears to take place between 2.5 and 3.5 hr following a meal and by 4.0 hr postprandial most ingesta has moved out of the forestomach. Endoscopy has revealed that motility in the dolphin forestomach is unique. We have observed these contractions at a rate of 3-5 contractions per min. Mucosal health, degree of forestomach insufflation, and sedatives can influence motility rates and patterns detected endoscopically.

Pathology and Treatments: We continue to see many of the same gastrointestinal diseases that have been documented in the literature over the past 25 yr. Retrospective case reviews over the past 5 yr have shown the following: bacterial enteritis, ulcerative gastritis documented in all three stomachs (forestomach, fundic stomach, pyloric stomach), ulcerative esophagitis, pica, and idiopathic hemorrhagic colitis all to have been documented in dolphins. Specifically in regards to ulcerative gastritis, the role of *Helicobacter pylori* has been questioned and is being investigated.

Treatment of dolphin gastrointestinal tract disease has traditionally been empirically based on human therapeutic modalities and doses. Because of the anatomical and physiological differences these kinds of extrapolations may not result in efficacious treatment regimens. Below we have listed a
few of the commonly used drugs and dosages used in an average 160 kg adult *Tursiops truncatus*. 

1. Cimetidine, 600-1000 mg p.o. b.i.d.
2. Ranitidine, 600-900 mg p.o. b.i.d. (limited usage to date)
3. Omeprazole, 60 mg p.o. b.i.d. (limited usage to date)
4. Sucralfate, 1 gm p.o. b.i.d./t.i.d.

**Resumen**

Una gran población de delfines nariz de botella en el Programa de Mamíferos Marinos de la Marina es mantenida en NCCOSC (NRaD) en San Diego, CA. En fechas recientes, los veterinarios clínicos del NRaD han enfocado sus esfuerzos hacia la mejora de los métodos de tratamiento y diagnóstico en lo que concierne al tracto gastrointestinal de cetáceos (GI). Contando con los reportes previos de modalidades y nuevas técnicas de investigación, los veterinarios clínicos del NRaD intentan responder a algunas de las preguntas en lo que se refiere a enfermedades, fisiología y medicina. Este resumen describe nuestros hallazgos en cuanto a técnicas de diagnóstico, parámetros fisiológicos normales, patología y tratamientos.

**Técnicas de Diagnostico**: La historia clínica continua siendo nuestra herramienta inicial de diagnóstico. La anorexia parcial o completa, el vómito, los cambios en las características de las heces son, entre otros signos, los que más nos pueden guiar a intervenir en el caso. Los procedimientos de diagnóstico que pueden ser empleados en estos casos están en la siguiente lista:

- Hematología y química sanguínea
- Gastroendoscopía del esófago, preestómago y estómago fúndico
- Ultrasonido abdominal
- Radiología
- Colonoscopía

**Valores Fisiológicos Normales**: Existe un cierto número de pruebas clínicas diseñadas para responder a preguntas en cuanto a lo que es “normal” para el tracto gastrointestinal de nuestros animales. Hemos demostrado que el pH del preestómago está entre 1.22 y 1.80 (n=43), y que ese pH aumenta entre 3.0 y 4.0 dentro de los siguientes 15 minutos después de una comida de pescado, y se mantienen en este mismo pH por aproximadamente 3 horas. El vaciado del preestómago ocurre entre las 2.5 y 3.5 horas después de una comida, y a las 4 horas postprandiales la mayor parte de la ingesta se ha desplazado fuera del preestómago. La endoscopía ha revelado que la motilidad del preestómago es única. Hemos observado estas contracciones a una velocidad de 3 a 5 contracciones por minuto. El estado de la mucosa, el grado de insufilación del preestómago y los sedantes pueden influir en la frecuencia de motilidad y los patrones detectados endoscópicamente.

**Patología y Tratamientos**: Continuamos observando muchas de las enfermedades gastrointestinales que han sido reportadas en la literatura desde hace más de 25 años. Las revisiones retrospectivas de los casos de los últimos 5 años han demostrado lo siguiente: Enteritis bacteriana, gastritis ulcerativa documentada en los 3 estómagos (preestómago, estómago fúndico y pilórico), esofagitis
ulcerativa, pica y colitis hemorrágica idiopática, han sido documentadas en delfines. Específicamente en los que se refiere a la gastritis ulcerativa, el papel de *Heliobacter pylori* ha sido cuestionado e investigado.

El tratamiento de las enfermedades del tracto gastrointestinal del delfín ha sido tradicionalmente empírico basado en terapias médicas humanas. Debido a las diferencias anatómicas y fisiológicas, esta clase de extrapolaciones pueden resultar en regímenes de tratamiento ineficaces. A continuación se han listado algunos de los medicamentos más comunes y las dosis usadas en *Tursiops truncatus* adultos de 160 kg en promedio:

1. Cimetidina, 600-1000 mg p.o. b.i.d.
2. Ranitidina, 600-900mg p.o. b.i.d. (con uso limitado por la fecha)
3. Omeprazole, 60 mg p.o. b.i.d. (con uso limitado por la fecha)
4. Sucralfate, 1 mg p.o. b.i.d./t.i.d.

**Introduction**

A large population of bottlenose dolphins in the Navy Marine Mammal Program are kept at NCCOSC (NRaD) in San Diego, CA. Recently NRaD veterinary clinicians have been focusing on advancing diagnostic and treatment methods concerning the cetacean (bottlenose dolphin, *Tursiops truncatus*) gastrointestinal (GI) tract. Relying on previously reported modalities and investigating new techniques, clinicians at NRaD’s Vetlab are attempting to answer some questions regarding disease, physiology, and medicine. This abstract describes our findings regarding diagnostic techniques, normal physiologic parameters, pathology and treatments, and finally future research interests concerning the dolphin GI tract.

**Diagnostic Techniques**

Clinical history continues to be our initial diagnostic tool. Partial or complete anorexia, vomiting, or changes in feces character are among the signs that may lead us to a work-up of the case. Diagnostic procedures that may be employed in a case workup are listed as follows:

1. Hematology and serum chemistries--Complete blood counts (CBC) and serum iron values are often useful in the diagnosis of hemorrhage and inflammation of the GI system. Inflammatory leukograms, decreasing serum iron and alkaline phosphatase levels, and most importantly mild to moderate anemia which may or may not be regenerative are all cause for further diagnostics, especially endoscopy.

2. Esophageal, forestomach, and fundic stomach gastroscopy--The use of endoscopy in marine mammals continues to increase and is tolerated well with moderate restraint in most animals. Vetlab most often uses a 280 cm long by 9.8 mm diameter video endoscope manufactured by Pentax for these procedures. Observation of mucosal appearance, gut contents, forestomach motility patterns, and mucosal biopsies are all facilitated through the use of an endoscope.
Abdominal ultrasound--By using a 3.5 MHz, 17 cm linear array transducer abdominal anatomy is effectively visualized. Gastrointestinal motility, gas/fluid presence, foreign objects, and gut wall appearance can be evaluated.

Radiology--Plain films of the GI tract within the abdomen have been of limited use. It is our impression that this is due to compression of abdominal organs and the lack of peritoneal fat and subsequent contrast, delineation of organs is difficult. Currently we are evaluating the usefulness of contrast studies.

Colonoscopy--By utilizing a smaller diameter fiber endoscopes (cystoscope which is 65 cm long by 4.9 mm diameter or pyelo-nephroscope which is 70 cm long by 3.5 mm diameter) we are able to visualize rectal/colonic mucosa and obtain biopsies.

Normal Physiologic Values

There are a number of ongoing clinical trials designed to answer questions regarding what is “normal” for the gastrointestinal tract of our animals. We have demonstrated that the fasting pH of the forestomach is between 1.22 and 1.80 (n=43), and that the pH climbs to between 3.0 and 4.0 within 15 min following a fish meal and maintains this pH for approximately 3 hr postprandial. Forestomach emptying appears to take place between 2.5 and 3.5 hr following a meal and by 4.0 hr postprandial most ingesta has moved out of the forestomach. Endoscopy has revealed that motility in the dolphin forestomach is unique. A focal circumferential constriction begins at the proximal portion of the forestomach, travels toward the distal apex of the forestomach, and finally returns toward the proximal portion. We have observed these contractions at a rate of 3-5 contractions per min. Mucosal health, degree of forestomach insufflation, and sedatives can influence motility rates and patterns detected endoscopically. Clinicians have observed the retrograde reflux of digestive fluids from the fundic stomach into the forestomach and subsequent mixing with ingesta due to these peristaltic waves.

At the time of the writing of this abstract, data was not yet complete on mucosal biopsies. We are currently investigating H & E, electron microscopy, and culture results from clinically normal animals. Post mortem samples from forestomachs have yielded the following organisms: *Staphylococcus* spp., *Vibrio* spp., *Escherichia coli*, *Streptococcus* spp., *Clostridium* spp., and *Candida* spp.

Pathology and Treatments

We continue to see many of the same gastrointestinal diseases that have been documented in the literature over the past 25 yr. Retrospective case reviews over the past 5 yr have shown the following: Bacterial enteritis, ulcerative gastritis documented in all three stomachs (forestomach, fundic stomach, pyloric stomach), ulcerative esophagitis, pica, and idiopathic hemorrhagic colitis all to have been documented in dolphins. Specifically in regards to ulcerative gastritis, the role of *Helicobacter pylori* has been questioned. The bacteria has been demonstrated in humans, pigs, ferrets, cheetahs, cats, and dogs. We have utilized the CLO-test, Delta West Pty Ltd. and have received both positive and negative results. Positive results have not been confirmed via light or electron microscopy to date. One important note is that the CLO-test is simply a +/- test for the
presence of urease. The prevalence of other urease + microorganisms in the dolphin upper GI tract has not been determined and is currently being worked on at NRaD Vetlab.

Another area of investigation at NRaD is the development of forestomach foreign object retrieval tools. Within our population we have identified a 5-7% incidence in the ingestion of sea grasses and kelp. At times, these materials can pose a significant health problem and this warrants their removal. To date, construction of a large three pronged retrieval forcep has proven most effective in extraction of this foreign material.

Treatment of dolphin gastrointestinal tract disease has traditionally been empirically based on human therapeutic modalities and doses. Because of the anatomical and physiological differences these kinds of extrapolations may not result in efficacious treatment regimens. Below we have listed a few of the commonly used drugs and dosages used in an average 160 kg adult *Tursiops truncatus*.

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4. Sucralfate, 1 gm p.o. b.i.d./t.i.d.

**Future Research Efforts**

The clinical staff is interested in continuing to evaluate new diagnostic techniques for the GI tract. One such technique is endoscopic ultrasonography with transluminal fine needle aspiration. A technique which has shown promise within the human medical field, it offers the marine mammal veterinarian access to various thoracic and abdominal organs through the upper GI tract. Mucosal health and it’s influence on motility will also be an area of interest. Preliminary endoscopic observations have raised the question as to the diagnostic value of forestomach motility in relation to mucosal disease. Continued mucosal observations with microbiologic and histopathologic evaluation will hopefully provide some answers to this question.
OVERVIEW OF AQUATIC MAMMAL MORBILLIVIRAL DISEASES

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Abstract

Recently, morbilliviruses have emerged as causes of major epizootics that have affected populations of aquatic mammals from waters in and around three continents. In 1987, there was a ten-fold increase in strandings of bottlenose dolphins (Tursiops truncatus) along the Atlantic coast of the United States from New Jersey to Florida. Although an initial investigation implicated a red tide algal bloom as the likely cause, a later study found syncytial cells, eosinophilic intranuclear and intracytoplasmic inclusion bodies, and lymphoid depletion in many of the fatally affected dolphins. These findings are highly characteristic of morbilliviral infection. The diagnosis was confirmed by immunohistochemical and polymerase chain reaction tests. Later in 1987, a morbillivirus closely related or identical to canine distemper virus killed thousands of Baikal seals (Phoca siberica) in Lake Baikal, Siberia. In 1988, approximately 17,000 harbor seals (Phoca vitulina) died in northwestern Europe during an epizootic caused by a newly recognized morbillivirus related to, but distinct from, canine distemper virus. Morbilliviral disease was also documented in a small number of harbor porpoises (Phocoena phocoena) that died in European waters in 1988. Morbilliviral infection killed thousands of striped dolphins (Stenella coeruleoalba) in the Mediterranean Sea in 1990 and 1991. The porpoise and striped dolphin morbilliviruses are more closely related to peste des petits ruminant virus than to other known morbilliviruses. During 1993 and 1994, the most recent aquatic mammal morbilliviral epizootic caused a wave-like increase in bottlenose dolphin strandings in the Gulf of Mexico from western Florida to Texas. Serologic studies suggest that morbilliviral infection is enzootic in some pinniped and cetacean species; other species may be subject to periodic epizootics. Although unknown a few years ago, aquatic mammal morbilliviral diseases are now recognized as the most important causes of mass mortality of seals and small cetaceans.

Resumen

Los morbillivirus han emergido como causa de grandes epizootias recientes que han afectado las poblaciones de mamíferos acuáticos de alrededor de tres continentes. En 1987, hubo un incremento 10 veces mayor en encallamientos en delfines nariz de botella (Tursiops truncatus) a lo largo de la costa del Atlántico en los Estados Unidos, desde Nueva Jersey hasta Florida. Aunque una investigación inicial reveló la implicación de un florecimiento de algas de marea roja como causa probable, un estudio posterior encontró células sincitiales, cuerpos de inclusión eosinófilos intranucleares e inclusiones intracitoplasmáticas, y depresión linfótica en muchos de los delfines fatalmente afectados. Estos hallazgos son altamente característicos de una infección por morbillivirus. El diagnóstico fue confirmado con pruebas inmunohemáticas y de reacción en cadena de polimerasa. Más tarde, en 1987, un morbillivirus muy relacionado o idéntico al virus del Moquillo Canino mató a miles de focas Baikal (Phoca siberica) en el lago Baikal, Siberia. En 1988, aproximadamente 17,000 focas de puerto (Phoca vitulina) murieron en el noreste de Europa durante una epizootía provocada por un morbillivirus, recientemente reconocido y relacionado, aunque de
distinta morfología, al virus del moquillo canino. Las enfermedades morbillivirales fueron también reportadas en un pequeño grupo de belugas de puerto (*Phocoena phocoena*) que murieron en aguas europeas en 1988. Las infecciones morbillivirales mataron miles de delfines rayados (*Stenella coeruleoalba*) en el mar Mediterráneo en 1990 y 1991. Los morbillivirus de belugas y delfines rayados están más relacionados con el virus de la peste de pequeños rumiantes que con otro morbillivirus conocido. Durante 1993 y 1994, una epizootía causó el encallamiento de un gran número de delfines nariz de botella a lo largo de la costa del golfo de México, desde el oeste de Florida hasta Texas. Los estudios serológicos sugieren que las infecciones por morbillivirus son enzoóticas en algunas especies de Pinípedos y Cetáceos. Otras especies pueden ser sujetas a periodos epizoóticos. Aunque desconocidas hasta hace poco tiempo, las enfermedades morbillivirales en mamíferos acuáticos están ahora reconocidas como la causa de mortalidad masiva más importante en focas y pequeños cetáceos.

**LITERATURE CITED**


THE VETERINARY MANAGEMENT OF SOUTHERN ELEPHANT SEALS (Mirounga leonina) AT TARONGA ZOO

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Abstract

Southern elephant seals (Mirounga leonina) have a circumpolar distribution, concentrated on and around sub-Antarctic islands near the Antarctic convergence. There have been reports of vagrant animals at mainland Australia, New Zealand, Africa and South America. Southern elephant seals have been held in zoos in Europe, North America, South Africa and Australia. Taronga Zoo has held four southern elephant seals: three in its collection and a vagrant under-yearling, which was released back into the wild after treatment and rehabilitation. Her movements after release were monitored using satellite telemetry. Experience has been gained in captive husbandry, nutrition, disease, anesthesia, rehabilitation and release of these animals. Ocular anterior segment disease and oral candidiasis have resulted in significant veterinary input into the management of these animals. The identification of factors, such as water quality, nutritional deficiencies ultraviolet light, trauma and stress and the use of appropriate anti-inflammatory and antimicrobial therapy have reduced the incidence of these diseases. Five anesthetics on three animals have been carried out. Based on experiences with wild southern elephant seals, tiletamine-zolazepam was chosen as the sole anesthetic agent. Heavy sedation or light anesthesia was achieved and no complications were encountered.

Resumen

Los elefantes marinos del Sur (Mirounga leonina) tienen una distribución circumpolar, concentrados en y alrededor de las islas subantárticas, cerca de la convergencia de la Antártida. Se tiene información de animales errantes en Australia, Nueva Zelanda, Africa y Sudamérica. Los elefantes marinos sureños han sido mantenidos en los zoológicos de Europa, Norteamérica, Sudáfrica y Australia. El zoológico de Taronga ha tenido 4 elefantes marinos, 3 de su colección y uno errante de un año de edad, mismo que fue liberado a la vida silvestre después de su tratamiento y rehabilitación. Sus movimientos después de su liberación fueron vigilados, utilizando telemetría por vía satélite. Se ha obtenido experiencia en crianza, nutrición, enfermedades y anestesia, así como en rehabilitación y liberación de esos animales. El tratamiento de la enfermedad del segmento anterior del ojo, así como la candidiasis oral, han supuesto un importante avance veterinario en el manejo de estos animales. La identificación de factores tales como la calidad del agua, deficiencias nutricionales, luz ultravioleta, traumatismo y estress, así como del uso adecuado de los antiinflamatorios y de los tratamientos antimicrobianos han reducido la incidencia de éstas enfermedades. Se han llevado a cabo 5 anestesias en 3 de los animales. En base a experiencia con elefantes marinos silvestres, la Tiletamina-Zolazepam fue elegida como agente anestésico único. Una sedación fuerte o una anestesia ligera se logró sin que se presentaran complicaciones.

Introduction
Southern elephant seals (*Mirounga leonina*) have a circumpolar distribution, concentrated on and around sub-Antarctic islands near the Antarctic convergence. Births occur on these sub-Antarctic islands, but have also been recorded in Antarctica, and prior to exploitation of the animals for oil, on the north-western coast of Tasmania and on King Island in Bass Strait (Australia). There have been various reports of vagrant animals at mainland Australia, New Zealand, Africa and South America.\(^1\)

The world southern elephant seal population was estimated at 750,000 in 1985.\(^1\) There is growing evidence, however, that some island populations are declining.\(^3,6,10\) The species’ natural history, form and function have been described in detail.\(^13\) They have an annual life cycle which varies with age and sex, are pelagic and only come ashore to breed, give birth and molt.\(^4\)

Southern elephant seals have been kept in zoos in Europe, North America, South Africa and Australia. One of the earliest reports of a captive southern elephant seal is from Philadelphia Zoo in 1933-1934. An adult male, which usually traveled with a circus, “was housed in the outdoor elephant bathing pool, where he spent most of the day and night submerged.”\(^18\) Wilhelma Zoo in Stuttgart, West Germany, kept and bred southern elephant seals for 40 yr. Several animals survived to over 20 yr and 4 pups were produced. All pups died, however, one lived for 14 mo (Rietschel, personal communication).

Taronga Zoo has held four (1.3) southern elephant seals. Three (1.2) were wild caught (under permit from Tasmanian National Parks and Wildlife Service) at Macquarie Island. They arrived at the zoo on 15 December, 1987 and were approximately 12-14 mo-old. One female died on 28 December 1992 and the male died on 8 January 1995. The fourth, a vagrant 10-mo-old female was treated and rehabilitated over a period of 13 mo before release. One adult female remains in the collection.

Despite the abundance of literature available on wild southern elephant seals, there is little information, however, regarding captive southern elephant seals. This report describes Taronga Zoo’s experiences with captive husbandry, nutrition, diseases, anesthesia, rehabilitation and release of southern elephant seals.

**Husbandry and Nutrition**

Captive southern elephant seals have been kept in either salt or fresh water, with or without varying types of filtration or sterilization of water. A range of diets and supplements has also been used.

The southern elephant seals at Taronga Zoo spend most of the year in an enclosure with a 400,000 L pool, which has a maximum depth of 3 m and a surface area of 290 m\(^2\). Two adjacent pools (180,000 L and 79,000 L) and smaller off-exhibit holding pools are also used. The southern elephant seals have been held together (up to 3 in the larger pool), separately, or with New Zealand fur seals (*Arctocephalus forsteri*) or harbor seals (*Phoca vitulina richardsi*). Sea water pumped from Sydney Harbour is used in the pools. The water is filtered through high pressure sand filters with an approximate turnover rate of 3-4 hr. Only top filtration is available. Water is chlorinated using sodium hypochlorite to approximately 0.3-0.4 ppm total chlorine. A disadvantage of using sodium hypochlorite is that when it oxidizes and binds chemically with
organic material it forms chloramines, and when the hypochlorite ion (OC\textsuperscript{1}\textsuperscript{-}) reacts with bromide ions (Br\textsuperscript{-}), hypobromous acid (HOBr) is produced. Chloramines and hypobromous acid are irritant to mucous membranes, skin and the cornea. The pools are drained and cleaned every 4-6 wk (more frequently in summer).

Sections of the pools and haul-out areas are covered with shade cloth in summer. The pools are painted with a non-reflective, UV absorbent, dark green paint.

Haul-out areas with a sand substrate are frequently used. During the molt animals may spend 4-6 wk hauled out. The haul-out areas are well-drained and the substrate completely changed after each molt.

Little is known about the diet of southern elephant seals. Feeding has not been observed and quantitative studies of diet have not been performed.\textsuperscript{13} Remains of fish, cephalopods, and invertebrates have been found in stomach contents. The diet varies with age and sex, and detailed studies of foraging behavior have been carried out.\textsuperscript{11}

The diet fed to animals at Taronga Zoo includes bull mullet (\textit{Mugil cephalus}), yellow-tail (\textit{Trachurus novaeezelandiae}), herring (\textit{Arripis georgianus}), red-spot whiting (\textit{Sillago flinders}), yellow-eyed mullet (\textit{Aldrichetta forsteri}), blue mackerel (\textit{Scomber australasicus}) and arrow squid (\textit{Loligo spp.}). Preferences for different species vary between individuals and despite the fact that squid is thought to be a significant food item in the wild, animals at Taronga Zoo accept fish more readily. The male refused to eat squid.

The food is frozen for at least 6 wk and stored for no longer than 6 mo. It is thawed in air at 16-21\textdegree C. The diet is supplemented with a multivitamin tablet (BVR Marine tabs, Biochemical Veterinary Research, 229 Hume Highway, Mittagong, 2575, Australia) (1 tablet/100 kg body weight daily), retinyl palmitate paste (Biochemical Veterinary Research) (500 IU/kg body weight daily) and omega-3 fatty acids (Biochemical Veterinary Research) (4 ml/100 kg body weight daily).

The daily food intake varies with age, sex and time of year. Southern elephant seals eat little or nothing during the molt. As their new pelage develops their appetite increases.

\textbf{Diseases}

Ocular anterior segment disease and oral candidiasis have resulted in significant veterinary input into the management of these animals.

Other problems such as skin wounds are seen occasionally. Keeping pairs of animals together during the breeding season is an unnatural situation (in the wild harems of 20-600 females occur and males may mate many times). Overmating in captivity, resulting in death of the female has been reported (Irvine, personal communication).\textsuperscript{18} At Taronga Zoo, the male showed great interest in the females from its adjacent pool and it was considered that if kept together this may have resulted in trauma to the females. For this reason the male was separated from the females during the breeding season and we do not recommend housing these animals together at this time.
Ivermectin (Ivomec liquid for sheep, MSD Agvet, 54-68 Ferndell Street, South Granville, 2142, Australia) (10 µg/kg) is administered monthly as heartworm (*Dirofilaria immitis*) prophylaxis.

**Ocular anterior segment disease** (OASD) is common and has been described in many species of captive pinnipeds. Many causes have been proposed (Figs. 1 and 2). A survey of the prevalence of OASD in captive pinnipeds in Europe showed that 66.7% of southern elephant seals examined were affected. Southern elephant seals held at Port Elizabeth Oceanarium in South Africa over a period of 15 yr were rarely free of OASD (Kobus, personal communication). OASD has been reported in wild southern elephant seals (Burton recorded this disease at Macquarie Island and Round, at the Falkland Islands, personal communication). OASD is the most significant disease of southern elephant seals at Taronga Zoo.

This species may be more susceptible to OASD than other pinnipeds. The large corneal surface area and the fact that these animals spend approximately 95% of their lives at sea, often at depths of 600 m or more, means their eyes may be more adapted to very low light and high pressures and are probably less able to adapt to a captive environment.

The clinical entities seen at Taronga Zoo include corneal opacity (varying from central to diffuse), corneal ulceration, chronic keratitis, corneal scarring with neovascularization and ruptured descemetocele. Lesions are usually unilateral and associated with intense blepharospasm. The conjunctiva and nictitating membrane are normally red and often difficult to visualize, therefore the presence of conjunctivitis is difficult to evaluate. Cataracts and dilated pupils have not been seen. One animal had a small area of depigmentation of the iris.

There appears to be a definite correlation with increase in light intensity, ambient and water temperature (i.e., summer) and the incidence and severity of OASD in Taronga Zoo’s southern elephant seals.

Clinical examination of the pinniped eye has been described. Thorough examination of the southern elephant seal eye in the conscious animal is difficult due to the strong orbicularis muscle, which holds the eye tightly closed. Closer examination has been facilitated by the use of general anesthesia and palpebral nerve blocks.

The most significant case of OASD occurred in the male. After 2 mo of severe blepharospasm which was unresponsive to treatment, the eye opened and revealed a descemetocele. This ruptured and the cornea healed over quickly with scarring and neovascularization. A deep corneal ulcer developed in the animal’s other eye 3 yr later. A descemetocele again developed and ruptured. A conjunctival pedicle graft was performed under general anesthesia to repair the defect. This detached 2 mo later, leaving a central scar in an otherwise normal eye.

A great deal of effort has been put into the control of factors which contribute to OASD. These include the reduction of UV light, dietary supplementation with vitamin A and C, and omega-3 fatty acids, improving water quality and reduction of stress induced by interaction with other animals. Supplementation with omega-3 fatty acids is thought to decrease the omega-6:omega-3 ratio and thus decreasing inflammation. The peroxidation of unsaturated fatty acids in membrane phospholipids, caused by increased free radicals in corneal stoma and Descemet’s membrane would
support the use of ascorbic acid (a free radical scavenger) and vitamin E (an antioxidant) supplements. The provision of additional vitamin E above the animal’s multivitamin supplement may be of use and is to be investigated.

The use of the non-steroidal anti-inflammatory copper indomethacin (Cu-algesic oral paste, Biochemical Veterinary Research) at dose rates varying from 0.2-0.6 mg/kg s.i.d. to once a week was the most effective treatment to reduce corneal opacity and blepharospasm. Prednisolone was used occasionally in refractory cases with some success. Flunixin meglumine (Finadyne, Schering-Plough/Heriot, 8 Mosrael Place, Rowville, 3178, Australia) had little effect and some animals became anorectic whilst on the drug. Topical preparations were of little use as affected eyes were often closed or the animal quickly closed its eyes when the preparation was administered. Most topical preparations would also be washed out of the eye once the animal entered the water.

**Oral candidiasis** characterized by inflammation, ulceration and plaque formation around the teeth, particularly the canines and incisors was common. Lesions were either discrete or diffuse and occasionally occurred on the sublingual, lingual or palatine mucosa.

Of 20 microbiological investigations of mouth lesions, *Candida albicans* was isolated on 14 occasions (70%). Other organisms isolated included ß-hemolytic *Streptococcus* (20%), *Vibrio* spp. (45%) and other mixed aerobes and anaerobes which were probably normal flora or contaminants. Candidiasis caused by *C. albicans* is a common finding in captive pinnipeds. The lesions described in phocids include inflammation of the mucocutaneous junctions, especially the commissures of the mouth, and the perioral, perivulvar and perianal areas. One report describes candidiasis in southern elephant seals, where lesions were cutaneous, conjunctival, as well as oral. Beta-hemolytic streptococci were also isolated in these cases. All lesions in Taronga Zoo’s southern elephant seals have involved only the oral mucosa, although *C. albicans* was cultured from a swab taken from the male’s eye. Mucocutaneous and oral lesions of unknown aetiology have been observed in wild southern elephant seals, particularly toward the end of the molt (Woods, personal communication).

It has been speculated that candidiasis in pinnipeds, as is the case in many other species is associated with immunosuppression, skin or mucous membrane damage and concurrent disease. Treatment with ketoconazole (Nizoral, Jannesen-Cilag, 706 Mowbray Road, Lane Cove, 2066, Australia) (3-10 mg/kg p.o. s.i.d. for 7-21 days) resulted in temporary regression of lesions. Concurrent use of clavulenic acid/amoxicillin (Clavulox, Pfizer Animal Health, Wharf Road, West Ryde, 2114, Australia) (12.5-20 mg/kg p.o. b.i.d.) appeared to give better results. More rapid and longer regression of lesions appeared to occur with the use of topical mycostatin (Nilstat, Lederle Laboratories, Cyanamid Australia, 5 Gibbon Road, Baulkham Hills, 2153, Australia) applied to the lesions daily with a paintbrush for up to 30 days.

**Deaths**

A female aged 5 yr and weighing 570 kg, died after a short illness while molting. Necropsy revealed multiorgan involvement with congestion and hemorrhage the most common finding. Hemorrhages and swelling ranging from 1-10 cm, where present in the skin. Histopathology also revealed multiorgan involvement with lymphoid depletion and bacterial colonies in lung and kidney. A lack
of inflammatory response indicated that this may have been a terminal septicaemia. Extensive inflammatory cell infiltration, necrosis and masses of bacterial colonies were present in the skin. Mixed gram-negative rods were isolated. The stress of the molt, severe bacterial dermatitis and cellulitis and resultant toxaemia were the likely cause of death.

A male aged 8 yr and weighing 1,033 kg, was found dead at the bottom of its pool 30 min after being fed. Necropsy revealed fish lodged in its nasopharynx and left terminal bronchus in an otherwise healthy animal. Histopathology was normal apart from the left eye which showed vacuolation of the cornea with focal congestion and mild deep mononuclear perivascular cuffing and focal thinning of the surface epithelium.

**Anesthesia**

Anesthesia of wild southern elephant seals has been described.\(^1,20,21\) Five anesthetics on three southern elephant seals have been performed at Taronga Zoo. Based on experiences with wild southern elephant seals, tiletamine-zolazepam (Zoletil 100, Vibac Australia, 15 Pritchard Place, Peakhurst, 2210, Australia) was chosen as the sole anesthetic agent.

All animals were fasted for 24 hr and weighed (or mass estimated) prior to anesthesia. Induction doses were administered into the lumbar muscles by projectile dart or remote injection. The latter method involves the insertion of a needle into the animal to which a length of drip tubing is attached. The drug is injected and flushed through the tube allowing the operator to stand up to 2 m away from the animal.

A relatively low induction dose (1.04 mg/kg [0.77-1.25 mg/kg]) was given in each case with the intention of inducing sedation in order to facilitate the placement of a needle into the extradural intravertebral vein. This was then used to administer further doses to deepen sedation and anesthesia if necessary. None of the animals were intubated and oxygen was administered at 20 L/min via an intranasal cannula. Limited physiological data were collected due to the light levels of sedation/anesthesia, the short duration of procedures and difficulty in attaching vital signs monitoring devices (particularly an SpO\(_2\) probe). The mean heart rate and respiratory rate for one animal were 81 and 8.3 respectively. Apnea has been reported in wild southern elephant seals (up to 40 min) anesthetized with tiletamine and zolazepam. The longest period of apnea we experienced was 5 min. Rocking animals from side to side appeared to induce respiration.

Hyperthermia has been reported as a potential concern during southern elephant seal anesthesia.\(^21\) Regular rectal temperatures were difficult to take at the levels of sedation and anesthesia we obtained. The highest temperature recorded was 39°C. Hyperthermia was avoided by carrying out procedures early in the morning, hosing the animal’s with cold water and packing ice around the flippers.

During anesthesia blood samples were taken for hematology and biochemistry (Table 1). Recovery in all cases was uneventful with the longest recovery being approximately 2 hr. The five anesthetic events are summarized in Table 2.

**Rehabilitation and Release**
A female southern elephant seal estimated to be 10-mo-old and weighing 54 kg was found hauled out on a Sydney Beach on 16 August 1994. She was brought to Taronga Zoo for treatment and rehabilitation. The animal was emaciated, dehydrated, had an abscess on its ventral left thorax and its respiration appeared labored. Her condition initially improved with intensive therapy, however, she became dyspneic and anorectic a month after initial presentation. Thoracic radiography and ultrasonography demonstrated a diffuse pneumonia, increased pleural fluid and a small amount of air in the thoracic cavity. It recovered after intensive treatment.

After 13 mo it was released at a beach in southern Tasmania and weighed 140 kg. Its movements were monitored using satellite telemetry. Signals were received for 25 days and the animal traveled over 1,500 km. Freeze branding and flipper tagging were also used to facilitate post-release monitoring.

ACKNOWLEDGMENTS

The authors thank veterinary ophthalmologists Dr Jeff Smith and Dr Anna Deykin, Taronga Zoo’s Marine Mammal staff, in particular Mr Andrew Irvine for providing much of the husbandry and dietary information, and Vanessa Di Giglio for her assistance in the preparation of this manuscript.

LITERATURE CITED

Table 1. Hematology and biochemistry, means (±S.D.) for N replicates from two healthy southern elephant seals (*Mirounga leonina*).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>S.D.</th>
<th>N</th>
</tr>
</thead>
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<td>White blood cell count</td>
<td>x10^9/L</td>
<td>±</td>
<td>5</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>gm/L</td>
<td>±</td>
<td>4</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>%</td>
<td>±</td>
<td>5</td>
</tr>
<tr>
<td>MCHC</td>
<td>gm/dl</td>
<td>±</td>
<td>4</td>
</tr>
<tr>
<td>Segmented neutrophils</td>
<td>x10^9/L</td>
<td>±</td>
<td>5</td>
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<tr>
<td>Neutrophilic bands</td>
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</tr>
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<td>±</td>
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<td>x10^9/L</td>
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<td>5</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>x10^9/L</td>
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<td>4</td>
</tr>
<tr>
<td>Estimated platelet count</td>
<td>/HOIF</td>
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</tr>
<tr>
<td>Glucose</td>
<td>mmol/L</td>
<td>±</td>
<td>5</td>
</tr>
<tr>
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<tr>
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<tr>
<td>Total protein (refractometer)</td>
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</tr>
<tr>
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Table 2. Summary of southern elephant seal (*Mirounga leonina*) anesthetics at Taronga Zoo using tiletamine and zolazepam.
<table>
<thead>
<tr>
<th>Case</th>
<th>Dosage (mg/kg)</th>
<th>Dose* (I,S)</th>
<th>Route³ (i.m., i.v.)</th>
<th>Effect⁸</th>
<th>Time taken to effect (min)</th>
<th>Mass (kg)</th>
<th>Sex (♂,♀)</th>
<th>Reason for anesthesia</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1.06</td>
<td>I</td>
<td>i.m.</td>
<td>1</td>
<td>32</td>
<td>800</td>
<td>♂</td>
<td>Blood collection</td>
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<tr>
<td></td>
<td>0.625</td>
<td>S</td>
<td>i.m.</td>
<td>1</td>
<td>7</td>
<td></td>
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</tr>
<tr>
<td>2</td>
<td>0.769</td>
<td>I</td>
<td>i.m.</td>
<td>3</td>
<td>10</td>
<td>650</td>
<td>♀</td>
<td>Blood collection. (unsuccessful)</td>
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<tr>
<td>3</td>
<td>1.25</td>
<td>I</td>
<td>i.m.</td>
<td>1</td>
<td>15</td>
<td>800</td>
<td>♂</td>
<td>Conjunctival pedicle graft</td>
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<td></td>
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<td>2</td>
<td>16</td>
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<tr>
<td></td>
<td>0.313</td>
<td>S</td>
<td>i.v.</td>
<td>3</td>
<td>2</td>
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<tr>
<td>4</td>
<td>0.993</td>
<td>I</td>
<td>i.m.</td>
<td>1</td>
<td>16</td>
<td>141</td>
<td>♀</td>
<td>Glue platform transmitter terminal, brand, flipper tag, blood taking, skin biopsy, ocular exam.</td>
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<tr>
<td>5</td>
<td>1.13</td>
<td>I</td>
<td>i.m.</td>
<td>1</td>
<td>17</td>
<td>141</td>
<td>♀</td>
<td>Attach platform transmitter terminal (sutured on)</td>
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<td></td>
<td>0.709</td>
<td>S</td>
<td>i.m.</td>
<td>1</td>
<td>5</td>
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</table>

* I = Immobilizing dose, S = Supplemental Dose.

³ i.m. = intramuscular (either hand injected or projectile syringe), i.v. = intravenous (into extradural intravertebral vein).

⁸ P 1 = mild sedation, 2 = heavy sedation, 3 = light anesthesia.

§ A further 160 mg supplemental dose was given intravenously over 34 min, maintaining heavy sedation.

¶ A further 180 mg supplemental dose was given intravenously over 39 min, maintaining heavy sedation.
Figure 1  Factors suspected of contributing to the occurrence of ocular anterior segment disease (OASD) in southern elephant seals (*Mirounga leonina*).

- High ambient and water temperatures
- Fresh water
- Ocular infection
- Haul-out substrate (quality and contamination)
- Water quality (bacteria, particulate matter, chloramines)
- Nutritional deficiencies (Vitamins E, C and A)
- UV light (200-295 nm)
- Trauma
- Stress
- Immune suppression (concurrent disease, malnutrition)
Figure 2  A proposed mechanism for the formation of corneal oedema in pinnipeds by oxidative compounds, ultraviolet light and high temperatures.

Oxidative Compounds (chlorine, ozone, iodines) → Depletion of free radical scavengers in corneal epithelium

+ Ultraviolet light (200-295 nm) → Increase in free radicals in corneal stroma and Descemet’s membrane

+ High water and ambient temperatures → Peroxidation of unsaturated fatty acids in membrane phospholipids

Corneal oedema
EFFECTS OF LEUPROLIDE ACETATE IN DEPOT SUSPENSION ON TESTOSTERONE LEVELS, TESTICULAR SIZE AND SEMEN PRODUCTION IN MALE ATLANTIC BOTTLENOSE DOLPHINS (Tursiops truncatus)

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Abstract

There are few available methods of chemical contraception in marine mammals, and none frequently used in cetaceans. To properly manage any species, the ability to regulate the reproduction of the animals is essential. In many cases there is a desire to increase reproductive capacities of certain individuals or groups while in other cases there is a desire to decrease or stop reproduction in an individual or group. To facilitate the proper management of Atlantic bottlenose dolphins (Tursiops truncatus), a GnRH agonist, leuprolide acetate in depot suspension (Lupron®), was used to evaluate effect on testosterone levels, testicular size, semen production and ultimately, its function as a practical contraceptive.

This study was conducted at three sites. The Chicago Zoological Society (CZS) maintains two breeding populations of dolphins. One at its Brookfield Zoo facility in Brookfield, Illinois, USA and the second is at its off-site breeding and research facility, the Dolphin Connection at Hawk’s Cay Resort and Marina, Duck Key, Florida, USA. The United States Navy maintains dolphins at Naval Command Control and Ocean Surveillance Center, RDTE Division (NRAoD) in San Diego, California, USA.

The study group of animals consisted of ten animals, eight treatment and two controls. Of the ten animals, six were in the CZS group and four were at the Navy facility. All animals received the ultrasonic evaluations (Table 1). All CZS animals were in the treatment group, while two of the Navy animals were treated and two were controls. Semen was consistently collected on three of the animals at the Dolphin Connection facility (CZS group).

The treatment dolphins were injected with 0.075 mg/kg Lupron® every 28 days for 6 mo. The control animals were injected with an equal volume of the copolymer suspension without the active ingredient, leuprolide acetate. During this period, blood samples were taken as noted in Table 1. Blood was collected by venipuncture of the fluke vessel and the whole blood was placed in a thrombin tube. After the clot had formed the tube was spun in a centrifuge and the serum was decanted into plastic vessels for storage in a -70°C freezer until analysis. Ultrasound and semen collection were performed at least monthly.

Testosterone levels were determined by using the radioimmunoassay 125I testosterone test kit. During the study period, the testosterone levels dropped from 15-40 ng/ml to consistent levels of <
0.45 ng/ml, testicular size was reduced during the period, and semen production dropped from levels as high as 3.0 X 10⁹ sperm /ml to 0-10,000 sperm/ ml, for the test animals. The sonographic image of the testes changed from that of a distinct, well-circumscribed tissue to a very diffuse pattern that made both visualization and measurements difficult. Upon cessation of treatments, the size of the testes, the testosterone levels, and the sperm production returned to pre-trial levels. There were no changes noted in the two control animals.

**Resumen**

Existen pocos métodos actuales de anticonceptivos químicos en los mamíferos marinos, y ninguno frecuentemente utilizado en los cetáceos. Para el manejo adecuado de las especies, la habilidad para regular la reproducción de los animales, es considerada esencial. En muchos casos existe el deseo de aumentar la capacidad de reproducción de algunos individuos o grupos en tanto que en otros casos existe el deseo de disminuir o detener la reproducción de algún grupo o individuo. Para facilitar el manejo adecuado de los delfines nariz de botella (*Tursiops truncatus*) se utilizó un agonista GnRH, el acetato de leuproide en suspensión de depósito (Lupron®), para evaluar su efecto sobre los niveles de testosterona, tamaño de testículos, producción de semen y, finalmente, su función como un anticonceptivo práctico.

Este estudio fue llevado cabo en tres sitios, La Sociedad Zoológica de Chicago, USA (CZS) que mantiene dos grupos de delfines reproductores; uno en sus instalaciones en el Zoológico de Brookfield, Illinois, USA y el segundo en sus instalaciones de reproducción e investigación distantes, llamado Conexión de Delfines en Hawk’‘s Key Resort and Marina), en Duck Key, en Florida, USA. La Marina de los Estados Unidos, alberga delfines en la comandancia naval del centro de control y de vigilancia del océano, en la División RDTE (NReD) en San Diego California USA.

Los grupos de estudio estaban compuestos por 10 animales, ocho en tratamiento y dos testigo (control). De los 10 animales, seis se encontraban en el grupo de Chicago y cuatro en las instalaciones de la Marina. Todos los animales fueron sujeto de evaluaciones ultrasonográficas (cuadro 1). Todos los animales de la Sociedad Zoológica de Chicago se encontraban en el grupo de tratamiento, así como dos animales del grupo de estudio de la Marina. Los otros dos animales se mantuvieron como testigo (control). Se recoleció semen repetidamente en tres de los animales en las instalaciones del grupo de la Sociedad Zoológica de Chicago.

Los delfines bajo tratamiento fueron inyectados con 0.075 mg/kg de Lupron® cada 28 días durante seis meses. Los animales testigo fueron inyectados con un volumen equivalente de una suspensión de co-polímero sin el ingrediente activo: acetato de leuproide. Durante este periodo se tomaron muestras sanguíneas, como se señala en cuadro número uno. La sangre fue obtenida mediante la punción de vena de los vasos de la aleta y el volumen total de la sangre obtenida fue colocada en un tubo para trombina. Después de formado el coágulo, cada tubo fue centrífugado, y el suero fue decantado en tubos de plástico para su almacenamiento y congelación a menos 70º C hasta su análisis. La recolección del semen y la utilización de ultrasonido, se realizaron por lo menos cada mes.

Los niveles de testosterona, fueron determinados utilizando la técnica de radioinmunoensayo 1²⁵ del
kit de pruebas de testosterona. Durante el periodo de estudio los niveles de testosterona disminuyeron de 15 a 40 ng/ml hasta niveles consistentes de <0.45 ng/ml, en el que el tamaño del testículo fue reducido durante este periodo y en que la producción de semen disminuyó de niveles tan altos como 3.0 x 10⁹ espermatozoides/ml a 0-10,000 espermatozoides/ml para los animales en tratamiento. La imagen sonográfica de los testículos cambio de un tejido diferenciado y bien circunscrito a uno de patrón muy difuso que hizo muy difícil su visualización y medida. Después de suspender el tratamiento, el tamaño de los testículos, los niveles de testosterona y la producción de esperma retornaron a los niveles previos al estudio. No fue detectado ningún cambio en los animales testigo.

Summary

Lupron® effectively decreases testosterone levels, testicular size, and sperm production in cetaceans. The Brookfield collection has continued four of the males on the Lupron® for as long as 24 mo in a potential breeding situation with multiple proven breeder females and there have been no pregnancies. The males will even go through ritualistic type matings, but no females have become gravid. Following the described trial, one of the study males in the Brookfield group died from an unrelated illness, and upon examination of the testes of this animal, no pathology nor sperm production was found.

The use of this product has lead to an effective means of male contraception. Based upon the physiologic response to Lupron® withdrawal it is expected that contraception will be reversible.

ACKNOWLEDGMENTS

We would like to thank Dr. Jack Briit and Vicki Hedgepeth at the Department of Animal Science, North Carolina State University for evaluating the serum samples for testosterone levels and thank the trainers at all facilities who took the extra time to work the animals and maintain behaviors during the study. We would also like to thank Marley Herrin of TAP Pharmaceutical (Deerfield, Illinois) for the donation of the Lupron®. Without the collaboration of all the contributors, the study would not have been possible.
Table 1. Protocol for Lupron® study.

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<td>X</td>
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1. Blood collection was performed by phlebotomy of the fluke vein. Serum was obtained from the sample after centrifugation.
2. Ultrasound: all animals in the study received the ultrasonic examination. The Navy dolphins were examined by removing the animals from the water and examining them on the dock. The CZS animals were examined in the water where they stationed in a lateral layout.
3. Animals received deep intramuscular injections via a 3 inch, 18-ga spinal needle.
4. Three of the males at the Dolphin Connection facility were trained for semen collection.
TREATMENT OF STEREOTYPIC BEHAVIOR IN THE POLAR BEAR (Ursus maritimus)

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Animal Health Centre, Calgary Zoo, P.O.Box 3036, Station B, Calgary, Alberta, T2M 4R8, CANADA

Abstract

Stereotypic behaviors are described as excessive, invariant and repeated production of one type of motor act in which no obvious goal or function is apparent. They have some commonalities with the human condition of obsessive compulsive behaviors (OCD). Between 55-100% of captive polar bears are believed to express some degree of stereotypic behavior usually in the form of pacing. This study involved a 26-yr-old captive born polar bear that had expressed varying degrees of pacing over the last 24 yr. The stereotypic behavior of this bear included a facial tic and a “huffing/cough” usually apparent simultaneously at the time of a change in direction of a pacing bout. At the time of the study this bear was housed with another female bear. The habitat is 836 m² in total with 14% of this being a 170,000 L pool. The facility had been enhanced by providing alternative materials such as wood chips, boulders, rocks, tree trunks, and pebbles in place of a smooth concrete surface. Behavioral enrichment activities included occupational feeding such as: scatter feeding; feeding in the pool; food filled logs; fish or fruit “sickles” involving freezing food items in 20 L pails and feeding in the pool; irregular feeding times; and altering access to the main feeding areas. Other activities included removal and reintroduction of old “toys” (barrels and rings) into novel locations. Other than occasional reoccurring problems with alopecia and pruritus which were treated with ivermectin at 300 µg/kg (Ivomec, Merck Agvet, Merck Frosst Canada Inc., Kirkland, Quebec, Canada) p.o. once a month, the bear was considered healthy. The study involved continuous videotaping for nine daylight hours, on an average of every three days, for a total of 94 days over a period of the 300 days of the study. The observation periods were broken down to a 32 day pre trial period, 105 days of fluoxetine (Prozac, Eli Lilly & Co. Indianapolis, Indiana, USA) therapy, and post treatment observation of 163 days. During the pretrial phase the bear was found to exhibit stereotypic pacing behavior 68.6% of the day. Fluoxetine is a second generation anti-depressant compound that along with its major metabolite, norfluoxetine, functions as a potent selective 5HT-reuptake inhibitor. This action serves to make the neurotransmitter serotonin more available to the neural pathways. An initial dose of 1.32 mg/kg p.o. was used for seven days, but was reduced based on consultation and changed to an allometrically scaled dose of 0.62 mg/kg which was then used until the last 21 days of the drug trial when the dosage was increased to 1.0 mg/kg. The bear was immobilized on days 83,138, and 178 of the trial, with a tiletamine zolazepam combination at 4.5 mg/kg (Telazol, Fort Dodge Laboratories Inc., Fort Dodge, Iowa, USA) for physical exams, weights, and to obtain blood samples for complete blood counts (CBC’s) and serum biochemistry profiles, as well to run serum levels for fluoxetine and its metabolites. In the 6th wk of the drug therapy a steady decrease in the stereotypic pacing became evident which at 16 wk had dropped to zero and was maintained at that level for the period of the drug therapy. At the conclusion of the drug therapy there was no reoccurrence of pacing until day 14 and was considered sporadic until day 104 by which time the pacing was considered comparable to pretreatment levels. Other “normal behaviors”
remained unchanged throughout the trial. Physical exams, weights, and CBC and chemistry profiles were considered within normal ISIS reference ranges except for cholesterol which was mildly elevated. The drug levels reported (see Table 1) were within ranges reported therapeutic for humans, although there was a transition from the predominant fluoxetine at the low dose to the metabolite norfluoxetine at the high dose suggesting that the active pharmacological agent may be the metabolite norfluoxetine. Medical treatment with fluoxetine (Prozac), when used in combination with behavioral and habitat enrichment, was an effective inhibitor of stereotypic behavior in this bear.

**Resumen**

La conducta esterotipada se describe como la excesiva, invariable y repetida producción de un tipo de acto motor sin aparente función o meta. Tiene algo de común con la condición humana de comportamiento obsesivo compulsivo (OCD). Entre el 55-100% de los osos polares en cautiverio, se cree que expresan algún grado de comportamiento esterotipado usualmente en la forma de andar. Este estudio involucró a un oso polar de 26 años nacido en cautiverio que había mostrado varios grados de este tipo de conductas en los últimos 24 años. La conducta esterotipada incluía un tic facial con resoplos y toses que generalmente aparecían simultáneamente en el tiempo que cambiaba de dirección al andar. En el tiempo de estudio este oso se encontraba albergado con una osa. El hábitat era de 836 metros cuadrados en su totalidad, siendo el 14% una alberca de 170,000 L. El lugar había sido enriquecido, proporcionando materiales alternativos como: pedazos de madera, rocas, peñascos, troncos de arboles y superficies rugosas en lugar de superficies de concreto liso. Las actividades para el enriquecimiento de la conducta incluyeron la alimentación ocupacional como: alimentación en el estanque, comida dispersada, trozos de madera y superficies de concreto liso. Los horarios irregulares de alimentación y accesos alternos al área principal de alimentación también se incluyeron. Actividades alternas incluyeron la remoción y reintroducción de “juguetes viejos” (barriles y aros) en lugares nuevos. Aparte de problemas ocasionales y recurrentes de alopecia y prurito, que fueron tratados con Ivermectina 300µg/kg (Ivomec, Agvet, Merck Frosst Canadá Inc., Kirkland, Quebec, Canadá), por vía oral una vez al mes, el oso estaba considerado sano. El estudio incluyó videos continuos durante 9 hrs. diurnas, en un promedio cada tercer día, por un total de 94 días filmados en un periodo de 300 días de estudio. Los periodos de observación se dividieron en un periodo de 32 días de pre-estudio, 105 días de terapia con Fluoxatina (Prozac, Eli Lilly Co., Indianapolis, Indiana, USA.) y un periodo de observación de 163 días post-tratamiento. Durante la fase de pre-estudio se encontró que el oso presentaba la conducta esterotipada de andar durante el 68.6% del día. La fluoxatina es un compuesto antidepresivo de segunda generación que, junto con su metabolito, la norfluoxatina, funciona como un inhibidor selectivo de la re-captación de 5-HT. Esta acción permite que la serotonina esté más disponible a los receptores nerviosos. Durante los 7 primeros días se utilizó una dosis inicial de 1.32 mg/kg por vía oral, pero se redujo en base a consultas a una dosis medida halométricamente de 0.62 mg/kg, la cual se utilizó hasta los últimos 22 días de administración del tratamiento, cuando la dosis fue incrementada a 1 mg/kg. El oso fue inmovilizado los días 83, 138 y 178 del tratamiento con una combinación de tiletamina/zolazepam a razón de 4.5 mg/kg. (Telazol, Fort Dodge Laboratories Inc., Fort Dodge, Iowa, USA). Se le practicó un exámen físico, se obtuvo el peso corporal, y se tomaron muestras de sangre para evaluar cambios celulares (CBC’s), y los perfiles bioquímicos en suero.
Además se examinaron los niveles de Fluoxatina y sus metabolitos en suero. En la sexta semana de terapia se hizo evidente una disminución en el andar estereotipado, el cual desapareció totalmente a la semana 16 y siguió sin aparecer durante el período de tratamiento con la droga. Al finalizar la terapia con la droga no hubo recurrencia del paso de andar estereotipado hasta el día 14 y fue considerado esporádico hasta el día 104. Hasta ese día fué que el andar volvió a ser considerado comparable a los niveles de pre-tratamiento. Otras “conductas normales” permanecieron sin cambios durante el experimento. Los exámenes físicos, pesos y los perfiles químicos y de CBC fueron considerados dentro de los valores normales de referencia de ISIS excepto para el colesterol, el cual resultó medianamente elevado. Los niveles de droga reportados (ver Tabla 1) estaban dentro de los rangos reportados en terapias para humanos. Hubo una transición de la fluoxatina, predominante a la dosis baja, al metabolito Norfluoxatina a la alta dosificación, lo cual sugiere que el agente farmacológico activo puede ser el metabolito de Norfluoxatina. El tratamiento médico con Fluoxatina (Prozac), cuando se usó en combinación con el enriquecimiento de la conducta y el hábitat, fue un inhibidor efectivo en el comportamiento estereotipado en este oso.

**Table 1.** Serum level (ng/ml) for the R and S enantiomers of fluoxetine (F), norfluoxetine (NF), and for trifluoromethylphenol (TM), at three different dose rates.

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<th>F-S</th>
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<td>160</td>
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<td>69.2</td>
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<td>260</td>
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Abstract

One male in a group of seven Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) managed in the same water system as four beluga whales (*Delphinapterus leucas*) and four harbor seals (*Phoca vitulina*) exhibited signs of lethargy and depression over a 6-10 hr period, culminating in death. None of the other animals exhibited clinical signs.

Gross examination showed the animal was in excellent nutritional condition, and the only significant finding was moderately enlarged mesenteric lymph nodes. Histopathology revealed generally massive numbers of gram-positive, frequently filamentous, bacilli, usually intravascular, in all tissues. Bacteria were both extracellular and present in macrophages, monocytes and neutrophils. Mesenteric lymph nodes were moderately reactive. Aerobic bacterial culture of lung, liver, kidney, and spleen yielded pure cultures of *Erysipelothrix rhusiopathiae*.

Based on clinical course, histopathology, and bacteriology, a diagnosis of acute *Erysipelothrix rhusiopathiae* septicemia was made. Organism source and route of infection were not determined. Interestingly, months before the onset of clinical signs this animal had a 1/100 anti-*Erysipelothrix rhusiopathiae* titer detected by enzyme-linked immunosorbent assay (Middlebrooks, B.L., Univ. So. Miss., personal communication). Infection secondary to immunosuppression was deemed unlikely as evidence suggesting immunocompetence, including reactive lymph nodes, good body condition, and the presence of an anti-*Erysipelothrix* titer, were observed.

Erysipelas in captive marine mammals can present as cutaneous vasculitis similar to “diamond back” in swine, or as peracute to subacute septicemia. The disease is frequently sporadic, with only single or few animals in a group or water system affected. Definitive source and transmission of this organism in marine mammals has not been ascertained. Likely sources of infection include contaminated feed fish and water. Ingestion of contaminated feedstuffs and opportunistic colonization of wounds are plausible modes of transmission. Clinical signs of erysipelas are frequently non-specific, including anorexia, depression, and lethargy. Peracute to acute septicemia occurs with none, or only a short duration of non-specific clinical signs, as in this case. Diagnosis is frequently made postmortem. Characteristic cutaneous lesions, positive blood cultures, and possibly, rising serum anti-*Erysipelothrix* titers are the typical clinical means of confirming infection. Titers should be interpreted with consideration of other clinical data. Successful treatment of cutaneous and septic erysipelas has been reported. Penicillins and related antibiotics, such as cephalosporins, are considered the drugs of choice.
Resumen

Un macho de un grupo de 7 delfines de costado blanco del pacífico (Lagenorhynchus obliquidens) manejado en el mismo sistema de agua que 4 ballenas belugas (Delphinapterus leucas) y de 4 focas de puerto (Phoca vitulina) mostró signos de letargia y depresión durante un periodo de 6 a 10 horas culminando en la muerte del animal. Ninguno de los otros animales mostró signos clínicos.

Un examen macroscópico mostró que el animal estaba en excelente estado nutricional, y el único hallazgo significativo fue el crecimiento de los ganglios linfáticos mesentéricos. El examen histopatológico reveló un gran número de bacilos gram positivos, frecuentemente con filamentos, y usualmente intravasculares en todos los tejidos. Las bacterias se encontraban en el espacio extracelular y en macrófagos, mononcitoides y neutrófilos. Los nódulos linfáticos mesentéricos estaban moderadamente reactivos. Los cultivos de bacterias aeróbicas de pulmón, hígado, riñón y bazo mostraron sólo crecimiento de Erysipelothrix rhusiopathiae.

Basados en el curso clínico, histopatología y bacteriología, se hizo el diagnóstico de septicemia aguda por Erysipelothrix rhusiopathiae. El origen y la ruta de infección no fueron determinados. Meses antes de presentar los signos clínicos, este animal tenía títulos anti-Erysipelothrix de 1/100 detectados por ELISA (Middlebrooks, B. L., Univ. So Miss., comunicación personal). No fue posible determinar una infección secundaria por inmunosupresión, ya que como evidencia de una inmunocompetencia, se encontraron los nódulos linfáticos reactivos, la buena condición corporal y la presencia de títulos anti-Erysipelothrix rhusiopathiae que fueron observados.

La Erysipela en mamíferos marinos en cautiverio puede presentar una vasculitis cutánea, similar a las manchas en diamante de los cerdos. O como una septicemia de per-aguda a subaguda. Esta enfermedad es frecuentemente esporádica, con solamente uno o pocos animales en un grupo o con el sistema de agua afectado. Definitivamente, el origen y transmisión de este organismo en mamíferos marinos no ha sido determinado. El origen probable de la infección, incluye el pescado para la alimentación y agua contaminada. La ingestión de alimentos contaminados y heridas contaminadas por agentes infecciosos oportunistas, son factores probables de transmisión. Los signos clínicos de la Erysipela no suelen ser específicos, incluyendo norexia, depresión y letargia. Una septicemia de per-aguda a aguda ocurre sin ningún signo o sólo con signos clínicos de corta duración y no específicos como en este caso. El diagnóstico es frecuentemente hecho postmortem. Las lesiones cutáneas características, los cultivos sanguíneos positivos y títulos elevados de suero anti-Erysipelothrix son los hallazgos clínicos típicos para confirmar la infección. Los títulos deberán ser interpretados con consideración de otros datos clínicos. El tratamiento exitoso de erisipela cutánea y séptica han sido reportados. Las penicilinas y otros antibióticos como las cefalosporinas son consideradas las drogas de elección.

ACKNOWLEDGMENTS

The authors wish to acknowledge the excellent technical assistance of Dr. Timothy Walsh, Allen Feldman, and the Hines Veterans Administration Hospital Histopathology Laboratory.

LITERATURE CITED


CIRCULATING GESTATIONAL PROGESTERONE AND ESTRADIOL CONCENTRATIONS IN BELUGA WHALES (Delphinapterus leucas)

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Abstract

Blood samples were obtained between 1990 and 1995, during 13 gestations, from ten female beluga whales (Delphinapterus leucas) (nine nulliparous and four primiparous), housed at four institutions. Whales were approximately 6-20 yr-old at conception. Five to 51 samples were collected per gestation on an irregular sampling interval by a trained fluke presentation behavior or in conjunction with medical or management procedures. Serum or heparinized plasma samples were analyzed by radioimmunoassay for progesterone (207 samples) and estradiol (93 samples) levels.

Gestation was 15-17 mo but more precise determination was limited by infrequent sampling in the postovulatory period. Pooled mean monthly gestational hormone levels ranged from 0.97 ± 1.14 ng/ml to 42.86 ± 12.0 ng/ml for progesterone and from 13.93 ± 11.62 pg/ml to 30.62 ± 12.43 pg/ml for estradiol (Table 1). There was no significant correlation between circulating progesterone and estradiol levels. There was no significant difference between progesterone levels of nulliparous compared to primiparous whales, although there were few primiparous whales sampled. Early gestational progesterone levels showed significant temporal variation (p=0.016) with earlier levels significantly higher than later levels. Limited data for estradiol did not demonstrate temporal variation (p=0.40).

Beluga whales are seasonally polyestrous with conceptions occurring between February and June and births from July through September. Lactational anestrus occurs for the first postpartum breeding season and estrus activity resumes in the following breeding season with a resulting interbirth interval of 3 yr.

Resumen

Las muestras sanguíneas fueron obtenidas entre 1990 y 1995 durante 13 gestaciones de 10 hembras de ballena beluga (Delphinapterus leucas) (9 nulíparas y 4 primíparas), albergadas en cuatro instituciones. Las ballenas tenían aproximadamente entre 6 y 20 años de edad en el momento de la concepción. Por cada gestación se recogieron de 5 a 51 muestras, en un muestreo irregular por intervalos según el comportamiento o en conjunción con procedimientos médicos o de manejo. Las
muestras de suero o plasma heparinizado fueron analizadas por radioinmunoensayo para los niveles de progesterona (207 muestras) y niveles de estradiol (93 muestras).

La gestación fue de 15 a 17 meses, (no se pudo hacer una determinación precisa debido al muestreo infrecuente en el periodo postovulatorio). Los niveles mensuales de la hormona gestacional variaron en un rango de 0.97 ± 1.14 ng/ml a 42.86 ± 12.0 ng/ml de progesterona y un rango de 13.93 ± 11.62 pg/ml a 30.62 ± 12.43 pg/ml para estradiol (Tabla 1). No hubo una correlación significativa entre la progesterona circulante y los niveles de estradiol. No hubo una diferencia significativa entre los niveles de progesterona de nulíparas comparadas con ballenas primíparas, aunque hubo pocas ballenas primíparas muestreadas. Los niveles tempranos de progesterona gestacional mostraron una variación temporal significativa (p= 0.016) con niveles tempranos altos comparados con los niveles tardíos. Los datos limitados del estradiol no demostraron variaciones temporales (p= 0.40).

Las ballenas beluga son poliéstricas estacionales, ocurriendo la concepción entre febrero y junio, y los nacimientos desde julio hasta septiembre. El anestro por lactación ocurre en la primera temporada de apareamiento postparto y la actividad estral comienza en la siguiente temporada de apareamiento, resultando un intervalo entre nacimientos de 3 años.

ACKNOWLEDGMENTS

The authors thank the marine mammal staffs and technicians at each of the aquaria without whom this project would not have been possible and Drs. Peter Walsh and James Gibbs for statistical assistance.
Table 1. Beluga whale (*Delphinapterus leucas*) pooled monthly mean gestational progesterone (13 gestations) and estradiol (4 gestations) concentrations for ten beluga whales.

<table>
<thead>
<tr>
<th>Month Prepartum</th>
<th>Progesterone (ng/ml) mean ± SD</th>
<th>gestations (n)</th>
<th>Estradiol (pg/ml) mean ± SD</th>
<th>gestations (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-17</td>
<td>0.97 ± 1.14</td>
<td>9</td>
<td>30.62 ± 12.43</td>
<td>3</td>
</tr>
<tr>
<td>-16</td>
<td>4.35 ± 3.58</td>
<td>8</td>
<td>13.93 ± 11.62</td>
<td>3</td>
</tr>
<tr>
<td>-15</td>
<td>42.86 ± 12.0</td>
<td>5</td>
<td>17.15 ± 9.96</td>
<td>4</td>
</tr>
<tr>
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<td>6</td>
<td>15.72 ± 4.91</td>
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<tr>
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</tr>
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<tr>
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THE ALL TAXA BIODIVERSITY INVENTORY IN THE GUANACASTE
CONSERVATION AREA, GUANACASTE PROVINCE, COSTA RICA

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Abstract

An All Taxa Biodiversity Inventory (ATBI) is an inventory of all species in a large conserved wildland. The significance of the ATBI is based on the assumption that the developing tropical societies will conserve their biodiversity if it generates intellectual and economic benefits that pay for its maintenance and contribute to national economic growth. An ATBI brings together national and international taxonomic and administrative expertise to determine: (1) which species are present and how to tell them apart, (2) where they can be found, (3) how they can be obtained when needed, and (4) what is their basic natural history--and all of this computerized into the Internet public domain. By thus making biodiversity available to a multitude of users, conservation of the site’s biodiversity can now occur through managed sustainable use, and integration of wildland biodiversity into the host nation’s economic and intellectual framework. User groups include industry (ecotourism, pharmaceuticals, biotechnology, agriculture), educational and scientific institutions (elementary schools to universities and museums), public, livestock, and agricultural health experts, environmental monitoring and restoration programs, and economists and development agencies. For zoo veterinarians, an ATBI represents an unparalleled opportunity to study the health, reproductive, and nutritional requirements of a large number of exotic species in a fully natural, yet fully protected and defined environment. This known universe will provide an essential background to understand both the hosts and their parasites and diseases in a zoo environment. INBITTA (Inventario de la Biodiversidad de Todos los Taxa) is a specific ATBI of the 110,000 ha Guanacaste Conservation area (GCA) in northwestern Costa Rica. The GCA contains Costa Rica’s highest diversity - several hundred thousand species - with habitats representing all of its ecosystems found between 400 and 2000 m elevation. It is well-prepared to become a site for major biodiversity development, having a trained and experienced staff, a well-established Conservation Area strategy, five biological stations, and a Regional Council.

Resumen

Un Inventario de biodiversidad de todos los Taxa (ATBI por sus siglas en ingles) es un inventario de todas las especies en una gran zona destinada a la conservación. El significado de ATBI está basado en la idea de que el desarrollo de las sociedades tropicales conservarán su biodiversidad si generan beneficios económicos e intelectuales que puedan sostener su mantenimiento y contribuyan al crecimiento económico nacional. Un ATBI une taxones nacionales e internacionales con la pericia administrativa para determinar: 1) qué especies están presentes y cómo distinguirlas, 2) dónde se pueden encontrar, 3) cómo pueden ser obtenidas cuando se necesita, y 4) cual es su historia natural. Toda esta información es computarizada y puesta a disposición del público a través de Internet.

Poniendo así la biodiversidad al alcance de una multitud de usuarios, haciendo también que los sitios de conservación de biodiversidad puedan ahora ser mantenidos, y la integración de la biodiversidad
en estado libre al equipo de trabajo económico intelectual del país involucrado. El grupo de usuarios incluye a la industria (ecoturística, farmaceútica, biotecnológica, agrícola), instituciones educativas y científicas (desde escuelas primarias hasta universidades y museos), en instituciones públicas, ganaderos y expertos en salud, monitoreo ambiental y reestructuración de programas y agencias de economía y desarrollo. Para veterinarios de zoológicos, un ATBI representa una maravillosa oportunidad de estudiar los requerimientos de salud, reproducción y nutrición de un gran número de especies exóticas en una área completamente protegida y definida. Este universo conocido proveerá unos antecedentes esenciales para entender tanto a huéspedes como a sus parásitos y las enfermedades propias de un ambiente de zoológico. INBITTA (Inventario de la Biodiversidad de todos los Taxa) es una ATBI de 110,000 ha en el área de conservación de Guanacaste (GCA) al noroeste de Costa Rica. El GCA contiene la más grande diversidad con cien mil especies - con sus hábitats representando todos sus ecosistemas que se encuentran entre 400 y 2,000 m de elevación. Está bien preparado para llegar a ser el sitio con mayor desarrollo de biodiversidad, teniendo un equipo de trabajo entrenado y con experiencia, una estrategia de conservación bien establecida, cinco estaciones biológicas y una junta de consejo regional.

Introduction

During these tough economic times, discussions about the future inevitably include references to ‘down-sizing’ and ‘tradeoffs’. For example, residents of ‘developed’ countries tend to accept that economic development always occurs at the expense of preserving biodiversity. For them, continued economic development must lead to at least some loss of species, and at issue is the appropriate level of tradeoff between development and conservation. But it goes much further than that. If you live in a ‘developing’ country, this assumption means that the dreams of a better life for your children or grandchildren cannot be achieved without massive economic growth, implying massive loss of biodiversity--hence, there is little incentive for preserving biodiversity. If preservation of biodiversity and economic development are diametrically opposed, residents of ‘developed’ countries face a moral and ethical dilemma of biblical proportions with respect to ‘developing’ countries: Are we the custodians of the earth (do we simply protect biodiversity) or are we our brothers’ keepers (do we help improve the lives of people in developing countries)?

Fortunately, the world has Costa Rica. Lying between Panama and Nicaragua, the country has little in the way of mineral resources, few large ports, and almost no large industries. Much of the population is rural and agrarian. And yet, Costa Rica has a long tradition of political stability, no military, a 98% literacy rate among its people, a growing economy, and an excellent national health care system. They have also set aside 25% of their land area as national parks and nature reserves. How could this happen if biodiversity preservation comes at the expense of economic development? Costa Rica had challenged the assumption that economic development and biodiversity preservation are opposing forces. It has opted for economic development driven by sustainable management of its biodiversity resources. The current government has embarked on an audacious plan to base Costa Rican economy on principles of sustainable development.

The Costa Rican landscape consists of urban areas, agroecosystems producing one set of economically valuable products, and wildlands maintained for another set of products derived from their biodiversity. About 25% of Costa Rica consists of wildlands conserved as biodiversity
preserves. They contain about 500,000 species of wild plants, animals and microorganisms. These species, their genes, and their natural histories are distributed from the nearly desert-dry forest habitat in the northwest to the very wet rain forest habitats of the remainder of Costa Rica lowlands, to the 3000-plus meter tall mountain ranges. If we can assume that a tropical society will conserve a major portion of its wild biodiversity if protected areas can generate enough intellectual and economic income for its own upkeep, then Costa Rica’s biodiversity - about 4% of that of the terrestrial world - is a potentially powerful engine for intellectual and economic development based on sustainable use of renewable natural resources. Accomplishing these goals requires first a complete inventory of Costa Rica’s biodiversity.

The Instituto Nacional de la Biodiversidad (INBio)

The institutional entity that is responsible for Costa Rica’s biodiversity-based economic development is the Instituto Nacional de la Biodiversidad (INBio). On June 5, 1989, a Presidential Executive Decree established the INBio Planning Commission, which recommended that INBio be created as a non-profit, private organization for the public good. INBio’s mandate is based on 2 principles: (1) that only by understanding biodiversity can we protect it, manage it, and help society use it without destroying it; and (2) such understanding requires a focused, on-site, day-in and day-out examination of the biodiversity in a country’s conserved wild lands carried forth with care, enthusiasm, dedication and perseverance by the people of the nation where this biodiversity lives. INBio carries out the processes of: (1) a national biodiversity inventory, (2) biodiversity prospecting and biodiversity information, (3) management, and (4) dissemination.

The National Biodiversity Inventory, ATBI, and INBITTA

Costa Rica’s national biodiversity inventory is intended to gather information about all species and to involve broad national participation in the process. The first goal of the national inventory is accumulating specimens necessary to elucidate the taxonomy of Costa Rica’s biodiversity in both a national and international context, including the knowledge of at least one site of occurrence in Costa Rica for each species. These efforts will take the form of identified reference collections, field guides and electronic identification services. In the long-term, the inventory will establish species’ ranges in more detail and begin the process of understanding their natural history and other properties. The basic field work is conducted by a small army of lay people called “parataxonomists”. Working from “biodiversity offices” scattered across the country’s habitats and conserved wildlands, parataxonomists supply the specimens and other field data which flow into INBio to be processed into the collections of the National Biodiversity Inventory and the National Biodiversity Information Management System, used to develop local expertise, and into the international network of taxonomists and collections throughout the world. Residents of the communities in and around Costa Rican wildlands, parataxonomists also teach their neighbors about their local environment.

In April 1993, 57 biodiversity administrators, systematists, and biodiversity computer specialists from the United States, Canada, Brazil, Australia, Mexico, Norway, England, and Costa Rica met at a workshop in Philadelphia sponsored by the US National Science Foundation. They came together to discuss the feasibility, technology, protocols, products, costs and sociology of a timely and thorough inventory of the biodiversity of a large and biologically rich terrestrial wildland. The
result was the All Taxa Biodiversity Inventory (ATBI), an enormous collaborative project that brings international and national taxonomic expertise to bear on determining what is in a wildland site and organizing that information for use by society. It is meant to contribute to conservation of the biodiversity of the inventoried site and the integration of biodiversity into the host nation’s economic and intellectual framework through sustainable use. The workshop participants estimated that an ATBI would cost as much as $120 million, but methods, protocols, and strategies established by the first one should decrease the costs of subsequent ATBI’s in other parts of the world. Given the structure of INBio, Costa Rica was an obvious choice for the world’s first ATBI.

INBITTA (Inventario de la Biodiversidad de Todos los Taxa) is an ATBI of the 110,000 ha Guanacaste Conservation Area (GCA) in Guanacaste Province, northwestern Costa Rica. Now part of the Costa Rican National Biodiversity Inventory, INBITTA is moving through the pre-planning phase and gearing up to commence intense activities in early to mid-1997. The GCA comprises Santa Rosa National Park, Rincón de la Vieja National Park, Guanacaste National Park, Horizontes Experimental Forest Station, Junquillal Recreation Area, and Isla Bolaños Wildlife Refuge. The GCA extension and its particular physical location in the country has made this wildland area notable for containing the highest ecosystem diversity in the country making it ideal for the world’s first ATBI. The GCA contains dry forest, cloud forest, and rain forest ranging over approximately 36 different habitats and more than 120 micro-habitats representing all of Costa Rica’s ecosystems found between 400 and 2000 m elevation.

The INBITTA represents an unparalleled opportunity for zoo veterinarians to study the health, reproductive, and nutritional requirements of a large number of exotic species in a fully natural, yet fully protected and defined environment. This known universe will provide essential background to understand both the hosts and their parasites and diseases in a zoo environment. The Vertebrate Taxonomic Working Group (TWIG) associated with INBITTA is expected to complete its inventory of the approximately 1,000 species of vertebrates living in the GCA fairly quickly. Information obtained from that TWIG will help the efforts of the four TWIGs concerned with viruses, bacteria, fungi, and the protist, helminth and arthropod parasites of vertebrates. The species studied by those four TWIGs serve as sensitive bio-indicators of the overall health of the ecosystem, and are the baseline for assessing health risks to humans, livestock and wildlife, including the possibility of emerging diseases. Of particular interest is the potential use of these species as model systems for studying disease in other parts of the world. For example, in mid-February 1996, a team representing the Parasite TWIG conducted a training course at the GCA, teaching parataxonomists how to find, collect, and preserve a variety of parasites. During that 6-day course, a number of new species were found. One of them, a relative of species of *Plasmodium*, which cause malaria, lives in the blood of a locally common large iguana, *Ctenosaura similis*. The new parasite does not multiply within the red blood cells of its host, causing the periodic mass destruction of blood cells and resulting acute malarial attacks of chills and fever. It seems reasonable that pharmaceutical and biotechnology companies would be interested in species related to malaria that do not cause the same degree of disease.

The major costs of INBITTA are being borne by the government of Costa Rica through its economic development plans in association with the World Bank and other international development agencies. Other sources have provided significant support. Funding for the first year of pre-planning for INBITTA was provided by a grant from the National Science Foundation of the USA.
Subsequently, Norway (through NORAD) donated funds to help defray the costs of 2 yr of planning conferences. In the fall of 1995, Canada and Costa Rica signed a debt-swap agreement, whereby half of the forgiven debt would be used by INBio for parataxonomists salaries and for the national inventory. On March 20, 1996 the Dutch government announced a grant of $12,000,000 US to help support the costs of INBITTA. All of this support represents investments in the future of the world’s biodiversity, and a vote of confidence in INBio’s ability to utilize biodiversity inventory information to accomplish its other 3 mandates, briefly discussed below.

**Biodiversity Prospecting**

Costa Rica’s wildlands are in effect an enormous, and largely unread, library. Through biodiversity prospecting activities, INBio intends to help the world’s pharmaceutical, medicinal and agricultural fields advance significantly and to help Costa Rica by generating financial support for its protected wildlands and its economic development. These efforts presently focus on the search for chemicals produced by plants, insects and microorganisms that may be of use to pharmaceutical, medicinal and agricultural industries. Bioprospecting efforts are carried out in collaboration with local and international research centers, universities, and the industrial sector. They offer opportunities to train Costa Ricans for laboratory and field work. They also generate income to support the country’s conservation activities and promote market driven research and sustainable economic development, and contribute to the care of Costa Rican conserved wildlands. Typically bioprospecting agreements include at least 10% overhead to the Ministry of Natural Resources, Energy and Mines. The remainder of the research budget supports the actual bioprospecting efforts, including the activities of the parataxonomists. The Ministry of Natural Resources, Energy and Mines receives royalties if a successful research product results from a bioprospecting venture. The landmark partnership between INBio and Merck & Co., Inc., is an example of this.

**Biodiversity Information Management**

INBio’s needs to become highly capable in information management for its internal operations and to present information in appropriate formats for a wide range of users throughout the world. In this way, Costa Rica can become a net exporter of scientific information. INBio’s rapidly growing biodiversity information, when coupled with topographic maps, soil maps, climate data, and patterns of land use, is extremely complex. No biodiversity users in the world today are capable of analyzing, managing, and distributing and integrating such a data base. Through a partnership with Intergraph Corporation (Huntsville, Alabama) and ongoing efforts at computerizing and networking biodiversity information, INBio is bringing cutting edge technology in Geographic Imaging Systems (GIS) and data base management and development to bear on this problem.

**Biodiversity Information Dissemination**

Few people are aware of the economic potential of the emerging biodiversity industry. For INBio this is a problem that can be solved by enhancing biological literacy and increasing appreciation of what biodiversity information can offer. In this manner, Costa Rica is confronting the dual potential threats of the loss of the tropics’ massive biodiversity and the loss of biological literacy by promoting the development of a society whose ethical and moral values will be rooted in respect for nature and the wise management of natural resources. Programs being developed include outreach
to educational institutions, training the staff of conservation areas, consultancies to political and economic policy-making efforts, and hosting national and international conferences.

**Discussion**

The Costa Rican attitude with respect to biodiversity is radically different from that in most countries. Extinction is not a regrettable but necessary by-product of economic development. It is the irreplaceable loss of economic potential and opportunity. This attitude is based on the notion that all species have value. From that follows 2 propositions: (1) What we do not value, we will not protect and preserve--therefore, we must determine the value of each species. (2) What we do not understand, we cannot value—therefore, we must understand each species. US Vice-President Al Gore has said, “The world needs Costa Rica.” Never has the truth of those words been more evident.

**ACKNOWLEDGMENTS**

My thanks to Dr. Susan Mikota for the invitation to present these ideas to the AAZV. My thanks also to Dr. Dan Janzen, who invited me to participate in the 1993 NSF Workshop, thereby introducing me to ‘nuestra esperanza para el futuro’, and to all the people of INBio I have met and with whom I have had the honor of working.

**LITERATURE CITED**

WILDLIFE RESCUE IN FRENCH GUIANA: OBJECTIVES, METHODOLOGY AND PRELIMINARY RESULTS

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Abstract

Between January 1994 and July 1995, 310 km² of a primary rainforest were flooded at the Petit Saut dam site on the Sinnamary river (French Guiana). A wildlife rescue was organized and followed various objectives: translocate threatened vertebrates, build a biological bank and database on Guianan wildlife, carry out a post release survey including ecological studies and provide public awareness on Guianan wildlife conservation. A total of 3278 non flying mammals (47 species), 799 snakes (68 species) and 1386 tortoises were captured and examined at the veterinary facility before release in a safe area. Biological samples and data were collected on most of them. Fifty animals of several target species were radio-collared including well-studied species like red howler monkeys (Alouatta seniculus), common but poorly known species like sloths (Bradypus tridactylus and Choloepus didactylus) and brocket deers (Mazama gouazoubira) or uncommon and poorly known species like white-faced sakis (Pithecia pithecia). A large majority survived and remained close to the release site. Moreover, social animals like howlers frequently integrated resident groups. A large amount of original data is now in the process of being analyzed and published, many studies are still going on but the results of the translocation, the first scientific results and the interest of the scientific community for our samples and data confirm our initial conviction that the operation was worthwhile.

Resumen

Entre enero de 1994 y julio de 1995, 310 km² de selva fueron inundados por la presa de Petit Saut en el Río Sinnamary (Guyana Francesa). Un rescate silvestre fue organizado con varios objetivos: 1) Translocar vertebrados amenazados, 2) construir un banco biológico y una base de datos de la vida silvestre de Guyana, 3) conducir una post-liberación de los sobrevivientes incluyendo estudios ecológicos y 4) proveer información al público para la conservación de la vida silvestre en Guyana. Un total de 3,278 mamíferos terrestres (47 especies), 799 serpientes (68 especies) y 1,386 tortugas fueron capturadas y examinadas en las instalaciones veterinarias antes de su liberación en zonas seguras. Muestras biológicas y bases de datos fueron obtenidos de la mayoría de ellos, 50 animales de varias especies claves fueron radio-colectadas. Incluyendo aquellas especies bien estudiadas como el mono aullador (Alouatta seniculus), especies comunes pero poco conocidas como el perezoso (Bradypus tridactylus y Choloepus didactylus) y venados como (Mazama gouzozybira), o las especies poco comunes y poco conocidas como el saki cara blanca (Pithecia pithecia). La gran mayoría sobrevivió y permanece cerca del sitio de liberación. Aún más, los animales muy sociables, como los aulladores, frecuentemente han integrado grupos residentes. Un gran número de datos originales está ahora en el proceso de ser analizados y publicados. Muchos estudios se están llevando a cabo. Los resultados de la translocación, los primeros resultados científicos y el interés de la comunidad científica por nuestra muestra y datos, confirma nuestra convicción inicial de que esta operación era digna de realizarse.
Introduction

One of the goals of conservation biology is to restore ecological damages. The damage can be accidental (oil spill, introduction of an alien species, appearance of a disease, etc.) or planned and directly linked to human economical development (road or hydroelectric power plant building, logging, agriculture development, etc.). In the case of an extended ecological damage, wildlife rescues are organized. Such operations have several things in common--they need energy, money and are controversial. After an accident, it is sometimes possible to partially restore the damage but, very often, increasing human development is responsible for irreversible changes and damages to natural ecosystems that can only be minimized. This is the case of numerous hydroelectric dams built in neotropical forest areas. After the closing of the dam, water rises on large surfaces and threaten wildlife by drowning or starvation. Human sensitivity is very important and mainly for this reason, starting in 1964 in Surinam, wildlife rescues were regularly organized during the flooding of forest areas. Surprisingly, all rescues of this type are poorly documented and scientific literature giving results or discussing interest and efficiency is scarce. Contrary to an oil spill rescue, in this case, wildlife veterinarians and biologists have more time to plan the operation, discuss the methodology and interest, give scientific orientations, set up scientific collaborations and document their action carefully. Thus, a larger interest can be given to the operation. This is what we attempted to do in French Guiana.

Context

Between January 1994 and July 1995, 310 km² of a primary rainforest were flooded at the Petit Saut dam site on the Sinnamary river. The dam was built following economical and political decisions made several years ago. The flooding was known to have a great impact on the forest. A rescue was organized by Electricité de France, the French company building the dam in order to translocate threatened animals. Many people blamed the building of the dam and of course, the operation was controversial given it was considered as an alibi for the company.

Our purpose here is not to be polemic. The only other alternative to the rescue was to watch and do nothing. Most of all, we considered this inevitable situation as an opportunity to undertake extensive studies (ecology and behavior of rare or unknown species, wildlife diseases, genetics, etc.) which are impossible to carry out under normal field conditions. In French Guiana, such studies are scarce although necessary for a better knowledge of Guianan wildlife and useful for the development of efficient conservation strategies and tools.

Interest

We were convinced that the rescue could increase our knowledge of an ecosystem which is still largely undisturbed and covers more than 90% of the Guianan territory, and thus be more effective in the conservation of its remaining parts. It was seen as a worthwhile undertaking for the following reasons:
- if many aspects of Guianan wildlife remain unknown, it is mainly due to our limited capture techniques and a poor access to live animals. The operation gave an opportunity to capture animals
impossible to catch in another context;
- funding is scarce for wildlife research and the operation also allowed the creation of a large research program investigating new aspects of local wildlife in French Guiana;
- the possibility to discover new species and to increase our knowledge of local biodiversity;
- translocation already proved to be an efficient tool in terms of conservation; it was an occasion to translocate a large number of species, most of which are not currently endangered, and the experience could be useful for the management of threatened species in the neotropics. Moreover, the adaptation of translocated animals was poorly documented in similar operations in the neotropics and we had no idea of rescue efficiency;
- studies on the ecology of game species would be very useful in the establishment of hunting regulations which do not exist in French Guiana, partly because of our poor knowledge of local wildlife;
- the access to a large number of potential disease reservoirs and vectors would allow biomedical research programs;
- the opportunity to create a serum bank which could be very useful in the case of an emerging disease or to investigate medical explanations to decreasing populations; and
- the interest in a “spectacular” and “mediatized” operation would attract a lot of people, and increase sensitivity to environmental problems of French Guiana would be possible. Moreover, due to the difficulty of filming or photographing Guianan species, there are very few documentaries or photos.

Objectives

The following objectives were defined:
- capture and translocate threatened animals,
- keep the animals the shortest possible time in captivity,
- document the post-release adaptation of several target species,
- build a biological bank and database on Guianan wildlife,
- centralize and share information and material with the international scientific community, and
- provide public awareness on Guianan wildlife conservation to our numerous visitors.

Method

A release area was established very close to the dam in order to avoid or limit potential disease transmission and genetic pollution and also reduce animal stress during transportation. The selected area was already partially disturbed by logging and heavily hunted. The limited accessibility to the area made it possible to control. For the first time in French Guiana a rainforest area was protected by law against hunting and allowed us to carry out our post-release surveillance under normal conditions.

Up to 40 people worked on the operation; the team was composed of veterinarians, biologists, vet students and local workers.

The rescue focused on medium and large size mammals, tortoises and snakes. Various methods were used to capture animals: live traps, nets, hands, etc. The animals were caught either on the ground on isolated islands or in the trees above water by climbing or cutting down the shelter tree. Very few
animals could be darted.

After correct species identification, a full set of data (capture location, sex, weight, body dimensions, results of clinical exam) were recorded and biological samples (blood, parasites, skin biopsies, venom) were collected from a large majority of them. Blood smears and exams for trypanosomiasis or filariasis were performed. Serum was stored at -80°C in our lab. Other samples were sent to various collaborators for identification (parasites), investigation (retroviruses, leishmaniasis, genetics, etc.) or analysis (hematological and biochemical parameters). All the animals were anesthetized; a large number of immobilizations were performed and documented.

Every animal was tattooed and visually identified with colored tags. Some individuals of several target species were radio-collared.

Results

A total of 3278 mammals (47 species), 799 snakes (68 species) and 1386 tortoises were captured and examined at the veterinary hospital. Seventy six animals (23 species) are included but were captured or collected along the roads in various areas of french Guiana in order to increase the species diversity of our biological bank and database and the genetic diversity within taxa (Tables 1-3).

As regards chemical immobilization, medetomidine/ketamine associations proved to be very efficient on a large variety of neotropical mammals.

Fifty individuals of several target species were radio-collared: red howler monkeys (Alouatta seniculus), white-faced sakis (Pithecia pithecia), two-toed sloths (Choloepus didactylus), three-toed sloths (Bradypus tridactylus) and grey brocket deers (Mazama gouazoubira).

The proportion of threatened animals that could be captured and translocated is impossible to assess. The initial density of many species, and the number of animals which escaped or died in the flooded forest remain unknown. We can state that a large majority of radio-tracked mammals survived after translocation. Moreover, social animals like howler monkeys integrated into resident groups and one birth was recorded. The overall observed mortality (animal found dead on the lake, death during capture or captivity and after release) was slightly above 5%.

A large number of studies are still going on (parasitology, virology, ecology, genetics, etc.) and very few results are currently available or are about to be published. We can mention the discovery of a new rodent species (genus Isothrix) or new records of snakes species for French Guiana, showing again that neotropical ecosystems still hold secrets in term of vertebrate species diversity.

Conclusion

The positive results of the translocation, the large amount of original data, the interest of scientists for our samples confirm our initial conviction that this operation was a worthwhile one. A large number of biological samples are now available for the international scientific community and we think that they could be very useful to people working on the health and conservation of neotropical ecosystems.
ACKNOWLEDGMENTS

Table 1. List of captured mammals.

<table>
<thead>
<tr>
<th>Orders</th>
<th>Species</th>
<th>Common name</th>
<th>Orders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodents</td>
<td>Coendou prehensilis</td>
<td>Brazilian porcupine</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Coendou melanurus**</td>
<td>hairy dwarf porcupine</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Myoprocta acouchy</td>
<td>red acouchy</td>
<td>355</td>
</tr>
<tr>
<td></td>
<td>Agouti paca**</td>
<td>paca</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Dasyprocta agouti**</td>
<td>red-rumped agouti</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Hydrochaeris hydrochaeris*</td>
<td>capybara</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sciureus aestivalis</td>
<td>Guianan squirrel</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Sciurillus pusillus</td>
<td>neotropical pigmy squirrel</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Proechimys sp.</td>
<td>spiny rat</td>
<td>463</td>
</tr>
<tr>
<td></td>
<td>Echimys chrysurus</td>
<td>white-faced tree rat</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Echimys armatus</td>
<td>red-nosed tree rat</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Isothrix sinnamariensis</td>
<td>brush-tailed rat</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mesomys hispidus</td>
<td>spiny tree rat</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Oryzomys sp.</td>
<td>rice rat</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Rhipidomys sp.</td>
<td>climbing rat</td>
<td>1</td>
</tr>
<tr>
<td>Opossums</td>
<td>Didelphis marsupialis**</td>
<td>common opossum</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td>Didelphis albiventris</td>
<td>white-eared opossum</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Philander opossum**</td>
<td>gray four-eyed opossum</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>Metachirus nudicaudatus</td>
<td>brown four-eyed opossum</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Caluromys philander</td>
<td>hare-tailed wooly opossum</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Micoureus cinereus</td>
<td>wooly mouse opossum</td>
<td>1</td>
</tr>
<tr>
<td>Xenarthra</td>
<td>Bradypus tridactylus**</td>
<td>three-toed sloth</td>
<td>647</td>
</tr>
<tr>
<td></td>
<td>Choloepus didactylus**</td>
<td>two-toed sloth</td>
<td>321</td>
</tr>
<tr>
<td></td>
<td>Tamandua tetradactyla**</td>
<td>tamandua</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Cyclopes didactylus*</td>
<td>silky anteater</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dasypus novemcinctus</td>
<td>nine-banded armadillo</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>Dasypus kappleri</td>
<td>long-nosed armadillo</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Cabassous unicinctus*</td>
<td>naked-tailed armadillo</td>
<td>2</td>
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</table>
Table 1. List of captured mammals (continued).

<table>
<thead>
<tr>
<th>Orders</th>
<th>Species</th>
<th>Common name</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primates</td>
<td><em>Alouatta seniculus</em></td>
<td>red howler monkey</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td><strong>Saguinus midas</strong></td>
<td>golden-handed tamarin</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td><em>Pithecia pithecia</em></td>
<td>white-faced saki</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td><em>Ateles paniscus</em></td>
<td>black spider monkey</td>
<td>1</td>
</tr>
<tr>
<td>Carnivores</td>
<td><em>Nasua nasua</em></td>
<td>coati</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><strong>Potos flavus</strong></td>
<td>kinkajou</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><strong>Eira barbara</strong></td>
<td>tayra</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td><strong>Galictis vittata</strong></td>
<td>grison</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><strong>Felis pardalis</strong></td>
<td>ocelot</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td><strong>Felis wiedii</strong></td>
<td>margay</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>Felis yagouarundi</em></td>
<td>jaguarundi</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Felis concolor</em></td>
<td>puma</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>Panthera onca</em></td>
<td>jaguar</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><em>Procyon cancrivorus</em></td>
<td>crab-eating raccoon</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><em>Speothos venaticus</em></td>
<td>bush dog</td>
<td>1</td>
</tr>
<tr>
<td>Artiodactyla</td>
<td><em>Mazama gouazoubira</em></td>
<td>gray brocket deer</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td><em>Mazama americana</em></td>
<td>red brocket deer</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><em>Tayassu tajacu</em></td>
<td>collared peccary</td>
<td>10</td>
</tr>
<tr>
<td>Manatees</td>
<td><em>Trichechus manatus</em></td>
<td>West Indian manatee</td>
<td>1</td>
</tr>
</tbody>
</table>

* species captured only outside the dam (mainly road kills) for which partial data or samples were collected

** at least one specimen in this species was collected outside the dam
**Table 2.** List of material sampled on mammals.

<table>
<thead>
<tr>
<th></th>
<th>number of specimens</th>
<th>number of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum / Plasma</td>
<td>1933</td>
<td>33</td>
</tr>
<tr>
<td>Blood smears</td>
<td>2446</td>
<td>36</td>
</tr>
<tr>
<td>DNA samples (skin biopsies)</td>
<td>2106</td>
<td>37</td>
</tr>
<tr>
<td>DNA samples (organ samples)</td>
<td>58</td>
<td>28</td>
</tr>
<tr>
<td>Hemolysates</td>
<td>1131</td>
<td>18</td>
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<tr>
<td>Ectoparasites</td>
<td>1469</td>
<td>35</td>
</tr>
<tr>
<td>Endoparasites</td>
<td>47</td>
<td>23</td>
</tr>
<tr>
<td>Animal specimens</td>
<td>133</td>
<td>31</td>
</tr>
<tr>
<td>Cryopreserved cells</td>
<td>60</td>
<td>23</td>
</tr>
</tbody>
</table>

**Table 3.** List of material sampled on snakes.

<table>
<thead>
<tr>
<th></th>
<th>number of specimens</th>
<th>number of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum / Plasma</td>
<td>204</td>
<td>36</td>
</tr>
<tr>
<td>Blood smears</td>
<td>312</td>
<td>48</td>
</tr>
<tr>
<td>DNA samples (organ samples)</td>
<td>33</td>
<td>24</td>
</tr>
<tr>
<td>Ectoparasites</td>
<td>93</td>
<td>34</td>
</tr>
<tr>
<td>Endoparasites</td>
<td>56</td>
<td>28</td>
</tr>
<tr>
<td>Venom</td>
<td>85</td>
<td>8</td>
</tr>
<tr>
<td>Animal specimens</td>
<td>79</td>
<td>49</td>
</tr>
</tbody>
</table>
ZOOMAT: RESEARCH, ENVIRONMENTAL EDUCATION, CONSERVATION, AND SOMETHING MORE

Carlos Alberto Guichard Romero
Departamento de Zoología, Instituto de Historia Natural de Chiapas, Mexico

Abstract

The Miguel Alvarez del Toro Regional Zoo is located in the extreme Southeast of México, in the city of Tuxtla Gutiérrez; the capital of the state of Chiapas. The zoo belongs to the Instituto de Historia Natural (Natural History Institute), which is a decentralized institution of the government of the State of Chiapas dedicated to the conservation of the natural resources of the region.

The zoo was founded in 1942 and has developed steadily ever since. It is presently located in a natural tropical forest area, and exhibits a total of 220 different species; mammals, birds, reptiles, and invertebrates of the region.

The zoo has an environmental education program that reaches more than 15,000 children per year. There are also numerous research, wildlife rehabilitation, and conservation programs dedicated to regional fauna.

Resumen

El Zoológico Regional Miguel Alvarez del Toro se ubica en el extremo sureste de México, en la ciudad de Tuxtla Gutiérrez, en el estado de Chiapas. Pertenece al Instituto de Historia Natural, que es un organismo descentralizado del gobierno del Estado de Chiapas dedicado a la conservación de los recursos naturales de la región.

El zoológico fue fundado en el año de 1942, y ha mantenido una trayectoria ascendente. Actualmente se encuentra ubicado en un bosque tropical natural y exhibe 220 especies distintas entre mamíferos, aves, reptiles, e invertebrados de la región.

En las instalaciones del zoológico se mantiene un programa de educación ambiental en el que se atienden a más de 15,000 niños al año. Asimismo, se llevan a a cabo diversos programas de investigación, rehabilitación y conservación de la fauna de la región.

Introduction

The basic objective of the following presentation is to speak of the Miguel Alvarez del Toro Regional Zoo, more widely known by its Spanish acronym of ZOOMAT. To better understand its operation, it is necessary to first speak of the structure and function of the Institute to which the zoo belongs.

I have the privilege of working for one of the few institutions in Mexico which has been able to
continuously maintain its work and involvement with the people who sustain it. The Natural History Institute of Chiapas (I.H.N.) has, over its half century of existence, contributed to the knowledge and conservation of that state’s biotic resources. The I.H.N.’s operation is synthesized in each of the following lines of action.

**Protection Of Our Natural Heritage**

Understanding conservation as a dynamic process that allows for the continuity of environmental conditions which permit the sustainable development of a population; the Institute’s primary goal is to conserve representative samples of the different ecosystems of the State of Chiapas, as well as their biological wealth and diversity. It promotes the preservation of strategic sites for the maintenance and development of the community’s economy, which is based mainly on the potential of its natural resources. Thus, proposals for establishing new protected areas are made, and plans for the management and operation of the reserves fall under the I.H.N.’s responsibility. The necessary means are offered so different management activities can take place, such as area patrolling and security, construction of necessary infrastructure, the area’s assigned material and personnel resource management, and the coordination of interinstitutional functions that guarantee continuous operation.

Another important aspect of the INH is the generation and supervision of projects which are meant to tighten the human-nature bond. The objective of these projects is to instill a change in the attitude of the human population towards the reserves themselves, or to the areas which they affect.

Presently, there are permanent protective and operative activities taking place in the Biospheric Reserves of El Triunfo and La Encrucijada, the Faunistic and Forest Protected Area of El Ocote, the Educational Park Laguna Bélgica, and the Environmental Reserve of El Zapotal.

**Environmental Education**

This area’s goal is to establish a connection between the state’s population and its natural resources, creating a culture of proper use, conservation, and appreciation; not only for the resources’ possible uses, but also for their aesthetic and cultural value.

To meet this objective, environmental education programs and materials are developed to modify and direct the population’s behavior. Thus, since 1980 the institute has had programs directed towards children and adolescents involving summer school activities and year round school programs. Over 15000 children are reached a year, and counseling and training courses are offered to preschool and elementary school teachers.

The future programs are meant to extend this effort to rural areas as a means of support of the conservation and development of natural protected areas; to extend the counsel offered to the local government in environmental matters, and to participate in planning the focus and contents of the State’s formal education programs.

**Research**

To be able to effectively preserve natural resources, we must know and support that which we wish
to conserve. Following this precept, the INH has always promoted the disciplined observation and interpretation of nature. It has undertaken many studies, sometimes with modest means, and subsequent limited results. At other times with greater resources, producing studies which have led to more substantial achievements.

Research activities have followed two main lines: creating a catalogue or inventory of the flora and fauna of the different ecosystems of the zone, with a special emphasis on the reserve areas, and the study and preservation of the rare, threatened, or endangered plant and animal species, including socioeconomically important species, in an effort to stimulate their proper use.

Many and diverse projects are being developed. The resource inventories undertaken in the areas protected by the IHN, as well as the study of the biology of the flora and fauna contained in the zoo and in the botanical gardens, and numerous observations of the same species in the wild state, allow us to devise reproductive, re-introduction, and wildlife management strategies which will ensure the conservation of these species.

Studies in the traditional use of natural resources, socioeconomic diagnoses, and land use have been initiated as a result of the growing interaction of the local human population with the reserves. All this has allowed us to increase our knowledge of the biodiversity of Chiapas, enriching the scientific collections, which are a product of internal work and exchange with other institutions, both nationally and abroad. Besides, it is worth mentioning that INH’s continuity of work in the natural sciences has meant a parallel formation of professional human resources, many of which are presently working with similar organizations in the state of Chiapas.

**Information Management**

Computers offer many possibilities for the management of information. The Institute has developed the basic infrastructure needed to handle available information on the flora, the fauna, and the general natural resources of the region. This has been achieved through the structuring of several data banks of native species, and the operation of two systems of geographical information, which constitute a solid foundation for consultation, research analysis, and decision making. The data center’s importance is based on the analysis and handling of the information regarding natural resources, and the value in their preservation and sustainable use.

The INH has deposited this data into the four libraries it operates. These libraries, initially meant for internal use only, have gradually been opened to the public. The bibliographic data bank of the biotic resources of the state of Chiapas now has 1440 registered entries; the herbal data base presently has 15,150 plant and fungal specimens, of which 9,500 have been entered; the flora databank, which is managed by the Information Department, has 4,035 entered specimens, and the fauna databank (limited to terrestrial vertebrates) has 26,317 entries for the state. Two other banks are presently being organized; the protected areas data bank, which contains physical, biological, and cultural data for each protected natural area, and the geographic information system, in which digitized cartographic information of the six areas the INH is managing.

**Communication and Information Dissemination**
The communication and information dissemination program was established to support the INH’s conservation efforts of the state’s natural resources. It tends to the demands for information made by the media, while it promotes the Institute’s relationship with the government, private groups, or the public in general. To achieve this, the INH uses several different means of communication. Scientific papers, audiovisual materials, press releases, radio and television programs and spots are all produced to obtain an effective communication with society. The editorial project is dedicated to the publication of findings pertaining to the flora and fauna of the region, and the results of research in them. Publication is achieved with minimal resources, and occasionally with the support of other institutions. Most published material is distributed for free in rural and urban areas of the state.

Regional Delegation

For several years the INH had contemplated the need to expand its activities to other cities in the state. It wasn’t until 1990 that the Regional Delegation San Cristobal, in Los Altos de Chiapas (the state’s mountain region) was created. The Delegation was established to meet the demands of individuals and institutions, as well as the rural communities, in matters dealing with environmental protection and rational use of biotic resources. Diffusion and environmental education programs were set into place in accordance with the area’s specific needs. A 16 ha lot with a house was donated to the Institute in the vicinity of San Cristobal de las Casas. The property has housed the Regional Delegation’s offices since 1993. This facility houses the largest scientific collection of terrestrial vertebrates in the country’s Southeast. Due to the wealth of information this collection contains, it is considered not only the state’s heritage, but also that of Mexico, and the world.

Exhibition

One of the focuses of work at INH, that has made it unique in Mexico and Latin America, is to merge the scientific labor with a strong effort to immediately communicate and diffuse information to the public by all means available. This practice has remained uninterrupted since the opening of the old museum of natural history and zoological garden, in 1942. This was followed by the opening of what was originally called the Botanical Institute, in 1949. Today, the cultural spaces of the INH; the Parque Zoológico Regional Miguel Alvarez del Toro (ZOOMAT), the Jardín Botánico Regional Faustino Miranda, and the Museo Botánico, have stood out because of their contribution to the knowledge of the native flora and fauna of the region. This work has reached its society, and improved the understanding and worth of Chiapas’ natural wealth.

ZOOMAT exhibits specimens of 205 species, which best represent the diversity of the fauna of Chiapas. The zoo’s design and the husbandry practiced on its animals has won it international recognition. The zoo is visited by more than 600,000 persons per year, which make it one the largest tourist attractions of Tuxtla Gutierrez. The zoo’s sections entertain as well as educate the visitors by offering correct information on the biology and habits of the species exhibited in a simple and entertaining manner.

The Jardín Botánico Dr Faustino Miranda is considered the oldest, as well as one of the most active, botanical gardens in the country. With over 50,000 local, national, and international visitors, it is considered an important cultural and entertainment center. It is also important to higher education,
since it contains not only regional, but also nationally and internationally introduced plants. At present its catalog includes 539 different species, of which over 90% are native.

The state of Chiapas, where the INH is located, is strategically situated in the extreme Southeast of the Mexican Republic. Due to its privileged geographic location, its topographic, climatic, and edaphic diversity, it is a florally and faunistically megadiverse territory. It contains around 8,500 plant species, 696 avian species, 184 species of mammals, and 220 known species of reptiles. The Chiapas Zoo, known also as ZOOMAT, has a long history, since it first opened in 1942. At that time its was located on a small triangular shaped plot of land, with an area of barely 3000 m². It contained a little over 20 species and a small museum of natural history.

By 1949, the zoo changed its location for the first time. It was re-located to the Eastern part of the city, and on to a 5 ha lot. It remained at that location for over 30 yr gathering, in small and rustic enclosures, an important collection of fauna of Chiapas. It gained prestige due to its original design, its exhibits, and its active participation in the state’s conservation. In 1981 the zoo was moved to its present location, to the South of the city. Its name was changed to Zoologico Regional Miguel Alvarez del Toro by presidential decree, in acknowledgment of its only director for the past 54 yr, and his accomplishments as a researcher and conservationist.

The zoo’s main objective is to maintain a permanent exhibit of the most representative species of the fauna of the state of Chiapas, and in so doing, to contribute to the conservation, research, and dissemination of knowledge of the biology of this rich state. The public is to receive a pleasant visit with important biologic and popular information, as well as the unforgettable experience of being in a natural tropical jungle.

ZOOMAT is located in the outskirts of the city of Tuxtla Gutierrez, in a 100 ha reserve of semihumid tropical forest known as El Zapotal. Its altitude is of 630 m above sea level, and the median temperature is 24.7° C. Its facilities occupy a total of 30 ha, and the remaining 70 ha are maintained as a small reserve. The reserve is inhabited by numerous local species that do not represent any danger to the visiting public. It contains: howler monkey (Alouatta palliata), black guaqueque (Dasyprocta mexicana), white tailed deer (Odocoileus virginianus), curacao (Crax rubra), Chachalaca (Ortalis vetula), and cojolita (Penelope purpurascens), among many others. The installations are designed to take maximum advantage of the topography of the area for the enclosure’s location. The animals are kept in more or less spacious areas that imitate, as best is possible, their natural habitat.

The zoo only exhibits regional fauna which, besides simplifying the animals’ adaptation, allows for the exhibit of the regions’ natural resources as a means of promoting their conservation and avoiding their destruction. Its exhibit areas highlight animals of the tropical areas; noticeably harpy eagle, quetzal, tapir, temazate deer, and other species which are not exhibited anywhere else. The zoo also has a nocturnal animal area, a vivarium, and a herpetomuseum.

Thirty-eight species of mammals are exhibited in open areas within fenced or moated limits. Of particular notice are the Central American tapir (Tapirus bairdii), spider monkey (Ateles geofroyii), jaguar (Panthera onca), and temazate deer (Mazama americana). Other mammals are exhibited in enclosed areas, such as the tigrillo (Felis weidii), or the ocelot (Felis pardalis). The nocturnal house
is a closed building, with a naturalistic setting, and a light reversal system. Nocturnal animals, such as the golden opossum (*Caluromys derbianus*), nine-banded armadillo (*Dasypus novencinctus*), skunk, and bats are exhibited.

Chiapas is rich in avian species, so ZOOMAT exhibits over 80 different species in naturalistic settings. Of notice are the quetzal (*Pharomachrus mocinno*), Pavón (*Oreophasis derbianus*), harpy eagle (*Harpia harpyja*), water birds, macaws, and parrots. There is also a large free flight aviary which contains 30 different species of birds.

ZOOMAT has a herpetarium with large terrariums which contain natural plants. The facility exhibits reptiles which have medical relevance, such as royal nauyaca (*Bothrops asper*), water moccasin (*Agkistrodon bileniatus*), tropical rattlesnake (*Crotalus durissus*), coral snake (*Micrurus brownii*), and others. Also exhibited are reptiles about which there are numerous beliefs or species easily recognized by their bright coloration or odd shape; mountain turipache (*Corytophanes hernandezi*), and green vine snake (*Oxybelis fulgidus*). Other areas are dedicated to iguanas, tortoises, crocodiles, and there is a herpetomuseum, where visitors can observe casts made from animals which are difficult to maintain in captivity.

The invertebrates are exhibited in a building designed just for that purpose, the Vivarium. Invertebrates are exhibited in enclosures that simulate each species’ habitat. The collection contains spiders such as velludas, black widows, fishing spiders, and other invertebrates, including millipedes and walkingsticks.

Conservation is the INH’s primary concern, so numerous activities take place to enhance this function. Breeding of captive endangered wild animals, rehabilitation and reintroduction of important injured wildlife, scientific research regarding species of whose biology little is known, monitoring wildlife activity throughout the state, release of rehabilitated wildlife, a permanent education program directed primarily at school children, and the education of individuals living near protected areas are all activities the INH pursues.

Due to the critical state of affairs of tropical natural resources, it is important to foster the active participation of zoos in the diffusion of information, the education, and the conservation of our heritage. The Chiapas Zoo has remained committed to this principle for the past 50 yr. The work being done by the INH throughout its history has been prosperous and productive. Nevertheless, it could be said that the product has been insufficient if compared to what is needed. Chiapas, in the Mexican Southeast, is considered one of the globes’ areas with the richest biodiversity. Unfortunately, it is also one of the regions with the greatest indexes of loss of natural resources, which makes it necessary for us to double our efforts to obtain even greater results.
RECREATING A TROPICAL RAINFOREST INDOORS: AN EXERCISE IN INTENSIVE CARE

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Abstract

The Biodome of Montreal, in operation since June 1992, is an ecological museum whose goal is to stimulate in the visitor a passion for nature and the environment. A second goal is to educate the public about natural ecosystems, their diversity, their fragility, and how humans interact with them. It attempts to do this by putting the visitor in direct contact with nature by way of representations of four new-world ecosystems.

The “ecosystem” theme is represented in the Biodôme’s educational and museological activities, which emphasize interactions between organisms rather than simply identifying the various plants and animals. There are advantages and disadvantages to this strategy. Coming out of the Biodome, the visitor has a better grasp of what an ecosystem is but may sometimes express a certain frustration at not being able to put a name to the different elements of the habitat.

There are many difficulties to recreating a large (40,000 ft²) tropical forest in a northern climate. The cost of construction was minimized by the fact that the building itself already existed but the on-going energy costs are substantial ($3 million/yr). Because of the Biodome’s ecosystem approach, the plants are an integral part of the exhibit rather than simply a backdrop for the animals and considerable effort and money is expended each year to maintain and increase the collections of about five to six hundred plant species. Five production greenhouses are used for this purpose and a considerable budget is allocated to biological control of plant pests both in the exhibit and the greenhouses. The large species diversity is used to recreate several types of forest habitat within the exhibit: primary, initial and advanced secondary, and inundated forests. The large trees (up to forty feet in height) were brought from Florida by truck and are for all practical purposes irreplaceable in the short term.

Because of the ecosystem concept and the importance of the plant collections, the number of animals is lower than in a traditional zoo and the choice of animals is cause for continual compromise. The animal must be true to the habitat represented, be non-predacious because of the free ranging birds, and not destructive of the vegetation. The most numerous group are the birds and much effort is made to enhance their reproduction.

The job of a veterinarian in this kind of institution is complex and he must rely to a great extent on the zookeepers’ competence and their observations of the animals. Some species are difficult to observe and to capture. A good prophylactic program is essential.

The research department plays an important role in linking the Biodome to other institutions. We have several research projects in progress (tropical fruit bat nutrition, plant soil relationships, tropical agroforestry) that are conducted within the Biodome’s facilities and in the field.
In the 4 yr since the Biodome’s opening most of our efforts in the field of conservation have been confined to educational activities. Because of space limitations our conservation projects will concentrate on small animals such as macaws and other birds, small primates (we are presently enrolled in the golden lion tamarin and cotton top SSPs) and reptiles.

**Resumen**

El Biodome de Montreal, en operación desde 1992, es un museo ecológico cuya meta es estimular en el visitante la pasión por la naturaleza y el ambiente. Un segundo objetivo es educar al público acerca de los ecosistemas naturales, su diversidad, su fragilidad, y cómo los humanos interactúan con ellos. Se intenta lograr esto poniendo al visitante en contacto directo con la naturaleza en un camino que contiene representaciones de los 4 ecosistemas del nuevo mundo.

El tema “ecosistema” es representado en las actividades educativas y museológicas de Biodome, las cuales enfatizan la interacción entre organismos en vez de simplemente identificar las diferentes plantas y animales. Hay desventajas y ventajas en el uso de ésta estrategia. Saliendo del Biodome, el visitante tiene una mejor perspectiva de lo que es un ecosistema pero puede expresar a veces cierta frustración al no ser capaz de nombrar a los diferentes elementos del hábitat.

Hay muchas dificultades al tratar de recrear un gran bosque tropical (de 40,000 pies cuadrados) en un clima del norte. El costo de construcción fue minimizado por el hecho de que el edificio en sí ya exista, pero los costos de energía subsecuentes son verdaderamente substaniales (3 millones de dólares al año). Debido al parecido de los ecosistemas del Biodome, las plantas son una parte integral del exhibidor, en lugar de sólo ser simples adornos para los animales y un gran esfuerzo y dinero se invierte cada año para mantener e incrementar la colección de cerca de 600 especies de plantas. Cinco invernaderos de producción son usados para éste propósito y un presupuesto considerable utilizado para el control biológico de plagas ya sea dentro del exhibidor ó en los mismos invernaderos. La gran diversidad de especies es utilizada para recrear varios tipos de bosque dentro del exhibidor: primario, secundario inicial, avanzado, y bosque inundado. Los grandes árboles (de más de 40 pies de altura) fueron traídos desde Florida en camiones y son, por cuestiones prácticas, irremplazables a corto plazo.

Debido al concepto de ecosistema y a la importancia de las colecciones botánicas, el número de animales es menor que en los zoológicos tradicionales, y su elección es causa de un compromiso continuo. Los animales deben estar acordes al hábitat representado, no deben ser predadores debido a que hay pájaros volando en libertad, y no deben destruir la vegetación. El grupo más grande son las aves y muchos esfuerzos se llevan a cabo para mejorar su reproducción.

El trabajo de un veterinario en este tipo de instituciones es complejo y debe realmente tener experiencia de competencia con otros animaleros y en cuanto a sus observaciones de los animales. Algunas especies son difíciles de observar y capturar. Un buen método profiláctico es esencial.

El departamento de investigación juega un papel importante conectando al Biodome con otras instituciones. Tenemos varios proyectos de investigación en proceso (nutrición de un murciélago frugívoro tropical, relación planta-suelo, trabajo agroforeste tropical) que están siendo conducidos
dentro de las instalaciones del Biodome y en campo.

En los cuatro años desde que el Biodome se abrió, la mayoría de nuestros esfuerzos en el campo de la conservación han sido confinados a actividades educativas. Debido a las limitaciones de espacio, nuestro proyectos de conservación se concentran en animales pequeños tales como guacamayos y otras aves, pequeños primates (actualmente estamos involucrados con el tamarín dorado y el cotton top SSP) y reptiles.
THE ZOO CONSERVATION OUTREACH GROUP: LINKING ZOO-BASED CONSERVATION EFFORTS IN THE AMERICAS

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Abstract

A global approach to species and habitat conservation demands active participation of the professional zoo community in initiatives that extend beyond zoo gates and country borders. In recognition of this fact, the World Zoo Organization’s (WZO) “Zoo Future 2005” calls upon zoos and zoo professionals throughout the world to become part of a “conservation continuum” in which their skills and resources contribute to the long-term viability of threatened ecosystems and wildlife populations. Specifically, the document urges zoos and zoo professionals from developed countries to cultivate working relationships with zoos and zoo colleagues in regions where wildlife is most threatened by human encroachment, habitat loss, and fragmentation.

Since 1988, the Zoo Conservation Outreach Group (ZCOG) has facilitated cooperative zoo-based conservation initiatives in Mesoamerica. Mesoamerica is one of many biologically diverse regions currently threatened with massive species and habitat loss. Mesoamerican zoos, with annual visitation rates of well over 25 million people, possess a tremendous potential to interest, educate, and motivate the public towards positive conservation action in this region. Yet in many cases, these zoological institutions lack the resources necessary to maintain basic animal health care and facility operation. Mesoamerican zoo staffs also possess limited opportunities to obtain appropriate professional training. Consequently, animal collections often lack adequate living conditions and proper medical attention, and zoo staffs are often undertrained and unprepared for the challenge of zoo practice. Opportunities to develop and implement comprehensive education and conservation programming are therefore, limited. As a result, the potential of Mesoamerican zoos to impact regional conservation efforts remains untapped.

ZCOG is a consortium of North American zoos, zoo professionals, and corporate sponsors dedicated to assisting Mesoamerican zoos in their quest to become regional centers of environmental education and conservation training. ZCOG’s primary goal is to empower Mesoamerican zoos in their efforts to provide high quality animal care, effective environmental education programs, and scientific research that contributes to regional and global conservation efforts. We have sought to achieve this goal by pooling technical and financial resources of North American zoos and zoo professionals, and delivering this assistance to zoo colleagues in Mexico and Central America. In accordance with these ideals, ZCOG sponsors regional training workshops in zoo management, animal husbandry, conservation education, and veterinary care; organizes equipment and material donation drives; and facilitates Sister Zoo relationships to provide long-term support.

All of ZCOG’s projects and programs are cooperative zoo-based efforts. ZCOG has been one of the primary catalysts in creating working relationships and collaborative zoo-based conservation programs between North American zoos, Meso American zoos, and regional organizations such as the Asociación Mesoamericana de Zoológicos (AMAZOO), the Asociación de Zoológicos,
Criaderos, y Acuarios de la República Mexicana (AZCARM), and the Asociación Latinoamericana de Parques Zoológicos y Acuarios (ALPZA). Acting as a central clearinghouse of ideas and information, ZCOG promotes and coordinates the development of active partnerships among zoological institutions, zoo-related organizations, and zoo professionals from the two regions. As such, ZCOG plays a critical role in linking collaborative zoo-based conservation initiatives throughout the Americas.

By establishing these linkages, ZCOG also presents an opportunity for North American zoo professionals to actively support the transfer of training, technology, and information to Mesoamerican zoo professionals. Veterinarians from United States zoos play a major role in these cooperative, zoo-based conservation efforts. Zoo veterinarians assist in developing training practica and workshops, organize equipment and material donation drives, oversee transfers of appropriate information and technology, and act as consultants/advisors to ZCOG-sponsored ex situ animal management programs. Veterinarians who take part in staff exchanges or the Working Vacation program also provide important training in the areas of preventative medicine, treatments and procedures, nutrition, and animal husbandry.

ZCOG recognizes that broader conservation goals will only gain acceptance and credibility if zoo animals in regions like Mesoamerica are perceived to be well-cared for and thriving. We, therefore, remain committed to helping zoos in Mesoamerica raise their standards of animal keeping, husbandry, and veterinary medicine. Only through thoughtful coordination of local and regional conservation programs, however, can these important goals be achieved. ZCOG, therefore, remains committed to promoting cooperation among zoos throughout the Americas, advancing environmental education and research, and enhancing the quality of both ex situ and in situ conservation programs. By linking zoo research and educational outreach with in situ projects, ZCOG seeks to ensure the future success of contemporary global conservation efforts.

Resumen

Un acercamiento global a la conservación de las especies y su hábitat exige una participación activa de la comunidad profesional del zoológico en iniciativas que se extiendan más allá de las puertas del mismo zoológico y de las fronteras del país. Reconociendo este hecho, el “Zoológico del Futuro 2005” (“Zoo Future 2005”) de la Organización Mundial de Zoológicos (WZO por sus siglas en inglés) hace un llamado a los zoológicos y a los profesionistas de zoológicos alrededor del mundo a venir a formar parte de una “conservación continua” en la cual sus habilidades y recursos contribuyan en al viabilidad a largo plazo de los ecosistemas amenazados y en las poblaciones silvestres. Específicamente, el documento insta a los zoológicos y profesionistas de zoológicos de países desarrollados a cultivar relaciones de trabajo con zoológicos y colegas en regiones donde la vida silvestre está en mayor amenaza por la invasión humana, la pérdida de su hábitat, y la fragmentación.

Desde 1988 el Zoo Conservation Outreach Group (ZCOG), ha facilitado la cooperación basada en zoológicos con iniciativas en conservación en Mesoamérica. Mesoamérica es una de muchas regiones biológicamente diferentes altamente diferenciadas amenazadas con pérdidas masivas de especies y hábitats. Los zoológicos mesoamericanos con un índice anual de visitantes de más de 25
millones de personas, poseen un tremendo potencial de interés, educación y motivación al público a favor de acciones de conservación positivas en la región. Sin embargo en muchos casos, éstas instituciones zoológicas carecen de recursos necesarios para sostener las necesidades básicas en cuanto a salud de los animales y de instalaciones operativas. El personal de los zoológicos mesoamericanos también tienen oportunidades limitadas para obtener un entrenamiento profesional adecuado. Como consecuencia, la colección de animales frecuentemente carecen de condiciones de vida adecuados y de atención médica apropiada, mientras que el personal del zoológico esta frecuentemente bajo entrenamiento e impreparado para el reto que representa la práctica en el zoológico. Las oportunidades de desarrollo y la implementación de educación, de comprensión y programas de conservación son entonces limitados. Como resultado, el potencial de los zoológicos de mesoamérica para impactar a favor de la conservación regional permanece sin explorar.

El ZCOG es un consorcio de los zoológicos de Norteamérica, profesionistas de zoológicos, y corporaciones patrocinadoras dedicadas a ayudar a zoológicos mesoamericanos en la búsqueda por llegar a ser verdaderos centros de entrenamiento para la educación ambiental. La meta principal del ZCOG es autorizar a los zoológicos mesoamericanos en sus esfuerzos para proveer un cuidado adecuado a los animales de alta calidad, unos programas de educación ambiental efectivos, e investigaciones científicas que contribuyan a los esfuerzos de conservación globales y regionales. Nosotros hemos buscado alcanzar esta meta uniendo tanto recursos técnicos como financieros de los zoológicos de Norte América y de sus profesionistas, y otorgando esta ayuda a colegas en otros zoológicos de México y Centro América. De acuerdo a estos ideales, los patrocinadores de la ZCOG encargados de los talleres de entrenamiento regional en el manejo de zoológicos, la crianza de animales, la educación en el área de conservación, el cuidado veterinario, organizar, equipar, donar, y facilitar las relaciones de hermandad entre zoológicos proveyendo de un sostenimiento a largo plazo.

Todos los proyectos y programas de la ZCOG son esfuerzos de cooperación entre zoológicos. La ZCOG ha sido uno de los principales catalizadores creando relaciones de trabajo y de colaboración en los programas de conservación entre zoológicos de Norteamérica, Mesoamérica y otras organizaciones regionales tales como la Asociación Mesoamericana de Zoológicos (AMAZOO), la Asociación de Criaderos y Acuarios de la República Mexicana (AZCARM), y la Asociación Latinoamericana de Parques Zoológicos y Acuarios (ALPZA). Haciendo el papel de centro de conjunción de ideas e información, la ZCOG promueve y coordina el desarrollo del compañerismo activo entre las instituciones zoológicas, organizaciones relacionadas con zoológicos y profesionistas de zoológicos de las dos regiones. ZCOG juega un papel crítico entrelazando iniciativas de colaboración en pro de la conservación a través de toda América.

Estableciendo uniones, ZCOG también presenta la oportunidad a los profesionistas de zoológicos de Norteamérica para apoyar activamente con entrenamiento, tecnología e información a los profesionistas de zoológicos de Mesoamérica. Los veterinarios de los Estados Unidos juegan un papel importante dentro de estos esfuerzos de cooperación en cuanto a la conservación. Los veterinarios de zoológicos ayudan a desarrollar entrenamiento práctico y talleres de trabajo, organizan donaciones de equipo y material, vigilan la transferencia apropiada de información y tecnología, y actúan como consultores/consejeros en los programas de manejo de animales ex situ. Los veterinarios que toman parte en el equipo de trabajo de intercambio o en el programa de trabajo en vacaciones también brindan un importante entrenamiento en áreas de medicina preventiva,
tratamientos y procedimientos, nutrición y crianza de animales.

La ZCOG reconoce que las metas más amplias en cuanto a conservación sólo ganarán aceptación y credibilidad si los animales de zoológicos en regiones como Mesoamérica claramente se ven con mejoras en su cuidado y aprovechamiento. Nosotros, por lo tanto continuamos con nuestro objetivo de ayudar a los zoológicos de mesoamérica e incrementar sus estándares de cuidado y crianza de animales y medicina veterinaria. Solo a través de la generosa coordinación en los programas de conservación regional y local, éstas importantes metas pueden ser alcanzadas. La ZCOG sin embargo, permanece en su cometido de ser un promotor de cooperación entre los zoológicos a lo largo de toda América, progresando en investigación ambiental y educativa y enriqueciendo la calidad ya sea ex situ o in situ de los programas de conservación. Uniendo las investigaciones de zoológicos y el trabajo social educativo con proyectos in situ, la ZCOG busca asegurar el futuro de los esfuerzos de conservaciones actuales a nivel mundial.
THE MESO-AMERICAN FAUNA INTEREST GROUP: REGIONAL IN SITU PROJECT AND CONSERVATION TRAINING

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Abstract

Fauna Interest Groups (FIGs) were established by AZA to supplement its taxon-oriented conservation programs, e.g., Species Survival Plans (SSP) and Taxon Advisory Groups (TAG) with regional/ecosystem programs, and to support the AZA mission to develop zoo-based in situ conservation programs. In keeping with AZA guidelines, the Meso-American Fauna Interest Group, encompassing the region from Mexico through Panama, has four major objectives: 1) create and maintain a database of AZA institutions active in conservation projects in the Meso-American region, 2) promote in situ conservation projects in the region, 3) facilitate collaboration between AZA institutions and the zoos and conservation agencies in the region, and 4) incorporate conservation education into all FIG-related programs and projects. The last point includes not only conservation education for the public, in support of regional projects, but training for Meso-American zoo staff and other wildlife biologists.

Although there is some overlap in mission with the Zoo Conservation Outreach Group (ZCOG), especially in the area of conservation education, ZCOG and the FIG work cooperatively and complementarily. In general, ZCOG supports zoos in the region with equipment, supplies, linkages with sister zoos in the U.S., and personnel exchanges, whereas the FIG primarily focuses on in situ programs and training. However, the FIG intends that in situ projects be coordinated with the regional zoo(s), to enhance their credibility as zoological and conservation institutions and to facilitate coordination and collaboration between the zoos and the universities and wildlife agencies in the area.

Currently, the FIG is accepting proposals for in situ projects, concentrating especially on projects listed for species in the region that are part of SSP and TAG Five-Year Plans. Support for projects will be sought through zoo partnerships and appropriate funding agencies. FIG member Linda Wachsberg, of the Detroit Zoo, has established a computer database, with information on projects, agencies and personnel in the region, that is being used as a model for other FIGs.

The FIG continues to work with AMAZOO and AZCARM, the zoo associations for Central America and Mexico. The 1995 workshop, held in San José, Costa Rica, focused on the role of zoos in international conservation and resulted in a resolution to establish the margay as a model for creation of a studbook and regional management plan. The theme of the 1996 workshop, to be held in Cancun, Mexico, is Conservation Education Masterplanning. These workshops have been the result of a collaboration between Yolanda Matamoros (current AMAZOO president and director of the Simón Bolívar Zoo in Costa Rica), the St. Louis Zoo, and Wildlife Preservation Trust International.

Resumen
Los Grupos de Interés en Fauna (FIGS por sus siglas en Ingles) fueron establecidos por el AZA para suplementar sus programas de conservación orientados en Taxones, por ejemplo: Los planes de sobrevivencia de especies (SSP) y grupos de consejo sobre el Taxón con programas de ecosistemas regionales, y para apoyar la misión de la AZA para desarrollar programas de conservación in situ usando como base los zoológicos. De acuerdo con las guías de AZA, el Grupo de Interés en Fauna Mesoamericana, que abarca la región de México hasta Panamá, tiene cuatro objetivos principales: 1) crear y mantener una base de datos de instituciones que pertenecen al AZA y que son activas en proyectos en la región Mesoamericana, 2) promover proyectos de conservación in situ en la región, 3) facilitar colaboración entre instituciones de la AZA, los zoológicos y agencias de conservación en la región, y 4) incorporar educación sobre la conservación en todos los programas y proyectos relacionados con el FIG. El último punto incluye no sólo educación en conservación para el público en apoyo de proyectos regionales, sino entrenamiento para personal de zoológicos mesoamericanos y otros biólogos en vida silvestre.

Aunque hay una cierta duplicidad en misiones con el Grupo de Conservación en Zoológicos (ZCOG), especialmente en el área de educación en la conservación, ZCOG y FIG trabajan en cooperación y complemento uno con el otro. En general ZCOG apoya a zoológicos en la región con equipo, provisiones, conexiones con zoológicos hermanos en los Estados Unidos, y en intercambios de personal, mientras FIG se enfoca primordialmente en programas in situ y entrenamiento. Sin embargo, FIG tiene el deseo de que los proyectos in situ sean coordinados con los zoológicos regionales, para confirmar su credibilidad como instituciones zoológicas y de conservación y para facilitar la coordinación y colaboración entre los zoológicos y universidades así como agencias de fauna silvestre en el área.

En la actualidad, FIG está aceptando propuestas para proyectos in situ, dando atención especial a proyectos que están en la lista para especies dentro de la región que son parte de los planes a cinco años de SSP y la TAG. Se buscará apoyo para proyectos a través de sociedades y agencias que otorguen los fondos apropiados. Un miembro de la FIG, Linda Wachsberg, del Zoológico de Detroit, ha establecido una base de datos de computadora con información sobre proyectos, agencias y personal en la región, que se está usando como modelo para otros FIGs.

FIG continúa trabajando con AMAZOO y AZCARM, las asociaciones de zoológicos para América Central y México. El taller de 1995 que se llevó a cabo en San José, Costa Rica, fue enfocado sobre el papel de los zoológicos en la conservación internacional y dio como resultado establecer el tigrillo (margay) como modelo de un “studbook” y para un plan de manejo regional. El tema del taller de 1996 que se llevó a cabo en Cancún, México fue el de Establecimiento de Planes Maestros de Educación para la Conservación. Estos talleres han sido el resultado de una colaboración entre Yolanda Matamoros (actual presidente de AMAZOO y directora del Zoológico Simón Bolívar en Costa Rica), el Zoológico de Saint Louis, y el Fideicomiso Internacional para la Preservación de la Fauna Silvestre.
TROPICAL ECOSYSTEMS AND THE CONSERVATION OF THE LIVING DEAD

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Abstract

Throughout the Latin American and Caribbean region, human activities are threatening the integrity and existence of biodiversity. From the temperate forests and Patagonian grasslands of the southern cone through the cerrado savannas and Atlantic forests of Brazil and high-altitude formations of the Andes to the Central American dry forests and the Caribbean mangroves, biodiversity at all levels, and in almost all places is suffering.

Awareness of the need to conserve biodiversity has spurred efforts to create systems of parks and protected areas. Major advances have been made, though not all types of ecosystems are yet represented in these protected area systems. Concomitant with these efforts has been an increasing interest in sustainable development which has resulted in strong advocacy for the concept of conservation through use and resultant increase in the area declared as being protected.

The integration of conservation and use has been the result of a lack of appreciation for the ecological impacts of human use. This, combined with the dynamic nature of biological systems, is threatening the integrity of the areas set aside to conserve biodiversity. Many species and systems are being conserved in sizes and configurations that do not hold promise for long-term conservation -- they are the “living dead.” Opportunities still exist to change this situation, but must be taken in the very near future.

Resumen

A lo largo de toda la región Latinoamericana y del Caribe, las actividades humanas están amenazando la integridad y la existencia de la biodiversidad. Desde los bosques templados y pastizales de la Patagonia en el cono sur, hasta las sabanas y bosques a la orilla del Atlántico de Brasil y las formaciones de gran altitud en los Andes hasta los bosques secos de Centro América, y los manglares del Caribe; la biodiversidad a todos los niveles y en casi todos los lugares, está amenazada.

Conscientes de la necesidad de conservar la biodiversidad hemos alentado los esfuerzos por crear sistemas de parques y áreas protegidas. Se han realizado grandes esfuerzos, aunque no todos los tipos de ecosistemas están representados en este sistema de áreas protegidas. Aunado a estos esfuerzos ha habido un incremento de intereses en el desarrollo del proyecto, lo cual ha resultado en un fuerte apoyo al concepto de conservación a través de un uso y del mejor resultado en el área declarada como protegida.

La integración de conservación y su uso, ha sido el resultado de la falta de apreciación del impacto ecológico por el hombre. Este hecho, combinado con la dinámica natural de los sistemas biológicos,
está amenazando la integridad de las áreas apartadas para la conservación de la biodiversidad. Muchas especies y sistemas están siendo conservados en tamaños y configuraciones que no mantienen la visión de conservación a largo plazo, estos son los llamados “muertos vivientes”. Las oportunidades se siguen dando para cambiar esta situación, pero deben ser tomadas en un futuro inmediato.
IMMOBILIZATION OF ELD’S DEER (Cervus eldi): MEDETOMIDINE-KETAMINE VERSUS CARFENTANIL

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Abstract

This study was undertaken to evaluate and compare carfentanil vs. medetomidine and ketamine for immobilization of Eld’s deer (Cervus eldi). Nineteen adult deer were used in the study, eight females weighing 72.9±11.5 (56-86) kg, and 11 males weighing 118±8.5 (104-133) kg. Five deer received carfentanil (Wildnil, Wildlife Pharmaceuticals, Incorporated, Fort Collins, CO 80524, USA) and 14 received medetomidine-ketamine (Wildlife Pharmaceuticals, Incorporated), given by projectile syringe (Telinject USA, Incorporated, Saugus, CA 91350, USA). The dose of carfentanil was 1.0 or 1.2 mg per animal and was based on previous experience with members of the herd. The initial intended doses of medetomidine and ketamine were 50 µg/kg and 1.6 mg/kg, respectively, and were calculated using estimated weights.

Carfentanil at 14-16 µg/kg (three females) and 9.2 and 10.6 µg/kg (two males) produced rapid immobilization (7-13 min). Muscle relaxation and analgesia were poor. Ketamine at 1.3 and 1.5 mg/kg i.v. given to two deer improved relaxation, but bradypnea or apnea and cyanosis occurred and respiratory support was necessary. Naltrexone at 100 mg/mg carfentanil given i.m. after 17-20 min of immobilization, produced rapid recovery to a standing posture (4-5 min in deer receiving carfentanil alone and 8-15 min in deer given carfentanil and ketamine). Residual ataxia was present in those deer receiving ketamine, but renarcotization was not observed. During immobilization, the deer were moderately to severely hypercarbic, hypoxemic, and hypertensive. Lactic acidosis was present and one deer exhibited paroxysmal tachycardia.

Medetomidine in doses of 50-66 µg/kg with ketamine at 1.5-2.2 mg/kg did not produce rapid or complete immobilization in four female deer, and additional ketamine (1.1-2.8 mg/kg i.v.) was required. In one additional female and nine males, 84.6-100 µg/kg medetomidine with 2.2-3.2 mg/kg ketamine produced immobilization in 6-22 min. Muscle relaxation and analgesia were good. After 19-36 min of immobilization, atipamezole, 4 mg/mg medetomidine was given either ¼ i.v. and ¾ s.c., or ½ i.v. and ½ s.c. After atipamezole, deer stood in 3-16 min. Deer receiving only ¼ the atipamezole dose i.v. exhibited more marked residual ataxia than those receiving ½ the atipamezole i.v. Mild hypercarbia and significant hypoxemia were present in some deer during immobilization with medetomidine and ketamine, but heart rhythm, arterial blood pressure, and plasma lactate levels were normal.

It is concluded that carfentanil alone does not provide satisfactory immobilization, muscle relaxation, or analgesia in adult Eld’s deer. Ketamine i.v. improved relaxation but produced life threatening respiratory depression. Despite significant physiologic alterations, recovery after
naltrexone was rapid, and no permanent injury was apparent. Medetomidine-ketamine, in sufficient dosage, provided good immobilization, muscle relaxation, and analgesia with maintenance of normal acid base status and only mild respiratory depression. Ataxia after recovery was attributed to insufficient i.v. atipamezole and residual effects of ketamine.

Most immobilized deer were hypoxemic (PaO₂ <60 mm Hg), even though they were maintained in a sternal posture. These results suggest that all immobilized deer should be given supplemental O₂ during immobilization, even when respiration appears clinically to be adequate.

Resumen

Este estudio se emprendió para evaluar y comparar el Carfentanil contra Medetomídina-Ketamina para la inmovilización de los venados Eld. Diecinueve venados adultos fueron utilizados en el estudio, 8 hembras pesando 72.9 ± 11.5 (56-86) kg, y 11 machos pesando 118 ± 8.5 (104-133) kg. Cinco venados recibieron Carfentanil (Wildnil, Wildlife Pharmaceuticals, Incorporate, Fort Collins, CO 80524, USA), y catorce recibieron Medetomídina-Ketamina (Wildlife Pharmaceuticals, Incorporated), suministrados con un proyectil inyectable (Telinject USA, Incorporated, Saugus, CA, 91350, USA). La dosis de Carfentanil fue 1.0 ≤ 1.2 mg por animal y se basó en la experiencia previa con los miembros de la manada. La dosis inicial propuesta de Medetomídina y Ketamina fue 50 µg/kg y 1.6 mg/kg respectivamente, y calculada usando pesos estimados.

El Carfentanil a dosis de 14-16 µg/kg (tres hembras) y 9.2 y 10.6 µg/kg (2 machos) produjo una inmovilización rápida (7-13 minutos). La relajación muscular y la anestesia fue pobre. La Ketamina a 1.3 y 1.5 mg/kg i.v. dio a dos venados mejor relajación, pero se observaron bradipnea, disnea y cianosis, y fue necesaria la respiración asistida. Naltrexona a una dosis de 100 mg/mg de Carfentanil administrada i.m. después de 17 a 20 minutos de inmovilización, produjo una rápida recuperación (4 a 5 minutos en el venado que recibió sólo Carfentanil y de 8 a 15 minutos en el que se le dio Carfentanil y Ketamina). La ataxia residual se presentó en los venados que recibieron Ketamina, pero la renarcotización no fue observada. Durante la inmovilización los venados presentaron hiperacapnia moderada a severa, hipoxia, e hipertensión. Se presentó acidosis láctica y uno de los venados mostró taquicardia paroxística.

La Medetomíndia en dosis de 50 a 66 µg/kg con Ketamina de 1.5 a 2.2 mg/kg no produjo una inmovilización rápida o completa en cuatro hembras, por lo que fue necesario adicionar Ketamina (1.1 a 2.8 mg/kg i.v.). En una hembra adicional y en nueve machos, 84.6 a 100 µg/kg de Medetomíndia con 2.2 a 3.2 mg/kg de Ketamina produjeron inmovilización en 6 a 22 minutos. La relajación muscular y la analgesia fueron buenas. Después de 19 a 36 minutos de inmovilización, se revitló la sedación con atipamezole, a razón de 4 mg/mg de medetomíndia dado ¼ i.v. y ¾ s.c., o bien ½ i.v. y ½ s.c. Después del atipamazeo, el venado se levantó en 3 a 16 minutos. El venado que recibió solo ¼ de la dosis de atipamezole i.v exhibió una ataxia residual más marcada que el que recibió ½ dosis de atipamezole i.v. Una leve hiperacapnia y una hipoxemia marcada se presentaron en algunos venados durante la inmovilización con medetomíndia y ketamina, pero el ritmo cardiaco y la presión sanguínea, así como los niveles de lactato en plasma fueron normales.

Este trabajo tiene como conclusión que el Carfentanil por sí sólo no produce una inmovilización, relajación muscular o analgesia satisfactoria en venados Eld adultos. La ketamina i.v. mejoró la
relajación, pero produjo una depresión respiratoria que puso en riesgo la vida del animal. A pesar de tan significantes alteraciones fisiológicas, la recuperación mediante la naltrexona fue rápida, y no produjo daño aparente. Medetomidina-ketamina en dosis suficientes produjeron buena inmovilización, relajación muscular, y analgesia, manteniendo normal el equilibrio ácido básico y produciendo solo una ligera depresión respiratoria. La ataxia en la recuperación fue atribuida a una dosis insuficiente de atipamezole i.v. y a los efectos residuales de la Ketamina.

La mayoría de los venados inmovilizados mostraron hipoxia (PaO₂<60 mm Hg) a pesar de mantenerse en posición esternal. Estos resultados indican que toda inmovilización de venados deberá ser suplementada con O₂ durante la inmovilización; aun cuando la respiración sea aparentemente adecuada.

Introduction

This study was undertaken to evaluate and compare carfentanil vs. medetomidine and ketamine for immobilization of Eld’s deer. The immobilizations were carried out at the Wildlife Conservation Park-Bronx Zoo in mid-November 1995, to accommodate physical examination, identification procedures, hoof care, and vaccinations.

Methods

Nineteen adult deer were used in the study, eight females weighing 72.9±11.5 (56-86) kg, and 11 males weighing 118±8.5 (104-133) kg. Five deer received carfentanil (Wildnil, Wildlife Pharmaceuticals, Incorporated, Fort Collins, CO 80524, USA) and 14 received medetomidine-ketamine (Wildlife Pharmaceuticals, Incorporated), given by projectile syringe (Telinject USA, Incorporated, Saugus, CA 91350, USA). The dose of carfentanil was 1.0 or 1.2 mg per animal and was based on previous experience with members of the herd. The initial intended doses of medetomidine and ketamine were 50 µg/kg and 1.6 mg/kg, respectively, and were calculated using estimated weights.

While immobilized, the deer were restrained in a sternal posture. Ketamine was given i.v. if necessary to maintain immobilization or muscle relaxation. At the conclusion of the procedures, the deer were given the appropriate antagonist, and hand held until they were able to maintain a sternal posture. They were observed until they could stand and walk without difficulty and then checked periodically for signs of resedation.

Samples for plasma lactate determination (YSI Sport 1500 Lactate Analyzer, YSI Incorporated, Yellow Springs, OH 45387, USA) were drawn immediately after immobilization occurred, and just before administration of antagonists. Samples for blood gas and pH measurement (StatPal II, Sen Dx Medical, Incorporated, Carlsbad, CA 92009, USA) were drawn from 24-ga x 1 in catheters (Surfl o, Terumo Medical Corporation, Elkton, MD 21921, USA) placed in an auricular artery.

Auricular arterial blood pressure and electrocardiogram were continuously monitored during immobilization (Propaq 106, Protocol Systems, Incorporated, Beaverton, OR 97005, USA).

Data are reported as mean ± SD.
Results

Carfentanil

The dose of carfentanil, based on actual body weight, was 14-16 µg/kg in the three females, and 9.2 and 10.6 µg/kg in the two males. Immobilization occurred in 10.0±2.8 (7-13) min, but muscle relaxation and analgesia were poor. Two deer were given ketamine, 1.3 and 1.5 mg/kg i.v., to improve immobilization. One became bradypneic and cyanotic after ketamine and was given doxapram 80 mg i.v. (Dopram, Fort Dodge Laboratories, Incorporated, Fort Dodge, IA 50501, USA) and intranasal oxygen. In the other deer, apnea occurred after ketamine administration, and O₂ was given via endotracheal tube and demand valve (Hudson Respiratory Care, Incorporated, Temecula, CA 92589, USA). Naltrexone, 100 mg/mg carfentanil, was given 3 min after immobilization had occurred. In the other four deer, naltrexone was given i.m. 17-20 min after immobilization. Deer not receiving ketamine stood 4-5 min after naltrexone administration, and those receiving ketamine stood in 8 and 15 min. Residual ataxia was present in those deer which had received ketamine, but no renarcotization was noted. Early and late plasma lactate levels were 7.0±4.4 and 10.9±4.9 mM/L in deer receiving carfentanil. The PaCO₂, pH, and arterial base excess in four deer receiving carfentanil were 69.7±19.4 (52-97) mm Hg, 7.17±0.10 (7.03-7.24), and -5.6 ± 4.5 (-0.7 to -9.8) mEq/L, respectively. PaO₂ in the three deer breathing ambient air was 39.2-48.1 mm Hg. Average mean arterial blood pressure during the immobilization period was 129-212 mm Hg, heart rate was 57-176 beats per minute, and respiratory rate was 7-36 in the three deer not requiring respiratory support. Irregular breathing patterns were noted, and periods of paroxysmal tachycardia occurred in one deer (heart rate 176) but could not be further characterized due to motion artifact on the electrocardiogram (ECG) tracing. Rectal temperature of three deer receiving carfentanil was 39.8±1°C.

Medetomidine-Ketamine

The first five deer receiving medetomidine-ketamine were females. Of those, four received 56.2±6.96 (50-66) µg/kg medetomidine and 1.76 ±0.3 (1.5-2.2) mg/kg ketamine, based on actual body weight. The induction period was long, immobilization was incomplete, and all four deer received additional ketamine (1.1-2.8 mg/kg i.v.). The 5th female received 98.6 µg/kg medetomidine and 2.5 mg/kg ketamine was completely immobilized and required no additional ketamine. Time from injection of medetomidine-ketamine to immobilization was 24.4±6.9 (17-35) min in females.

The nine males received 87.7±6.7 (84.6-100) µg/kg medetomidine and 2.7±0.3 (2.2-3.2) mg/kg ketamine. Immobilization occurred in 10.7±6.4 (6-22) min, and only one male required additional ketamine (1.2 mg/kg i.v.). In that animal, the initial drug injection had been incomplete. Muscle relaxation and analgesia during medetomidine-ketamine immobilization were good.

Atipamezole was given after 27.6±6.5 (19-36) min of immobilization at a rate of 4 mg/mg medetomidine, either ¼ of the dose i.v. and ¾ s.c. or ½ i.v. and ½ s.c. After atipamezole administration, two animals stood out as having very slow recoveries (22-24 min to stand). This was attributed to incomplete i.v. injection of the intended atipamezole. Times to recovery of standing posture in the other 12 deer were 7.6±4.0 (3-16) min in six deer given ¼ the total dose of...
atipamezole i.v. and 7.7±3.7 (4-12) min in six deer given ½ the atipamezole i.v. The animals receiving ¼ atipamezole i.v. and ¾ s.c. were noted to be markedly atactic after standing, while those receiving ½ the atipamezole i.v. and ½ s.c. exhibited mild to moderate ataxia.

Early and late plasma lactate levels were 2.1±1.7 and 1.9±1.2 mM/L, respectively. The PaCO₂, pH, arterial base excess, and PaO₂ in 13 deer receiving medetomidine-ketamine were 50.9±4.5 (46-58) mm Hg, 7.39±0.03 (7.33-7.44), + 5.5±1.7 (+ 2.8 to + 7.7) mEq/L, and 58.5±15.5 (33.5-94.4) mm Hg, respectively. Average mean arterial pressure in 12 deer was 118±13.7 (98-142) mm Hg. Heart rates ranged from 40-71 beats per minute, and ECG indicated regular sinus rhythm or sinus arrhythmia. Respiratory rates were 7-14 when regular. Periods of irregular breathing were noted in some deer, with respiratory rates of 3-28 respirations per minute. Rectal temperature of 14 deer receiving medetomidine-ketamine was 38.7±0.9 C.

Discussion and Conclusions

Carfentanil alone, in the dosages used, did not provide satisfactory immobilization, muscle relaxation or analgesia in adult Eld’s deer. Ketamine, given i.v., improved immobilization and muscle relaxation but was followed by life threatening respiratory depression. Animals receiving carfentanil also developed significant lactic acidosis, hypertension, and arrhythmias. Despite these complications, recovery after naltrexone was rapid, and no permanent injury was apparent.

Medetomidine-ketamine produced good immobilization in adult male deer in doses of 84-100 µg/kg medetomidine and 2.2-3.2 mg/kg ketamine. The required dose of medetomidine-ketamine for immobilization of females was not determined. In four females, doses of 50-66 µg/kg medetomidine and 1.5-2.2 mg/kg ketamine were insufficient, while one female receiving 98.6 µg/kg and 2.5 mg/kg was completely immobilized. Medetomidine-ketamine provided good muscle relaxation and analgesia, and the deer maintained normal acid base balance with mild respiratory depression. No significant arrhythmias, hypertension, or hypotension were noted. The deer remained somewhat sedated and atactic after atipamezole administration. Ataxia was attributed to insufficient i.v. atipamezole and residual effects of ketamine.

Most of the immobilized deer in this study were hypoxemic (PaO₂ <60 mm Hg), even though they were maintained in a sternal posture. These results suggest that all immobilized deer should be given supplemental O₂ during immobilization, even when respiration appears clinically to be adequate.
COMPARISON OF TWO METHODS OF CHEMICAL IMMOBILIZATION IN FALLOW DEER (*Cervus dama*): MEDETOMIDINE-TILETAMINE-ZOLAZEPAM VERSUS XYLAZINE-TILETAMINE-ZOLAZEPAM

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Abstract

Eighteen fallow deer were chemically immobilized with two different methods on 21 occasions; all were completely immobilized and of good physiological quality. On 11 occasions animals were injected with a mixture of tiletamine-zolazepam-xylazine (TZX) and in 10 animals, with tiletamine-zolazepam-medetomidine (TZM). Both methods produced a level of sedation adequate for capture in all fallow deer and adequate for minor surgical procedures in 63% of the animals treated with TZX and in 100% of the animals treated with TZM. Although no significant differences were observed in the two groups for most of the blood gas parameters and cardiorespiratory values, TZX resulted in much higher values of lactate in arterial blood. No statistically significant differences were found in serum enzymes between groups, although a mild increase in creatinine kinase was seen in TZX.

Resumen

Dieciocho venados dama fueron inmovilizados químicamente con dos métodos en 21 ocasiones. Todos fueron completamente inmovilizados mostrando adecuadas respuestas fisiológicas. En once ocasiones los animales fueron inyectados con una mezcla de tiletamina-zolazepam-xilacina (TZX) y en diez animales con tiletamina-zolazepam-medetomidina (TZM). Ambos métodos produjeron niveles de sedación adecuados para la captura en todos los venados y adecuado para procedimientos quirúrgicos menores en el 63% de los animales tratados con TZX y en el 100% de los animales tratados con TZM. Aunque no se observaron diferencias significativas en los dos grupos para la mayoría de los parámetros de gases sanguíneos y valores cardiorespiratorios. TZX resultó con altos valores de lactato en sangre arterial. No se encontraron diferencias estadísticas significativas en las enzimas del suero entre los grupos, aunque un leve incremento en la creatinino-quinasa fue observado en el grupo TZX.

Introduction

Different drug combinations have routinely been used for chemical immobilization of fallow deer, being mainly based on xylazine (X), tiletamine-zolazepam (TZ), etorphine, ketamine and medetomidine.4,7,8,9 Of these, TZ is widely used at present in zoo animals for handling and surgery procedures, but in fallow deer, the dosages reported are as high as 33 mg/kg7 and even then, this anesthetic combination provides no acceptable analgesia and anesthesia. However, in combination
with xylazine (a more potent sedative which blocks presynaptic central \(\alpha_2\)-receptors\(^9\)) the quality of the anesthesia can be improved and the dosages of TZ diminished.

Medetomidine, a more potent and selective \(\alpha_2\)-adrenoceptor agonist than xylazine, has been used alone and in combination with ketamine to immobilize nondomestic ruminants,\(^4\) but references to chemical immobilization of fallow deer with medetomidine, alone or in combination, are scarce.\(^4\)

The aim of the present study was to assess the efficacy of medetomidine, TZ and xylazine combinations for immobilizing fallow deer.

**Material and Methods**

Eighteen healthy fallow deer (seven males and nine females) were immobilized on 21 occasions at the Barcelona Zoo during different procedures, including health assessment, transportation, prophylactic treatments, etc. Eight animals were injected on 11 occasions with a mixture of xylazine (Rompun\(^\text{®}\), Bayer, Leverkusen, Germany) supplied as dry powder and tiletamine-zolazepam (Zoletil\(^\text{®}\), Virbac, Carros, France) 100 mg/ml. Eight other animals were immobilized on ten occasions by means of medetomidine 10 mg/ml (Orion Corporation\(^\text{®}\), Farmos, Turku, Finland) and Zoletil. Plastic darts (Dan-inject\(^\text{®}\), International GmBH, Germany) were injected in the higher part of the hind limb by means of a gas rifle (G.U.T. 50, Telinject\(^\text{®}\), Römerberg, Germany). Approximate doses were based on sex, age or size and calculated by estimating each animal’s weight by eye, based on previous experience with this species.

If anesthesia was insufficient to achieve complete muscle relaxation, a blindfold was used to keep the animals calm. During immobilization, animals were always monitored for heart rate and oxygen saturation (N-20P, Nellcor\(^\text{®}\), Pleasanton, CA, USA) with a C-clamp applied to the tongue. All animals were weighed during immobilization. Reflexes, rectal temperature and respiratory rate were checked every 5 min.

A total of 14 arterial blood samples were collected for determinations of blood gases and acid-base status 35 min after darting. All samples were collected into 5 ml heparinized syringes, placed in an ice bath and analyzed within 1 hr using a blood gas analyzer (IL 1302 model). All values were corrected to the rectal temperature of each animal by the instrument used.

Serial venous blood samples from 16 animals were removed from the jugular vein approximately 15 and 45 min after darting. Three milliliter aliquots were placed in vials with EDTA-K as anticoagulant, refrigerated with ice and sent to the laboratory for hematological purposes which were completed within 4 hr. Also, with the same conditions, 1 ml aliquots were placed in vials with EDTA-F for lactate analyses. The largest portion of the blood sample was cooled and allowed to coagulate, and the serum separated by centrifugation. The tubes containing serum were immediately frozen and stored at -28°C until used for the biochemical analyses. Whole blood samples were analyzed for hematocrit, red blood cells, hemoglobin concentration and white blood cells as described in a previous paper.\(^5\) Serum samples were analyzed for glucose (Glucofix Menagent, Menarini, Italia), serum glutamate oxalacetate transaminase (GOT) (12150 Granutest, Merck, Germany), serum glutamate pyruvate transaminase (GPT) (12166 Granutest, Merck), creatine kinase (CK) (12134 Granutest, Merck) and lactic dehydrogenase (LDH) (3349 Merck-1-Test, Merck) using
specific commercial kits.

Induction time was the interval between injection and the time deer could be handled with minimal physical response. The plane of anesthesia was recorded using the Jalanka scale from 0-3; being 0: no effect, 1: insufficient, 2: moderate and 3: complete immobilization.3

Differences between both groups were analyzed using one-way analysis of variance (ANOVA) test, and statistically significant differences were evaluated by unpaired student’s t-test. Results of the serial determinations were compared by paired students t-test. Significance was set at p<0.05, and all p values reported are two-tailed.

Results

Tables 1, 2 and 3 summarize the recordings and determinations of clinical, blood gas, hematological and biochemical values for the fallow deer as well as the mean, standard deviation and the statistically significant differences found, between sampling groups.

Both combinations resulted in rapid development of signs of sedation with complete immobilization in lateral recumbency, in most cases, after a single dose. Arterial blood gases and acid-base status were largely similar in deer immobilized with medetomidine-Zoletil and xylazine-Zoletil combinations. Although the results indicate mild depressed ventilation, no hypoxemia (PO2<80) or extreme alterations of acid-base values were recorded. Some hematological values show a statistically significant decrease during period of anesthesia as a consequence of adrenolytic properties of anesthetics. Nevertheless, no variations of serum enzymes activities are found during sedation which can not be ruled out by injuries during immobilization.

Discussion

Induction periods were calm and animals became sedated without apparent stress. Although the analgesic effects of these combinations were not experimentally tested, it appears that they can be used for minor surgical procedures. Both methods resulted in induction time within acceptable ranges (8.1±4.1 min for TZX and 5.8±1.7 min for TZM), although medetomidine shortened them considerably.

Both methods used in this study produced an adequate level of sedation for capture in all fallow deer but adequate level of sedation for minor surgical procedures in only 63% of the animals with TZX but 100% of the animals with TZM. Xylazine combined with a low dose of tiletamine/zolazepam allowed us to perform all the clinical procedures but rarely reached the maximum effect of anesthesia.

In our study, effective immobilization was achieved with a mean dose of 1.5 mg/kg of tiletamine/zolazepam with 1.6 mg/kg of xylazine and with 99 µg/kg of medetomidine with 0.5 mg/kg of tiletamine/zolazepam. Dosages of xylazine alone or in combination with ketamine presented in the literature are as high as 5-8 mg/kg,5,6 whereas those for tiletamine reach 33 mg/kg.7 On the other hand, with medetomidine combined with ketamine other authors needed higher dosages (100-150 µg/kg).4 Thus the combination with TZ offers an alternative.
As mentioned before, animals were monitored during the immobilizations. The degree and quality of sedation and immobilization were evaluated following the scale proposed by Jalanka from 0-3. With the dosages used in the study the animals injected with TZM over all reached a better degree of anesthesia (2.9 in TZM vs 1.8 in TZX).

Fallow deer immobilized with medetomidine-ketamine showed respiratory rates from 10-25 breaths per minute, and respirations were deep with strong thoracic movements. With our two mixtures we recorded higher frequencies of respiration with a mean of 16 breaths per minute for TZX and 13 for the TZM. Although no significant differences were observed in the two groups for most of the blood gas parameters and cardiorespiratory values, TZX resulted in much higher values of lactate in arterial blood (41 mg/dl in TZX vs 15.7 mg/dl in the TZM). Apart from that, the results of determination of arterial blood gas and acid-base status parameters generally indicated good physiological quality during the procedures.

The authors had earlier experiences with immobilization of fallow deer using ketamine-xylazine and etorphine-xylazine combinations. The two methods described in this paper offered a better alternative for clinical procedures with more effective sedation and anesthesia.

The duration of action of medetomidine is long, especially after high doses, much longer than the time needed for usual clinical procedures and therefore, a reversing agent would be potentially advantageous in allowing the veterinarian to shorten the recovery period of animals sedated with the compound. Atipamezole and tolazoline have been shown to be a highly potent, selective and specific antagonists of centrally and peripherally located α₂-adrenoceptors. In both drug combinations, the immobilization time can be effectively reduced by using tolazoline or atipamezole as reversal agents, although the data are not included in this paper; many animals were reversed with tolazoline (in TZX) or with atipemazole (in TZM) resulting in both cases in complete and effective reversal in all animals tested.

In order to know the effect of anesthetics on the physiology of this species, some hematological and biochemical parameters were obtained during procedures. In this study, an elevated serum glucose concentration was found in animals anesthetized by two methods that was statistically significant higher 45 min than 15 min after darting. This hyperglycemic effect by α₂-adrenoceptor agonists, xylazine or medetomidine, has been reported for several species and is mediated by receptors in the pancreatic ß-cells that inhibit insulin release and/or by an increase of hepatic glucose production. No statistically significant differences were found in serum enzymes between groups, although a mild increase in CK was seen with TZX. Certain enzymes such as CK, GOT and LDH can be very high values due to injuries or vigorous exercise before blood sampling. The absence of an increase of these enzymes during sedation indicates that anesthesia was performed with a minor trauma of musculature.

The statistically significant decrease which was noted in the hematocrit, hemoglobin concentration and red blood cell count comparing the second with the first bleeding, has been reported in other animals. Adrenolytic properties of α₂-adrenoceptor agonist results in a splenic relaxation with the subsequent withdrawal of erythrocytes from the peripheral vasculature leading to a decreased hemoglobin, hematocrit and red blood cell numbers.
ACKNOWLEDGMENTS

We thank the staff and keepers at Barcelona Zoo who helped us during this trial and also Conrad Enseñat, curator/veterinarian at Barcelona Zoo who started to use the TZX in fallow deer, for guiding us with his previous experiences. This study was partially supported by Lab Dr. Echevarne (gas studies). Dr. Beatriz Fernández from Lab Echevarne helped us with the analysis of blood gases.

LITERATURE CITED

Table 1. Mean ± standard deviation of anesthetic data in fallow deer anesthetized with Zoletil/xylazine (TZX) and medetomidine/Zoletil (TZM) combinations. N = number of animals; & = dose is given in mg/kg except in medetomidine in which is given in µg/kg. Plane of anesthesia is registered using the Jalanka scale from 0-3.

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<td>Weight (kg)</td>
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<td>Plane (1-3)**</td>
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*p<0.05, **p<0.01

Table 2. Mean ± standard deviation of blood gas, acid base parameters and cardiorespiratory values in 14 fallow deer anesthetized with Zoletil/xylazine (TZX) and medetomidine/Zoletil (TZM) combinations. N = number of animals.

<table>
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<th>Parameter</th>
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<th>Medetomidine/Zoletil</th>
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<tbody>
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<td>N</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Time (min)</td>
<td>34 ± 7</td>
<td>37 ± 12</td>
</tr>
<tr>
<td>pH</td>
<td>7.378 ± 0.024</td>
<td>7.350 ± 0.052</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>47.1 ± 2.4</td>
<td>50.0 ± 7.7</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>94.2 ± 11</td>
<td>83 ± 22</td>
</tr>
<tr>
<td>HCO₃ (mmol/L)</td>
<td>26.4 ± 1.4</td>
<td>26.0 ± 1.8</td>
</tr>
<tr>
<td>Saturation (%)</td>
<td>97 ± 2</td>
<td>89 ± 10</td>
</tr>
<tr>
<td>Body Temp. (°C)</td>
<td>38.8 ± 0.5</td>
<td>38.8 ± 0.5</td>
</tr>
<tr>
<td>Resp. rate (bpm)</td>
<td>16 ± 5</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Heart rate (bpm)*</td>
<td>44 ± 7</td>
<td>35 ± 6</td>
</tr>
<tr>
<td>Lactate (mg/dl)**</td>
<td>41.0 ± 7.4</td>
<td>15.7 ± 5.6</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01

Table 3. Mean ± standard deviation of some hematological and biochemical parameters in 16 fallow deer anesthetized with Zoletil/xylazine (TZX) and medetomidine/Zoletil (TZM) combinations. N = number of animals; HC = hematocrit (%); RBC = red blood cells (cells/mm³ x10⁶); HB = hemoglobin (g/dl); WBC = white blood cells (cells/mm³ x10⁶); CK = creatine kinase (IU/L); LDH = lactate dehydrogenase (IU/L); GOT
= glutamate oxalacetate transaminase (IU/L); GPT = glutamate pyruvate transaminase; glucose (mmol/L).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Zoletil/Xylazine</th>
<th>Medetomidine/Zoletil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Time</td>
<td>13±3</td>
<td>42±5</td>
</tr>
<tr>
<td>HC**</td>
<td>37.8 ± 4.2</td>
<td>34.8 ± 3.8</td>
</tr>
<tr>
<td>RBC**</td>
<td>9.58 ± 1.08</td>
<td>9.07 ± 1.08</td>
</tr>
<tr>
<td>HB**</td>
<td>13.9 ± 1.7</td>
<td>12.8 ± 1.5</td>
</tr>
<tr>
<td>WBC</td>
<td>4.14 ± 0.94</td>
<td>3.84 ± 0.82</td>
</tr>
<tr>
<td>CK</td>
<td>128 ± 60</td>
<td>172 ± 129</td>
</tr>
<tr>
<td>LDH</td>
<td>432 ± 69</td>
<td>390 ± 79</td>
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<tr>
<td>GOT</td>
<td>38.8 ± 5.9</td>
<td>38.4 ± 7.7</td>
</tr>
<tr>
<td>GPT</td>
<td>29.4 ± 4.8</td>
<td>27.2 ± 2.9</td>
</tr>
<tr>
<td>Glucose**</td>
<td>8.44 ± 1.51</td>
<td>10.2 ± 1.6</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01
IMMOBILIZATION OF FREE RANGING WOODLAND CARIBOU (Rangifer tarandus caribou) WITH MEDETOMIDINE-KETAMINE AND REVERSAL WITH ATIPAMEZOLE

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Abstract

Thirteen woodland caribou were immobilized with medetomidine-ketamine during the winters of 1993, 1994, and 1995. Two methods were used to dart the animals. One method consisted of chasing the animal onto a frozen lake and darting it from the helicopter. The other method was to herd animals toward a camouflaged individual on the ground, who would dart the caribou. Body weight of the animals was determined by weighing the animals in a sling, or calculating the weight from chest girth measurements, using regression analysis. Initially the animals received an estimated dose of 70 µg/kg of medetomidine +1 mg/kg of ketamine. When this dose proved to be ineffective the dose was increased to approximately 250 µg/kg of medetomidine + 2.5 mg/kg of ketamine. An effective dose was defined as a dose that produced immobilization in < 20 min. Reversal of sedation was achieved with atipamezole administered at 3 times the medetomidine dose. Table 1 shows data from the 10 animals that received an effective dose.

Animals maintained a hemoglobin saturation of 83±8 %, a heart rate of 47±7 beats/min, a respiratory rate of 15±4 breaths/min and a body temperature of 40±4°C. Quality of immobilization was good, with adequate muscle relaxation, and no additional drugs were required following administration of an effective dose. Pulse oximetry demonstrated the presence of mild-moderate hypoxemia. Medetomidine-ketamine can be used for safe, effective immobilization of woodland caribou.

Resumen

Trece caribues de bosque fueron inmovilizados con medetomidina-ketamina durante los inviernos de 1993, 1994, y 1995. Dos métodos fueron usados para inyectar a los animales. El primero consistió en perseguir a los animales sobre un lago congelado y disparar la droga desde un helicóptero. El otro método consistió en acercar la manada a un individuo que estaba camuflado sobre el campo, quien dispararía el dardo al Caribú. El peso de los animales fue determinado en un cabestrillo o calculándolo con la medición de la cinta torácica, usando análisis de regresión. Inicialmente los animales recibieron una dosis estimada de 70 µg/kg de medetomidina mas 1 mg/kg de ketamina. Cuando esta dosis demostró ser inefectiva, la dosis se aumentó a aproximadamente 250 µg/kg de medetomidina + 2.5 mg/kg de ketamina. Una dosis efectiva fue definida como aquella que produce inmovilización en < de 20 min. La reversion de la sedación fue llevada acabo con
Introduction

Medetomidine is a potent, selective, \( \alpha_2 \)-adrenoreceptor agonist, which can be combined with a relatively low dose of ketamine to produce immobilization in a wide variety of wildlife.\(^3\) Medetomidine is readily reversible with atipamezole, a potent, selective \( \alpha_2 \)-adrenoreceptor antagonist. The low dose of ketamine usually has little residual effect following reversal of medetomidine, and arousal is generally good following medetomidine reversal. The following report details the use of medetomidine-ketamine for immobilization of free ranging woodland caribou.

Background

Caribou in this study were immobilized as part of a habitat selection study. Thirteen caribou were immobilized with medetomidine-ketamine during the winters of 1993, 1994, and 1995 in north-central Saskatchewan. Two methods were used to facilitate dart placement. One method consisted of pursuing the animals with a Jet Ranger helicopter onto a frozen lake, and darting the animals from the air with a Zulu arms dart rifle. An attempt was made to limit pursuit times to 2-3 min. The other method was to place a camouflaged individual on the ground, and using the helicopter, to slowly herd animals toward the concealed darter on the ground. Animals were darted in the gluteals or semimembranosus-semitendinosus muscle mass. Once the animals were immobilized they were blindfolded, and samples of blood, feces, and hair were obtained. Body measurements were made, and a radio collar was placed on the animal. Several of the animals were weighed.

Materials and Methods

Initial dosages were extrapolated from medetomidine-ketamine dosages used in forest reindeer (\textit{Rangifer tarandus fennicus}),\(^3\) 70 \( \mu \)g/kg of medetomidine + 1 mg/kg of ketamine. These animals also received hyaluronidase at a dose of approximately 1 unit/kg. The dose was increased to approximately 250 \( \mu \)g/kg of medetomidine + 2.5 mg/kg of ketamine when the initial dose proved to be ineffective. Three of the animals receiving the effective dose also received hyaluronidase at a dose of 1 unit/kg. An effective dose was defined as a dose that produced immobilization in < 20 min. In animals that were not weighed, body weight used for calculation of effective dose, was calculated from chest girth using multiple regression ([SPSS for Windows] Rettie, unpublished data); an actual weight was used when it was available. Immediately following dart placement the time was noted and the helicopter retreated to a safe distance for observation of the animal. Time to immobilization (TI) was taken as the time from dart placement to the time that the animal assumed lateral
recumbency. Once the animal was down, the helicopter landed, and the animal was approached. The animal was blindfolded and maintained in sternal recumbency throughout immobilization. Heart rate (HR) was taken from auscultation of the heart or from palpation of the femoral arterial pulse. Respiratory rate was determined by observation of chest excursions. Body temperature was measured rectally with a digital thermometer. Oxygen saturation (SaO₂) was obtained using a Sure-Sat™ pulse oximeter with a Nellcor Oxisensor II D-25 probe attached to the tongue. Once the animal was processed, atipamezole was administered at a ratio of approximately three times the medetomidine dose. Half of the atipamezole was administered i.v. and half was administered i.m.

Time to reversal (TR) was the time from administration of the reversal agent to the time the animal assumed a standing position. Down time (TD) was from the time the animal assumed lateral recumbency to the time the animal was fully awake.

Results

Three animals did not receive an adequate dose for immobilization. The first animal received 73 µg/kg of medetomidine + 1.3 mg/kg of ketamine and 150 units of hyaluronidase. This animal ran a long distance when it was approached, it was finally wrestled to the ground 1 hr after dart placement, its legs were hobbled and it was processed. The next animal was darted on the ground following a failed attempt to capture it with a net gun. The animal received 110 µg/kg of medetomidine + 1.5 mg/kg of ketamine and 150 units of hyaluronidase. This animal became sternally recumbent but attempted to rise after it was approached. The animal was eventually wrestled to the ground and 1 mg/kg of ketamine was administered i.v. Following ketamine the animal was easy to process. The third animal received 73 µg/kg of medetomidine + 1.2 mg/kg of ketamine and 75 units of hyaluronidase. The first dart struck the animal in the caudal lumbar region and was probably a subcutaneous injection. A second dart was delivered 55 min following the first dart. This dart contained 150 µg/kg of medetomidine + 1.5 mg/kg of ketamine. The animal assumed lateral recumbency 2 min post injection. The results for the 10 animals that received an effective dose are listed in Table 1. Physiological data is illustrated in Table 2.

Discussion

Initial dose was based on the dose used in captive forest reindeer. A substantially higher dose was required to produce sufficient immobilization in woodland caribou. A similar situation was seen when the dose requirements were compared in semidomesticated Norwegian reindeer (Rangifer tarandus tarandus) and Svalbard reindeer (R. t. platyrhynchus). Hyaluronidase was added to the combination in all of the animals that received an ineffective dose and in three of the animals that received an effective dose. The data set was too small to determine if the addition of this drug had a significant effect on induction times. The ambient temperature during the majority of immobilizations was -20°C to -30°C. Two problems were noted as a result of the cold temperature. The atipamezole had been formulated at a concentration of 25 mg/ml, the drug tended to come out of solution very rapidly as soon as it was exposed to the cold. The atipamezole was subsequently formulated at a concentration of 10 mg/ml, to maintain the drug in solution. The pulse oximeter was powered by three C cell batteries. Battery life was very short when the oximeter was exposed to the cold and the monitor could only be used for intermittent measurement.

No complications were noted as a result of the immobilization. Body temperature remained stable.
throughout the immobilization. Hemoglobin saturation was good initially, but decreased to hypoxemic levels between 15-30 min post immobilization. Studies in mule deer and mule deer-white tailed deer hybrids have demonstrated that these animals developed mild hypoxemia following immobilization with medetomidine-ketamine.\textsuperscript{2} A study to determine the accuracy of pulse oximetry in mule deer and mule deer-white tailed deer hybrids demonstrated that erroneously low pulse oximeter readings are obtained from these animals when they are immobilized with medetomidine-ketamine.\textsuperscript{1} The true hemoglobin saturation may actually have been higher than the value reported by the pulse oximeter. Reversal of medetomidine with atipamezole was smooth and uneventful. Seven of the animals that received an effective dose of medetomidine-ketamine were fitted with satellite collars. Data obtained from these animals demonstrated that they were still alive at least 1 yr post immobilization.

**Conclusion**

Data from this trial demonstrates that medetomidine-ketamine will produce adequate immobilization of free-ranging woodland caribou. Mild to moderate hypoxemia may result during immobilization, and supplemental oxygen would be beneficial, if available. Dose requirements are relatively high in this species, and under dosing can result in an animal that appears to be immobilized, but flees when approached. Reversal of medetomidine was rapid and smooth following administration of atipamezole, and satellite data demonstrated a good survival rate in these animals 1 yr post immobilization.

**ACKNOWLEDGMENTS**

The authors would like to thank Farmos Pharmaceuticals for the medetomidine and atipamezole used in this study. The authors also thank Karen Machin DVM, Susan Tedesco DVM, Murray Woodbury DVM, MSc and Judit Smits DVM, MVetSc for their assistance in gathering data for this study.

**LITERATURE CITED**


**Table 1.** Drug dosages, times to immobilization, down time and time to recovery (mean±sd).

<table>
<thead>
<tr>
<th>Medetomidine (µg/kg)</th>
<th>Ketamine (mg/kg)</th>
<th>Atipamezole (µg/kg)</th>
<th>TI\textsuperscript{1} (min)</th>
<th>TD\textsuperscript{2} (min)</th>
<th>TR\textsuperscript{3} (min)</th>
</tr>
</thead>
</table>

1996 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS
Table 2. Oxygen saturation, heart rate, respiratory rate and body temperature.

<table>
<thead>
<tr>
<th>time from immobilization</th>
<th>% saturation (mean±SD)</th>
<th>heart rate beats/min (mean±SD)</th>
<th>resp. rate breaths/min (mean±SD)</th>
<th>temperature °C (mean±SD)</th>
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</thead>
<tbody>
<tr>
<td>0-15 min</td>
<td>90±6 (n=5)</td>
<td>51±7 (n=6)</td>
<td>12±5 (n=5)</td>
<td>40.4±0.1 (n=3)</td>
</tr>
<tr>
<td>15-30 min</td>
<td>77±7 (n=5)</td>
<td>45±7 (n=5)</td>
<td>15±3 (n=8)</td>
<td>40.4±0.7 (n=4)</td>
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<tr>
<td>30-60 min</td>
<td>80±2 (n=3)</td>
<td>40±4 (n=3)</td>
<td>18±3 (n=5)</td>
<td>40.4±0.2 (n=3)</td>
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<tr>
<td>POOLED DATA</td>
<td>83±8 (n=13)</td>
<td>47±7 (n=14)</td>
<td>15±4 (n=18)</td>
<td>40.4±0.4 (n=10)</td>
</tr>
</tbody>
</table>

1 Time to immobilization (time from dart placement to lateral recumbency)
2 Time down (time from assuming lateral recumbency to standing)
3 Time to reversal (time from administration of atipamezole to standing)
COMPARATIVE CARDIOPULMONARY EFFECTS OF MEDETOMIDINE-KETAMINE AND TELAZOL® IN POLAR BEARS (Ursus maritimus)

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Abstract

Five polar bears (Ursus maritimus) were immobilized in a crossover design to compare the cardiopulmonary effects of medetomidine-ketamine (MK) and Telazol® (TZ; Fort Dodge Laboratories, Incorporated, Fort Dodge, IA 50501, USA). Animals were immobilized with 159±34 µg/kg of medetomidine + 4.0±0.8 mg/kg of ketamine (all reported values are mean±SD). Telazol® was administered at a dose of 8.2±1.3 mg/kg. Immobilization was produced 3.9±2.4 min following administration of MK, and 4.6±1.5 min following the administration of TZ. Once the animals were immobilized they were placed in dorsal recumbency and a femoral arterial line was placed for direct pressure measurement, and for the removal of arterial blood gas samples. A lead II electrocardiogram was monitored for arrhythmias. Rectal temperature was determined with a digital thermometer. Respiratory rate was determined by observation of chest excursions. A Wilcoxon Signed Rank Test was used to determine if differences between and within treatment groups were significant. Table 1 compares physiological data obtained at 30 min post immobilization with each treatment.

<table>
<thead>
<tr>
<th></th>
<th>HR 1</th>
<th>RR 2</th>
<th>MAP 3</th>
<th>T 4</th>
<th>pH 5</th>
<th>BE 6</th>
<th>PaO2 7</th>
<th>PaCO2 8</th>
<th>Hb 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK</td>
<td>39±9</td>
<td>4±3</td>
<td>205±28</td>
<td>37±0.7</td>
<td>7.3±.05</td>
<td>-5.6±2</td>
<td>70±16</td>
<td>40±9</td>
<td>160±16</td>
</tr>
<tr>
<td>TZ</td>
<td>82±27</td>
<td>9±3</td>
<td>143±11</td>
<td>37±1.2</td>
<td>7.3±.05</td>
<td>-3.4±2</td>
<td>94±26</td>
<td>37±11</td>
<td>139±6</td>
</tr>
</tbody>
</table>

1heart rate, beats/min; 2respiratory rate, breaths/min; 3mean arterial pressure, mm Hg; 4rectal temperature, °C; 5pH; 6base excess; 7PaO2, mm Hg; 8PaCO2, mm Hg; 9hemoglobin concentration, g/L.

When the two treatments were compared there were significant differences (p<0.05) in heart rate, mean arterial pressure and hemoglobin concentration. There was a trend towards increased PaO2, respiratory rate, and decreased PaCO2 in TZ compared to MK. Animals immobilized with MK demonstrated hypertension and bradycardia; this is a common finding in animals immobilized with this combination. Ventilation and oxygenation was excellent with both combinations particularly considering that they were large animals maintained in dorsal recumbency. After 1 hr of
immobilization medetomidine was reversed with atipamezole at a dose of 631±140 µg/kg. Animals stood 7.6±4 min after atipamezole administration. Animals were allowed to recover spontaneously from TZ. Both combinations produced safe, effective immobilization.

Resumen

Cinco osos polares fueron inmovilizado con un diseño de mezclas para comparar los efectos cardiopulmonares de medetomidina-ketamina (MK) y tiletamina-zolazepam (Telazol; Fort Dodge Laboratories, Incorporated, Fort Dodge, IA 50501, USA). Los animales fueron inmovilizados con 159±34 µg/kg de medetomidina + 4.0±0.8 mg/kg de ketamina (todos los valores reportados están expresados en medias de DS). El telazol fue administrado a una dosis de 8.3±1.3 mg/kg. La inmovilización se produjo a los 3.9±2.4 min después de la administración de la MK, y 4.6±1.5 min. después de la administración de TZ. Una vez que los animales se inmovilizaron fueron puestos en recumbencia dorsal y se les colocó una línea en la arteria femoral para medir directamente la presión, y para la obtención de muestras de gases arteriales. Se monitorearon con electrocardiogramas para detectar arritmias. La temperatura fue determinada con un termómetro rectal. La frecuencia respiratoria fue determinada por la observación de la inspiración. La prueba de Wilcoxen Signed Rank fue utilizada para determinar si las diferencias entre y dentro de los grupos eran significativas. La tabla 1 compara los datos fisiológicos obtenidos 30 min. post inmovilización con cada tratamiento.

Cuando los dos tratamientos fueron comparados hubieron diferencias significativas (p<0.05) en la frecuencia cardiaca, presión arterial y concentración de hemoglobina. Hubo tendencia al incremento de PaO₂, frecuencia respiratoria y disminución de PCO₂ en TZ comparado con MK. Los animales inmovilizados con MK mostraron hipertensión y bradicardia. Este es un hallazgo común en animales inmovilizados con esta combinación. La ventilación y la oxigenación fueron excelentes en ambas combinaciones considerando que eran animales de gran tamaño mantenidos en recumbencia dorsal. Después de una hora de inmovilización, la medetomidina fue antagonizada con atipamezole a una dosis de 631±140 µg/kg. Los animales se incorporaron 7.6±4 min después de la administración de atipamezole. Se les permitió incorporarse espontáneamente después de la inmovilización con TZ. Ambas combinaciones produjeron una inmovilización efectiva y segura.

Background

Telazol® (zolazepam + tiletamine; Fort Dodge Laboratories, Incorporated, Fort Dodge, IA 50501, USA) has become the drug of choice for immobilization of polar bears. Reasons for the popularity of this drug combination include relatively small drug volume, rapid induction and safe reliable immobilization.3,7 The major disadvantage of Telazol® is lack of reversibility and prolonged recovery. Medetomidine is a potent α₂-adrenoceptor agonist that has been used in combination with ketamine, to produce immobilization in a variety of domestic and nondomestic animals.6 The sedative effects of medetomidine are readily reversed with atipamezole, a potent α₂-adrenoceptor antagonist. A “reversible” combination, such as medetomidine-ketamine, is desirable in some situations. Medetomidine-ketamine combinations have been shown to produce mild to moderate hypoxemia in ruminants.1,2,4 Hypoxemia was not apparent in snow leopards.5 Although Telazol® is commonly used for immobilization of bears, the cardiopulmonary effects of Telazol® have not been reported in this
genus. The objective of this study was to determine the cardiopulmonary effects of these two combinations in polar bears.

Materials and Methods

The bears immobilized in this study were five captive polar bears that were captured in Churchill, Canada, during October 1995, 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.

Five bears were immobilized in a crossover design. 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 120 µg/kg, this was combined with ketamine at an estimated dose of 3 mg/kg. These dosages were based on field studies of this combination (Cattet, unpublished data). 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,3,7 and on a field study of this combination.

Once the bear was immobilized it was removed from the cage and an 18-ga x 2 in 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 x 2.25 in catheter was placed in the jugular vein, and blood was removed for complete blood count and hemoglobin determination. A lead 2 electrocardiogram 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 medetomidine-ketamine, was reversed with atipamezole, and bears receiving Telazol® were maintained in sternal recumbency and recovered spontaneously. A Wilcoxon Signed Rank Test was used to compare differences within and between treatments. 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 159±34 µg/kg of medetomidine plus 4.0±0.8 mg/kg of ketamine. Reversal was achieved with a 631±140 µg/kg of atipamezole. The time from drug administration to immobilization was 3.9±2.4 min following administration of medetomidine-ketamine. The time to standing was 7.6±4 min following administration of atipamezole. The actual dose of Telazol®
administered was 8.2±1.3 mg/kg. The time from drug administration to immobilization was 4.6±1.5 min. Quality of immobilization was good with both combinations. 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 medetomidine-ketamine. Sinus arrhythmia was commonly seen following medetomidine-ketamine administration. One bear developed a bigeminal rhythm 50 min following administration of medetomidine-ketamine.

Physiological data is listed in Tables 1 and 2. There were significant differences in heart rate, mean arterial blood pressure, hemoglobin concentration and respiratory rate, between treatments. Comparison of the 15 min and 60 min measurements within treatments revealed a significant increase in PaO₂, and respiratory rate, and with a decreased PaCO₂ during immobilization with Telazol®. Hemoglobin concentration decreased significantly during medetomidine-ketamine immobilization.

Discussion

Hypertension, bradycardia and sinus arrhythmia are common findings in animals anesthetized with medetomidine-ketamine, and polar bears are not an exception. Hypertension results from peripheral activation of α₂-receptors. Bradycardia is likely due to reflex increase in vagal tone. Bradycardia can result in decreased cardiac output and oxygen delivery. Immobilization with Telazol® resulted in a heart rate and blood pressure that we would consider more “normal” for an animal of this size. Unfortunately without baseline data it is difficult to comment on these parameters. Oxygenation was excellent with both combinations. Ruminants immobilized with medetomidine-ketamine generally develop hypoxemia. We were surprised how well animals maintained their PaO₂ particularly since they were maintained in dorsal recumbency without supplemental oxygen. There was a trend towards better oxygenation during immobilization with Telazol®, but the actual difference in oxygen content between treatments would be minimal. Respiratory depression was minimal. Normal PaCO₂ for most species ranges between 35-45 mm Hg. Animals in this study consistently demonstrated PaCO₂ values within this range. Increased hemoglobin was noted during immobilization with medetomidine-ketamine. We are not sure of the mechanism underlying this increase. Splenic contraction can cause increased hemoglobin in some species. Splenic contraction may have occurred during attempts to administer the drugs, but it should have occurred following Telazol® administration as well. Increased hemoglobin may have resulted from changes in intravascular volume produced by the pronounced vasoconstriction that is seen with medetomidine. Further studies are required to explain the increased hemoglobin concentration. Application of a hemostat to the nail bed resulted in a pronounced spike in heart rate and blood pressure during immobilization with Telazol®. Heart rate and blood pressure did not change 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 may be lacking during immobilization with Telazol®.

Conclusion

Both of these combinations can be used to produce safe, effective immobilization, for at least 1 hr in polar bears. Oxygenation is adequate, and supplemental oxygen was not required in these animals. It would be good clinical practice to monitor oxygen saturation with a pulse oximeter, and to have
equipment available to deliver supplemental oxygen, if necessary. Reversal of medetomidine-ketamine induced sedation was smooth, and rapid, following the administration of atipamezole. Further studies are required to better characterize the analgesic properties of these combinations.

ACKNOWLEDGMENTS

The authors would like to thank the Manitoba Department of Natural Resources 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. 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. Physiological data following the administration of medetomidine + ketamine (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 beats/min</td>
<td>34±7</td>
<td>39±9\textsuperscript{B}</td>
<td>37±11\textsuperscript{B}</td>
<td>36±6\textsuperscript{B}</td>
</tr>
<tr>
<td>resp. rate breaths/min</td>
<td>8±2</td>
<td>4±3</td>
<td>5±3</td>
<td>6±1</td>
</tr>
<tr>
<td>mean art. press. mm Hg</td>
<td>192±28</td>
<td>205±28\textsuperscript{B}</td>
<td>206±27\textsuperscript{B}</td>
<td>205±13</td>
</tr>
<tr>
<td>temperature °C</td>
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<td>37.1±0.7</td>
<td>37.3±0.8</td>
<td>37.4±0.8</td>
</tr>
<tr>
<td>pH</td>
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<td>7.31±0.05</td>
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<td>7.32±0.02</td>
</tr>
<tr>
<td>BE</td>
<td>-5.1±1.4</td>
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<td>-4.8±0.7\textsuperscript{B}</td>
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<tr>
<td>PaO\textsubscript{2} mm Hg</td>
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<td>70±16</td>
<td>79±18</td>
<td>77±4</td>
</tr>
<tr>
<td>PaCO\textsubscript{2} mm Hg</td>
<td>39±5</td>
<td>40±9</td>
<td>39±3</td>
<td>41±3</td>
</tr>
<tr>
<td>Hemoglobin g/L</td>
<td>162±18\textsuperscript{B}</td>
<td>160±16</td>
<td>154±21</td>
<td>154±21\textsuperscript{W}</td>
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\textsuperscript{W}= significant difference within treatment (difference between 15 and 60 min measurement).
\textsuperscript{B}= significant difference between treatments at this time point.
Table 2. Physiological data following the administration of telazol (mean±sd).

<table>
<thead>
<tr>
<th>time from immobilization</th>
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<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
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<tr>
<td>heart rate (beats/min)</td>
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<td>resp. rate (breaths/min)</td>
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<td>mean art. press. (mm Hg)</td>
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<td>143±11^B</td>
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<td>156±22</td>
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<tr>
<td>temperature (°C)</td>
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<tr>
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<td>PaO₂ (mm Hg)</td>
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<td>PaCO₂ (mm Hg)</td>
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<td>Hemoglobin (g/L)</td>
<td>139±7^B</td>
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<td>140±6</td>
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</table>

^W= significant difference within treatment (difference between 15 and 60 min measurement).
^B= significant difference between treatments at this time.
ORAL ANESTHETIC INDUCTION OF CHIMPANZEES (Pan troglodytes) WITH DROPERIDOL AND CARFENTANIL CITRATE

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Abstract

Traditional means of immobilizing chimpanzees have often involved the use of remote injection delivery systems. Chimpanzee intelligence can make dart delivery particularly stressful for the animals, veterinarians, and keeper staff involved. Transmucosal administration of opioids is currently used in human medicine to reduce the stress of anesthetic induction, particularly in pediatric cases. Due to its high concentration and relatively minimal cost per animal, carfentanil citrate (Wildnil™, Wildlife Laboratories, Incorporated., Fort Collins, CO 80524, USA.) was selected for study in transmucosal anesthetic induction of chimpanzees.

Initial trials were performed with carfentanil citrate as the sole induction agent. Doses of 0.8-4.0 µg/kg were offered to five chimpanzees in food items selected to maximize contact with the oral mucosa. Two animals immediately swallowed the food items, providing minimal time for transmucosal drug absorption. Neither animal showed any signs of sedation; however, one demonstrated severe pruritus approximately 60 min later. The third chimp received a carfentanil/marshmallow creme mixture applied directly to the mucosa with a tongue depressor, while two other animals received carfentanil directly onto their oral mucosa. Complete induction was achieved in all three of these chimps within 20 min of drug administration; however, all showed respiratory depression resulting in cyanosis. Anesthetic reversals were performed at the time of initial contact via i.m. injections of naltrexone HCl (INADA 6277) at a dose of 100 mg naltrexone per 1 mg carfentanil administered. Recoveries were complete within 1-2 min.

For subsequent inductions, chimpanzees were offered 2.5-5.0 mg droperidol in fruit juice 45 min prior to carfentanil administration. Doses of carfentanil administered were 2.0 and 1.0 µg/kg. Induction was again achieved by 20 min post-administration. Mucous membranes remained pink throughout these trials. Respiratory depression gradually increased throughout the maintenance phase of anesthesia, however. Unacceptable levels of respiratory depression occurred when animals were maintained on this regimen longer than 40 min post-administration of carfentanil. In one case this effect was extremely severe at 40 min and proved irreversible. Necropsy showed no evidence of pre-existing disease and the cause of death was presumed to be anesthetic related.

The final study involved anesthetizing five chimpanzees using 2.5 mg oral droperidol followed by 2.0 µg/kg transmucosal carfentanil citrate. A combined injection of naltrexone and 3 mg/kg tiletamine and zolazepam (Telazol™, Fort Dodge Laboratories, Incorporated, 800 Fifth Street Northwest, Fort Dodge, IA, 50501, USA) was administered once the animal could be safely hand injected. In cases where full induction was not achieved by 25 min post-administration of carfentanil,
animals were injected by remote delivery. Following tiletamine and zolazepam (T/Z) induction, chimps were monitored for 40 min via electrocardiogram, pulse oximetry, indirect blood pressure readings, arterial blood gases, temperature, pulse, respiratory rate, and mucous membrane color. Readings were recorded every 10 min. Anesthesia under T/Z was supplemented with i.m. injections of ketamine as needed. A physical examination, complete blood count, chemistry panel, and chest radiographs were performed on each animal.

Results from the first three animals in the final study showed chimps becoming gradually more sedate during the induction phase and eventually reclining into recumbency. Pink mucous membrane color was maintained throughout the procedures. Animals remained recumbent during the transition from induction to maintenance of anesthesia; one individual showed head movement when stimulated during the initial 2 min following the naltrexone/T/Z injection. Initial blood gas readings showed elevated pCO₂ values with decreased pO₂, but readings improved throughout the 40 min maintenance phase. Anesthetic recoveries were uneventful.

Resumen

El método tradicional de inmovilización de chimpancés ha involucrado a menudo el uso de sistemas de inyección remota. La inteligencia del chimpancé puede hacer que la inyección del dardo sea particularmente estresante para el animal, como para los veterinarios y trabajadores involucrados. La administración transmucosal de opioides es usado frecuentemente en humanos para reducir el estres de la inducción a la anestesia, particularmente en casos pediátricos. Debido a su alta concentración y relativamente bajo costo por animal, el citrato de carfentanil (WildnilTM, Wildlife laboratories, Incorporated, Fort Collins, CO 80524, USA) fue seleccionado para el estudio como inductor de anestesia transmucosal en chimpancés.

Los ensayos iniciales fueron realizados con citrato de carfentanil como el único agente de inducción. Dosis de 0.8-4.0 µg/kg fueron administradas a los chimpancés con alimentos seleccionados para maximizar el contacto con la mucosa oral. Dos animales ingirieron inmediatamente los alimentos, transcurriendo un mínimo tiempo para la absorción transmucosal de la droga. Ningún animal mostró signos de sedación; sin embargo uno de ellos mostró prurito intenso aproximadamente 60 min después. El tercer chimpancé recibió una pasta con una mezcla de carfentanil y bombones aplicada directamente en la mucosa con un abate lenguas, mientras otros dos animales recibieron carfentanil directamente en la mucosa oral. La inducción completa se alcanzó en los tres animales dentro de los 20 min de la administración de la droga. Sin embargo, todos presentaron depresión respiratoria dando como resultado cianosis. La reversión de los efectos anestésicos se inició al momento del contacto inicial mediante la inyección i.m. de Naltrexona HCl (INADA 6277) a una dosis de 100 mg de Naltrexona por 1 mg de Carfentanil administrado. La recuperación se completó en 1-2 min.

Para inducciones subsecuentes, se les ofreció a los chimpancés 2.5-5.0 mg de Droperidol en jugo de frutas 45 minutos antes de la administración de Carfentanil. La dosis administrada de Carfentanil fue 2.0 y 1.0 µg/kg. La inducción se realizó nuevamente a los 20 minutos de su administración. Las membranas mucosas permanecieron rosadas durante toda la prueba. Sin embargo, hubo un incremento gradual en la depresión respiratoria durante toda la fase de mantenimiento de la anestesia. Niveles inaceptables de depresión respiratoria ocurrieron cuando los animales fueron
manteñidos con este régimen durante más de 40 minutos posteriores a la administración de Carfentanil. En un caso este efecto fue extremadamente severo a los 40 minutos y resultó irreversible. La necropsia no demostró evidencia de una enfermedad preexistente y la causa de muerte fue relacionada a la anestesia.

El estudio final involucró la anestesia de cinco chimpancés usando 2.5 mg de Droperidol Oral seguido de 2.0 µg/kg de citrato de Carfentanil transmucosal. Una inyección combinada de naltreoxona y 3 mg/kg de Tiletamina y Zoletil (Telazol™, Fort Dodge Laboratories, Incorporated, 800 Fifth St. Northwest. Fort Dodge, IA, 50501, USA) fue administrada una vez que el animal pudo ser manipulado con seguridad. En los casos en que la inducción total no pudo conseguirse después de 25 minutos de la administración de Carfentanil, los animales fueron inyectados a distancia. Después de la inducción con Tiletamina y Zoletil (T/Z), los chimpancés fueron monitoreados durante 45 minutos con electrocardiograma, oxímetro de pulso, lectura indirecta de presión sanguínea, gases arteriales, temperatura, pulso, frecuencia respiratoria y color de la membrana mucosa. Las lecturas fueron tomadas cada 10 minutos. La anestesia bajo T/Z fue suplementada con inyecciones i.m. de Ketamina cuando fue necesario. Un examen físico, biometría hemática, química sanguínea y radiografías torácicas fueron realizadas en cada animal.

Los resultados finales de los primeros tres animales muestran que los animales llegaron a estar más sedados durante la inducción y eventualmente cayeron en recumbencia. Las membranas mucosas se mantuvieron de color rosa durante todo el procedimiento. Los animales permanecieron en recumbencia durante la transición de las fases de inducción a mantenimiento; un individuo mostró movimientos de cabeza cuando fue estimulado durante los dos primeros minutos siguientes a la inyección de Naltrexona/T/Z. Las lecturas iniciales de los gases sanguíneos mostraron valores de pCO₂ con una disminución de pO₂, pero las lecturas mejoraron durante los 40 minutos de la fase de mantenimiento. La recuperación de la anestesia se desarrolló sin eventualidades.

ACKNOWLEDGMENT

The authors thank Dr. Rita McManamon of Zoo Atlanta for suggestions related to this study.
ACUTE LYMPHOCYTIC LEUKEMIA IN AN INFANT WESTERN LOWLAND GORILLA
(Gorilla gorilla gorilla)

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Abstract

A 6-mo-old, hand raised, male western lowland gorilla was diagnosed with acute lymphocytic leukemia based on complete blood count (CBC) and bone marrow cytology. Clinical signs of the disease presented as pyrexia, abdominal distention, splenomegaly and lethargy. Acute lymphocytic leukemia has rarely been reported in this species and therapy was based on human oncology protocols. Remission induction chemotherapy resulted in complete clearing of leukemia cells from the bone marrow. Consolidation and maintenance chemotherapy followed. In spite of initial success, the gorilla relapsed 120 days into treatment. Therapy was facilitated by the use of an infusion port, for intravenous treatments, and an indwelling lumbar catheter, for intrathecal therapy. Side effects associated with chemotherapy were inappetence, moderate alopecia, pancytopenia resulting in sepsis, and bleeding tendency. Intensive care by the animal staff was a key factor in the treatment of this gorilla.

Resumen

A una hembra gorila de seis meses de edad criada a mano, se le diagnosticó leucemia linfocítica aguda, basada en el recuento completo de sangre (CBC) y un examen citológico de médula ósea. Los signos clínicos presentados en el enfermo fueron pirexia, distensión abdominal, esplenomegalia y letargo. La leucemia linfocítica aguda ha sido reportada raramente en esta especie y la terapia se basó en protocolos de oncología en humanos. La inducción con quimioterapia dio como resultado una completa desaparición de células leucémicas en médula ósea. La consolidación y el mantenimiento siguieron con quimioterapia. Y a pesar del éxito inicial, la gorila recayó 120 días después de iniciado el tratamiento. La terapia se facilitó con el uso de un implante de infusión para tratamientos intravenosos y un catéter intralumbar para terapia intratecal. Los efectos asociados a la quimioterapia fueron inapetencia, alopecia moderada, pancytopenia causando sepsis y tendencias a hemorragias. Los cuidados intensivos por parte del grupo que trabajó con el animal fue un factor clave en el tratamiento de este gorila.

Introduction

Acute lymphocytic leukemia (ALL) is the most commonly reported neoplasia in human pediatrics
comprising approximately one third of all childhood cancers. It is categorized into subtypes based upon immunologic phenotypes i.e., precursor B-ALL, B-cell, and T-cell leukemia. Sixty-five percent of ALL is precursor B-ALL. The cure rate of precursor B-ALL is 65-75% except in children less then 1 yr of age when the cure rate is 20-35%. A cure is only possible with intensive treatment.2

The treatment approach is divided into three phases: induction therapy, to get the patient into remission, consolidation and maintenance therapy. The total duration of treatment, if successful, is usually 2-3 yr. Local treatment of the central nervous system (CNS) is necessary, either by radiation or intrathecal treatment (or a combination of both) in order to treat occult central nervous system leukemia. This report describes treatment of leukemia in an infant gorilla using a treatment protocol developed for human infants with ALL.

Case report

On 12 December 1995, a 6-mo-old, nursery raised gorilla weighing 5.2 kg was examined for lethargy and partial anorexia of 2 days duration. Significant findings included pyrexia, 38.6 C (101.5 F), abdominal distention and a firm abdominal mass. Radiographs and ultrasound of the abdomen revealed a markedly enlarged, homogeneous spleen. The spleen extended from the left pelvic bone across the midline to the right iliac crest, filling about two-thirds of the palpable abdomen. Significant laboratory studies included severe anemia (PCV 17%), and thrombocytopenia (24,000/ml³). The white blood cell count (WBC) was 6,700/ml³ with 19% pathologic cells. The chemistry profile was normal except for an elevated LDH (2983 IU/L).

The bone marrow sample was aspirated from the posterior left iliac crest. Cytological evaluation showed predominantly medium to large sized blast cells with folded nuclei, smudgy or finely granular chromatin containing one or two (rarely three) nucleoli. These cells contained a moderate amount of cytoplasm with small cytoplasmic vacuoles. Occasional coarse, purple (Wright’s stain) granules were observed in the cells. Special stains, to differentiate acute myelogenous leukemia were negative.

Slides of cultured leukemic cells were examined for chromosomes analysis. The karyotype was arranged according to Presumptive Homologies for the Great Apes in the International System for Human Cytogenetic Nomenclature (ISCN, 1985). The G-banded karyotypes for Gorilla gorilla are arranged according to length and centromere position. The chromosomes of the leukemic cells demonstrated a repeated deletion of chromosome arm 5q and additional chromatin on chromosome arm 9q. A translocation between these two chromosomes cannot be ruled out. There also appeared to be an additional band on chromosome 13, the human homologue of which is chromosome 14.

Immunophenotyping was attempted with the intention of identifying the cell line of the leukemic cells (B-cell or T-cell). This test was inconclusive. Therefore, the diagnosis of ALL was established morphologically. Treatment in this case followed the Children’s Cancer Study Group Protocol developed for human infants suffering with precursor B-ALL.3

Following the diagnosis an initial plan was drafted to begin chemotherapy.

Treatment
The protocol is divided into three phases: induction, consolidation, and maintenance. Table 1 compares the protocol for human infants with that given this gorilla. Therapy for the gorilla had to be modified immediately because of thrombocytopenia, poor venous access and anticipated severe complications (i.e., mouth sores, pancytopenia and sepsis). In addition treatment against occult CNS leukemia, via lumbar puncture, was postponed because of thrombocytopenia not corrected by a platelet transfusion. One intrathecal treatment (methotrexate and hydrocortisone) was administered during induction therapy, after the platelet level rose above 80,000/ml³ (day 12). Other lumbar taps were unsuccessful.

Prior to the initiation of chemotherapy the gorilla received several treatments of packed red blood cells acquired from the gorilla’s mother and processed by a local blood institute into 75 ml units. Red blood cells were given at 1-2 units per day over 3 days along with double maintenance intravenous fluids (0.45% NaCl, 2.5% dextrose, 10 mEq/L KCl, 30 mEq/L NaBicarb). Allopurinol was also given (10 mg/kg daily) to prevent uric acid nephropathy, anticipated from leukemic cell lysis.

From the onset of the disease the gorilla was placed on 24 hr care. The dedication of the keeper staff was essential for the success of treatment which included assisting the veterinary staff in monitoring intravenous lines and giving medications. Because peripheral venous access was necessary for safe and reliable treatment, an indwelling femoral catheter was placed for the first 2 wk and was replaced by an indwelling infusion port (Chemo-port, HDC Corporation, San Jose, CA, USA). This port was placed in the subclavian vein with the injection disc located caudal to the left axilla.

The first clinical sign of improvement was rapid decrease in the size of the spleen. At the time of remission the spleen was estimated as 4 cm long. The first response to treatment was observed as an increased platelet count after 10 days. The WBC count returned to normal levels 18 days after the onset of treatment. Side effects consisted of anorexia and moderate alopecia.

Consolidation therapy started on day 32 (see Table 1). During this phase there were complications which necessitated stopping therapy at times. Intrathecal medication against occult CNS leukemia is essential during this phase. After three unsuccessful spinal tap attempts the spinal fluid space in the lumbar area was evaluated with magnetic resonance imaging. Because this space was small, an indwelling lumbar catheter, with an access port placed right of midline (EDM Lumbar Catheter, Ventricular Access Port, PS Medical Corporation, Goleta, CA, USA) was placed by a pediatric neurosurgeon. Unfortunately, the catheter had to be removed because of severe bleeding most likely caused by a heparin overdose. This was a setback which interrupted the schedule of thioguanine and cytarabine for 10 days. On day 52 the gorilla developed neutropenia and fever (WBC 1,100/ml³). A blood culture, taken through the infusion port grew *Staphylococcus epidermidis*, sensitive to ciprofloxin and vancomycin in vitro. Ciprofloxin (6 mg/kg q 12 hr) was replaced with vancomycin (25 mg/kg q 8 hr, 1 hr infusion) when repeat cultures remained positive. Vancomycin was given for 16 days and was stopped when blood cultures had been negative for 10 days. During this period the absolute granulocyte count was consistently low. A bone marrow aspirate at this time revealed a normal cell population with no pathological cells.

Consolidation therapy was further complicated by poor appetite and low fluid intake resulting in gradual weight loss and decreased muscle mass. On day 53 intravenous hyperalimentation (840 ml/
24 hr) was initiated and continued for 23 days until a second central line infection necessitated removal of the infusion port. Because the CBC was in the normal range, a nasogastric tube could be placed allowing reliable feeding for the duration of the case. The gorilla then began to gain weight.

The plan for maintenance therapy was modified because at that time there was no venous or lumbar access. On day 82, maintenance therapy began with asparaginase (1000 IU i.m.) in four daily doses. The infusion port and indwelling lumbar catheter were replaced on day 87. Thioguanine was given for 9 days which was followed by reinduction therapy with prednisolone, doxorubicin, and cyclophosphamide. The gorilla also received weekly intrathecal injections of methotrexate and hydrocortisone (see Table 1).

The therapy was well-tolerated. Vomiting was associated with cyclophosphamide and with intrathecal methotrexate treatments. This side effect was controlled with Phenergan (0.5 mg/kg i.v.). In addition to the described chemotherapy, the gorilla was also given prophylactic trimethoprim-sulfa (20 mg/kg b.i.d., 3 days/wk) beginning on day 30.

On day 110 (day 28 of maintenance therapy) an enlarged spleen was once again noted on physical exam. Bone marrow aspirate performed 2 days later demonstrated recurrence of the disease. Chemotherapy was discontinued and 7 days later the gorilla was euthanatized when respiratory distress developed.

Gross postmortem findings revealed a markedly enlarged spleen and liver. There was no obvious lymphadenopathy and the thymus was normal size. Histopathological data was not available at the writing of this report.

Discussion

Leukemia has rarely been reported in great apes. Fatal ALL has been diagnosed in a chimpanzee.\(^1\) The morphology of the pathological cells in this infant gorilla were compatible with ALL but immunophenotyping of the cells was not successful. The results of the chromosome study, which became available only several months after treatment was initiated, revealed that the pathologic cells in the bone marrow resembled those of the mature B-cell lineage. The observed anomaly at chromosome 13q is interpreted as analogous to 14q in humans. Band 14q is usually associated with B-cell leukemia.\(^2\) The treatment protocol was tailored for a human infant with precursor B-ALL. In infants the prognosis is generally poor (less than 30% cure).

Consideration to treat this gorilla involved many factors. It was of primary importance to have enough manpower and other resources to provide care. It was also crucial to get input from oncologists, diagnosticians, intensivists and surgeons. The difficulties of administering intravenous chemotherapies also needed to be overcome in order to expect success in this extended treatment. It was unknown how well a gorilla of this age would tolerate chemotherapy. For that reason, chemotherapy was chosen which would not cause too severe side effects. Of special concern was therapy which would cause long lasting bone marrow suppression as blood products were not abundantly available. It is unknown how well frequent blood transfusions are tolerated.

Side effects during therapy included pancytopenia (resulting in sepsis), poor appetite and partial hair loss.
loss. Poor appetite was an unexpected complicating factor during treatment, especially considering corticosteroid therapy was given. There was some improvement in appetite when treatment was changed from liquid prednisolone formulation (0.5 mg/kg p.o. t.i.d.) to prednisone tablets (1 mg/kg p.o. t.i.d.). Alopecia was an anticipated side effect of several of the chemotherapeutic drugs. There was considerable thinning of the hair and the areas that were clipped did not grow back, but the gorilla did not completely lose its coat.

The therapy chosen produced good partial remission. Human leukemias of the mature B-cell lineage do not respond well to this drug combination and, for that reason, it is thought this infant gorilla had precursor B-ALL. Therapeutic plans to continue with rather intensive treatment in short intervals were hampered by complications. This may be why there was an early relapse.

In summary, a gorilla with ALL can be treated if there is a commitment by the zoo to provide the appropriate resources and there is willing professional community support for extensive, long term treatment. Treatment plans can be hampered by complications of venous access and access to the CSF. It is especially important to have dedicated personnel willing to provide the intensive, 24 hr care necessary to treat a gorilla of this age.

ACKNOWLEDGMENTS

The authors thank the Nursery keeper staff, Susan Ragsdale and Mark Valentine of St. Jude Research Hospital (NIH Cancer Center Cytogenetics Core Grant #CA21765), Dr. David Tuggle, Dr. Cameron Mantor, Dr. Don Horton, Dr. Valerie Rigual, Linda Chandler, and Mick Martin of the Oklahoma Blood Institute, and Charla Prentis and Misti Leeper of Addison Option Care, for their remarkable support in this case.

LITERATURE CITED

1. Calle, P. Personal communication.

Table 1. Therapy.

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<th>Drug</th>
<th>Dose</th>
<th>Human Infant Protocol</th>
<th>Gorilla Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide</td>
<td>40 mg/kg i.v.</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Prednisolone</td>
<td>2 mg/kg p.o.</td>
<td>1-28</td>
<td>0-28</td>
</tr>
<tr>
<td>Danorubicin</td>
<td>2 mg/kg i.v.</td>
<td>2,3</td>
<td>0, 7, 14</td>
</tr>
<tr>
<td>Asparaginase</td>
<td>200 IU/kg i.m.</td>
<td>15-28 (3 x/wk)</td>
<td>3 x/wk (7trt)</td>
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</table>

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<table>
<thead>
<tr>
<th>Medicine</th>
<th>Dosage</th>
<th>Days</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vincristine</td>
<td>0.05 mg/kg i.v. weekly x 5</td>
<td></td>
<td>weekly x 5</td>
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<tr>
<td>Cytarabine</td>
<td>20 mg i.t.</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>6 mg i.t.</td>
<td>15, 29</td>
<td>11 (5 mg)</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>5 mg i.t.</td>
<td>15, 29</td>
<td>11</td>
</tr>
</tbody>
</table>

**Consolidation Therapy** (day 32 - 82 of gorilla treatment)

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Dosage</th>
<th>Days</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine</td>
<td>5mg/kg s.c.i.v. 0-7</td>
<td>0-2, 13-18</td>
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</tr>
<tr>
<td>Thioguanine</td>
<td>2.5 mg/kg p.o. 0-7</td>
<td>0-2, 13-17</td>
<td></td>
</tr>
<tr>
<td>Prednisolone</td>
<td>Taper</td>
<td>0-14</td>
<td>0-16</td>
</tr>
<tr>
<td>Asparaginase</td>
<td>200 IU/kg i.m. 8-19</td>
<td>19-20</td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0.3 mg/kg i.v. 20-24</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>6 mg i.t. weekly x 4</td>
<td>7</td>
<td></td>
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</table>

**Maintenance** (day 82-114 of gorilla treatment)*

<table>
<thead>
<tr>
<th>Medicine</th>
<th>Dosage</th>
<th>Days</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparaginase</td>
<td>1000 IU, i.m. -</td>
<td>0-3</td>
<td></td>
</tr>
<tr>
<td>Thioguanine</td>
<td>10 mg/kg p.o. 0-3</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>40 mg/kg i.v. 4</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.05 mg/kg i.v. 11,18,25</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Prednisolone</td>
<td>6 mg/kg p.o. 11-17</td>
<td>19-33</td>
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<tr>
<td>Doxyrubricin</td>
<td>0.5 mg/kg i.v. 39,40</td>
<td>-</td>
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<tr>
<td>Cytarabine</td>
<td>1.2 mg/kg q12 hr s.c.</td>
<td>41-43</td>
<td>-</td>
</tr>
<tr>
<td>Thioguanine</td>
<td>1 mg/kg q12 hr p.o.</td>
<td>41-43</td>
<td>-</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>6 mg i.t. 56</td>
<td>5,14,21</td>
<td></td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>5 mg i.t. 56</td>
<td>5,14,21</td>
<td></td>
</tr>
</tbody>
</table>

*In human infants, maintenance therapy occurs in 56 day cycles following the consolidation phase.*
Abstract

Gastrointestinal parasitism is considered to be one of the health problems that affects most of the nonhuman primates found in zoological parks around Mexico. At the Chapultepec zoo, 100% of the primates were found to host at least one species of gastrointestinal parasite. Parasitism has been a problem in almost every gorilla collection in the world. *Balantidium coli* is considered to be one of the few ciliated protozoans pathogenic to primates. Some of the drugs used in the treatment for amebiasis have proved to be effective against *B. coli*. At Chapultepec Zoo, treatment of balantidiasis has been accomplished with different drugs: metronidazole, diiodohydroxyquin, tinidazole, oxytetracycline, and doxycycline. The rejection of oral medication is a major difficulty in treating balantidiasis so injectable medication has been chosen on some occasions. Dehydroemetine dihydrochloride (Dehidroemetina, Roche) at a daily dose of 1 mg/kg for 10-15 days and Hemezol (Hemestal, Silanes) at a daily intramuscular dose of 5.5 mg/kg followed by an oral dose of 3.3 mg/kg b.i.d., have been used in treating balantidiasis. Diarrhea and dysentery caused by *Balantidium coli* have improved with both injectable treatments but eradication of the parasite has not yet been obtained. *Salmonella* usually causes an enteric disease, but it sometimes turns into a bacteremia or septicemia and can affect other organs. One of the main heavy metal poisoning in zoo animals is lead poisoning. This has been reported in a wide variety of primates and has been characterized by anemia and epileptiform seizures. A case of balantidiasis, salmonellosis and lead poisoning of unknown origin was seen in a 35 yr male gorilla causing the animal’s death. This individual developed enteritis during several periods of his life caused by different agents: *Salmonella* spp., *Shigella* spp., *Strongyloides* spp., *Balantidium coli*, *Entamoeba histolytica*, and *Enteromonas* spp., among others, and was successfully treated. On the last case, immunosuppression probably played an important role.
Introduction

Chapultepec Zoo received a male gorilla in 1987 as a gift from the Memphis Zoo; one year later a female was donated by the Cincinnati Zoo. The male was considered sterile and the female was out of any reproductive program due to its age (27 yr). In September 1990 they were introduced and 12 mo later a male gorilla was born.

Gastrointestinal parasitism is considered to be one of the health problems that affects most of the nonhuman primates found in zoological parks around Mexico. It may cause problems with reproduction and appearance of the animals, detrimental effects in two of the main purposes of a modern zoo. Also some of these parasites are considered to be important zoonosis that affect human beings causing a serious public health problem.

At the Chapultepec zoo, 100% of the primates were found to host at least one species of gastrointestinal parasite. Entamoeba histolytica was found in 62.7% of the total population of primates, Trichuris trichiura was found in a 40.6%, Entamoeba coli (30.5%), Endolimax nana (23.7%), Enterobius vermicularis (13.5%), Strongyloides stercoralis (13.5%), Ancylostoma duodenale (11.8%), Taenia spp. (11.8%), Giardia lamblia (10.1%), Ascaris lumbricoides (3.3%), Hymenolepis diminuta (3.3%), Balantidium coli (3.3%), and Iodamoeba butschlii (3.3%).

Parasitism has been a problem in almost every gorilla collection in the world. Balantidium coli is considered to be one of the few ciliated protozoans pathogenic to primates. It colonizes the large intestine of many nonhuman primates, especially the great apes: orangutans (Pongo pygmaeus), chimpanzees (Pan troglodytes), and gorillas (Gorilla gorilla) and also wild and captive baboons.
(Papio cynocephalus), rhesus monkeys (Macaca mulatta), etc.

*Balantidium coli* causes colitis and ulceration of the colonic mucosa; it inhabits the large intestine of a great number of mammals and the gastrointestinal tract of some birds and arthropods. There are two stages in the life cycle of this parasite: the trophozoite and the cyst. The trophozoite normally exists as a free living commensal in the large intestine, and the cyst is a more environmentally resistant form. Ingestion of trophozoites or cysts may lead to infection. Domestic swine are considered to be the main natural reservoir.

Despite the important health problems that this organism may cause, less than 100 nonhuman primate cases of clinical disease caused by *Balantidium coli* had been reported by 1988. Nonhuman primates may remain as carriers of *Balantidium coli* for years. There are some predisposing factors such as poor sanitation, a large amount of inoculum, polyparasitism, malnutrition, association with one or more agents capable of causing enteric disease, and presumed inadequate immune mechanisms associated with youth, extreme age, or concomitant illness. If the disease remains untreated, it may be fatal, owing to dehydration and shock or to intestinal perforation and peritonitis.

Several drugs have been produced to treat amebiasis in humans in Mexico, where a high percentage of its population is a carrier of this disease. Some of these are effective against balantidiasis. Treatment of balantidiasis has been accomplished with different products: paromomycin sulfate, metronidazole, tetracycline, doxycycline, iodoquinol, oxytetracycline and mebendazole together with supportive therapy.

Members of the genus *Salmonella* are gram negative bacteria in which the fecal oral route is the most common way of transmission. *Salmonella* usually causes an enteric disease, but it sometimes turns into a bacteremia or septicemia and can affect other organs. The enteritis caused by this bacteria has three steps: a) gut colonization, b) invasion of the intestinal epithelium and c) induction of lost electrolytes.

One of the main heavy metal poisoning in zoo animals is lead poisoning. This has been reported in a wide variety of primates and has been characterized by anemia and epileptiform seizures. Lead is absorbed through the lungs or gut, and affects three important systems in the body: nervous, urinary and hematopoietic. In humans (children), the central nervous system (CNS) is the target organ in lead poisoning while in adults, signs are related to peripheral neuropathies. The most common sources of lead available to the caged primate are painted cage bars and other cage furniture. Eosinophilic intranuclear inclusion bodies are found in the proximal convoluted tubular epithelium at necropsy. Renal and hematologic manifestations may be reversible but the damage to the CNS is irreversible.

In the present report, we describe clinical signs, laboratory findings and two different antiprotozoal therapies used in the treatment of *Balantidium coli* infection in gorillas. We also describe a case in which *Salmonella* and lead poisoning were associated with this parasite causing the death of an adult male gorilla.

**Case Report**

The Chapultepec Zoo used to have an exhibit for lowland gorillas that consisted of one large grass
covered outdoor enclosure and three indoor enclosures. Two adult gorillas (male and female) and a young male were maintained together during the day time, and in the afternoon the male was placed in a separate night den. Later on, a new enclosure was built, consisting in a large grass covered outdoor exhibit, with a dry moat in the front and back, an indoor exhibit and five indoor enclosures or night dens.

When the male arrived in 1987, *Balantidium coli* was not found in its fecal direct smears; after the female arrived, trophozoites started to be seen in fecal smears of both gorillas. At Chapultepec Zoo, treatment of balantidiasis has been accomplished with different drugs: metronidazole, diiodohydroxyquin, tinidazole, oxytetracycline, and doxycycline. The drug of choice is oral metronidazole combined with diiodohydroxyquin (Flagenase 400, Liomont). This syrup has been used successfully to treat balantidiasis in different species including gorillas.

**Case 1**

A 4-mo-old gorilla was noted to be lethargic, he frequently passed small amounts of brown watery feces, and for more than 12 hr he was not seen suckling; *B. coli* trophozoites were seen in a direct smear. Although an initial parenteral treatment was initiated, the situation continued for more than 24 hr. It was decided to sedate the female and treat the infant. Intravenous fluid therapy (160 ml/kg of body weight/day) was administered for 4 days to correct dehydration and electrolyte imbalance. Oral fluids were given only after 12 hr of fasting. Oral metronidazole was given at a dose of 35 mg/kg/day for 7 days to treat balantidiasis; gentamicin (7.5 mg/kg/day) and ampicillin (100 mg/kg/day) were given i.v. for 5 days to control a secondary bacterial infection caused by *Escherichia coli*. After 7 days of physical separation from its parents (he was always maintained in visual contact), the infant gorilla was reintroduced.

**Case 2**

The rejection of oral medication is a major difficulty in treating balantidiasis so injectable medication has been chosen on some occasions. Dehydroemetine dihydrochloride (Dehidroemetina, Roche) at a daily dose of 1 mg/kg for 10-15 days and Hemezol (Hemestal, Silanes) at a daily intramuscular dose of 5.5 mg/kg followed by an oral dose of 3.3 mg/kg b.i.d., have been used in treating balantidiasis. Dehydroemetine dihydrochloride acts against some protozoans by degenerating the nucleus and the cytoplasmic reticulum; it is believed that it inhibits trophozoite multiplication. Hemezol is a nitroimidazol derivative whose action against protozoans has not been well-described.

A 31-yr-old female gorilla developed diarrhea, 10 days after parturition. She frequently passed small amounts of brown watery feces. Fecal flotation (Faust method) was negative for parasitic eggs, larvae, or protozoan cysts. A direct smear showed numerous trophozoites of *Balantidium coli* and trichomonads. Metronidazole was given orally but it was not well-accepted. The following day, it was decided to treat the female with an injectable product. Dehydroemetine dihydrochloride was injected intramuscularly at a daily dose of 120 mg (1 mg/kg). Watery feces continued to be passed up to 5 days post-treatment and trophozoites were seen (every day in lesser amounts) up to day 10. Treatment continued for 5 more days (day 15). No side effects were seen. Since then, the female has passed normal feces and occasionally a few *B. coli* trophozoites have been seen in direct smears; oral medication with metronidazole has been used to control proliferation of *B. coli*. 
Case 3

A 32-yr-old male gorilla was noticed to have profuse diarrhea. Results of fecal flotation (Faust method) were negative and a direct fecal smear showed numerous *B. coli* trophozoites and trichomonads. He refused any oral medication so it was decided to inject him with dihydroemetine dihydrochloride, 150 mg daily dose, for 10 days. Trichomonads were not seen after day 3. Feces were formed by the 5th day but *B. coli* trophozoites were still seen in direct smears by day 10. Large amounts of mucous were seen in the feces during these days. It was decided to stop the treatment with this product because of its cardiotoxicity and continue treatment with injectable hemezol at a daily dose of 840 mg i.m. for 5 days followed by 500 mg p.o. b.i.d. for 7 days. Trophozoites were not seen after day 17 (immediately following hemezol oral medication). Long acting oxytetracycline was injected to prevent any secondary bacterial infection on days 1, 3, and 5. Once therapy was discontinued, *B. coli* trophozoites were seen occasionally in the stools.

This same individual subsequently developed signs of colitis due to *B. coli* on several occasions. One month later, treatment for balantidiasis included dihydroemetine for 6 days and hemezol for 11 days at the same rate described earlier. This seemed to control the problem. Balantidiasis was seen on four more occasions and hemezol and/or oral metronidazole (whenever this individual accepted oral medication) therapy was given.

Two years later, a severe colitis associated with *B. coli* was seen. Anorexia was observed and an inferior molar was extracted because it appeared to be causing him pain while eating. A few days later this gorilla developed pseudomembranous colitis thought to be related to the administration of long acting oxytetracycline. *Entamoeba histolytica* was seen in fecal smears and also *Edwardsiella* spp. was cultured. Four months later, *Salmonella arizona* was cultured from his feces for the first time. Recovery from an enteritis was uneventful on this occasion.

During the preceding 3 yr, the male gorilla developed a form of neuritis in all arms and legs. Sometimes an arm or leg seemed to be completely paralyzed; treatment with analgesics solved the problem each time.

Almost a year later, this male gorilla developed an enteritis in which *B. coli* was associated. Treatment was started right away but he refused any oral medication. The diarrhea worsened daily and 11 days after initial onset of the problem he was anesthetized. Blood analysis showed renal failure, elevated hepatic enzymes, leukopenia and positive reaction of Typhic O antibodies. Six days later the male gorilla died. Macroscopic lesions showed hemorrhages in the lungs and intestine, ulcers in the oral mucosa from which *Candida albicans* was isolated, hepatitis and hemorrhages in the kidneys. Some of the lesions could have been associated with the uremia. A preliminary diagnosis included renal failure/uremia, colitis, salmonellosis and balantidiasis.

Routine methods were used to fix and stain tissue samples with H/E and Zielh-Neelsen; semi-fine samples were taken and electronic microscopy was also performed. The histopathological analysis revealed tubular necrosis, nephrocalcinosis, and round structures within the cell nucleus of the proximal convoluted tubules, that could be diagnosed as inclusions. The liver presented with coagulative necrosis (typhoid nodules) and round structures were found in most of the liver cells that could suggest inclusions. Wide areas of necrosis could be observed in the colon, together with
inflammatory cells in its mucosa (monocytes, lymphocytes and plasmacytic cells). Thrombosis was seen in blood vessels belonging to the submucosa and in the organ’s lumen, abundant protozoans (B. coli) were seen. Kidney and liver samples were stained with Zielh-Neelsen, and the round structures were found within the nucleus of the epithelium cells of both organs. These structures can be diagnosed as inclusions. The ultrastructure of liver and kidney revealed electrodense inclusions within the cell nucleus.

Lead levels in the liver were 28.5 ppm; levels in the kidneys were 0.93 ppm. Lead levels in two different serum samples were less than 0.2 ppm; lead always binds itself mainly to red blood cells. Some toxicology results are still pending at the writing this manuscript.

Discussion

Clinical findings suggest that Balantidium coli was the primary pathogen in this cases of diarrhea. Balantidium coli trophozoites were seen in large quantities in the fecal samples of the two adult gorillas, and then disappeared after treatment with both injectable and oral products. Escherichia coli was isolated on several occasions but was discarded as a possible primary pathogen. Salmonella arizona was also isolated and in the case of the adult gorilla, probably played an important role in his disease.

Amebiasis is one of the most common diseases in Mexico and its incidence is high: 27% of the total human population suffer from this disease. Several drugs have been investigated to treat amebiasis; in 1912 emetine was discovered and it had good results in the treatment of this disease in humans. In 1956, metronidazole was discovered, and it is still considered the drug of choice in the treatment of amebiasis. It was not until 1975 that hemezol was used, having some advantages over treatment with metronidazole. Some of the drugs used in the treatment for amebiasis have proved to be effective against B. coli.

Injectable products can be one of the options in the treatment of intractable patients. It has to be pointed out that toxicity problems with dihydroemetine has been reported in the human literature. Secondary effects in humans include diarrhea (which can be mistaken as an exacerbation of the infection), and acute lesions in heart, liver, kidney, intestine, and muscle tissues. The main problem is the cardiotoxicity, which can be accumulative so the product should not be used for more than 15 days in a row and should not be repeated before 45 days have passed after the last treatment. For all these reasons, it should only be considered as a last option and should not be used in patients that have a suspected cardiac problem.

Hemezol has a direct action on Entamoeba histolytica trophozoites and cysts. It has not been reported as a treatment for balantidiasis. It is effective against trichomonads, giardias and amoebas. Hemezol is also effective against some aerobic bacteria (Campylobacter spp., and Haemophilus vaginalis) and against most anaerobic bacteria (Bacteroides fragilis, Fusobacterium spp., Peptococcus spp., Gaffkya spp., Peptostreptococcus spp., gram negative cocci, and Clostridium spp.). Hemezol has been reported to have less side effects than metronidazole or other imidazole derivatives. Oral treatment with hemezol has one disadvantage: it has an unpleasant flavor, so it has to be offered with palatable foods. Blood levels of oral hemezol remain higher than ones obtained with the injectable presentation; this is probably the reason why better results are obtained with the oral treatment.
Oxytetracycline’s major effect in the treatment of balantidiasis is probably the disruption of the normal bacterial flora of the intestine, upon which the protozoa feed. In some cases where invasive balantidiasis is already present, it is probable that the antiparasitic treatment alone will be ineffective, so surgical resection of the affected portions of the gastrointestinal tract is recommended.

It is difficult to decide when to treat an animal if *Balantidium coli* trophozoites are seen occasionally in direct fecal smears and there are no clinical signs of the disease; the rapid onset of diarrhea caused by the parasite cannot be predicted so it is not possible to give any treatment before this moment. Diarrhea and dysentery caused by *Balantidium coli* have improved with both injectable treatments but eradication of the parasite has not yet been obtained.

It is important to mention that the adult male gorilla developed enteritis during several periods in his life caused by different agents: *Salmonella* spp., *Shigella* spp., *Strongyloides* spp., *Balantidium coli*, *Entamoeba histolytica*, and *Enteromonas* spp., among others, and was successfully treated. On the last case, immunosuppression probably played an important role. Lead poisoning of unknown origin was present. Levels in liver were above 20 ppm which the literature reports as diagnostic. Low levels of lead in kidney tissues could have been due to the sample collection; it included cortex and medulla. Lead usually attaches to the cortex and levels in renal medulla are minimal. Paint from cages, the most common source of lead poisoning, could have been the source of lead. This individual was never seen to bite bars or mesh from the cage but he sporadically licked the bars. Paint from his last cage was supposed to be “ecological” paint with low lead levels and was also supposed to bind to bars without allowing any paint to come off. It is important to mention that this zoo is located in a city with significant levels of air pollution. One of the most common heavy metals which pollutes the air is lead. Lead is emitted into the atmosphere when combustibles are consumed by cars and industries. Lead could have been slowly accumulating in different tissues (like bone) and released into the blood stream when disease was present. Some of the signs of lead poisoning like weakness and paralysis of legs and arms (peripheral neuritis) were seen in this gorilla. A reported case of lead poisoning occurring in a gorilla was characterized by paralysis of the lower part of the arms.

**ACKNOWLEDGMENTS**

The authors wish to thank David Berrón H., Gustavo Ramírez Z., Miguel Peña R., Jose Luis González M., Alfonso Delgado O., Carlos Sánchez R., Adriana Gallegos T., Jorge Guzmán R. (members of the Veterinary Staff of Chapultepec Zoo) and Raul Villar (Senior Keeper) for their assistance in the treatment of all the gorillas and their collaboration with this study. Special thanks to Ma. Claudia Suárez de Gual for her help in translating and correcting this manuscript.

**LITERATURE CITED**


ABERRANT DIROFILARIASIS IN THE PALE-HEADED SAKI MONKEY (*Pithecia pithecia*)

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Animal Internal Medicine Clinic, 2353 Royal Lane, Dallas, TX 75220, USA

Abstract

The Dallas Zoo has exhibited pale-headed saki monkeys since the 1950s. Four animals, imported in 1982 through a Florida dealer, died in 1982 (n=2), 1983 (n=1) and 1985 (n=1). Three of these specimens presented with multiple organ disease and evidence of microfilaremia at necropsy. Specific identification was not possible as adult nematodes were never found. Whole blood samples were subsequently screened in the surviving saki monkeys (n=5).

Large numbers of microfilaria were found in one pair in 1985 and another pair in 1987. All animals were screened negative by 1989. Four of these animals have since died but none demonstrated microfilarial infections. In 1989, the troop was shipped from Dallas and included a young, Dallas Zoo born male that eventually transferred to Florida in May 1992. This animal returned to the Dallas Zoo collection to begin a new troop in 1994.

During the quarantine exam for this male, a whole blood sample was screened due to the prior history for this species in the collection but was microfilaria negative. The animal completed quarantine uneventfully and remained healthy on exhibit. A year later, this animal was again screened when new saki monkeys (1.1) were obtained. In all animals a standard exam and whole blood screening were performed; additionally, an occult *Dirofilaria immitis* antigen test (Pet Chek, IDEXX Laboratories, Incorporated, One IDEXX Drive, Westbrook, ME 04092, USA) was performed for detecting possible microfilaria (source). All three animals were negative on direct screen but the male was positive by the occult test.

Further diagnostics were performed with this animal including repeated occult testing (Dirocheck, Synbiotics Corporation, 11011 Via Frontera, San Diego, CA 92127, USA), thoracic radiography, and hemograms. The test results were supportive of a diagnosis of dirofilariasis in an aberrant host. Ultimately, an echocardiogram demonstrated parallel linear opacities within the right ventricle consistent with adult *D. immitis*.

Patent canine dirofilariasis has not been reported in the nonhuman primate literature and, in human medicine, infection with this parasite typically presents as pulmonary emboli. Therefore, this aberrant infection was suspected as most comparable to feline heartworm disease (i.e., lower host infection rate in endemic area, decreased life span of nematode in aberrant host). Treatment options for affected felines center on minimizing clinical signs and relying on the host to clear the disease; adulticide treatment in felids often produce more side effects than reliable elimination of the nematodes. Without clinical signs in this saki monkey, no specific treatment was elected.

The animal remained free of clinical disease and was successfully introduced to the new specimens.
At 6 mo post diagnosis, the affected animal was rechecked. Although the occult antigen test remained positive, an echocardiogram did not find evidence of patent dirofilariosis.

Resumen

El zoológico de Dallas ha exhibido monos saki cabeza pálida desde 1950. En 1982 fueron importados cuatro ejemplares a través de un comerciante de animales con Florida. En 1982 murieron dos, en 1983 uno y en 1985 uno. Tres de ellos presentaron una enfermedad que afectó múltiples órganos y la evidencia de microfilaremia en la necropsia. La identificación específica no fue posible ya que los nemátodos adultos nunca fueron encontrados. Muestras de sangre completa fueron subsecuentemente analizadas en los sakis sobrevivientes.


Durante los exámenes de la cuarentena, se obtuvo una muestra de sangre completa para analizarla debido al historial clínico en esta especie, pero los exámenes de microfilaria fueron negativos. El animal completó su cuarentena sin contratiempos y permaneció saludable en exhibición. Un año más tarde, el animal fue de nuevo sometido análisis cuando se adquirió una pareja más de sakis. En todos los animales se verificó un examen general y un análisis de sangre completa; adicionalmente se realizó una prueba de antígenos ocultos de *Dirofilaria immitis* (Pet check, IDDEX Laboratories Inc., One IDDEX Drive, Westbrook, Maine, 04092, USA) para detectar posibles microfilarias (el origen). Los tres animales fueron negativos en el examen directo, pero el macho resultó positivo en la prueba oculta.

Pruebas diagnósticas adicionales se realizaron en este animal, mismas que incluyeron pruebas de parásitos ocultos (Dirocheck, Synbyotics, San Diego, CA 92127, USA), radiografías torácicas y hemogramas. Los resultados fueron concordantes con el diagnóstico de dirofilariosis en un huésped aberrante. Finalmente, un ecocardiograma demostró opacidades lineales paralelas dentro del ventrículo derecho que eran consistentes con adultos de *Dirofilaria immitis*.

La dirofilariosis canina no ha sido reportada en la literatura de primates no humanos, ni en la medicina humana, y la infección con este parásito típicamente se presenta como una embolia pulmonar. Sin embargo esta infección aberrante se sospechó más bien relacionada con la dilrofilariosis felina (la tasa de infección de huéspedes es baja en áreas endémicas, y hay una longevidad disminuida del nemátodo en huéspedes aberrantes). Las opciones de tratamiento para felinos afectados se centran en la minimización de signos clínicos y recaen en el huésped para la erradicación de la enfermedad; el tratamiento para eliminar parásitos adultos en felinos a menudo produce más efectos colaterales que la eliminación adecuada de nemátodos. En vista de que no existían signos clínicos en este mono Saki no se llevó a cabo ningún tratamiento.

El animal permaneció clínicamente libre de la enfermedad, y se introdujo satisfactoriamente con los
nuevos especímenes. A los seis meses del diagnóstico el animal se volvió a revisar. No obstante que la prueba del antígeno oculto permaneció positiva, en el ecocardiograma no se encontró evidencia de una dirofilariosis patente.
MANAGEMENT OF A RECTAL PROLAPSE IN A JUVENILE BLACK RHINOCEROS

(*Diceros bicornis*)

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Abstract

An 11-mo-old female black rhinoceros (*Diceros bicornis*) presented with an 18 cm long rectal prolapse. Within 2 hr, condition of the prolapsed tissue deteriorated which required surgical correction. Postoperative care included feeding mineral oil and fruits to keep the stool consistency soft, and anti-inflammatories. The animal was asymptomatic for 3 mo. One short episode of prolapse was observed at 3 mo. It resolved spontaneously but mineral oil was reinstituted prophylactically. No etiology could be determined for the prolapse.

Resumen

Un rinoceronte negro hembra de 11 meses de edad presentó un prolapso rectal de 18 cm. de longitud. Dos horas después la condición del tejido prolapso se deterioró al grado de requerir corrección quirúrgica. Los cuidados postquirúrgicos incluyeron alimentación con aceite mineral y frutas para mantener suave la consistencia de las heces. Además, se administraron anti-inflamatorios. El animal estuvo asintomático durante tres meses. Un pequeño prolapso fue observado a los 3 meses. Este se resolvió espontáneamente pero el aceite mineral fue reinstituido profilácticamente. La etiología del prolapso no ha sido determinada.

Case Report

An 11-mo-old female black rhinoceros (*Diceros bicornis*) presented one morning with an acute onset of rectal prolapse. The juvenile was housed with her dam; they had access to a yard during the day and to a cement barn at night. There was no previous history of medical problem except intermittent soft stools. The soft stools were attributed to various “treats” (apples, bananas, corn-on-the-cob) that had been recently eliminated from their diet. Fecal analysis for ova and parasites had been consistently negative.

Visual examination revealed an 18 cm long rectal prolapse; the mucosa was bright red in color with no sign of necrosis. Fresh blood was observed on the tail. The animal appeared to be straining intermittently and feces were observed passing through the center of the prolapsed mucosa. The rhinoceros was otherwise alert, bright, and responsive.

Food and water were withheld in the event of a surgical intervention. Initial treatment was aimed at reducing the swelling and allowing the animal to retract the mucosa on its own. It included
application of a 50% dextrose solution (The Butler Co., Columbus, OH, USA) using a spray bottle. This was done twice at 30 min intervals with no significant improvement. Preparation H® (Whitehall Laboratories, Incorporated, New York, NY, USA) in mineral oil was also sprayed, but with no visible effect. Two hours after the initial discovery of the problem, the prolapse increased to 30 cm and shortly after, a few black spots started to appear on the rectal mucosa, indicating loss of vascularization. Immobilization for examination and reduction was elected.

The dam was sedated using a combination of butorphanol (Fort Dodge Laboratories, Incorporated, Fort Dodge, IA, USA) 30 mg i.m. and detomidine (Pfizer, Incorporated, West Chester, PA, USA) 20 mg i.m. via dart in the neck. Eighteen minutes later, she was walked into an adjacent pen and separated from her offspring. The juvenile was induced with carfentanil (Wildlife Laboratories, Incorporated, Fort Collins, CO, USA) 0.9 mg i.m. via dart in the neck. She became ataxic within 3 min; head pressing occurred at 4 min. A blindfold was placed at 6 min and the animal became recumbent. She was positioned in lateral recumbency with several pads under her shoulder to prop her up. The neck was extended and oxygen lines were placed into each nares (15 L/min). Two intravenous catheters were placed, one in the left radial vein and the other in the right ear vein.

Examination of the prolapsed tissue suggested that the mucosa was viable and replacement was elected over resection. Direct application of granulated sugar to the inflamed mucosa reduced the swelling within 7 min and allowed manual reduction of the prolapse. Two layers of purse string sutures were placed using #2 Dexon (Davis and Geck, Incorporated, Manati, PR). A 6 cm diameter opening was secured.

During the procedure, the animal received 250 mg flunixin meglumine (Banamine®, Schering-Plough Animal Health Corporation, Kenilworth, NJ, USA) i.v., 3,600 units vitamin E (Schering-Plough Animal Health Corporation, Kenilworth, NJ, USA) i.m., 12.0 ml BoSe® (Schering-Plough Animal Health Corporation, Kenilworth, NJ, USA) i.m., 5.4 million units penicillin G benzathine/procaine (The Butler Company, Columbus, OH, USA) i.m., 1,500 units tetanus antitoxin (Sanofi Animal Health Incorporated., Overland Park, KS, USA) i.m., and 1.0 ml tetanus toxoid (Bayer Corporation, Shawnee Mission, KS, USA) i.m. Blood was collected for complete blood count, chemistry panel and serum banking. Reversal was achieved with 40 mg (¼ i.v., ¾ s.c.) naltrexone (Wildlife Laboratories Incorporated, Fort Collins, CO, USA). The rhinoceros was standing within 4 min. The mother was partially reversed with yohimbine (Lloyd Laboratories, Shenandoah, IA, USA) 48 mg i.m. and was reunited with her baby shortly after.

Post surgical treatment was aimed at reducing inflammation (flunixin meglumine, 250 mg p.o. b.i.d.) and maintaining soft stool consistency (mineral oil 1 L mixed with pelleted diet, bananas and apples as laxatives). Tenesmus and diarrhea occurred for the first 48 hr. Tenesmus continued for an additional 18 hr without producing feces. Mineral oil was increased to 1.2 L b.i.d. and stools were passed again. No straining was observed after day 4. A short prolapse (2-3 cm long) was observed on postoperative days 7 and 8 with very loose stools. Mineral oil was decreased to 1 L b.i.d. Flunixin meglumine was discontinued on day 11. By day 21, the stools had a normal consistency and mineral oil was decreased to 250 ml b.i.d. Mineral oil was decreased to 125 ml b.i.d. on day 23, to 125 ml s.i.d. on day 25 and discontinued on day 36. At this time, fecal consistency was normal and no prolapse was observed. On day 97, the keeper reported an 8 cm prolapse of bright red mucosa. They washed her twice and the prolapse retracted spontaneously within 5 min. She was placed under
observation for 48 hr and 1 L of mineral oil was given s.i.d. on pellets. The recurring prolapse decreased progressively over the next 48 hr and resolved by the third day.

Discussion

Four types of rectal prolapse (I-IV) have been described, based on the severity of the condition and the structures involved (Table 1). In this case, a type III was suspected and evolved rapidly to a type IV (complete prolapse with intussusception of rectum through the anus). Standing reduction with sedation is recommended in domestic animals. However, general anesthesia was elected for easier access and manipulation of the animal. The reduction technique used was similar to the one described for domestic animals. However, because of the noncompliance of the patient, it was impossible to open the purse string four times per day to allow for defecation. The purse string is usually removed 2-4 days postoperatively. Instead, we allowed an opening of about 6 cm and used an absorbable material. Laxatives and anti-inflammatory drugs were administered as excessive straining was anticipated. Because of the length of the sphincter, two layers of sutures were placed 3 cm apart. No mucosal resection was necessary since the mucosa appeared viable and was easily reduced at the time of reduction.

Rectal prolapse may occur secondary to parasitism, pneumonia, dystocia, space occupying lesions, obesity, enteritis, colitis, liver disease, etc. Prognosis for rectal prolapse depends on early identification, rapid intervention, and on the amount of damage to the mucosa. Complications include recurrence, rectal strictures, pararectal abscess and obstipation. In our case, the cause of the prolapse remains obscure but the intermittent, chronic soft stool may be at the origin of the problem. Irritation of the colon by protozoans has also been considered.

LITERATURE CITED

Table 1. Classification of rectal prolapse.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>rectal mucosal and submucosal tissue protrudes through the anus</td>
</tr>
<tr>
<td></td>
<td>-small circular swelling</td>
</tr>
<tr>
<td>Type II</td>
<td>complete prolapse with eversion of part or all of the ampulla recti through the anus</td>
</tr>
<tr>
<td></td>
<td>-small circular swelling</td>
</tr>
<tr>
<td>Type III</td>
<td>complete prolapse with invagination of the small colon into the rectal canal but</td>
</tr>
<tr>
<td></td>
<td>it does not extend past the anus</td>
</tr>
<tr>
<td></td>
<td>-appears larger than Type I and II</td>
</tr>
<tr>
<td>Type IV</td>
<td>complete prolapse with intussusception of the rectum through the anus</td>
</tr>
<tr>
<td></td>
<td>-appears as a long tubular structure hanging from the anus.</td>
</tr>
</tbody>
</table>
CONGESTIVE HEART FAILURE SECONDARY TO MULTIPLE CONGENITAL HEART DEFECTS IN A SIX-DAY-OLD BLACK RHINOCEROS (*Diceros bicornis*)

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Abstract

Persistent truncus arteriosus (PTA) is a rare congenital heart defect (CHD) defined by one arterial trunk arising from the ventricular outflow tract which supplies the pulmonic, coronary and systemic circulations. The PTA usually overrides a ventricular septal defect (VSD). A 6-day-old black rhinoceros was diagnosed with PTA, VSD, and atrial septal defect (ASD) which lead to congestive heart failure and death. Persistent truncus arteriosus occurs when the embryonic aorticopulmonary septum fails to divide the truncus arteriosus into an aorta and pulmonary artery. The cause of PTA appears to be multifactorial, although in some breeds of animal and some human families it appears to be due to monogenetic inheritance. The pathophysiology of PTA ultimately leads to pressure and volume overload of the ventricles and congestive heart failure. This report represents the first documented case of PTA in a black rhinoceros.

Resumen

La persistencia del tronco arterial (PTA) es un defecto cardiaco congénito (CHD) poco común definido por un tronco arterial proveniente del tracto ventricular que provisiona a las circulaciones pulmonar, coronaria y sistémica. La PTA usualmente anula un defecto del septo ventricular (VSD). Un rinoceronte negro de seis días fue diagnosticado con PTA, VSD y defecto del septo atrial (ASD) que condujo a una falla cardiaca congestiva y muerte. La persistencia del tronco arterioso ocurre cuando el septo aórtico pulmonar embrionario falla en la división del tronco arterial a la arteria aorta y la arteria pulmonar. La causa de la PTA parece ser multifactorial aunque, en algunas razas de animales y algunas familias humanas, aparentemente es debido a herencia monogenética. La fisiopatología de la PTA a la larga conduce a la sobrecarga de volumen y de presión en los ventrículos y falla cardiaca congestiva. Este reporte representa el primer caso documentado de PTA en rinoceronte negro.

Introduction

Congenital heart defects are not uncommon in animals and man. In man, CHD are the most common form of birth defect. There are several types of CHD including patent ductus arteriosus, atrial and ventricular septal defects, conotruncal abnormalities, valvular abnormalities, and abnormal location of the entire heart (ectopia cordis) which occur at varying rates in animals and man. Clinical signs associated with CHD range from asymptomatic to incompatible with life depending on type and severity of the anomaly.
Persistent truncus arteriosus is a relatively rare CHD and consists of a single arterial trunk arising from the ventricular aspect of the heart. Usually there is an associated ventricular septal defect which allows the trunk to receive blood from both ventricles and supply the coronary, pulmonary, and systemic circulations. Persistent truncus arteriosus was first reported in man in 1798. Since then, PTA has been reported rarely in various species.

In this case, PTA occurred along with VSD, ASD, and hypoplastic pulmonary arteries with secondary right and left ventricular dilation. This combination of congenital cardiac defects has been rarely reported in domestic species and has never been reported in the black rhinoceros.

Case Report

On 18 December 1995, at approximately 1945 hr a male black rhinoceros was born at Caldwell Zoo. The birth, which was monitored via surveillance camera, was uneventful. The calf stood within 2 hr and nursed within 2.5 hr of birth. Initial and subsequent (12 hr post-birth) visual exams of both dam and offspring were within normal limits.

Due to past experience of a rhinoceros birth at the zoo, it was decided to take a “hands off” approach with respect to management of dam and calf. Since repeated visual exams were normal, and calf and dam appeared well-adjusted to each other, initial care provided to the calf was limited to spraying iodine on the umbilical stump two to three times per day. The calf and dam initially did well. The calf was nursing normally, often, and appeared to be gaining weight.

On the afternoon of 23 December 1995, zookeepers noted an increased respiratory rate in the calf. Visual exam revealed a bright, alert and responsive calf who was nursing and following its mother normally. Mucous membranes were noted to be pink. The respiratory rate was 84 breaths per minute and respiratory character was rapid and shallow. Differential diagnoses at that time included: pulmonary hemorrhage secondary to accidental trauma from dam; pneumonia or septicemia secondary to failure of passive transfer; diaphragmatic hernia, congenital or acquired secondary to trauma; and congenital heart or lung disease.

It was decided to monitor the calf overnight and if the condition remained unchanged or worsened, the calf would be separated from its mother in the morning (24 Dec 1995) for physical exam, thoracic radiology, blood work and potential treatment. Zookeepers monitored the dam and calf until 2350 hr and noted nothing unusual. The calf was found dead at 0700 hr 24 December 1995.

Gross necropsy revealed a persistent truncus arteriosus, delineated at its base by three semilunar valves. The PTA was in communication with the right ventricle, but was slightly overriding the high 2.1 x 3.0 cm, oval, VSD. The hypoplastic pulmonary arteries, of equal diameter, branched off of the caudolateral aspect of the truncus 4.0 cm distal to its origin. There was also a multifenestrated, round, 1.4 cm diameter, interatrial septal defect. The left and right ventricular chambers appeared enlarged. The atrioventricular valves and chordae tendineae were unremarkable.

Other lesions included 300 ml of pericardial effusion, a partially foam filled trachea, severe pulmonary edema, and 500-700 ml of peritoneal fluid. Fluid analysis of pericardial and peritoneal fluids were characterized as modified transudates. There were no histopathologic lesions of major
organs. Bacteriology of the above mentioned fluids and lung revealed few contaminant bacteria. All other organ systems were within normal limits. The baby was in good flesh and weighed 47.7 kg (105 lb) at necropsy.

Discussion

Conotruncal abnormalities include a group of CHD in which malformations of the ventricular outflow region occur. Persistent truncus arteriosus, tetralogy of Fallot and subarterial ventricular septal defects are types of conotruncal abnormalities. Persistent truncus arteriosus results from a defect in formation of the aorticopulmonary septum (Fig. 1). The aorticopulmonary septum also participates in the formation of the membranous portion of the ventricular septum, thus it is logical that most PTA occur along with a VSD. The cause of conotruncal abnormalities appears to be multifactorial; however, there are reports in certain breeds of animals and in some human families of monogenetic inheritance.

The consequence or pathophysiology of PTA results in mixing of oxygenated and deoxygenated blood within the atria (if an ASD is present, as in this case) and ventricles (in most cases) (Fig. 2). Also, the pressures within the ventricles are elevated, especially within the right ventricle, because both ventricles are working against the combined pressures of the systemic and pulmonary circulations. Blood entering the PTA has a relatively low oxygen content and high carbon dioxide content. Therefore, the coronary and systemic circulations receive hypo-oxygenated blood. As the animal grows, the increased tissue oxygen demand is not met which sets up a vicious cycle of cardiac compensatory mechanisms, increasing the workload on an abnormal heart. This set of consequences leads to volume and pressure overload of the ventricles and eventually to congestive heart failure.

In conclusion, this is the first report of PTA, a rare form of CHD, in a black rhinoceros. Although there are no published reports of CHD in the black rhinoceros, two cases of full siblings with CHD have been identified (D. Agnew, personal communication). One female died at 10 days of age with a tricuspid valve defect and one male died at roughly 2.5 mo of age with a VSD and Salmonella enteritis. Moreover, another full sibling of the above two cases died at 18 days of age with mineralizing cardiomyopathy and mycotic pneumonia; however, these lesions do not appear to be congenital. Therefore, in cases where more than one full sibling is born with a CHD, serious consideration should be given to continued breeding of the parents to each other.

ACKNOWLEDGMENTS

The authors thank Caldwell Zoo for providing funding for this case report. The authors also thank the Graphics Departments at Caldwell Zoo and Texas A & M University, the Texas A & M University Medical Sciences Library staff, Sara Dean, Secretary, and the Large Mammal staff at Caldwell Zoo for their assistance with this case.

LITERATURE CITED

Figure 1. Schematic diagram demonstrating normal development of the aortico-pulmonary or spiral septum (from Latshaw 1987).
Figure 2. Diagram of the circulatory system in a normal (A) and in persistent truncus arteriosus (B). The luminal shading represents probable relative oxygenation (after Taussig 1960).
BONE MARROW COLLECTION IN THE ASIAN ELEPHANT (*Elephas maximus*)

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Abstract

The collection of bone marrow is a common practice for the diagnosis of a variety of disease in mammals and birds. These include diseases which can cause any type of blood dyscrasias, anemias, thrombocytopenias, and/or leukopenias. Examples of a few of these diseases include feline leukemia, feline lymphosarcoma, lymphoma, bovine leukosis, aplastic anemias of many etiologies, equine infectious anemia, and various types of leukemia. Dependent upon the species involved, marrow is routinely collected from a variety of locations, such as the crest of the tibia in psittacines, or the ribs, iliac crest or sternum in equids, to the dorsal spinous processes or sternum in the cow. Although in these species there is seldom a problem with collection, obtaining marrow from some of the megavertebrates such as the elephant or rhinoceros proves to be problematic due to both the restraint of the animals and their size. This paper describes a simple and effective way to obtain quality bone marrow samples from an Asian elephant (*Elephas maximus*) and thus likely applicable to other megavertebrate species.

Resumen

La recolección de médula ósea es una práctica común para el diagnóstico de una variedad de enfermedades en mamíferos y aves. Estas enfermedades incluyen aquellas que pueden causar algunos tipos de discracias sanguíneas, anemias, trombocitopenias y/o leucopenias. Ejemplos de unas pocas de estas enfermedades incluyen leucemia felina, linfosarcoma felino, linfoma, leucosis felina, anemias aplásticas de varias etiologías, anemia infecciosa equina y varios tipos de leucemia. Dependiendo de las especies involucradas, la médula es rutinariamente recolectada de una variedad de sitios, como la cresta de la tibia en psitácidos, las costillas, cresta ilíaca o esternón de los caballos, hasta la protuberancia de la espina dorsal o esternón de los bovinos. Aunque en esas especies rara vez hay problema con la recolección, la obtención de médula de algunos de los megavertebrados como elefantes y rinocerontes puede ser problemática, tanto debido a la contención de estos animales, como a su talla. Este documento describe un método simple y efectivo para obtener muestras de médula ósea de buena calidad de los elefantes asiáticos y que puede ser aplicable a megavertebrados.
**Procedure**

An adult, 47-yr-old, female, Asian elephant was placed in left lateral recumbency by trainer staff. Upon laying in left lateral recumbency, a catheter was placed in the ear veins and in injection of midazolam, 100 mg, followed by carfentanil, 3 mg i.v., induced plane III anesthesia. The heart and respiratory rate were monitored throughout the procedure.

The curvature of the last rib was palpated and we estimated its most caudal portion of each arc. A dorsally projecting tangential line was estimated and at the point where it would bisect the dorsal point of the back was considered to be the first lumbar vertebrae, as this was the case noted on articulated elephant skeletons. The top of the dorsal spinous processes were palpated and the skin was then prepped with a surgical scrub regimen of alternating povidone-iodine solutions with 70% alcohol. The area was then infused with 12 ml 2.0% lidocaine using an 18-ga x 1.5 in needle, to provide local analgesia. The lidocaine was slowly injected as the needle was advanced down to the point of contact with the plateau of bone at the most dorsal aspect of the dorsal spinous process. The lidocaine was allowed to react with the tissue for approximately 5 min.

The area was surgically scrubbed again. After donning sterile gloves, a 3 cm stab incision was made with a #10 scalpel blade at the point for placement of a Jamshidi needle (Baxter Hospital Supply Division, Deerfield, IL, 60015, USA). The Jamshidi was inserted into the incision site and advanced until it touched bone. A sterile hammer was used to drive the instrument into the bone. As the needle advanced, there was a notable change in the pitch of the sound of the hammering as the Jamshidi passed from cortical to cancellous bone thus indicating it had passed into the marrow cavity.

A 25 ml syringe had been prepared by washing its chamber with ethylenediaminetetraacetate (EDTA) and allowing approximately 0.5 ml to remain in the syringe. The sterile syringe was then attached to the Jamshidi and in a pulsing fashion, marrow was aspirated into the syringe. Small aliquots were then placed on a clean slide. The slide was then tilted to one side and excess fluids blotted at the bottom edge with a 4 x 4 cm gauze sponge. Another slide was then touched to the first and the granular appearance of the marrow noted. Highly granular area slides were then smeared as in preparing a blood smear.

Several aspirates were made from each site and multiple slides from each aspirate. A representative slide from each aspirate was stained with Diff Quik and examined for quality of the sample. Samples with sufficient marrow were then fixed with xylene and cover slips were placed. These slides were then stained with special stains.

**Summary**

This procedure proved to be a quick and apparently pain free procedure for the collection of bone marrow in Asian elephants. Although there are anatomic variations within a species and certainly between species, it is hoped the procedure could be applied to other megavertebrates, such as the African elephant (*Loxodonta africana*), white rhino (*Cerotherium simum*), and possibly most...
importantly, the black rhino (*Diceros* spp.). This project will continue to be done on other animals as the opportunity arises and it is felt with the ease and speed of the procedure, it may become a viable diagnostic aid in many blood disorders. Other sites for collection are also currently under investigation.

**ACKNOWLEDGMENTS**

The authors would like to thank David Blasko and the other elephant handlers of the Marine World Africa USA Elephant Encounter for the help provided during the procedure. We would also like to thank Marine World Africa USA for financial support of this project.
VAGINAL VESTIBULOTOMY IN AN ASIAN ELEPHANT (*Elephas maximus*)

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Abstract

Due to its dimensions, dystocia in elephants presents a difficult problem. This paper describes the delivery of a dead calf by surgical intervention. A vestibulotomy was performed under local anesthesia. Complications in wound healing resulted in a permanent fistula of the vestibulum. The difficulties in decision making and the interpretation of clinical signs are discussed.

Resumen

Debido a sus dimensiones, la distocia en elefantes presenta un gran problema. Este trabajo describe la expulsión de una cría muerta por medio de una intervención quirúrgica. Una vestibulotomía fue realizada bajo anestesia local. Las complicaciones en la cicatrización de la herida resultaron en una fístula permanente en el vestíbulo. Las dificultades en la toma de decisiones y la interpretación de los signos clínicos son discutidos.

Introduction

Dystocia in elephants is not uncommon in zoos. Cesarian sections have been performed on a few occasions, resulting in the death of both mother and baby. There is one report of a vestibulotomy performed on an Asian elephant (*Elephas maximus*) under general anesthesia. In that case recovery of the mother was disturbed by life threatening complications originating from a rupture in the dorsal vaginal wall. This report describes a vestibulotomy performed under local anesthesia.

Case Report

A 29-yr-old, approximately 3,750 kg, primiparous Asian elephant was monitored 24 hr per day by a video camera during the last 2 mo of pregnancy in 1993. Retrospective study of the video showed that labor had started unnoticed on March 3rd at 2100 hr, 677 days after conception. Labor activities were observed by the keeper at 0800 hr the following morning. The presence of the amniotic sac was visible halfway up the urogenital canal. A 20x30 cm piece of fetal membrane tissue was found in the enclosure. At this time the female was given 480 mg zuclopentixol p.o. (Cisordinol 40, Lundbeck, 1101 BM, NL). Contractions and labor activities were very heavy and frequent until 1000 hr (one per 3-5 min). After 1000 hr the frequency diminished to one per 10-15 min.

At 1200 hr the animal was chained on one foreleg and 50 IU of oxytocin (Oxytocine-S, Intervet, 5831 AP, NL) s.c. behind the ear to stimulate uterine contractions. A blood sample was taken to measure the plasma calcium level. This was 9.76 mg/dl. Rectal palpation clearly indicated that the calf was in a backwards position. The calf could easily be pushed backwards in between contractions. No reactions of the calf were felt during these manipulations. Palpation of the birth
canal was not tolerated at that time. The animal responded well to oxytocin. The uterine contractions and labor activities intensified during the next 30 min, but no progress was made.

At 1300 hr another 400 mg zuclopentixol was given. At 1400 hr, an i.v. infusion of 750 ml of Ca-Mg-borogluconate (Ca-Mg-infuus, AUV, 5431 SL, NL) was administered, containing 12 g calciumborogluconate. Another 50 IU of oxytocin s.c. was given at the same time. The keepers then managed to chain the animal on three of her legs and vaginal palpation became possible. Even after cutting the amniotic sac with a finger knife, the hind feet of the calf could not be reached, except during uterine contractions when the soles could be touched.

At 1600 hr 50 IU oxytocin was injected intravenously. A rubber rumen tube was advanced through the vestibulum and the tip of the tube was placed between the feet of the calf. Approximately 2 L of corn oil were flushed into the birth canal as a lubricant. Two people tried to push the hind legs into the vertical part of the vestibulum, to no effect, during the heavy labor activities that followed the intravenous oxytocin injection.

Local anesthesia was performed at 1700 hr by administering 5 injections of 20 ml lidocaine 2% + noradrenaline (A.U.V., 5431 SL, NL) intra-and subcutaneously in the midline of the perineum, starting 5 cm ventrally of the anus, with an interval of 10 cm. The perineum was brushed with a povidone iodine soap (Betadine scrub, Dagra Pharma B.V., 1112 AX, NL). A 25 cm long incision was made in the midline, starting 5 cm below the anus. The urogenital canal was located with the help of a rubber rumen tube that was inserted retrograde into the birth canal. The vestibulum wall was incised over this tube just below the anus. This incision was enlarged ventrally to a distance of 25 cm. The hind legs were not visible at that time and chains had to be fitted blindly. Parts of the thick amniotic sac had to be cut away for better attachment of the chains. No reaction from the calf was observed during these operations. Four people pulled the chains to achieve a better presentation of the hind legs in the wound. Nevertheless, the pelvic region of the calf became stuck in the maternal pelvis.

The chains attached to the calf’s hind legs were initially passed through the distal part of the vestibulum in an attempt to pull the legs through the natural birth canal. Pulling the chains essentially horizontally caused too much irritation on the vulva, so this procedure was abandoned.

The incision was then enlarged ventrally to 37 cm. Eight people, four on each hind leg, were allowed to pull. By pulling on one leg at the time and changing the direction during 45 min, the calf was rotated 90° and finally extracted. The umbilical cord could be palpated after the first 10 cm of the hind legs had passed the incision. The cord was twisted around the right hind leg and no pulsation was felt. The entire extraction time was about 30 min.

The vaginal/uterine wall was explored by manual palpation for lesions. No lesions were found in the birth canal. The complete placenta was pulled out within 2 min by flushing cold water into the uterus and pulling on the fetal membranes.

Amoxicillin (Clamoxyl, Beecham, 1185 TH, NL) was given at 5 mg/kg i.m. before closing the wound. At this time xylazine (Sedaject, Dopharma, 4940 AE, NL) was also given at a dose of 0.05 mg/kg i.m. to calm the animal during suturing of the wound.
The vestibulum wall was closed using atraumatic Dexon 0 (Cyanamid International, 4872 XL, NL). A nonperforating continuous suture, tied after every fifth stitch was made. No subcutaneous tissue was available for suturing. The skin wound was flushed with 10% diluted povidone iodine (Betadine, Dagra Pharma, 1112 AX, NL). The endodermis was sutured using atraumatic Dexon 1 with the same type of stitch. The skin was closed with Mersilene 4 (braided polyester, Ethicon, 2000, Germany) using 27 single stitches. The wound was sprayed with U.S.P. wound spray (Sanofi, 3144 EG, NL).

The xylazine sedation was reversed when the wound was closed, using RX821002A i.m. (experimental 2-methoxy derivative of idazoxan, Reckitt and Coleman Pharmaceutical Division, HU 87 DS Hull, UK) at a dose of 0.002 mg/kg as recommended by Kock. Ten minutes after injecting the reversal agent the elephant started eating.

The calf was a 110 kg female. This weight is within the normal range of 50-150 kg. The anterior height was 95 cm and posterior height was 105 cm which is slightly more then the 70-90 cm indicated by Benedict. At necropsy, numerous small hemorrhages indicative of asphyxia were found in the liver. No other gross lesions were found.

The animal was kept chained during the first 11 days after surgery to enable the parenteral administration of antibiotics. Postsurgical care consisted of spraying cold water on the perineal area every 2 hr during the first 2 days. From day 2-4 amoxicillin was given at a dose of 5 mg/kg i.m. s.i.d. On day 2 the animal had a reduced appetite, but by day 5 began eating normally again. Some swelling with a minor discharge was observed in the ventral third of the wound on day 3. A wound swab was taken for bacterial culture and an antibiotic sensitivity test.

On day 4, the results from the culture showed gram negative rods being sensitive only to gentamicin and enrofloxacin. Treatment was changed to enrofloxacin (Baytril 5%, Bayer, 3641 RT, NL) given at a dose of 1.33 mg/kg i.m. This treatment was continued until day 11 when the entire wound spontaneously opened. The body temperature, measured daily in fresh feces, remained between 36.5 and 37.5 °C throughout the recovery period.

The wound had to be sutured again under local anesthesia and xylazine sedation on four occasions at 8-12 wk intervals. The first attempt was made 8 wk after the vestibulotomy. The animal was given 480 mg zuclopentixol p.o. 1 hr prior to surgery. This changed her behavior in an undesired way; she became more alert and aggressive than was expected. Granulation tissue was removed and the wound was closed in two layers, using the same material used for the initial sutures.

The wound opened again within a week. The wound was cleaned again completely and the mucosa was separated from the underlying tissue. A nonperforating continuous stitch with thicker suture material, was used to close the wound; monofilamentous PDS-1 (Ethicon, 2000 Norderstedt, Germany) was used for the vestibulum, and braided PDS-1 for the submucosal/subcutaneous tissue. The skin was closed with a continuous mattress stitch with sheep’s Bühner tape (Ethicon) using a modified Gerlach’s needle. Each skin perforation was made 2-3 cm from the incision and protected by 3 mm thick rubber rings (3 cm diameter). The animal remained hobbled on both hind legs during the following 10 days. During this period she received 500 mg acepromazine (Vetranquil granulate, Sanofi, 3144 EG, NL) p.o. b.i.d., 50 mg butorphanol (Stadol, Bristol-Meyers, 1382 JZ, NL) p.o.
b.i.d. and 20 g amoxicillin (Amoxycilline-anhydrate, Dopharma, 4940 AE, NL) p.o. b.i.d.

The wound opened partly after a few days. Twelve weeks later a third attempt was made to close the two remaining fistulas. Only the mucosa of these vestibulum fistulas (5 cm and 0.8 cm respectively) were closed in three layers, using PDS-1.

Again these wounds opened after a few days. One more attempt was made to close the remaining fistulas, which were healing per secundum. A modified balloon catheter was inserted into the urethra during this intervention. The orificium urethrae could be reached by hand, just at the edge of the horizontal part of the birth canal. The balloon was filled with 50 ml of water. Only the vestibulum mucosa was stitched to reduce infection of the wound by accumulation of purulent material in the subcutaneous space. During the following 2 days all urine was passed through the catheter. On the third day, the urine passed through the wound again. The catheter has never been recovered. No more attempts to suture the wound have been made; to date two fistulas, 10 mm and 2 mm respectively are still present.

**Discussion**

When veterinary intervention is required in the birth of an elephant is difficult to determine exactly. Schmidt indicated that labor is usually completed within 1 hr after contractions are seen, but may extend over a 2-3 days period.6,7 Surgical intervention in the case of a Hannover Zoo elephant was performed after 65 hr. 2,3 The umbilical cord was twisted around a hind leg in that case as well. During delivery, pressure in the pelvic canal may result in compression of the umbilical cord, causing asphyxia and the death of the calf. Because of the anatomy of the cow and calf this complication can not be recognized until the extraction is almost completed.

The first 11 hr of labor of the Rotterdam cow were missed, despite a video surveillance. The likelihood that the calf was still alive when veterinary assistance was requested was small given that heavy labor activities had not resulted in significant progression of the calf’s passage. It was not clear how the piece of fetal membranes found at 0800 hr had been separated from the amniotic sac. It is possible that the elephant in the stall adjacent to the maternity stall had managed to tear it off with her trunk. The resulting exposure of the amniotic fluid to external microbial flora would have been a possible threat for infection of the calf had it been alive.

Dittrich2 reported the use of a dose of 400 IU oxytocin i.v. to deliver a stillborn calf after 40 hr of labor. In the Rotterdam cow a dose of 50 IU of oxytocin (0.013 IU/kg s.c.) induced strong uterine contractions, but when repeated subcutaneous administration proved to be insufficient for natural delivery of the calf, one intravenous injection of the same dose was given. The intensity of the contractions could not be measured, but uterine action followed almost immediately after administration and lasted for about 30 min. The dose of 50 IU oxytocin s.c. administered to this cow seemed to be the correct dose. Higher doses may have carried the risk of a uterine rupture or displacement of the placenta, resulting in fetal death.

The blood calcium level found during labor was low (9.76 mg/dl), but in the normal range of 10-11 mg/dl (MedARKS). It was nevertheless decided to administer calcium ions to make sure that hypocalcemia would not complicate the extraction of the calf.
Lang discussed possible methods to perform a vestibulotomy in 1963, but did not need to practice it. This surgical intervention was performed for the first time in Hannover. However, the animal had then been in labor for more than 2 days, making the prognosis for a quick recovery poor. The complication of a vaginal rupture resulted in a serious threat to the cow’s life. In my opinion, quick intervention, e.g. a vestibulotomy, must be considered when injection of oxytocin (s.c., i.m. or i.v.) results in increased labor activities and both legs can be palpated rectally, but when no progress in the calf’s passage occurs. In an nonaggressive animal, this can be performed under local anesthesia only. This is highly desirable as the cow must remain standing and fit to take maximal advantage of uterine contractions and gravity. A standing posture facilitates the operation significantly, thus in the absence of a crush device a low dose of a sedative should be given to a nonpliable animal for security reasons.

Placement of chains to both hind legs is highly recommended. A third ring positioned exactly between the two rings for the hind leg chains should be fixed in the floor of a maternity enclosure. This extra ring can be used to pass the ropes that are tied to the chains on the calf’s legs. The use of a catrol for each rope would enable pulling in a ventral direction, which might make it possible to extract the calf through the entire urogenital tract, which would require a much smaller incision. It would also avoid the obstruction formed by the skin fold ventrally of the anus, which really makes extraction through the incision more difficult.

The cow at Rotterdam Zoo was given 480 mg zuclopentixol p.o. at 0800 hr because of her aggressive character. This dose was the same as that given to another elephant in this zoo in order to separate her from her 2 yr calf. Although the effect is difficult to measure, all of the keepers involved considered the results on both occasions positive. The behavior of both cows remained normal, while vocalization and other expressions of excitement basically were suppressed. It is important to note that when zuclopentixol was administered again 8 wk later it seemed to have the opposite effect, as if the animal had lost her fear.

Xylazine was given prior to suturing procedures, once expulsion efforts by the mother were no longer required.

A postsurgical complication encountered in this case was the healing of the wound. Two factors play a role:

1. properties of the skin

As in the Hannover case, stitches in the perineal area cut completely through the skin (pers. comm. Brandt). The continuous movements of the cow’s hind legs and her tail, as well as the lack of elasticity and the heavy weight of the thick perineal skin make healing per primam very difficult, if not impossible. Secondary healing takes place, in the Hannover case resulting in a fistula of approximately 5 cm after 9 yr. At the time of writing, the Rotterdam animal has a 10 cm long skin scar and two vestibular fistulas approximately 10 mm and 2 mm in length respectively. These fistulas don’t seem to bother the animal. Although it might go against the grain with every serious veterinarian, it seems better to me to suture only the vestibulum in 2 or 3 layers, leaving the skin wound open.
2. pressure of the urine flow

It is very important to prevent urine passing through the wound. Once the vestibulum wall has healed per primam, the skin wound will heal quickly per secundum. Insertion of an adapted balloon catheter in the urinary bladder during healing of the vestibulum wound is strongly recommended. This can probably be most easily done immediately after extraction of the calf, when the distance from the wound to the bladder is minimal.

ACKNOWLEDGMENTS

I would like to thank Catherine King for correcting my “American” English.

LITERATURE CITED

PATHOLOGIES PRESENTED IN TWO CAPTIVE BAT COLONIES RELATED TO PROBLEMS WITH THE AIR EXCHANGE

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Abstract

Several deaths occurred during the course of 1 yr in two captive colonies of Jamaican fruit bats (*Artibeus jamaicensis*) and common vampires (*Desmodus rotundus*). Clinical signs started after a serious stressful situation that occurred due to a high CO₂ concentration; no adequate air exchange was available inside their enclosures. This brought about a state of immunosuppression which lead to different problems, from the manifestation of a dormant disease (rabies), to signs of intoxication, to the proliferation of opportunistic bacteria such as *Enterobacter agglomerans*. The hemograms showed a leukopenia with lymphopenia. Histopathology showed pulmonary congestion and intraseptal pulmonary edema, the presence of protein in the lumen of some renal tubules, and some degenerative changes in most of the hepatic cells, suggestive of fat.

Resumen

Durante un periodo de un año se presentaron varias muertes en dos colonias de *Artibeus jamaicensis* y *Desmodus rotundus*, mantenidas en cautiverio, los cuadros clínicos presentados fueron desencadenados por una grave situación de estress dentro de las dos colonias debido a una alta concentración de CO₂, producto de un mal intercambio de aire dentro de los albergues, lo cual originó un estado de inmunodepresión que desencadenó diferentes patologías, desde la presentación de un enfermedad latente (Rabia), signos de intoxicación, hasta la proliferación de bacterias oportunistas como fue el caso de *Enterobacter agglomerans* (entre otras). Los hemogramas de los animales demostraron una leucopenia con linfopenia. La histopatología reportó congestión y edema intraseptal pulmonar, presencia de proteínas en la luz de algunos tubulos renales además de cambios degenerativos en la mayoría de las células hepáticas sugestivo a grasa, asociando las entidades con un cuadro compatible a intoxicación de curso sobreagudo a agudo.

Case Report

In October 1993, Africam Safari Zoo received seven common vampires (*Desmodus rotundus*) and 14 Jamaican fruit bats (*Artibeus jamaicensis*), both species wild caught. In November 1994, 27 vampires and 44 fruit bats were received. During this period, problems occurred which were believed to be caused by an inadequate air exchange within the exhibit. This caused severe stress to the colonies, and as a result, the death of several animals at different times. The period of highest mortalities coincided with the rainy season which complicated the air exchange when the ventilation ducts became flooded. The exhibit is as follows: two artificial caves of 3.1 m x 2.0 m x 2.20 m (height); the caves are divided by 19 mm glass. The average temperature was kept at 25 °C with an average humidity of 70%. Ventilation was accomplished by a 10 cm duct that connected the exhibits to the exterior. The doors of the exhibits that were connected to the service hall had a number of holes on the lower portions over a 5 x 10 cm area. There was an inverted photoperiod. The diet of the vampires was bovine blood with vitamin supplements; the fruit bats were fed various seasonal...
fruits, cooked egg whites, and vitamin supplements.

The first case was noted in a member of the group of seven vampires, 283 days after their arrival at the park. A 22 g adult male was found on the floor, unable to fly. It seemed slow when trying to walk, had bilateral epiphora, was hypothermic and dehydrated. The animal remained hanging, made slight movements, but didn’t try to move to the food, so forced feeding was attempted; it ate very well. A partial paralysis of the anterior limbs was noticed, making the limbs slightly lax and paretic. The individual defecated and urinated normally. Its health remained unchanged for 7 days, and died on the eighth day. No signs of trauma or any other relevant pathology were observed upon necropsy or histopathology. The brain was tested for rabies; it turned out to be positive. The same day the male died, a female from the same group was reported as having the same clinical signs. It died during the first day of treatment, and no relevant pathologies were found at either necropsy or histopathology. The results of the rabies test came back negative. Due to the importance of this disease, it was decided to test the rest of the animals of both groups (the fruit bats and vampires) for rabies. The laboratory results came back negative for all the samples sent. The exhibit was disinfected and kept empty for 3 mo. In November of 1994, a second group of bats was received. The groups consisted of 27 vampire and 44 fruit bats. At this time, there was still no apparent relationship between the first two deaths and the inadequate air exchange, so the animals were kept in the same environment, and with the same diet. Just as with the first group, the animals rapidly became acclimated to their new captive environment. Four months after they arrived at the zoo, deaths began to occur in the fruit bat colony and later on, in the vampire colony. Several animals isolated themselves, were lethargic, had no interest in food, showed signs of being unable to fly, and had difficulty breathing; some showed distended stomachs with large amounts of gas in the stomach as well as in the intestines. Other animals died without showing signs.

Histopathology reports did not identify any disorder that could clarify the etiology of the deaths: pulmonary congestion and intraseptal pulmonary edema, the presence of protein in the lumen of some renal tubules, and degenerative changes in most of the hepatic cells suggesting fat; signs implicating acute or peracute toxicity.

A few days before the deaths started to occur, it was noted that the fruit presented as food had developed a white film, the air inside the caves was sour and very concentrated, and the glass that separates the exhibit from the public was more fogged than usual. It was decided to remove the animals from the exhibit. After a careful inspection of the caves, it was found that the duct that supplied 85% of the air to the caves was in the shape of a “U” and obstructed by water. During the first 14 days that followed the move to a new location, nine more animals perished without apparent signs. After this period no more deaths occurred.

With the intent of having hematology values to better evaluate the state of the Artibeus jamaicensis that died, blood samples were collected from wild bats of the same species and at the same place that ours were captured. A severe leukopenia and lymphopenia correlating with stress was found in the captive bats.3

Of the cultures from internal organs, Enterobacter agglomerans (being the most frequent), Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis were isolated.
Toxicology tests to determine the presence of aflatoxins and organophosphates were run on samples of food that were consumed the day the animals died as well as samples of their liver. All results came back negative.

Conclusions

The histopathology findings suggest a nonspecific toxicity. Pulmonary congestion and dilated and hemorrhagic cerebral vessels (observed during necropsy) would suggest a CO₂ intoxication.

Based on the alterations of the environment within the caves, the gross and histopathology results, the laboratory results, and the cessation of deaths once the animals were removed from that environment, we believe that the clinical cases presented were brought about by a the high CO₂ concentration, caused by an insufficient air exchange. This then may have caused an immunosuppressive state, resulting in different pathologies: the manifestation of a dormant disease (such as rabies) and the proliferation of opportunistic bacteria such as *Enterobacter agglomerans*. It should be noted that once an air extraction system was installed, making it possible to have a complete air exchange at a rate of eight times per hour, no problems have arisen.

Excitable, aggressive, or furious phases were not observed in the case that resulted in a positive diagnosis of rabies; only a partial paralysis was noted. Paralytic rabies has been reported in North American bats.¹ It also mentions an absence of rabies outbreaks, therefore suggesting a balance between this deadly virus and its host, with only immunosuppressed individuals suffering from this disease.

In captivity, one of the longest incubation periods for rabies was reported in *Eptesicus fuscus* that developed clinical signs of the disease 209 days after its capture.² It died 4 days after the onset. In the present case, clinical signs were presented 283 days after its capture, dying 8 days later.

LITERATURE CITED

ATYPICAL MYCOBACTERIAL INFECTIONS IN CAPTIVE LONG-FOOTED POTOROOS (Potorous longipes)

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Abstract

The colony of long-footed potoroos (Potorous longipes) held at Healesville Sanctuary is derived entirely from four wild caught individuals acquired in 1980 (one male and two females) and 1981 (another female). There has been a total of 18 mortalities in the period of January 1986 from February 1996. Since February 1992, there have been nine mortalities. Five of the deaths within this latter period have been directly associated with infection due to the bacterial agent Mycobacterium avium-intracellulare. Three cases were male and two were female. Ages ranged from 3-10 yr. Additionally, there is circumstantial evidence based upon review of autopsy records to suggest that other individuals may have died of the same cause as early as 1986. The first two cases were diagnosed at necropsy. In the subsequent three cases, diagnosis was made prior to death by recovery of acid-fast bacilli from tracheal washes in two cases and fine needle aspirate in the other. Combination chemotherapy was attempted without success in two of these cases. The therapeutic regime consisted of rifabutin at 11.0 mg/kg b.i.d., ethambutol at 26.0 mg/kg b.i.d. and clarithromycin at 20 mg/kg b.i.d. all delivered p.o. for up to 1 mo. Failure of therapy was attributable to both poor patient compliance and the advanced state of the disease by the time of diagnosis.

As yet, there has been no investigation undertaken to assess the immunological status of the surviving colony members. There may be factors present which predispose these animals to this disease but they are yet to be identified. The group is quite inbred; no new genetic material has been introduced since the foundation of the colony (with the exception of an abandoned male pouch young collected from the wild in November 1994) and the reproductive capacity has diminished to zero. No animals have been born since February 1992; prior to this, 19 animals were born in the period between January 1981 and February 1992.

Preliminary lymphocyte transformation testing has been done and the results suggest that all the surviving adult animals (five females ranging in age from 4-13 yr) have had significant levels of exposure to M. avium. The exception is the young male previously described. At present, there are no reliable means for the detection of subclinical carriers, all animals have been intradermally tested with avian PPD and none (even an animal known to have been infected at the time) showed any appreciable response. One attempt to isolated mycobacterial DNA using polymerase chain reaction amplification from a blood sample from a known case was also unsuccessful. There is at present no information at all on the incidence of this disease in wild populations.

This disease represents a major threat to the survival of the only captive colony of long-footed potoroos in existence; the wild population is classified as endangered due to its limited distribution in habitat which is subject to logging activity, fuel reduction burning which depletes specific dietary items (most notably hypogeal fungi) and predatory activity by introduced species. It is imperative,
therefore, that the underlying factors which may contribute to the expression of this disease in the captive population by identified and addressed as a matter of urgency. Reliable means of diagnostic screening and development of an effective vaccination are perhaps the highest priorities.

**Resumen**

La colonia de potoroos del santuario Healesville proviene enteramente de cuatro individuos silvestres capturados en 1980 (un macho y dos hembras) y en 1981 (otra hembra). Ha habido un total de 18 muertes en el periodo de 1986 a febrero de 1996. Desde febrero del 1992 ha habido nueve muertes. Cinco de las muertes en este periodo han sido directamente relacionadas a la bacteria *Mycobacterium avian-intracellulare*. Tres casos fueron machos y dos casos fueron hembras. Los rangos de edad se encontraron entre 3 y 10 años. En forma adicional, hay una evidencia circunstancial basada en la revisión de los reportes de las necropsias que sugieren que otros individuos pudieron haber muerto por la misma causa a principios de 1986. Los primeros dos casos fueron diagnosticados en la necropsia. En los tres casos subsecuentes, el diagnóstico fue hecho antes de la muerte por el aislamiento de bacilos ácido-alcohol resistentes obtenidos de lavados traqueales en dos casos y aspiración con aguja fina en un caso. La terapia con una combinación de productos fue utilizada sin éxito. El régimen consistió en Rifabutin 11.0 mg/kg b.i.d., Ethambutol 26.0 mg/kg b.i.d. y Claritromicina 20 mg/kg b.i.d. todos proporcionados en forma oral por un mes. El fracaso de la terapia fue atribuido a la pobre respuesta del paciente y al avanzado estado de la enfermedad al momento del diagnóstico.

Todavía no ha habido investigaciones para evaluar el estado inmunológico de los miembros de la colonia que sobreviven. Puede haber factores que predispongan a estos animales a padecer la enfermedad pero no han sido aun identificados. Existe bastante consanguinidad en el grupo; no ha habido una introducción de nuevo material genético a la colonia (con la excepción de un macho joven solitario colectado de vida silvestre en noviembre de 1994) y la capacidad reproductiva ha disminuido a cero. Ningún animal ha nacido desde febrero de 1992; anteriormente 19 animales nacieron en el periodo entero de enero de 1981 y febrero del 1992.

Una prueba de transformación preliminar de linfocitos ha sido realizada y los resultados indican que todos los animales adultos que sobrevivieron (5 hembras en un rango de edad de 4 -13 años) han tenido niveles significativos de exposición a *M. avium*. La excepción es el macho joven previamente descrito. Hasta ahora no hay datos significativos para la detección de portadores subclínicos. A todos los animales se les ha realizado la prueba intradérmica contra PPD aviar y ninguno (no obstante que un animal estaba infectado en ese tiempo) mostró respuesta alguna. Un intento de aislar el DNA micobacterial utilizando la prueba de reacción en cadena de la polimerasa a partir de una muestra de sangre de un caso conocido tampoco tuvo éxito. No hay información de la incidencia de esta enfermedad en poblaciones silvestres.

Esta enfermedad representa la mayor amenaza para la supervivencia de la única colonia de Potoroos en cautiverio que existe; la población silvestre está clasificada como en peligro de extinción debido a su limitada distribución en un hábitat que está sujeto a la actividad de los leñadores, incendios que agotan su dieta específica (principalmente hongos hypogeal) y la actividad depredadora de especies introducidas. Es imperativo, por lo tanto, que los factores que puedan contribuir a la manifestación
de esta enfermedad en poblaciones en cautiverio sean identificados y señalados con carácter urgente. Pruebas de diagnóstico eficaces y el desarrollo de una vacuna efectiva pueden ser quizás la más importante prioridad.
ENROFLOXACIN SIDE EFFECTS IN A GALAPAGOS TORTOISE (Geochelone elephantopus nigra)

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Abstract

An adult male Galápagos tortoise (Geochelone [elephantopus] nigra) weighing nearly 200 kg has been housed at Loro Parque in an outdoor enclosure for 8 yr. During this time, there has been no history of disease. In November 1995, after some days of cold weather, the tortoise showed signs of anorexia, lethargy and noisy respiration. Pneumonia was suspected and an antibacterial therapy with enrofloxacin as a broad spectrum antibiotic was initiated. On the first day of treatment, 1,000 mg of enrofloxacin (Baytril® 10%, Bayer AG, Leverkusen, Germany) was injected i.m. into its forelegs divided in two different areas. One hour later, the tortoise showed a severe hyperexcitation with uncoordinated movements, hypersalivation and profuse diarrhea. This clinical course lasted for about 2 hr, and the animal was very depressed after that. Since blood had been taken before from the foreleg, while the tortoise was inverted with the carapace side up, the possibility of an intestinal torsion was considered. The animal was transferred to an indoor enclosure with higher temperature (25 °C). Blood work was considered within normal limits.

Within 48 hr the tortoise seemed to recover, and its appetite was returning. A second lower dose of enrofloxacin (500 mg) using a different Baytril® presentation (Baytril® 5%) was given. One hour later, the tortoise developed the same condition, and remained totally exhausted for 3 days. Supportive care included supplemental heat, subcutaneous administration of fluids (lactated Ringer’s solution and 5% dextrose), and a parenteral multivitamin. Parasympatholytic drugs were not used.

Enrofloxacin administrated i.m. in Hermann’s (Testudo hermanni), Indian star (Geochelone elegans), and gopher (Gopherus polyphemus) tortoises reach peak blood concentration after 30 min, 30 min and 1 hr, respectively.3,4,5 Thus, enrofloxacin might also peak in Galápagos tortoises at 1 hr, which was the time interval between the injection of the antibiotic and the onset of clinical signs.

Fluoroquinolones are generally well-tolerated, although side effects have been reported in both mammals and birds, including articular defects in growing juveniles of certain species, gastrointestinal upset, and anorexia.1 However, little information is available about adverse effects in reptiles. Local pain and soft tissue swelling caused by the injection of enrofloxacin i.m. have been noted.2 This report describes an acute clinical course in a Galápagos tortoise, which appears to be related to the administration of enrofloxacin.

Resumen

Desde hace 8 años, un macho adulto de tortuga gigante de Galápagos (Geochelone [elephantopus] nigra) de unos 200 kg de peso se mantiene en unas instalaciones exteriores en el Loro Parque. No hay constancia de enfermedades durante este periodo de tiempo. En noviembre de 1995, después de unos días fríos, la tortuga mostró síntomas de anorexia, letargia y estertores. Se sospechó que
podría tratarse de una pneumonía, y se inició el tratamiento antibacteriano con el antibiótico de amplio espectro enrofloxacina. El primer día, se injectó en dos sitios diferentes de las extremidades delanteras una dosis total de 1,000 mg de enrofloxacina (Baytril® 10%, Bayer AG, Leverkusen, Alemania) por vía intramuscular. Una hora más tarde, la tortuga mostró una acusada hiperexcitación con movimientos incoordinados, hipersalivación y diarrea abundante. Esta sintomatología duró aproximadamente dos horas tras lo cual el animal quedó muy deprimido. Como la tortuga había sido previamente volteada para tomar una muestra de sangre de la extremidad delantera, se consideró la posibilidad de que se tratara de una torsión intestinal. El animal fue transladado a unas instalaciones interiores con temperatura más alta (25° C). Los valores sanguíneos se consideraron normales.

Tras 48 horas, la tortuga parecía recuperada y con apetito, por lo cual se aplicó una segunda dosis menor de enrofloxacina (500 mg) utilizando una presentación diferente de Baytril® (Baytril® 5%). Una hora más tarde, la tortuga desarrolló el mismo cuadro, y permaneció totalmente exhausta tres días, durante los que se le aplicó un tratamiento de mantenimiento: incremento de temperatura, administración de fluidos por vía subcutánea (lactato de Ringer y Dextrosa 5%) y un compuesto multivitamínico por vía parenteral. No fueron empleados fármacos parasimpaticolíticos.

La aplicación intramuscular de enrofloxacina en la tortuga mediterránea (Testudo hermanni), tortuga estrellada de la India (Geochelone elegans), y tortuga terrestre de Florida (Gopherus polyphemus) alcanza el pico de concentración sanguínea pasados 30 min., 30 min. y una hora respectivamente.3,4,5 Por ello, pensamos que la enrofloxacina en tortugas de las Galápagos podría alcanzar el pico de concentración en aproximadamente una hora, que fue el tiempo transcurrido entre la administración del antibiótico y el comienzo de los síntomas clínicos.

Generalmente las fluoroquinolonas son bien toleradas, aunque se han descrito efectos adversos en mamíferos y en aves, tales como defectos articulares en animales en crecimiento de algunas especies, desarragos gastrointestinales y anorexia.1 Sin embargo, hay poca información referente a efectos adversos en reptiles. Se han citado casos de dolor local e inflamación de tejidos blandos causados por la inyección intramuscular de enrofloxacina.2 En este informe se describe un cuadro clínico agudo en una tortuga gigante de las Galápagos, que parece estar relacionado con la aplicación de enrofloxacina.

LITERATURE CITED

PITUITARY ADENOMA IN A BLACK-HEADED PYTHON (Aspidites melanocephalus)

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Abstract

An 11-yr-old, captive born, male Black-headed python presented to the clinical department with poor muscle tone, fasciculations and abnormal righting reflex in 1990. In 1994, the clinical signs returned and progressively worsened. The most significant finding at necropsy was a 1 cm diameter, round, firm, red to tan mass in the location of the pituitary that displaced the brain dorsolaterally. There was also moderate bony remodeling of the sella turcica to accommodate the mass. Histologically, the mass was compatible with a sinusoidal type of a chromophobe adenoma of the pars distalis. Electron microscopy was performed to confirm the cell of origin. Immunohistochemistry results to determine the contents of the secretory granules were inconclusive. Pituitary adenomas are common tumors in dogs, horses and rats, but are considered uncommon or rare in other species. This is believed to be the first report of a pituitary tumor of any type in a snake.

Resumen

Una serpiente pitón cabeza negra macho de 11 años fue presentada al departamento clínico en 1990 debido a baja tonicidad muscular, fasciculaciones y reflejos anormales. En 1994 los signos clínicos regresaron y empeoraron progresivamente y el animal murió. El hallazgo más significativo en la necropsia fue una masa roja de 1 cm. de diámetro, redonda, firme, en la zona de la pituitaria que desplazó al cerebro dorsolateralmente. También había un moderado cambio en los huesos de la silla turca para dar cabida a la masa. Histológicamente la masa era compatible con un tipo de adenoma sinusoidal cromófobo de los pares distales. Se le realizó microscopía electrónica para conocer el origen de las células. Los resultados inmunohistoquímicos para determinar el contenido de los gránulos secretorios fueron inconclusivos. Los adenomas en la pituitaria son tumores comunes en perros, caballos y ratas, pero son considerados poco comunes o raros en otras especies. Pensamos que este es el primer reporte de un tumor en la pituitaria en cualquier tipo de serpiente.
A REVIEW OF THE NATURAL HISTORY AND PATHOLOGY OF FILOVIRUS INFECTIONS IN NONHUMAN PRIMATES

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Abstract

Ebola virus and Marburg virus, members of the family Filoviridae, have been responsible for several explosive outbreaks of lethal, hemorrhagic fever in humans and nonhuman primates. Since 1967, when the first reported cases of Marburg virus infection occurred in laboratory workers in Germany and Yugoslavia, at least 13 known outbreaks of disease caused by filoviruses have been confirmed by virus isolation. Typically, outbreaks have been sporadic, widely separated temporally and relatively self-limiting. Three of the outbreaks occurring in humans have been directly related to contact with infected nonhuman primates or nonhuman primate tissues. Nearly all filovirus outbreaks thus far have arisen in Africa, including incidents in Uganda, South Africa, Kenya, Zaire, Sudan, Ivory Coast and Gabon. Infected African green monkeys exported from Uganda were responsible for the initial Marburg outbreaks in Germany and Yugoslavia. Ebola-Reston virus, which was the cause of monkey infections in Virginia, Pennsylvania, Texas and Italy, appears to be the only filoviral disease to have originated outside of Africa. Outbreaks of Ebola-Reston virus were traced to a single source in the Philippines. Ebola-Reston virus is also unique in that it does not appear to cause disease in infected humans, although very few human infections have been documented. Exhaustive efforts have uniformly failed to provide evidence of the natural reservoirs of the filoviruses.

The incubation period for Ebola and Marburg viruses in nonhuman primates ranges from 4 to 16 days. Clinical signs include anorexia, fever, splenomegaly, swollen lymph nodes, conjunctivitis, serosanguineous nasal discharge, cutaneous rashes, dehydration and severe depression preceding death. Epistaxis, frank hemorrhage from body orifices, vomiting and diarrhea are not obvious clinical features of these diseases in nonhuman primates as they are in humans. Death generally occurs in nonhuman primates within 2 to 7 days after the onset of clinical signs. Clinicopathologic findings are characterized by leukocytosis due to neutrophilia, lymphopenia, thrombocytopenia, increased liver enzymes and increased BUN, serum creatinine and fibrin degradation products terminally. Important necropsy findings, which may vary depending on the species involved, include cutaneous rashes of the facial, axillary, thoracic, abdominal and inguinal regions, lymphadenopathy, a swollen and friable liver, and hemorrhages within the thoracic and peritoneal cavities and gastrointestinal tract. Multifocal necrosis, hemorrhage and evidence of disseminated intravascular coagulation are the main histologic lesions. Liver, spleen, lymph nodes, adrenal glands and lungs are important target organs.

The differential diagnosis for hemorrhagic fever in nonhuman primates includes Ebola and Marburg fevers, Simian hemorrhagic fever, yellow fever and Kyasanur Forest disease. In addition, measles virus, acquired through contact with humans, affects a variety of nonhuman primate species and may manifest as maculopapular rash, conjunctivitis or facial erythema. Callitrichid hepatitis virus can cause subcutaneous and intramuscular hemorrhage and hepatosplenomegaly, but is a disease of tamarins and marmosets.
Histopathologic examination of tissues is helpful in ruling out other causes of hemorrhagic fever in nonhuman primates, however definitive diagnosis of filovirus infection requires demonstration of viral antigen by virus isolation in cell culture, electron microscopy or antigen capture (ELISA). Also, demonstration of viral antigen in tissues, blood smears or tissue impression smears via immunocytochemistry is a valuable diagnostic test. The use of ELISA for the demonstration of serum antibodies to Ebola or Marburg virus antigens is useful if an animal recovers or survives long enough to mount an immune response.

Due to previous outbreaks of filovirus disease affecting humans and nonhuman primates following the importation of nonhuman primates into the United States and other countries, special permit requirements exist for the importation and quarantine of nonhuman primates in the United States. Any animal suspected of having hemorrhagic fever during the 31-day quarantine period must be reported to the Centers for Disease Control within 24 hr. In addition, field researchers must take necessary precautions when investigating mortality events affecting wild apes and other primates or handling any primate species demonstrating evidence of hemorrhagic disease. Ebola and Marburg viruses are classified as biosafety level 4 (WHO risk group 4) pathogens and laboratory work must be performed in maximum containment facilities.

Resumen

Los virus del Ebola y Marburg, pertenecientes a la familia Filoviridae, han sido los responsables de diversos brotes letales, con fiebres hemorrágicas en humanos y en otros primates. Desde 1967, cuando el primer reporte de infección con el virus Marburg ocurrido en trabajadores de laboratorios en Alemania y en Yugoslavia, por lo menos 13 brotes conocidos de la enfermedad causada por filovirus han sido confirmados por aislamiento vírico. Generalmente, los brotes han sido esporádicos, en temporadas ampliamente separadas y en zonas delimitadas. Tres de los brotes ocurridos en humanos han sido directamente relacionados con el contacto con primates infectados o con tejidos de éstos. Casi todos los brotes de filovirus ocurridos hasta ahora se han presentado en Africa, incluyendo incidentes en Uganda, Sudáfrica, Kenia, Zaire, Sudán, Costa de Marfil y Gabón. Monos verdes africanos infectados exportados de Uganda fueron responsables del brote inicial de Marburg en Alemania y Yugoslavia. El virus del Ebola-Reston que fue el causante de la infección de los monos en Virginia, Pennsylvania, Texas e Italia, parece ser la única enfermedad filoviral originada fuera de Africa. Se conoce un brote de Ebola-Reston localizado como caso aislado en Filipinas. Este virus es el único que no causa enfermedad en humanos infectados. Sin embargo ha sido muy poca la información documentada en humanos. A pesar de los exhaustivos esfuerzos, no se han obtenido evidencias de los reservorios naturales de los filovirus.

El periodo de incubación de los virus Ebola y de Marburg en primates no humanos tiene un rango de 4 a 16 días, los signos clínicos incluyen anorexia, fiebre, esplenomegalia, inflamación de nódulos linfáticos, conjuntivitis, descarga nasal, serosanguinolenta, erupción cutánea, deshidratación y severa depresión antes de la muerte. Epistaxis, hemorragia en orificios corporales, vómito y diarrea son hallazgos clínicos que no son tan obvios en primates no humanos como en los humanos. La muerte generalmente ocurre en monos entre los 2 y los 7 días después de presentarse los signos clínicos. En cuanto a la patología clínica, suele presentarse leucocitosis debido a la neutrófilia, linfopenia, trombocitopenia, incremento de las enzimas del hígado y en el BUN, creatinina sérica y degradación
de productos terminales de fibrina. Los hallazgos importantes en la necropsia pueden variar de acuerdo a la especie involucrada. Suele apreciarse erupción cutánea en cara, axilas, tórax, abdomen y en zonas inguinales, linfodenopatías, hepatomegalia y friabilidad hepática, hemorragias en cavidad torácica, peritoneal y gastrointestinal, necrosis multifocal, hemorragia y evidencias de coagulación intravascular diseminada, siendo las principales lesiones histológicas en hígado, bazo, nódulos linfáticos, glándulas adrenales y pulmones.

El diagnóstico diferencial con fiebre hemorrágica en primates no humanos incluye fiebre de Ebola y Marburg, fiebre hemorrágica de Simios, fiebre amarilla y enfermedad de Kyasanur. Añadiendo el virus del sarampión adquirido por contacto con humanos, los efectos son variables en especies de primates no humanos y pueden manifestarse con erupciones maculopapulares, conjuntivitis o eritema facial. El virus de la hepatitis en los Calitricidos puede causar hemorragias subcutáneas e intramusculares, hepatoesplenomegalia en tamarines y marmosetas.

Los exámenes histopatológicos y tisulares son útiles para descartar las causas de fiebre hemorrágica en primates no humanos, aunque el diagnóstico definitivo de una infección por filovirus requiere una demostración de antígenos víricos, aislamiento del virus en un cultivo celular, microscopio electrónico y captura de antígenos (ELISA). También la demostración de antígenos víricos en tejidos, frotis sanguíneos, frotis con impresiones de tejidos para inmunocitoquímica, siendo ésta una prueba de diagnóstico valiosa. Para la utilización de ELISA para la demostración de anticuerpos de los virus Ebola y Marburg en suero es necesario tener antígenos del virus recogido de animales que vivan lo suficiente como para presentar respuesta inmune.

Debido a brotes previos de enfermedades por filovirus que afectan humanos y primates no humanos después de la importación de monos dentro de los Estados Unidos y en otros países, se requiere obtener permisos especiales para la importación y cuarentena de los primates. Todo animal que se sospeche que haya tenido fiebre hemorrágica durante los 31 días del periodo de cuarentena debe ser reportado al Centro de Control de Enfermedades en las siguientes 24 hrs. Además en el campo de la investigación se deben tomar las precauciones necesarias como: frecuencia de mortalidad en antropoides silvestre y otros primates que han estado en contacto con otras especies de primates en los cuales se encuentren evidencias de enfermedades hemorrágicas. Los virus del Ebola y Marburg están clasificados en bioseguridad como patógenos de nivel 4, por lo tanto el laboratorio tienen que trabajar con un máximo de seguridad.
NOVEL HERPES-B LIKE INFECTION OF SILVER LEAF LANGURS (*Presbytis cristata*)

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Abstract

Sixty-seven serum or plasma samples were obtained from 34 silver leaf langurs (*Presbytis cristata*) at the Wildlife Conservation Park/Bronx Zoo over a 10 yr interval (1984-1994) and tested by ELISA for IgG reactivity to alphaherpesviruses. Seronegative samples were screened by ELISA for IgM reactivity. Thirteen (38.2%) of the langurs were seropositive. Antibodies were quantified and characterized by titration and competition ELISA and western blot analysis. Antibodies were highly cross-reactive with both human and nonhuman primate alphaherpesviruses (*herpesvirus hominus* 1 [HSV-1], *herpesvirus hominus* 2 [HSV-2], *Cercopithecine herpesvirus* II [SA8] and *Cercopithecine herpesvirus* 1 [herpes B]). The greatest amount of cross-reactivity was to polypeptides of herpes B virus followed by SA8. Silver leaf langur serum antibodies were uniquely reactive with a specificity clearly different from that obtained with homologous virus:antibody interactions. Both captive born and wild caught langurs were seropositive and both primary and recurrent infections were documented, as were long-term stable titers. One langur had penile and muzzle vesicles concurrent with serological evidence of a recurrent herpesviral infection.

Virus isolation was attempted from 139 oral and conjunctival swabs obtained from 22 langurs over a 2 yr period. A polymerase chain reaction (PCR) designed for the detection of herpes B and viral culture were performed on 25 CNS samples (trigeminal and sacral ganglia) obtained from 7 langurs over a 1 yr interval. All these results were all negative.

A novel herpesvirus, more closely related to herpes B than other alphaherpesviruses, was isolated from pharyngeal tissue of a silver leaf langur which died with pharyngeal edema, tonsillitis, pharyngitis, bronchopneumonia, sialoadenitis, and lymphadenitis. Virus was isolated in an established Vero cell line from frozen tissue obtained at necropsy. Cytopathic effect was noted during the second passage and was herpetic with cell rounding and some syncytia. Herpesvirus virions were demonstrated by EM. Sequence analysis of the isolate’s polymerase gene revealed that it was an alphaherpesvirus and was more closely related to herpes B virus than to SA8, HSV-1, or HSV-2.

This is the first isolation of an endemic herpesvirus from a langur. Hanuman langurs (*Presbytis entellus*) at two west coast research institutions and Proboscis monkeys (*Nasalis larvatus*) at the Wildlife Conservation Park/Bronx Zoo were also found to have herpes-B like seroreactivities. These findings suggest that there exists at least one novel endemic herpesvirus in populations of Southeast Asian folivorous primates. Based upon the serologic profiles of these animals, and the virus isolated from the silver leaf langur, it is more closely related to herpes B than to any other known alphaherpesvirus. While herpes B, the endemic herpesvirus of macaques (*Macaca* spp.), is widely
recognized for its zoonotic potential, there is little recognition for the potential of non-macaques to harbor viruses which may pose as great a human health risk. Proper precautions should be taken when handling other species of primates because of the possibility of transmission of viruses of unknown pathogenic potential.

**Resumen**

Sesenta y siete muestras de suero o plasma fueron obtenidos de 34 langures hoja plateada (Presbytis cristata) del Wildlife conservation Park/Bronx Zoo en un intervalo de 10 años (1984-1994) y estudiados con un sistema ELISA para buscar anticuerpos IgG contra el alfaherpesvírus. Las muestras seronegativas se sometieron a otro test ELISA para buscar reacción de la IgM. Trece (38.2%) de los langures fueron seropositivos. Los anticuerpos fueron cuantificados y tipificados por titulación y competición por ELISA y análisis con Western-blot. Los anticuerpos presentaron una alta reacción cruzada con los alfaherpesvírus tanto humanos como de primates no humanos (herpesvirus hominus 1 [HSV-1], herpesvirus hominus 2 [HSV-2], Cercopithecine herpesvirus II [SA8] y Cercopithecine herpesvirus 1 [herpes B]). La mayor cantidad de pruebas que dieron reacción cruzada fue para el polipéptido del virus herpes B, seguido por SA8. Los anticuerpos del suero del langur hoja plateada fueron reactivos en forma clara, exclusiva y evidente con una especificidad claramente diferente a los encontrados en la prueba de virus homólogos: interacción de anticuerpos. Tanto los nacidos en cautiverio como los silvestres mantenidos en cautiverio fueron seropositivos, y tanto infecciones primarias como recurrentes fueron documentadas, presentando por largo tiempo títulos estables. Un langur presentó vesículas en el hocico y en el pene de manera concurrente, con evidencias serológicas de una infección herpes vírica recurrente.

El virus se aisló de 139 muestras orales y conjuntivales obtenidas de 22 langures en un periodo de dos años. La reacción en cadena de la polimerasa (PCR), designada para detección de herpes B y un cultivo fueron realizados en 25 muestras de CNS (de los ganglios trigémino y sacro) obtenidos de 7 langures en un periodo de un año. Todos los resultados fueron negativos.

Un nuevo herpesvírus, más estrechamente relacionado al herpes B que a otros herpesvírus, fue aislado del tejido faríngeo de un langur que murió presentando edema faríngeo, tonsilitis, faringitis, bronconeumonía, sialoadenitis y linfadenitis. El virus fue aislado utilizando una línea celular Vero a partir de tejido congelado obtenido durante la necropsia. Los efectos citopáticos fueron observados durante el segundo pase y estos fueron de tipo herpético: con células redondas y algunos corpúsculos sincitiales. Los herpesvírus fueron demostrados por EM. El análisis secuencial del gen de la polimerasa aislado reveló que este era un alfaherpesvírus y más estrechamente relacionado con el herpesvirus B que con el SA8, HSV-1 o HSV-2.

Este es el primer aislamiento de un herpesvírus endémico de un langur. Hauman langurs (Presbytis entellus) de dos institutos de investigación de la costa oeste y monos narigudos (Nasalis larvatus) del Wildlife Conservation Park/Bronx Zoo fueron también encontrados con seroactividad similar al herpes-B. Estos hallazgos sugieren que existe por lo menos un nuevo herpesvírus endémico en poblaciones de primates folívoro del sureste asiático. Basados en los perfiles serológicos y en el aislamiento vírico en el langur hoja plateada, puede concluirse que este virus esta más cercanamente relacionado al herpes B que ningún otro alfaherpesvírus conocido. Mientras el herpes B, el virus
endémico de los macacos (Macaca spp.), es ampliamente reconocido por su potencial zoonótico, el poco conocimiento de huéspedes víricos no macacos puede ser un gran riesgo para la salud humana. Se debe tener cuidado al manipular otras especies de primates debido a la posibilidad de transmisión de virus con potencial patogénico desconocido.

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DISCOVERING AN EMERGENT DISEASE OF CAPTIVE NEW WORLD PRIMATES CAUSED BY LYMPHOCYTIC CHORIOMENINGITIS VIRUS, AND ITS SUBSEQUENT CONTROL

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Abstract

This report reviews the discovery, tracking and identity of a viral entity of captive callitrichids that emerged over the past two decades as sporadic outbreaks in zoos and wildlife parks in North America with one documented outbreak in the U.K. No cases or evidence of previous exposure to the virus were identified in a comprehensive serosurvey of wild golden lion tamarins in their natural habitat in Brazil. The disease was inadvertently promoted in part by management practices, and abated when feeding practices were changed. Control of CH can be achieved by vigilant rodent extermination; eradication of the disease requires zero-level contact of the callitrichids with wild mice.

Resumen

Este reporte es una revisión del descubrimiento, rastreo e identificación de un ente vírico en Calitricidos en cautiverio que surgió en las últimas dos décadas con brotes esporádicos en zoológicos y parques de Fauna Silvestre en Norteamérica y con un brote documentado en el Reino Unido. No fueron identificados ni casos ni evidencias de exposiciones previas al virus en un estudio del suero realizado en el Tamarín dorado en su hábitat natural en Brasil. Esta enfermedad fue estimulada, en parte por las prácticas de manejo, y fue abatida cuando se cambiaron las prácticas de alimentación. El control de CH se llevó a cabo gracias a la exterminación de roedores. La erradicación de la enfermedad requiere un nulo ontacto de los Calitricidos con ratones silvestres.

Overview

In 1980, an outbreak of a disease characterized by a fatal hepatitis with a viral pattern occurred at the Henry Doorly Zoo in which seven of 11 golden lion tamarins (Leontopithecus rosalia) died. Six of the losses occurred in little over a month. Preliminary studies ruled out hepatitis A and B, or other known hepatotropic viruses. Animals from this colony were on breeding loan from the National Zoological Park (NZP) and material submitted to the NZP’s Pathology Department showed a viral type of hepatitis with acidophilic (Councilman-like) bodies. Subsequently, two similar outbreaks occurred in golden lion and emperor (Saguinus emperor) tamarins at the Oklahoma City Zoo in 1984 and 1986 although no direct point of animal contact could be identified between the two zoos. Necropsy material was sent to the San Diego Zoo for viral isolation. Particles compatible with an enveloped RNA virus measuring 85-105 nm were detected by electron microscopy, but a specific agent could not be identified. Both corona and bunyaviruses were being considered as etiologic agents at the time.
The above cases plus a similar outbreak of hepatitis in 12 marmosets at the Bristol Zoo in England early in 1980\(^3\) prompted an all out effort to review the mortalities of golden lion tamarins at North American zoos using SSP and studbook records\(^1\) for further epidemiological evidence. Retrospectively, clusters of fatal hepatic disease were identified in approximately 40 additional Callitrichidae at nine other North American zoos or animal parks with a similar case definition characterized by an acute onset with variable jaundice, elevated liver enzymes and hepatitis with acidophilic bodies and lesser involvement of other parenchymal organs and lymphoid necrosis.\(^9\) Still, no pattern emerged as to how a disease targeted to marmosets and tamarins might have been transmitted nor was the source of the agent identified. It was named callitrichid hepatitis (CH), and to determine its infectivity and obtain needed study material, attempts were made in a limited trial to transmit the disease which proved successful in several common marmosets (Callithrix jacchus).\(^6\)

In 1990 the agent of CH was identified as an arenavirus closely related to lymphocytic choriomeningitis virus (LCMV), later determined to be 86% homologous with GC-P gene of two guinea pig laboratory strains of LCMV.\(^{12}\) LCMV is a zoonotic virus that usually causes a flu-like illness in humans, uncommonly progressing to aseptic meningitis and rarely fatal. The source of human infections has been via contact with laboratory or pet rodents—mainly mice and hamsters.\(^2\) There was never any evidence of human illness in any of the outbreaks in the zoo primates studied; however, two care-givers developed titers to LCMV and became serum donors for subsequent immunologic studies of the disease in the non-human primates.

In the spring of 1991, an outbreak in golden lion tamarins and pygmy marmosets (Callithrix pygmaea) held at two separate colonies at the Fort Worth Zoo (FWZ) was attributed to the ingestion of mice fed to them for protein supplementation.\(^5\) At the time, feeding neonatal mice was still a common practice in the management of marmosets and tamarins at some zoos. Outbreaks in both of the FWZ colonies were attributed to a single feeding of the primates with newborn mice that were inapparently infected with LCMV. We determined in that outbreak that agents isolated from livers of the feed-mice and FWZ primates were the same virus and were related to isolates from previous CH outbreaks and to laboratory strains of LCMV by serology and nucleic acid hybridization. Also, 2 surviving FWZ animals had developed antibody to other LCMV\(_{\text{ar}}\) isolates and to guinea pig strains of LCMV.\(^5\) A new clinical sign observed in this outbreak was seizing, with inflammatory lesions noted in the brains of most of the affected animals. Also, the pygmy marmosets had a later onset of the disease and liver lesions were subacute with less necrosis. It was determined from the Fort Worth outbreak that primate to primate transmission of CH probably did not occur since primates not fed mice, or observed not to ingest mice or only small amounts, survived the outbreak.\(^5\)

Eventually a total of 17 epizootics at 11 North American Zoos occurred between 1980 and 1993 resulting in the deaths of 75 callitrichids representing 11 species (including Callimico goeldii), with the highest prevalence in the endangered golden lion tamarin.\(^{4,9}\) Not all of the outbreaks were transmitted by feed-mice, as cases of CH occurred earlier at Marine World in Vallejo, CA\(^9\) which never fed mice. Several zoos experienced new cases of CH after halting the practice of feeding mice as supplements after 1990: these included the Henry Doorly Zoo, (HDZ)\(^4\) in 1992, and the Sedgwick County Zoo (SCZ) and Buffalo Zoo (BZ) in 1993. Callitrichids have been observed to hunt wild mice in their exhibits and eat them and even share them with their conspecifics. Evidence to implicate ingested wild mice as a source of infection was obtained from the new cases of CH that occurred at HDZ in 1992 in a new Jungle exhibit in which 25 of 45 wild mice trapped in and around the
enclosure came up seropositive for LCMV (unpublished data).

Callitrichid hepatitis has not been reported in marmosets or tamarins kept at primate research centers or in university colonies most likely because feeding rodent material was not practiced, and there is less opportunity for exposure to wild mice in these institutions. In a comprehensive serosurvey for LCMV, a low prevalence (<7%) occurred predominantly in zoos with previous outbreaks of CH and in a few species of non-callitrichids in zoos with no history of hepatic disease. A large segment of the golden lion tamarin population representing wild and reintroduced animals from a reserve in Brazil were all negative for antibody to LCMV. Although it is likely that primates with positive titers to LCMV have conferred immunity to this virus and are deemed safe to exchange between collections, any tamarins seropositive for LCMV have, and should continue to be eliminated from being reintroduced into their natural habitat.

Many of the above factors explained the sporadic nature of these outbreaks of LCMV-induced CH over the past two decades. Recommendations to eliminate mouse-feeding and to enhance rodent control in callitrichid colonies have been implemented through the golden lion tamarin global SSP announcements and via meetings and publications. This is believed to have had a significant influence on the overall reduction of cases of this fatal disease in zoo and wild park facilities. However, since strains of LCMV are ubiquitous and can infect wild mice latently, CH still remains a threat wherever captive colonies of callitrichids may harbor wild mice that can be caught and eaten.

The latest cases of CH in two golden lion tamarins occurred presumably from wild mouse ingestion at the Chafee Zoological Park (CZP) in Fresno, CA late in 1995. Both animals had typical clinical signs with seizuring and elevated liver enzymes along with histological changes in livers compatible with CH. Both tamarins were seronegative to LCMV but this is not unusual because of the often rapid course and fatal nature of the disease. Diagnosis was confirmed by a newly developed immunohistochemical (IHC) method employing a peroxidase labeled antibody to a guinea pig strain of LCMV performed on paraffin tissue blocks.

LCMV in callitrichids has been found to have some pathogenetic similarities to Lassa fever, a potentially fatal arenavirus infection of humans and therefore has some important comparative relevance. Development of the ICH test for use on formalin fixed tissue and the availability of routine diagnostic serology for LCMV assures that proper assessment can be made during suspected outbreaks of CH in susceptible primate species.

The opportunity to obtain and review comprehensive breeding and mortality records furnished mainly through the golden lion tamarin international studbook and global SSP, and the multi-institutional cooperation in providing pathology material were all extremely instrumental in bringing this heretofore unknown disease of New World primates to light.

In summary, this report reviews the discovery, tracking and identity of a viral entity of captive callitrichids that emerged over the past two decades as sporadic outbreaks in zoos and wildlife parks in North America with one documented outbreak in the U.K. No cases or evidence of previous exposure to the virus were identified in a comprehensive serosurvey of wild golden lion tamarins in their natural habitat in Brazil. The disease was inadvertently promoted in part by management practices, and abated when feeding practices were changed. Control of CH can be achieved by
vigilant rodent extermination; eradication of the disease requires zero-level contact of the callitrichids with wild mice.

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LITERATURE CITED

THE THREAT OF *Cowdria ruminantium* INFECTIONS IN CAPTIVE WILD RUMINANTS

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Abstract

Heartwater, caused by the Rickettsial agent *Cowdria ruminantium*, is one of the most devastating livestock diseases in sub-Saharan Africa. In addition to domestic cattle, sheep, and goats, a variety of wild ruminants can acquire subclinical and clinical infections. Recent epidemiological findings which demonstrate a long-term host carrier state in domestic and wild ruminants, intrastadial transmission by the tick vector (*Amblyomma* spp.), vertical transmission of the agent from cows to their calves, and the presence of both *C. ruminantium* and *A. variegatum* in the Caribbean suggest that the introduction of this exotic disease to the American mainland is a continual and significant threat. Veterinarians working with captive wild ruminants should be familiar with this disease and follow appropriate preventive measures to minimize the risk of infection in captive and wild populations of ruminants.

Resumen

El hidropericardio causado por la rikettsia *Cowdria ruminantium* es una de las enfermedades más devastadoras del sur del Sahara africano. Además del ganado, ovejas y cabras, una gran variedad de ruminantes silvestres pueden adquirir infecciones clínicas o subclínicas. Recientes hallazgos epidemiológicos que demuestran que tanto ruminantes domésticos como silvestres permanecen por largo tiempo como huéspedes portadores, y que existe la transmisión intra-estadios del vector (*Amblyomma* spp.). La transmisión vertical de la garrapata de las vacas a sus becerros, así como la presencia tanto de *C. ruminantium* como de *A. variegatum* en el Caribe, sugiere que la introducción de esta enfermedad exótica al continente Americano es una continua y significativa amenaza. Los veterinarios que trabajan con ruminantes silvestres cautivos deben estar familiarizados con esta enfermedad y seguir medidas preventivas apropiadas para minimizar el riesgo de infección en poblaciones de ruminantes cautivos y silvestres.

Introduction

Heartwater is a non-contagious tick-borne disease caused by *Cowdria ruminantium*, a gram-negative, intracellular, bacterium-like organism. *Cowdria ruminantium* is the only species in the genus *Cowdria* and is currently classified as a member of the *Ehrlichieae* tribe in the Family Rickettsiaceae, Order Rickettsiales.1

Once confined to the African continent south of the Sahara, heartwater has now been confirmed on
THE THREAT OF Cowdria ruminantium INFECTIONS IN CAPTIVE WILD RUMINANTS

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Abstract

Heartwater, caused by the Rickettsial agent Cowdria ruminantium, is one of the most devastating livestock diseases in sub-Saharan Africa. In addition to domestic cattle, sheep, and goats a variety of wild ruminants can acquire subclinical and clinical infections. Recent epidemiological findings which demonstrate a long-term host carrier state in domestic and wild ruminants, intrastadial transmission by the tick vector (Amblyomma spp.), vertical transmission of the agent from cows to their calves, and the presence of both C. ruminantium and A. variegatum in the Caribbean suggest that the introduction of this exotic disease to the American mainland is a continual and significant threat. Veterinarians working with captive wild ruminants should be familiar with this disease and follow appropriate preventive measures to minimize the risk of infection in captive and wild populations of ruminants.

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Introduction

Heartwater is a non-contagious tick-borne disease caused by Cowdria ruminantium, a gram-negative, intracellular, bacterium-like organism. Cowdria ruminantium is the only species in the genus Cowdria and is currently classified as a member of the Ehrlichieae tribe in the Family Rickettsiaceae, Order Rickettsiales.1

Once confined to the African continent south of the Sahara, heartwater has now been
confirmed on Madagascar, various small islands in the Indian and Atlantic oceans, and islands in the Caribbean. In Africa heartwater is one of the most important vector-borne diseases of livestock. Endemic stability is a common epidemiological state in most of sub-Saharan Africa where the *Amblyomma* vectors exist and indigenous livestock and wildlife are present. The epidemiology of heartwater in Africa is an example of the interplay between a vector/wild host cycle and a vector/domestic host cycle.

The pathophysiology of heartwater is poorly understood. The primary pathologic lesions associated with *C. ruminantium* are hydropericardium, hydrothorax, and ascites. Additional gross lesions may include brain edema, edema of the lymph nodes, and splenomegaly. It is presumed that the transudate and edema is caused by increased capillary permeability; however, this has yet to be proven. Damage to capillary endothelial cells of the alveoli is limited and the often mild cytopathic changes seen in endothelial cells parasitized by the organism suggest that the organism itself is not the cause of the increased vascular permeability. Clinical pathologic changes are often variable. The most frequently measured alterations are a progressive anemia, fluctuations in total and differential white cell counts, increased total bilirubin, and a drop in total serum proteins.

In domestic ruminants (cattle, sheep, and goats), heartwater varies from a subclinical to peracute disease. Clinical signs can range from a mild transient fever in subclinical cases to death without premonitory signs in peracute cases. The acute form, characterized by rapid onset of fever, tachypnea, inappetence, and neurological signs (hyperesthesia, high-stepping or unsteady gait, twitching eyelids, chewing, abnormal tongue movement, and individual muscle tremors), is the most common presentation in susceptible, naive hosts, and often results in death. In domestic cattle a hemorrhagic diarrhea has also been reported in some cases of the acute form. Subclinical disease is the most common manifestation of heartwater in wild ruminants. However, in those animals with clinical disease the acute form is the usual presentation.

Cattle, sheep and African buffalo that recover from heartwater can remain *C. ruminantium* carriers for 246, 223, and 161 days, respectively. The persistent, non-clinical carrier state in wildlife means that they could remain reservoirs of infection for ticks long after introduction into heartwater-free regions.

Recent work has demonstrated that vertical transmission of *C. ruminantium* from infected cows to their calves occurs and this in conjunction with vector transmission may be necessary for the establishment and maintenance of endemic stability. If vertical transmission occurs in wild ruminants, as it does in domestic cattle, offspring from carrier animals may have subclinical or clinical infections.

**Wildlife Hosts**

Many wildlife species, both ruminants and non-ruminants, can become infected with *C. ruminantium*. African hoofstock in which *C. ruminantium* antigen has been detected include African buffalo (*Syncerus caffer*), African elephant (*Loxodonta africana*), blesbok (*Damaliscus*...
albifrons), eland (Taurotragus oryx), giraffe (Giraffa camelopardalis), impala (Aepyceros melampus), Kafue lechwe (Kobus leche kafuensis), sitatunga (Tragelaphus spekei), springbok (Antidorcas marsupialis), tsessebe (Damaliscus lunatus), waterbuck (Kobus ellipsiprymnus), and wildebeest (Connochaetes gnou).3,4,6,7,8,9,10,11,12,13,14,15 Serological evidence of *C. ruminantium* in black rhinoceroses (*Diceros bicornis*) and white rhinoceroses (*Ceratotherium simum*) has been demonstrated in one study.16 The specificity of the diagnostic test used in this study is questionable. Therefore, the carrier status of these animals should be confirmed by isolation of the organism.

*Cowdria ruminantium* has been documented in non African ruminants including white-tailed deer (*Odocoileus virginianus*), fallow deer (*Dama dama*), rusa deer (*Cervus timorensis*), Barbary sheep (*Ammotragus lervia*), and bison (*Bison* spp.).3,6,17,18 Additionally, African Muridae (*Mastomys coucha* and *Rhabdomys pumilio*), guinea-fowl (*Numida meleagris*), leopard tortoise (*Geochelone pardalis*), and the scrub hare (*Lepus saxatilis*) can be infected subclinically and develop a rickettsemia.6,7 Although cases of clinical disease have been documented in a few wild ruminants (springbuck, eland, white-tailed deer, sitatunga, Kafue lechwe, rusa deer), an asymptomatic carrier state is more commonly encountered.

**Tick Vectors**

The only known vectors capable of transmitting *C. ruminantium* are 13 species of ticks in the genus *Amblyomma*.6 On a global scale the two most important vectors are *A. variegatum* and *A. hebraeum*. *Amblyomma variegatum* was introduced into Guadeloupe in the early 1800s and is now established on 15 islands in the Caribbean.2,6,19 The presence of *C. ruminantium* on three Caribbean islands in conjunction with the wide-spread distribution of *A. variegatum* presents a continual threat of introduction to the American mainland.19,20 Three American ticks (*A. maculatum*, *A. cajennense*, and *A. dissimile*) have been shown experimentally to transmit *C. ruminantium*.

*Amblyomma variegatum* is an efficient vector and reservoir of *C. ruminantium*. The rickettsial agent can survive in unfed instars for up to 15 mo and is transmitted transstadially from larvae to nymphs and nymphs to adults, and intrasstadially by adults.21 Individual ticks can therefore transmit disease to a number of hosts. Additionally, while adult *Amblyomma* primarily parasitize medium to large ungulate species, the immature stages infest mammals, birds, and reptiles.22

**Diagnosis, Treatment and Control**

The diagnosis of heartwater in the live animal has been hindered by the inability to culture the organism. Prior to the 1980s (first successful in vitro cultivation was in 1985), the standard method of diagnosis was based on clinical signs, post-mortem findings, and microscopic examination of brain material obtained by biopsy.6 Currently, serological tests include the indirect fluorescent antibody test, Western blot assay, and competitive enzyme linked immunoassay (cELISA).6 These serologic tests have poor specificity because of cross-reactive antigenic determinants to other infectious agents. The recent development of a recombinant MAP1-B based ELISA has significantly improved the ability to detect *C. ruminantium*-specific antibodies.23 Unfortunately, no serologic test is currently available for commercial use.
Experimental use of DNA probes and PCR assays have greatly advanced the detection of *C. ruminantium* in infected animals and ticks.\(^6,24\) Once available, these tests should provide a more sensitive and specific means for detecting carrier animals.

The control of heartwater is based on individual chemotherapy using a tetracycline derivative, artificial immunization by infection and treatment, and acaricide tick control.\(^6\) The treatment of clinically ill animals is of limited value because mortality is quite high once nervous signs are noted. Even if the treatment is successful, the host is unlikely to be cleared of the organism and can act as a subclinical carrier. Artificial immunization (“vaccination”) requires intravenous administration of virulent infected blood and may result in clinical heartwater. The potential dangers of immunization far outweigh the protective benefits of vaccinating zoological and wildlife species destined for heartwater-free regions.

**Threat of Introduction of Heartwater to the American Mainland**

The threat of heartwater introduction to the American mainland is associated with epidemiological aspects of the vector, organism, and hosts. The foci of *A. variegatum* and *C. ruminantium* in the Caribbean acts as a constant reservoir for introduction.\(^19\) Migrating cattle egrets from Guadeloupe, which may be infested with larvae and nymphs of *A. variegatum*, have been observed in Florida.\(^20\) Many areas of the American mainland have been shown to be climatically suitable for *A. variegatum* if the tick were introduced.\(^25\)

Asymptomatic carrier hosts imported to the American mainland are another mode for the introduction of *C. ruminantium*. These carrier hosts serve as reservoirs for American and exotic *Amblyomma* vectors. Additionally, the potential of vertical transmission from carriers to offspring would enable the disease agent to persist in the ruminant population even in the absence of tick vectors.\(^5\)

The large population of susceptible, indigenous white-tailed deer offers a mode for widespread transmission in North America.\(^17\) Eradication of the disease after introduction into these wild populations would be extremely difficult. This fact is most significant in relation to recent trends in free-ranging wildlife ranches in which exotic ruminants are translocated to areas where contact between exotic, indigenous, and domestic ruminants can occur.

The increase in the importation of exotic species for the zoological and pet trade, which can serve as carriers of the organism and exotic *Amblyomma* ticks, exemplifies another avenue of introduction.\(^26,27\) Precautions must be taken in countries of origin and import. Animals should be treated with appropriate acaricides both prior to export and again while in quarantine. In species known to be susceptible to the disease and originating from heartwater endemic countries, diagnostic tests to determine their *C. ruminantium* status would be beneficial; unfortunately, no valid tests are currently available for commercial use.

The importance of preventing *C. ruminantium* and exotic *Amblyomma* ticks from entering the American mainland cannot be over-emphasized. If heartwater became established in the United States, it could devastate the livestock industry, decimate populations of white-tailed deer, and prove fatal for some exotic ruminant species maintained in captivity. Control of the disease would be facilitated by the nature of transmission (non-contagious, vector-borne) but
complicated by the recent discovery of vertical transmission. Because indigenous Amblyomma ticks are relatively poor vectors in comparison to the exotic Amblyomma species, establishment of the rickettsial agent would be difficult to achieve without the introduction of these exotic vectors. Although the potential for control and eradication of heartwater exists, it remains a grave threat to American livestock and exotic/native hoofstock because of several factors: 1) the difficulty in ante-mortem diagnosis; 2) the proximity of the Caribbean foci; 3) the large population of susceptible white-tailed deer; and 4) the often subclinical presentation of the disease.

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Q FEVER IN TWO SPECIES OF EXOTIC RUMINANTS

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Abstract

Coxiella burnetii is a ubiquitous organism. Antibodies to this organism and the organism itself have been found in a wide range of animals including mammals, reptiles, amphibians, and birds. We report here several cases of coxiellosis in two species of exotic ruminants.

A necrotizing placentitis was seen in four Cuvier’s gazelles (Gazella cuvieri) and one greater kudu (Tragelaphus strepsiceros strepsiceros). The kudu died within 24 hr of birth. At necropsy there was evidence of fetal distress and the placenta had pale cotyledonary areas. The Cuvier’s gazelle cases occurred 1 yr after the kudu case. Two of the gazelles were stillborn, and one was a midterm abortion. The fourth gazelle case was the placenta from an animal which lived and was the twin to one of the stillborn animals. Gross lesions were not seen in these cases, but in two cases placenta was markedly autolyzed.

In all cases significant microscopic lesions were limited to the placenta. In H&E stained sections there were extensive areas of necrosis. Some chorionic epithelial cells contained blue-grey granular intracytoplasmic material. Gimenez stain revealed intracytoplasmic red bodies with a diameter of less than 1 micron. Immunohistochemistry stains performed at a commercial diagnostic laboratory (Washington Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Washington State University, Pullman, WA 99165-2037) indicated that the organism was Coxiella burnetii. Computerized necropsy records were reviewed and no other cases of coxiellosis were identified.

Serology for Q fever using complement fixation was performed at National Veterinary Services Laboratories (1800 Dayton Rd., Ames, IA 50010) on serum from two Cuvier’s gazelles which were dams of above mentioned animals. One female was evaluated 9 mo after the birth and was negative. Another female was examined for a vaginal discharge 7 mo after the birth. The discharge contained organisms morphologically compatible with Coxiella. At this time serology was negative, but repeat serology performed 2 mo later was positive.

In domestic ruminants infection with Coxiella typically results in reproductive failures the first year following infection. Thereafter, the organism is shed in milk and at parturition, but abortions and stillbirths are seen rarely. The source of the organism in our cases is not known. The kudu and gazelles were housed in separate exhibits which were approximately 1 km apart.
Coxiella is a common organism, but infection in a collection of captive ruminants has not been previously reported. Infection with Coxiella may be undiagnosed in a group of animals because: necropsies may not be performed routinely on all animals, placentae may not be routinely examined grossly and/or microscopically, placenta may be lost due to consumption by the dam or predators, placenta may be too autolyzed for adequate examination, and Coxiella serology is not routinely evaluated. Furthermore, coxiellosis generally manifests only as reproductive failure and in a small group of animals increased losses may be difficult to identify due to small sample size. Detection of Coxiella infection in a group of animals is important not only to determine causes of reproductive failure, but also because Coxiella is a zoonotic pathogen.

Resumen

Coxiella burnetii es un organismo cosmopolita. Han sido encontrados anticuerpos de este organismo y el propio organismo en un amplio rango de animales incluyendo mamíferos, reptiles anfibios y aves. Nuestro reporte consiste en casos de Coxiellosis en dos especies de rumiantes exóticos.

Una placentitis necrosante fue observada en cuatro gacelas cuvier (Gazella cuvieri) y en un Kudú (Tragelaphus strepsiceros strepsiceros). El Kudú murió en las primeras 24 hrs. de nacido. En la necropsia hubo evidencias del sufrimiento fetal y la placenta presentó áreas cotiledonianarias pálidas. El caso de la gacela Cuviere ocurrió aproximadamente un año después del Kudú. Dos de las gacelas nacieron muertas y una fue abortada a mitad de gestación. El caso de la cuarta gacela, Coxiella se aisló de la placenta de un animal vivo cuyo gemelo nació muerto. En estos casos no se observaron lesiones macroscópicas, pero en ambos casos la placenta presentaba autólisis marcada.

En todos los casos las lesiones macroscópicas significativas estaban limitadas a la placenta. En la tinción de H&E se observaron extensas áreas de necrosis. Algunas células epiteliales coriónicas contenían material granular intracitoplasmático azul-grisáceo. La tinción de Gimenez reveló cuerpos rojos intracitoplasmáticos de un diámetro de menos de una micra. La tinción inmunohistoquímica realizada en un laboratorio de diagnóstico comercial (Laboratorio de Diagnóstico de Enfermedades Animales de Washington, Colegio de Medicina Veterinaria, Universidad del Estado de Washington, Pullman, WA 99165-2037) indicó que el organismo fue Coxiella burnetii. Se revisaron los registros computarizados de necropsias y no fueron identificados otros casos de Coxiellosis.

Se realizaron estudios serológicos por fijación de complemento para detectar fiebre Q por el Laboratorio Nacional de Servicios Veterinarios (1800 Dayton Rd., Ames, IA 50010) en sueros de dos gacelas cuvier que fueron las madres de los animales antes mencionados. Una hembra fue evaluada 9 meses después del parto y resultó negativa. La otra hembra fue examinada por una descarga vaginal 7 meses después del parto. La descarga contenía microorganismos compatibles con Coxiella. Para ese entonces, la serología fue negativa, pero una repetición de serología dos meses más tarde tuvo resultados positivos.

En rumiantes domésticos la infección típica con Coxiella resulta en fracasos reproductivos en
el primer año después de la infección. Luego el organismo es transmitido en la leche y durante el parto, pero los abortos y los nacimientos de mortinatos son observados rara vez. El origen de este organismo en todos los casos es desconocido. Las gacelas y el Kudu estaban albergados en exhibidores separados aproximadamente por un kilómetro.

A pesar de que Coxiella es un organismo común, no se habían reportado, hasta la fecha, infecciones en una colección de rumiantes silvestres en cautiverio. Puede que no se diagnostiquen las infecciones por Coxiella en un grupo de animales, ya que las necropsias no se realizan de una forma rutinaria, y cuando se hacen la placenta generalmente no es revisada macroscópicamente y/o microscópicamente. La placenta se puede perder debido a la ingestión por la misma madre o por los predadores, además la placenta puede estar demasiado autolizada para realizar un examen adecuado. La serología contra Coxiella se evalúa rutinariamente, además la Coxiellosis sólo manifiesta pérdidas reproductivas y en un pequeño grupo de animales el incremento en las pérdidas puede ser difícil de identificar debido al pequeño tamaño del grupo.

La detección de infecciones por Coxiella en un grupo de animales no sólo es importante para determinar las causas de pérdidas reproductivas sino también porque Coxiella es un patógeno zoonótico.
Mycobacterium bovis EPIDEMIC IN FREE-RANGING WHITE-TAILED DEER

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Abstract

Mycobacterium bovis was isolated from a single hunter-killed white-tailed deer (Odocoileus virginianus) in northeastern Michigan in January, 1995. In response a multi-institutional cooperative effort, including personnel from the MI Dept. of Natural Resources, MI Dept. of Public Health, MI Dept. of Agriculture, U.S.D.A., and the Animal Health Diagnostic Laboratory, was formed to survey the free-ranging deer in this area for tuberculosis. During the winter hunting season of 1995, 354 hunter-killed deer were surveyed for lesions of tuberculosis. Sixteen animals were confirmed positive for M. bovis through a combination of gross and histologic lesions, acid-fast staining, mycobacterial isolation, and DNA probes (Table 1). Based on these results, an estimated 4.5% of the wild adult deer population is infected with tuberculosis. This is the first report of epizootic tuberculosis in free-ranging deer anywhere in North America. The area involved in this outbreak has been historically recognized as a food shortage area for deer for many decades. This natural problem combined with management practices which have served to congregate deer in high concentrations has seemingly resulted in a unique wildlife problem. Ongoing surveys to delineate the size of the disease focus, and to evaluate the effects of changes in management practices on disease incidence over time are currently in progress. This outbreak suggests that management practices relating to wild cervids may inadvertently result in disease situations which pose significant threats to both human and domestic animal health. Therefore, continuous monitoring of wildlife species by public agencies should be continued and expanded.

Resumen

El Mycobacterium bovis fue aislado de un venado cola blanca (Odocoileus virginianus) cazado en el noreste de Michigan en enero de 1995. En respuesta a este hallazgo, se realizó un esfuerzo cooperativo multiinstitucional incluyendo personal del Departamento de Recursos Naturales de Michigan, El Departamento de Salud Pública, el Departamento de Agricultura (U.S.D.A.) y el Laboratorio de Diagnóstico de Salud Animal, para el estudio de tuberculosis en venados silvestres en esta área. Durante la cacería de invierno de 1995, 354 venados fueron cazados e investigados en búsqueda de lesiones de tuberculosis. Se confirmaron como positivos a Mycobacterium bovis 60 animales, por una combinación de lesiones macroscópicas e
Histológicas, tinción de ácido-alcohol, aislamiento de *Mycobacterium* y pruebas de DNA (Tabla No.1) Basados en los resultados obtenidos se observó que el 4.5% de la población de venados silvestres adultos estaban infectados con tuberculosis. Este es el primer reporte de tuberculosis epizoótica en venados en estado silvestre en todo Norte América. El área involucrada en este brote ha sido históricamente reconocida como un área con escaso alimento para venados por muchas décadas. Este problema natural combinado con prácticas de manejo que han servido para congregar venados en altas concentraciones ha resultado, aparentemente, en un problema único de fauna silvestre. Se están llevando a cabo estudios para delimitar el tamaño del foco de infección, así como para evaluar los cambios en las prácticas de manejo y su efecto en la incidencia de la enfermedad. Este brote sugiere que las prácticas de manejo referentes a cérvidos salvajes pueden resultar, inadvertidamente, en trastornos patológicos que representan un riesgo para la salud humana y la de los animales domésticos. Por tal motivo, el continuo monitoreo de especies silvestres con organismos gubernamentales debe continuar y expandirse.

Table 1. Summary of tuberculosis positive deer data.

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JOHNE’S DISEASE IN CAMELIDS: AN EMERGING DISEASE?

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Abstract

*Mycobacterium paratuberculosis* is a potential emerging disease, as a consequence of its epidemiological characteristics and the limitations of current diagnostic assays. A recent outbreak of Johne’s disease (JD) in Australian alpacas (*Lama pacos*) highlights the potential for JD to become an emerging disease in South American camelids (SACs). Similarly, there is evidence that JD is endemic in some Bactrian camel (*Camelus bactrianus*) populations. Camelids in the later stages of disease are emaciated and have diarrhea, as is seen in domestic ruminants. The gross and histopathology of JD in camelids is also similar to JD in ruminants. However, lymphadenopathy and thickening of the small intestine is more variable in camelids than in ruminants. Interpretation of diagnostic tests for JD must include recognition of the uncertain or poor test accuracy of many assays for *M. paratuberculosis*. It is difficult to identify animals in the early stages of infection, and similarities with saprophytic *Mycobacteria* decrease test specificities. Culture of feces or tissues is probably the most reasonable strategy for testing camelids and zoological hoofstock for JD, at this time. The development of a nonspecies specific ELISA may be a useful diagnostic tool in the future. A DNA probe for use in feces is untested for nonbovine species, but may be of value for quickly identifying animals shedding large numbers of organisms. In addition to preventing contact between uninfected animals and those with JD, less obvious routes of transmission must be eliminated, such as exposure to contaminated soil and water, and bottle feeding milk to orphaned hoofstock.

Resumen

*Mycobacterium paratuberculosis* es una enfermedad de surgimiento potencial, como consecuencia de sus características epidemiológicas y las limitaciones de los análisis de diagnóstico actuales. Un reciente brote de la enfermedad de Johnes (JD) en alpacas australianas (*Lama pacos*) demuestra el notable potencial de la JD de convertirse en una enfermedad que puede surgir en camélidos de Sudamérica. De igual forma hay evidencias que JD es endémico en algunas poblaciones de camello bactriano (*Camelus bactrianus*).

Los camélidos en la última fase de la enfermedad están emaciados y con diarrea, al igual que como se ha visto en rumiantes domésticos. Las lesiones macroscópicas e histopatológicas de la enfermedad de JD son similares a la JD en rumiantes. Sin embargo la linfadenopatía y el engrosamiento del intestino delgado es más variable en camélidos que en rumiantes. La interpretación de las pruebas de diagnóstico para JD deben contemplar el reconocimiento poco certero o datos poco precisos en
muchos de los ensayos para *Mycobacterium paratuberculosis*. Es difícil identificar la enfermedad en animales que se encuentran en los primeros estadíos de la infección, y las similitudes con Mycobacterias saprofíticas reducen la especificidad de las pruebas. El cultivo de heces y tejidos es hasta hoy en día, probablemente la estrategia más razonable para determinar JD en camélidos y artiodáctilos en Zoológicos. El desarrollo de una prueba de ELISA no especifica pudiera ser en el futuro, una herramienta diagnóstica muy útil. La sonda de DNA para heces no ha sido checada en especies no bovinas, pero pudiera ser de valor diagnóstico en la determinación de animales que se hallen esparciendo grandes cantidades de organismos. Además de prevenir el contacto de animales sanos con animales infectados con JD, las rutas menos obvias de transmisión deben eliminarse, tales como la exposición al agua y suelos contaminados, o también la alimentación de leche en biberón a animales huérfanos.

**Introduction**

“Emerging” infectious diseases have been defined as “infections that have newly appeared in a population or have existed but are rapidly increasing in incidence or geographic range.” The factors responsible for disease emergence include ecological, environmental, and demographic factors that increase a microbe’s contact to a new host species or which promote dissemination. *Mycobacterium paratuberculosis* is a strong candidate to become an emerging disease in many populations because of its biological characteristics, the limitations of current diagnostic options, and the transfer of animals of uncertain infection status between herds. The biological characteristics of *M. paratuberculosis* include a long subclinical stage, during which a host can be shedding viable organisms in its feces. This can result in exposure and infection of much of a herd prior to recognition that the disease is present. Efforts to prevent the introduction of *M. paratuberculosis* into a herd by screening new additions are hampered by assays which fail to correctly identify the infection status of all animals tested. Similarly, these assay’s limitations hamper the effectiveness of disease control strategies.

Recent reports of Johne’s disease (JD) in South American camelids (SACs) have raised concern that JD could be an emerging disease in the SAC industry. The intent of this manuscript is to provide a brief review of what is known about JD in SACs and Old World camelids (OWCs). We will also address more general diagnostic and epidemiological considerations which are applicable to other hoofstock species.

**A Review of Johne’s Disease in South American Camelids**

The camelids diverged from other members of the Artiodactyla approximately 55 mybp. Although naturally acquired *M. paratuberculosis* infections have been documented in several families in the Artiodactyla, the susceptibility of camelids to JD was unclear until fairly recently. A presumptive report of a single juvenile llama (*Lama glama*) with JD and two recent culture confirmed cases of JD in a herd of llamas indicated that SACs are susceptible to JD under some circumstances. However, the first evidence that the epidemiology of JD in SACs is similar to that of domestic ruminants was provided by the recent and ongoing outbreak of JD in Australian alpacas (*L. paca*). In the Australian outbreak of JD, multiple alpacas on multiple farms have had *M. paratuberculosis* infections confirmed by culture. As in cattle, emaciation and diarrhea were apparent in the terminal
stages of infection, and subclinically infected alpacas were documented to be shedding *M. paratuberculosis* in their feces. Also as in ruminants, there were no pathognomonic signs of disease and routine laboratory tests results were nonspecific. Two North American llama herds with culture confirmed infections (Miller et al., in prep) and one presumptive case were probably infected by other hoofstock species housed on the same pasture. Although it is currently not possible to establish the susceptibility of SACs to *M. paratuberculosis* infection and disease in comparison to other species, it does appear that the disease is uncommon in North America (Miller et al., in prep).

Katic’s review of JD cited Russian language reports which described JD as endemic in working Bactrian camels (*Camelus bactrianus*). Clinical signs of JD generally developed in 2-3 yr camels, and emaciation and diarrhea were common clinical signs. A report of JD in a dromedary camel (*C. dromedarius*) at a zoological institution also presented with emaciation and diarrhea. Therefore, both SACs and OWCs can be regarded as susceptible to *M. paratuberculosis* infection and disease, with epidemiological characteristics and clinical signs similar to that of domestic ruminants.

**Diagnosis of Johne’s Disease**

Johne’s disease has been a diagnostic conundrum for over 100 yr. The pros and cons of various diagnostic options have been presented elsewhere in greater detail than we will address in this manuscript. The reality is that none of the diagnostic options are applicable to all circumstances, and the tools currently available often fall short of what is desired. When interpreting JD assay results, it is important to consider a test’s diagnostic sensitivity and specificity, and critically evaluate the application of these values to “new” species. Even assays with unrealistically high test sensitivities will fail to identify all infected animals, and false positives due to low test specificities are often of equal concern. Therefore, it is important to understand the strengths and weaknesses of the available assays. Although most evaluations of *M. paratuberculosis* assays were conducted on domestic ruminants, cattle in particular, we believe that the information which we present here to be generally applicable to most hoofstock, including the camelids.

An excellent opportunity for identifying *M. paratuberculosis* infections is at necropsy. Johne’s disease should be considered as a differential for all camelids with chronic wasting disease, with or without diarrhea. Although the presenting pathology of JD in camelids is similar to that seen in domestic ruminants, there is enough interindividual variation that a rigid adherence to specific criteria could result in failure to identify some infections. For instance, thickening of the ileum, as seen in domestic ruminants with clinical JD, has not been a consistently reported feature in SACs with clinical signs of JD. Furthermore, atrophy of small intestinal mucosa and thickening of large intestine mucosa have been reported for bactrian camels with clinical JD. Lymphadenopathy is a feature of JD in domestic ruminants and has been reported for alpacas and camels with JD, but has been inconsistently reported in llamas with JD. Therefore, the gross appearance of tissues should not be the sole basis for collection of tissues. The histopathologic presentation of JD in camelids appears to be more consistent with what is seen in ruminants with JD than the gross presentation, as granulomatous enteritis and lymphadenitis is common in SACs with JD. It is worth noting that acid-fast organisms can be found in many camelid organs in the later stages of disease.

Acid-fast stained tissue section and feces have been used to provide rapid presumptive diagnoses of JD. However, it is important to recognize that the species of *Mycobacteria* can not be determined by
acid-fast staining, which thereby reduces the specificity of this method for diagnosing JD. In addition, the sensitivity of acid-fast staining is decreased when low numbers of organisms are present or when stages of the organism lacking a cell wall are present in tissues.

For postmortem diagnosis, tissues which should be collected for acid-fast stained histopathology and culture include ileum, jejunum, ileocecal junction, large intestine, and mesenteric lymph node as a minimum database, with additional tissues recommended. However, it is obviously preferable to diagnose JD antemortem, especially if animals with infections can be identified prior to fecal shedding and exposure of other animals in a herd to infection.

Culture of *M. paratuberculosis* is considered the gold standard for diagnosing JD. The conventional culture media has been Herrold’s egg yolk (HEY) media. Identification of *M. paratuberculosis* in a culture with HEY requires that at least $10^2$ organisms be present in the sample, and 12-16 wk or longer is required to identify colonies. A radiometric culture method has been developed which identifies fewer (possibly as low as 3) organisms and provides quicker results (as soon as 3 days for samples with large numbers of organisms), but can still require 7 wk to yield a negative culture result. Regardless of the culture method selected, it is important to submit samples to a laboratory which perform large numbers of cultures for *M. paratuberculosis*. This ensures that the laboratory’s staff have been able to maintain their skills, which thereby maximizes the probability of isolating *M. paratuberculosis* when it is present. It is also important that the laboratory have DNA probes capable of correctly identifying culture isolates by species; the initial misidentification of a *M. paratuberculosis* isolate from a llama as *M. bovis* generated more attention from regulatory agencies than most clients (including zoos) and veterinarians would probably select by choice.

Both tissues and feces can be submitted for *M. paratuberculosis* culture. Tissues sample cultures can identify infections at earlier stages than can fecal sample cultures. However, tissue samples are limited to postmortem collection or surgical biopsies. Fecal samples can be diagnostic for subclinical shedders. However, fecal culture can not identify animals prior to fecal shedding and contamination of the environment. Furthermore, because of the organism’s heterogenous distribution in feces and other factors, not all animals shedding *M. paratuberculosis* will be detected with a single fecal collection. Whitlock et al. sampled dairy cattle every 2 wk to demonstrate intermittent detection of *M. paratuberculosis* in feces. Table 4 of Whitlock et al. illustrates that even some cattle which were shedding large numbers of organisms failed to yield positive cultures in a to ¼ of the fecal samples. To address the problem of intermittent detection while accounting for finite resources, a zoological collection with culture confirmed *M. paratuberculosis* infections collected three fecal samples for radiometric culture over five day periods for each animal in their hoofstock collection. In herds without clinically affected animals or a history of exposure, submitting a single fecal sample for each animal on an annual basis, similar to the protocol adapted for a bovine JD certification program, may be a more reasonable strategy.

Serology offers a quick and inexpensive means of diagnosing JD, but is plagued by problems with low test sensitivity and specificity. In particular, cross reactivity with saprophytic *Mycobacteria* is responsible for decreased test specificities. The complement fixation test (CF) has been required for imported animals by many governments, but is not standardized and is complex to perform correctly. The agar gel immunodiffusion test (AGID) does not have species specific reagents, but appears to identify infected animals only in the later clinical stages of disease. An absorbed ELISA assay for
cattle is useful for herd screening and identifying individual high risk animals. However, as the absorbed ELISA will only identify $\frac{1}{2}$ to $b$ of infected cattle and cross-reactivity with saprophytic Mycobacteria can occur, it does not provide a definitive diagnosis for individual animals. Furthermore, it requires a species specific conjugate and is not applicable to nonbovine species. A commercially offered ELISA for llamas (Allied Laboratories, Fayetteville, Missouri, USA) has a test specificity of only 49%, and does not appear to have been thoroughly evaluated before being offered as a service (Miller et al., in prep). We are currently evaluating a USDA approved ELISA for JD in cattle (IDEXX Laboratories, Portland, Maine, USA) which has been provisionally modified for use in SACs with an anti-llama IgG. In addition, preliminary results for a modification of the IDEXX ELISA with a nonspecies specific protein G conjugate are being conducted and have provided encouraging preliminary results.

A potential “ceiling” to improvement in serology test sensitivities is that substantial quantities of antibodies to \textit{M. paratuberculosis} are not produced until the later stages of disease, as there is primarily a cellular immune response during early stages of infection. Intradermal testing for \textit{M. paratuberculosis} has proven to be inaccurate, and the only in vitro assay for JD based on cellular immunity is a USDA approved assay for gamma interferon (\textit{-IFN} \gamma) (IDEXX Laboratories, Portland, Maine, USA) in cattle. However, this assay needs to be conducted on recently collected blood (< 16 h), is of uncertain diagnostic value, and future development of this assay for nonbovine species is uncertain.

A USDA approved gene probe (IDEXX Laboratories, Portland, Maine, USA) for diagnosing JD in cattle is based on the IS900 sequence and provides a fast means of identifying \textit{M. paratuberculosis} shed in feces. This assay has 100% specificity, but is limited by the relatively large numbers of organisms ($10^4$ - $10^6$) which need to be present for detection. In addition, although the assay can probably be applied to nonbovine hoofstock species, research supporting this application has not been conducted.

\section*{Conclusions}

For camelids and zoological collections, understanding the epidemiology of \textit{M. paratuberculosis} and the attributes of various diagnostic options is important for designing control strategies. Housing multiple species on a single pasture is probably a significant risk factor for the development of JD in camelids. When JD is diagnosed in a herd, it is important to eliminate infected animals from close proximity to uninfected animals. In addition, it is important to consider less obvious routes of infection. As \textit{M. paratuberculosis} may remain viable for weeks to months in soil and some water sources, camelids and other hoofstock should not be placed on pastures soon after animals with JD have been removed. \textit{M. paratuberculosis} also appears to be resistant to pasteurization. Therefore, the source of milk provided to orphaned hoofstock must be critically evaluated. There is evidence for intrauterine transmission of \textit{M. paratuberculosis}, and this should be considered when determining the fate of individuals with culture confirmed infections.

As part of JD screening strategies, the strengths and weaknesses of various diagnostic options need to be considered. As is common for many diseases in “exotics” and nontraditional livestock species, many of the assays available for JD diagnosis are of marginal or uncertain accuracy for use in camelids. In animals with chronic weight loss, with or without diarrhea, thorough evaluation of the
gastrointestinal tract and associated lymph nodes and culture of suspect cases is an effective strategy for identifying JD in hoofstock. However, antemortem testing for JD is generally preferable and is a means of limiting the exposure of herdmates. An ELISA with a nonspecies specific conjugate may be of value for camelids and zoological collections in the future. The IDEXX DNA probe assay developed for feces may provide a quick means of identifying animals which are shedding large numbers of *M. paratuberculosis*, but further evaluation in nonbovine species is needed. Culture of feces is generally the most reasonable means of providing an antemortem diagnosis of JD in camelids and zoological collections, at this time. It is important to realize that strong empirical evidence is not available to evaluate any fecal sampling strategies for any species, and that judgement and vigilance are needed for controlling JD.

**LITERATURE CITED**


ENDOTHELIAL INCLUSION BODY DISEASE: A NEWLY RECOGNIZED FATAL HERPES-LIKE INFECTION IN ASIAN ELEPHANTS

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Abstract

In December, 1993, the National Zoo heralded the birth of its first Asian elephant. Sixteen months later, she died following a brief illness lasting five days. The clinical signs included intermittent anorexia, lethargy, decreased stool production, mild colic, and lingual cyanosis terminally. Hemogram and serum chemical evaluation showed mild leukopenia with decreased platelets and mild renal dysfunction. Postmortem examination revealed oral ulcers, mesenteric and serosal hemorrhages and edema, extensive cardiac hemorrhages, and a swollen, pale-red liver. Pertinent microscopic findings included myocarditis, hepatitis and glossitis accompanied by basophilic intranuclear inclusion bodies within capillary endothelial cells, often associated with microhemorrhages. By electron microscopy, the endothelial inclusions contained intranuclear viral particles measuring 80-92 nm in diameter, with morphological features consistent with the Herpesviridae family. The generalized hemorrhagic diathesis was likely due to capillary endothelial cell damage by the virus, with subsequent focal necrosis and inflammation of affected organs. Attempts at virus isolation have so far been unsuccessful. Immunohistochemistry for herpes simplex virus, adenovirus, hantaan virus, and polymerase chain reaction for morbillivirus have all been negative. Additionally, several immunoassays utilizing anti-elephant IgG prepared at the University of Tennessee have been negative. Further cultivation trials and additional molecular techniques are currently underway to better classify the virus.

By reviewing studbook mortality records and available pathology specimens, we subsequently identified six additional, previously unrecognized cases of fatal, disseminated herpes-like infections in Asian elephants in North American zoos and wildlife parks. Five of these animals ranged in age from 18 mo to 7 yr, and one was 26-yr-old. There was no sex predilection. Currently, the overall mortality rate of captive Asian elephants born in North America is 30% within the first year of life. Our findings suggest that this herpesvirus infection may have contributed to a significant proportion of mortalities in young Asian elephants. In 1990, a similar fatal systemic herpesvirus-like disease was reported in a 3-yr-old Asian circus elephant in Switzerland. The virus could not be isolated; however subsequent serologic studies indicated that a herpesvirus immunologically related to bovine herpesviruses (BHV) may be highly prevalent in the circus herd and in other collections of Asian elephants in Switzerland and Germany. We, in turn found that one of our three adult Asian elephants had serum antibodies reactive to BHV. Serologic evidence of infection by other viruses have so far been negative. There is also some morphologic evidence of localized cutaneous herpesvirus-related lesions in both Asian and African elephants. Cases of “herpes nodules” in the lungs of a high percentage of wild African elephants has also been documented.

Further characterization of the virus and determination of the epidemiological aspects of the disease...
are necessary to develop guidelines for its prevention. Information gathering for some of these studies have been initiated through the Elephant Species Survival Plan. Possible sources of the virus include 1) an indigenous Asian elephant herpesvirus that causes a systemic disease in young, immunologically naive elephants, 2) a latent African elephant herpesvirus that is fatal to Asians (many zoos socialize Asian and African elephants in the same exhibit), or 3) a herpesvirus of an unrelated species that causes illness in young Asian elephants. The status of this disease in elephants in captive settings or natural habitat in Asian countries is currently unknown.

Resumen

En diciembre de 1993 el Parque Zoológico Nacional del Instituto Smithsonian anunció el nacimiento de su primer elefante asiático. Dieciséis meses después, esta cría muere tras una breve enfermedad de 5 días. Los signos clínicos incluyeron anorexia intermitente, letargia, disminución en la producción de excretas, cólico leve y cianosis en la punta de la lengua. La evaluación del hemograma y el estudio químico del suero demostraron leucopenia con disminución de plaquetas y leve disfunción renal. El examen postmortem reveló úlceras orales, edema y hemorragias mesentétricas y serosas, extensas hemorragias cardíacas, hepatomegalia e hígado rojo pálido. Pertinentes hallazgos microscópicos incluyeron miocarditis, hepatitis y glositis acompañada por cuerpos de inclusión intranucleares basófilos dentro de las células endoteliales de los capilares, a menudo asociado con microhemorragias. Al microscopio electrónico las inclusiones endoteliales contenían partículas virales intranucleares, midiendo 80-92 nm de diámetro, con características morfológicas compatibles con la familia Herpesviridae. Las hemorragias generalizadas fueron posiblemente debidas al daño en las células endoteliales de los capilares causado por el virus, con subsecuente necrosis focal e inflamación de órganos afectados. Se intentó aislar el virus sin ningún éxito. La inmunohistoquímica para el virus herpes simplex, adenovirus, virus de Hantaan y reacción en cadena de la polimerasa para morbilivirus fueron todos negativos. Se realizaron además inmuno ensayos utilizando IgG anti-elefante preparado en la Universidad de Tennessee resultando negativos. Pruebas adicionales de cultivos y técnicas moleculares están desarrollándose para lograr una mejor clasificación del virus.

Revisando los registros de mortalidad y los datos patológicos disponibles fueron subsecuentemente identificados seis casos más de herpesvirus que no habían sido reconocidos de infecciones letales en elefantes asiáticos en Parques de vida silvestre y zoológicos de Norte América. Cinco de esos animales estaban en un rango de edad de 18 meses a 7 años y el otro tenía 26 años. No había predilección de sexo. Comunmente la tasa de mortalidad de elefantes asiáticos en cautiverio nacidos en Norte América es del 30% dentro del primer año de vida. Nuestros hallazgos sugieren que esta infección por herpesvirus puede haber contribuido con una significante porción de mortalidad en elefantes asiáticos jóvenes. En 1990 una enfermedad sistémica fatal similar a la del virus herpes fue reportada en un elefante asiático de tres años de edad en un circo en Suiza. El virus no pudo ser aislado; sin embargo estudios serológicos subsecuentes indicaron que un herpesvirus inmunológicamente relacionado con el herpesvirus bovino (BHV) puede tener una alta prevalencia en el hato del circo y en otras colecciones en Suiza y Alemania. Nosotros encontramos que uno de nuestros tres elefantes asiáticos adultos tenía anticuerpos reactivos al BHV. Evidencias serológicas de la infección por otros virus han sido hasta ahora negativas. También hay algunas evidencias morfológicas de erupciones cutáneas relacionadas con herpesvirus en elefantes asiáticos y africanos. Casos de herpes nodular en los pulmones de un alto porcentaje de elefantes silvestres también han
sido documentados.

Caracterizaciones adicionales del virus y una determinación de los aspectos epidemiológicos de la enfermedad son necesarios para desarrollar protocolos para su prevención. La recolección de la información de algunos de estos estudios ha sido iniciada por el Plan de supervivencia de elefantes. Posibles fuentes del virus incluyen: 1) Un herpesvirus autóctono de elefantes asiáticos que causan una enfermedad sistémica en jóvenes inmunológicamente deprimidos, 2) Un virus latente de elefantes africanos que es fatal en elefantes asiáticos (algunos zoológicos albergan elefantes asiáticos y africanos en el mismo exhibidor), ó 3) Un herpes virus de una especie no relacionada puede ser el agente causal de la enfermedad en elefantes asiáticos jóvenes. El estado actual de esta enfermedad en elefantes asiáticos en cautiverio o en su hábitat natural en países asiáticos es hasta el momento desconocido.

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LITERATURE CITED

Campylobacter jejuni IN CAPTIVE AND FREE-LIVING CRANES

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Abstract

As part of the International Crane Foundation’s preventive medicine and pre-release screening programs, feces from 76 apparently healthy captive cranes, representing 10 species, were screened for Campylobacter bacteria. Feces were collected from the cloaca on calcium alginate swabs with charcoal Amies modified medium, inoculated within 12 hr on Campylobacter enrichment media, and incubated at 42°C. Campylobacter were identified by a latex agglutination test, and speciated as Campylobacter jejuni by hippurate hydrolysis.

Results are reported in Table 1. Campylobacter were isolated from 19 of 21 (91%) chicks (< 6 mo of age), and 8 of 55 (15%) adults. Forty-two of the 44 isolates made (from 119 submitted swabs) were identified as Campylobacter jejuni.

A small number of healthy wild Florida sandhill cranes (Grus canadensis pratensis) caught in Alachua and Lake counties, Florida, USA, were also screened. Using some pooled samples, Campylobacter jejuni were found in two or three of seven (29-43%) chicks and none of three adults tested.

This is the first report of Campylobacter in captive or free-living cranes. Campylobacter are frequently isolated from poultry species at prevalences similar to those found in the cranes. Campylobacter associated enteritis has not been identified in the studied cranes, and is not commonly reported in birds. Campylobacter is the most common bacterial agent isolated from humans and nonhuman primates with diarrhea. Though many human cases of Campylobacter jejuni enteritis are associated with food contamination, studies have also linked cases to contact with poultry, nonhuman primates, and a variety of domestic and laboratory animals. Given the high prevalence of Campylobacter shedding found in this study, personnel working with cranes should be made aware of the potential zoonotic risk.

Resumen

Como parte del programa de medicina preventiva de la Fundación Internacional de Grullas y de los programas de monitoreo de pre-liberación, se muestrearon heces de 76 grullas cautivas aparentemente saludables representantes de 10 especies, en búsqueda de bacterias del género Campylobacter. Las heces fueron colectadas de la cloaca con hisopos de alginato de calcio y transportadas en un medio modificado de carbón Amies. En las 12 hrs. subsecuentes se inocularon en un medio enriquecido...
para *Campylobacter* que se incubó a 42°C. Se identificó *Campylobacter* mediante la prueba de aglutinación en látex y se tipificó como *Campylobacter jejuni* por hidrólisis con hipurato.

Los resultados son reportados en la Tabla 1. El *Campylobacter* fue aislado en 19 de 21 pollos (91%) de menos de 6 meses de edad y en 8 de 55 adultos (15%). Cuarenta y dos de 44 aislamientos realizados (de 119 muestras enviadas), fueron identificados como *Campylobacter jejuni*.

Un número pequeño de Grullas canadienses silvestres (*Grus canadensis pratensis*) aparentemente sanas, capturadas en Alachua y los condados de los Lagos de Florida, USA fueron también muestreadas. Utilizando las muestras disponibles se encontró *Campylobacter jejuni* en dos o tres de siete polluelos (29-43%) y en ninguno de 3 adultos muestreados.

Este es el primer reporte de *Campylobacter* en grullas silvestres y en cautiverio. Esta bacteria es frecuentemente aislada en aves de corral y su prevalencia es similar a las encontradas en grullas. La enteritis asociada con *Campylobacter* no ha sido identificada en las grullas estudiadas, y no es comúnmente reportada en aves. Sin embargo es el agente bacteriano más frecuentemente aislado en las diarreas de humanos y primates. No obstante que muchos casos de enteritis por *Campylobacter jejuni* son asociados con la contaminación de alimentos, algunos estudios han sido vinculados con casos de contacto con aves de corral, primates y una amplia gama de animales domésticos y de laboratorio. Dada la alta prevalencia de *Campylobacter* encontrada en este estudio, el personal que trabaja con grullas debe ser consciente del riesgo potencial de una zoonosis.
Table 1. *Campylobacter* isolates from captive cranes at the International Crane Foundation. (Reported as positives/# of birds tested.)

<table>
<thead>
<tr>
<th>Species</th>
<th>Chicks (&lt;6 mo)</th>
<th>Subadult/Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demoiselle Crane <em>Anthropoides virgo</em></td>
<td>--</td>
<td>1/1</td>
</tr>
<tr>
<td>Blue (Stanley) Crane <em>Anthropoides paradisea</em></td>
<td>--</td>
<td>0/1</td>
</tr>
<tr>
<td>Wattled Crane <em>Bugeranus carunculatus</em></td>
<td>--</td>
<td>0/2</td>
</tr>
<tr>
<td>Siberian Crane <em>Grus leucogeranus</em></td>
<td>4/5</td>
<td>0/10</td>
</tr>
<tr>
<td>Sandhill Crane <em>Grus canadensis</em></td>
<td>--</td>
<td>0/4</td>
</tr>
<tr>
<td>Sarus Crane <em>Grus antigone</em></td>
<td>--</td>
<td>1/2</td>
</tr>
<tr>
<td>Eurasian (Common) Crane <em>Grus grus</em></td>
<td>--</td>
<td>0/2</td>
</tr>
<tr>
<td>Whooping Crane <em>Grus americana</em></td>
<td>13/14</td>
<td>5/30</td>
</tr>
<tr>
<td>Black-necked Crane <em>Grus nigricollis</em></td>
<td>2/2</td>
<td>--</td>
</tr>
<tr>
<td>Red-crowned Crane <em>Grus japonensis</em></td>
<td>--</td>
<td>0/2</td>
</tr>
<tr>
<td>Hybrid</td>
<td>--</td>
<td>1/1</td>
</tr>
</tbody>
</table>
EMERGING Helicobacter DISEASES

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Abstract

The last two decades have witnessed a remarkable increase in recognition of clinically relevant diseases associated with a family of gram-negative, spiral-shaped, flagellated bacteria of the genus Helicobacter. The discovery in 1979 that gastric ulcers and gastritis in humans were associated with Helicobacter pylori infections caused a re-examination of the pathogenicity of spiral bacteria in all species. Helicobacter-associated diseases are now recognized in many domestic, laboratory, and zoo animals. Most cases of gastric, enteric, and hepatic helicobacter infections are clinically silent. The most notable signs of gastric helicobacter infection are intermittent vomiting and weight loss. Intestinal helicobacters cause enteritis or colitis, but also have been associated with arthritis and cellulitis in humans. Humans chronically infected with H. pylori also sometimes develop gastric or duodenal ulcers and rarely lymphomas and gastric adenocarcinomas. Gastric ulcers and neoplasia associated with helicobacters are very rare in other species. Ammonia produced from bacterial ureases has direct toxic effects on cells. Helicobacters also produce other cytotoxins and some stimulate gastric epithelial proliferation. Because the inflammation often greatly exceeds gastric epithelial damage, the reaction may have an auto-immune component. Because infection with Helicobacter spp. is not always correlated with disease, urease tests, antibody tests or gastric lavages that demonstrate organisms are not optimal diagnostic tests. For gastrointestinal helicobacter, the gold standard continues to be histopathologic evaluation of endoscopically acquired biopsies. At this time, most other helicobacters are diagnosed histologically after necropsy. The source and transmission of helicobacters between animals is still not completely understood. Oral-oral and fecal-oral routes are considered most likely, although water and raw vegetable sources are also suspected. Risk factors known to be correlated with infection rates are crowding, poor sanitation and age. Both a genetic basis for susceptibility and for outcome of infection have been demonstrated in humans and this also may be true for animals.

Resumen

En las últimas dos décadas hemos sido testigos de un marcado incremento en enfermedades clínicas relevantes asociadas con una familia de gérmenes Gram-negativos, de forma espiral y flageladas del género Helicobacter. El descubrimiento en 1979 de que úlceras gástricas y gastritis en humanos estaban asociadas a la infección con Helicobacter pylori, causaron una revisión de la patogenicidad de las bacterias en espiral en todas las especies. Las enfermedades asociadas a Helicobacter son ahora reconocidas en muchos animales domésticos, de laboratorio y de zoológico. La mayoría de los casos de infecciones gástricas, entéricas y hepáticas debido a Helicobacter son clínicamente silenciosas. Los signos más notables de la infección gástrica causada por Helicobacter pylori son vómito intermitente y pérdida de peso. El Helicobacter intestinal causa enteritis o colitis, pero también ha sido asociado con artritis y celulitis en humanos. Humanos crónicamente infectados con H. pylori desarrollan algunas veces úlceras gástricas o duodenales y en algunos casos linfomas y adenocarcinomas gástricos. Úlceras gástricas y neoplasias asociadas con Helicobacter son muy raras.
en otras especies. El amonio producido por ureas bacterianas tiene efectos tóxicos directos en las células. *Helicobacter* también produce otras citotoxinas y estimula la proliferación epitelial gástrica. Debido que la inflamación a menudo excede el daño en el epitelio gástrico, es posible que la reacción tenga un componente autoinmune. Ya que la infección con *Helicobacter* spp. no siempre está relacionada con la presencia de enfermedad, la prueba de ureasa, anticuerpos o lavados gástricos que demuestran organismos no son prueba óptimas de diagnóstico. Para *Helicobacter* gastrointestinal la prueba de oro continúa siendo la evaluación histopatológica de biopsias adquiridas por endoscopia. Hasta ahora, otros *Helicobacter* son diagnosticados histopatológicamente después de la necropsia. El origen y la transmisión del *Helicobacter* entre animales no está completamente reconocida. Las rutas oral-oral y oral-fecal son consideradas las más comunes aunque el agua y los vegetales crudos son también sospechosos. Los factores de riesgo reconocidos como directamente relacionados con las tasas de infección son el hacinamiento, deficiente sanidad y la edad. Tanto factores genéticos de susceptibilidad y resultados de infección han sido demostrados en humanos y esto también puede ser cierto para animales.

**Introduction**

The last two decades have witnessed a remarkable increase in recognition of clinically-relevant diseases associated with a family of gram-negative, spiral-shaped, flagellated bacteria classified in the genus *Helicobacter*. The discovery in 1979 that gastric ulcers and gastritis in humans was associated with *Helicobacter pylori* infections caused a re-examination of the pathogenicity of spiral bacteria in all species. Helicobacter-associated diseases are now recognized in many domestic, laboratory, and zoo animals, and the prevalence and pathogenicity of these diseases appear to be increasing. The genus *Helicobacter* contains gastric and non-gastric types (intestinal, hepatic, and respiratory forms). All gastric helicobacters produce ureases which permit their survival in the acidic gastric environment and provide the basis for some diagnostic and therapeutic methods in humans.

The purpose of this report is to summarize current knowledge of *Helicobacter*-associated diseases in animals. Table 1 lists the type of *Helicobacter* spp. reported to infect each species and the associated clinical disease or pathologic finding. Initial concepts that *Helicobacter* spp. tend to be host specific are now being questioned.

**Clinical signs**

Most cases of gastric, enteric, hepatic, and respiratory helicobacter infections are clinically silent. The most notable signs of gastric helicobacter infection are intermittent vomiting and weight-loss from chronic gastritis. Intestinal helicobacters cause enteritis or colitis, but also have been associated with arthritis and cellulitis in immunocompromised humans. Humans chronically infected with *Helicobacter pylori* also sometimes develop gastric or duodenal ulcers and rarely lymphomas and gastric adenocarcinomas. Gastric ulcers and gastric neoplasia in association with helicobacter infection only rarely occur in other species.

**Pathologic basis of disease**

The precise basis for helicobacter pathogenicity is still under investigation and is best understood for
gastric helicobacters. Ammonia production from bacterial ureases has direct toxic effects on cells. Helicobacters also produce other cytotoxins. These bacterial products result in degeneration or necrosis of gastric epithelial cells. Some helicobacters also stimulate gastric epithelial proliferation. Most helicobacters incite a predominantly lymphoplasmacytic inflammatory response, whereas *Helicobacter pylori* also incites a neutrophilic reaction. Because the inflammation often greatly exceeds the extent of gastric epithelial damage, the inflammatory reaction may also have an autoimmune component. Circulating antibodies are produced, but this humoral response fails to clear the body of organisms. It is interesting to note that the inflammatory response and gastric hyperplasia persist after eradication of the organism with antibiotic treatment.

**Diagnostic tests**

Because the presence of *Helicobacter* spp. is not always correlated with disease, urease breath tests, antibody tests, or gastric lavages that demonstrate organisms are not optimal diagnostic tests. For gastrointestinal helicobacters, the gold standard continues to be histopathologic evaluation of endoscopically-acquired biopsies. At this time, most other helicobacters are diagnosed at necropsy. Most cases have no gross lesions or have gastric hyperplasia, atrophy, or ulcers. Definitive diagnosis requires histopathology, but the type of helicobacter cannot be determined morphologically. Organisms can be speciated by culturing and subsequent morphologic identification or sequence analysis. However, culturing is exceedingly difficult for most helicobacters and not possible for *H. heilmannii*.

**Epidemiology**

The source and transmission of helicobacters between animals is still not completely understood. Oral-oral or fecal-oral routes are considered most likely, although water and raw vegetable sources also have been suspected. Many animal helicobacter infections should be considered zoonoses, and conversely, the human pathogens, *Helicobacter heilmannii* and *Helicobacter pylori*, are proving to have a much broader species range than previously suspected.

Risk factors known to be correlated with high infection rates are crowding and poor sanitation. The prevalence of infection and the severity of disease increase with age. Both a genetic basis for susceptibility to and outcome of infection have been demonstrated in humans, and this also may be true for other species, such as the cheetah.

**LITERATURE CITED**


species isolated from poultry and from human patients with gastritis. Microbiology 140:3441-3449.
Table 1. Current reports of *Helicobacter* spp. infections and disease.

<table>
<thead>
<tr>
<th>Affected Species</th>
<th><em>Helicobacter</em> Species</th>
<th>Associated Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheetahs</td>
<td><em>H. heilmannii</em> (formerly <em>Gastrospirillum</em>-LO)  &lt;br&gt; <em>H. acinonyx</em>  &lt;br&gt; <em>H. felis</em></td>
<td>gastritis  &lt;br&gt; gastritis  &lt;br&gt; gastritis</td>
<td>7, 8, 24, 29</td>
</tr>
<tr>
<td>Primates (pig-tailed macaque, <em>M. rhesus</em>)</td>
<td><em>H. nemastriane</em>  &lt;br&gt; <em>H. heilmannii</em>  &lt;br&gt; <em>H. pylori</em>  &lt;br&gt; <em>H. cinaedi</em> (intestinal)</td>
<td>none?</td>
<td>6, 18, 29</td>
</tr>
<tr>
<td>Ferrets</td>
<td><em>H. mustelae</em></td>
<td>gastritis with ulcers  &lt;br&gt; adenocarcinomas</td>
<td>20, 29, 37</td>
</tr>
<tr>
<td>Mink</td>
<td><em>H. mustelae</em></td>
<td>gastritis</td>
<td>29</td>
</tr>
<tr>
<td>Birds (terns, gulls, sparrows)</td>
<td><em>H. spp.</em>  &lt;br&gt; <em>H. pametensis</em></td>
<td>hepatitis</td>
<td>29</td>
</tr>
<tr>
<td>Chickens</td>
<td><em>H. pullorum</em></td>
<td>hepatitis</td>
<td>32</td>
</tr>
<tr>
<td>Mice/Rats</td>
<td><em>H. hepaticus</em>  &lt;br&gt; <em>H. bilis</em>  &lt;br&gt; <em>H. muridarum</em>  &lt;br&gt; <em>H. pylori</em> (exp)  &lt;br&gt; <em>H. felis</em> (exp)  &lt;br&gt; <em>H. (Flexibacter) rappropi</em></td>
<td>hepatitis and carcinomas  &lt;br&gt; gastritis  &lt;br&gt; gastritis  &lt;br&gt; gastritis  &lt;br&gt; gastritis, MALTomas, proliferative gastritis</td>
<td>10, 11, 18, 20, 29, 35, 36</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td><em>H. pylori</em></td>
<td>gastritis</td>
<td>18, 19</td>
</tr>
<tr>
<td>Hamsters</td>
<td><em>H. cinaedi</em> (intestinal)</td>
<td>none?</td>
<td>29</td>
</tr>
<tr>
<td>Rabbits</td>
<td>Cilia-associated respiratory bacillus</td>
<td>upper respiratory tract infection</td>
<td>4</td>
</tr>
<tr>
<td>Cats</td>
<td><em>H. pylori</em>  &lt;br&gt; <em>H. felis</em>  &lt;br&gt; <em>H. heilmannii</em></td>
<td>gastritis  &lt;br&gt; gastritis  &lt;br&gt; gastritis</td>
<td>12, 14-16, 22</td>
</tr>
<tr>
<td>Dogs</td>
<td><em>H. felis</em>  &lt;br&gt; <em>H. heilmannii</em>  &lt;br&gt; <em>H. canis</em> (intestinal)  &lt;br&gt; <em>H. pylori</em> (exp)</td>
<td>gastritis  &lt;br&gt; gastritis  &lt;br&gt; gastroenteritis  &lt;br&gt; gastritis</td>
<td>12, 16, 29</td>
</tr>
<tr>
<td>Pigs</td>
<td><em>H. pylori</em> (exp)  &lt;br&gt; <em>H. heilmannii</em> (=<em>H. suis</em>?)  &lt;br&gt; <em>H. pametensis</em></td>
<td>gastritis with ulcers  &lt;br&gt; gastritis  &lt;br&gt; none?</td>
<td>29</td>
</tr>
<tr>
<td>Sheep</td>
<td><em>H. (Flexibacter) rappropi</em></td>
<td>placentitis, abortion</td>
<td>29</td>
</tr>
<tr>
<td>Calves</td>
<td><em>Helicobacter</em> spp?</td>
<td>gastritis</td>
<td>13</td>
</tr>
<tr>
<td>Humans</td>
<td><em>H. pylori</em>  &lt;br&gt; <em>H. heilmannii</em>  &lt;br&gt; <em>H. felis</em>  &lt;br&gt; <em>H. fennelliae</em>  &lt;br&gt; <em>H. canis</em> (intestinal)  &lt;br&gt; <em>H. cinaedi</em> (intestinal)  &lt;br&gt; <em>H. pullorum</em></td>
<td>gastritis  &lt;br&gt; gastritis  &lt;br&gt; gastritis  &lt;br&gt; enterocolitis  &lt;br&gt; enterocolitis  &lt;br&gt; arthritis, cellulitis  &lt;br&gt; gastroenteritis</td>
<td>3, 17, 21, 30</td>
</tr>
</tbody>
</table>
Preliminary Observations of a New Diet for Giant Anteaters
(Myrmecophaga tetradactyla)

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Abstract

Historically, diets fed to giant anteaters in captive settings tend to be very complex. A new diet was formulated which provides a more appropriate nutrient profile, eliminated feeds which may contribute to poor stool consistency, reduced the potential for feed spoilage, and increased the ease of preparation and flexibility of presentation.

Resumen

Históricamente, las dietas para alimentar a los osos hormigueros gigantes en cautiverio tienden a ser muy complejas. Se formuló una nueva dieta que provee un perfil de nutrientes más apropiado, eliminó alimentos que pudieran contribuir a una consistencia pobre en el excremento, redujo el potencial de desperdicio de alimento, e incrementó la facilidad de preparación y flexibilidad de presentación.

Introduction

As specialized feeders, giant anteaters (Myrmecophaga tetradactyla) present a unique challenge to the zoo nutritionist. Neither the natural diet, nor a suitable commercially available insect substitutes can be provided in appropriate quantities to deliver a nutritionally balanced ration. Additionally, the anatomy of the oral cavity and tongue dictate the need for a diet which is small in particle size and easily consumed.

As with many early diets for captive animals, giant anteater diets were unnecessarily complex. The basis for many of these diets included a type of dry dog kibble, however, the brand and thus nutrient density varied from institution to institution. In addition to the dry dog kibble, a wide spectrum of ingredients including baby cereals, honey, yogurt, milk, meats and numerous produce items were blended together with water to form a soupy mixture which these animals could consume. These diets are reminiscent of the panda gruels which were used until 1989.1

The stool produced by giant anteaters consuming this diet, unlike that of free-ranging animals, tended to be pasty to liquid in consistency.1 Although not surprising, considering the ingredients fed, no one has made an attempt to address the problem. In fact, these fecal characteristics are so prevalent, that many accepted them as “normal” or “typical”.

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1996 PROCEEDINGS AMERICAN ASSOCIATION OF ZOO VETERINARIANS
**Case Report**

Between 1992 and 1993, three (1.2) adult giant anteaters were received from separate institutions, by the Cleveland Metroparks Zoo (CMZ) to be housed in the new Rainforest Building. The following changes were made to the diets offered by the sending institutions in order to improve the nutrient concentrations, eliminate the presence of potentially harmful components, and provide a more consistent diet for these individuals. The dry dog kibble was replaced with a more digestible meat-based kibble formulated for cats. All milk, and fresh or frozen meat products were eliminated from the diet. Finally, a cellulose source was included to mimic dietary chitin.

The revised diet was formulated using a finely ground mixture of equal proportions of a dry cat kibble and a higher fiber primate diet. Selected nutrient concentrations of both ingredients and the final diet are provided in Table 1.

Initially the animals were offered the dry mixture, combined with adequate quantities of water, producing a thin paste consistency to encourage consumption. Over time, the water was removed until the animals were consuming only the dry diet.

Although no quantitative scoring was made to assess fecal consistency, the stool produced by the animals consuming the revised diet was a formed, cylindrical particle.

**Discussion**

Even though no specific nutrient requirements have been established for giant anteaters, as strict carnivores, they may have many of the same unique requirements described in felids. The complete feeds formulated for domestic felids provide those nutrients (e.g., taurine, arachidonic acid). Although these nutrients may be found in canine diets, the minimum concentrations are not typically guaranteed.

The elimination of milk products, including yogurt and fluid milk, removed the influence of lactose from the revised diet. Since giant anteaters cease to intake lactose post weaning, there is a potential for a lactose intolerance to occur. The undigested lactose will lead to osmotic changes in the lower gut, increasing the moisture levels of the gut contents, ultimately producing a loose stool.

The use of fresh or frozen meat products in these diets necessitates additional labor and storage conditions not needed by the dry ingredients. Additionally, giant anteaters may be more sensitive than other large carnivores to the bacterial organisms (i.e., *Salmonella* spp.) which are commonly cultured from these feeds. Elimination of the meat products also dramatically increases the time it takes for the mixed diet to spoil.

Like cellulose, chitin is a structural polysaccharide consisting of strait chains with a $\beta$-1,4 linkage. The chitinous exoskeleton of insects consumed by free-ranging giant anteaters may preform a role similar to that of cellulose in the digestive tract of herbivores by providing gut fill and maintaining fecal consistency. The leaf eater primate diet may be utilized as a cellulose source to assist in this function. Additionally, the high palatability of this product should increase the acceptance of the diet.
The simple formulation of the diet, as well as the presentation of dry material, dramatically increased the ease of diet preparation. The diet has been offered in both shallow pans, as well as in PVC tubes placed in a simulated termite mound in the exhibit area. This type of presentation extends feeding time and encourages natural feeding behavior and postures, while incorporating the animals’ prescribed diet in the exhibit enrichment program.

The results observed in these individuals supports a broader application of this diet in species with similar feeding strategies. Further examination of this diet, with giant anteaters and other strict insectivores, is currently on-going.

ACKNOWLEDGMENTS

The authors thank Don Kuenzer, and CMZ Rainforest and Hospital keeper staff for their assistance.

LITERATURE CITED

Table 1. Selected nutrient concentrations of cat food,\textsuperscript{a} leafeater primate diet,\textsuperscript{b} and revised diet (dry matter basis).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Cat Food, Dry</th>
<th>Leafeater Primate Diet, Dry</th>
<th>Revised Diet, Dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>7.25</td>
<td>8.60</td>
<td>6.51</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>36.28</td>
<td>25.44</td>
<td>30.90</td>
</tr>
<tr>
<td>Ether Extract (%)</td>
<td>23.81</td>
<td>5.43</td>
<td>14.68</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>6.22</td>
<td>6.39</td>
<td>6.30</td>
</tr>
<tr>
<td>Lysine (%)</td>
<td>1.94</td>
<td>1.33</td>
<td>1.64</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>1.08</td>
<td>1.05</td>
<td>1.07</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.97</td>
<td>0.71</td>
<td>0.84</td>
</tr>
<tr>
<td>Iron (ppm)</td>
<td>242.59</td>
<td>218.82</td>
<td>230.79</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>253.37</td>
<td>121.44</td>
<td>187.89</td>
</tr>
<tr>
<td>Vitamin A (IU/kg)</td>
<td>27385.44</td>
<td>8752.74</td>
<td>18137.39</td>
</tr>
<tr>
<td>Vitamin E (IU/kg)</td>
<td>113.21</td>
<td>280.70</td>
<td>196.34</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The Iams Company (Dayton, OH)
\textsuperscript{b}Marion Zoological, Inc. (Plymouth, MN)
SQUAMOUS CELL CARCINOMA OF THE PERINEAL SKIN IN TWO HAMADRYAS BABOONS (Papio hamadryas)

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Abstract

Two, aged, reproductively cycling, female Hamadryas baboons (Papio hamadryas) were diagnosed with ulcerative squamous cell carcinoma of the perineal skin. The first individual’s lesions were initially treated as infected bite wounds for a period of approximately 1 yr. Treatment consisted of a variety of broad spectrum antibiotics and superficial debridement. Progression of the lesions, lymphadenopathy, cachexia and deterioration of the animal’s quality of life eventually warranted euthanasia. Postmortem histopathologic examination of the affected perineal tissue revealed squamous cell carcinoma. The second individual also presented with what appeared to be infected bite wounds of the perineal skin. Initial treatment consisted of broad spectrum antibiotics and superficial debridement. Histopathologic results from the first animal and the progressive appearance of the wounds on the second animal prompted biopsy submission. Squamous cell carcinoma was confirmed. Several surgical attempts to adequately debulk the affected areas failed. The invasive nature of the neoplastic process prohibited adequate curative excision margins during three surgical procedures. The use of radiation and intralesional chemotherapies has proven impractical in this particular case however these options should be strongly considered for use in any additional cases.

Squamous cell carcinoma lesions vary in aggressiveness, predilection site and identifiable predisposing factors depending on the age, species, and hair/skin pigmentation in the affected individual. As a result, the biological behavior of and prognosis for squamous cell carcinoma varies with the affected species and location of the tumor. Although two cases of oral squamous cell carcinoma have recently been observed in Hamadryas baboons (Dr. Kathy Brasky, Southwest Foundation, personal communication), there is only one report of squamous cell carcinoma affecting the perineum of a Hamadryas baboon. The influence of environmental factors other than ultraviolet light exposure on non-pigmented skin surfaces have not been identified or quantified. Possible predisposing factors in these two baboons include age, consecutive cycling without conception, chronic irritation of the perineal skin, the use of chemical disinfectants on materials that directly contact the ischial skin and prolonged exposure to ultraviolet light. Although perineal skin wounds can be common findings in baboons housed in a captive troop, the presence of chronic, non-healing wounds on the perineal skin may be suggestive of a pre-neoplastic or invasive neoplastic process. It has long been assumed that the stages of initiation, promotion and progression of evolving SCC in small animal patients as well as humans are influenced by one or more genetic or epigenetic events. The confirmed appearance of this type of neoplasm in Hamadryas baboons elucidates the need to identify predisposing factors, circumstances surrounding unintentional intensification of these factors, and viable treatment options. Further investigation of all confirmed cases is warranted.
Resumen

Fueron diagnosticadas dos hembras de Babuino Hamadryas (*Papio hamadryas*) de edad avanzada y ciclando reproductivamente, con carcinoma ulcerativo de las células escamosas de la piel perineal. Las lesiones del primer individuo fueron tratadas inicialmente como heridas por mordedura infectadas por un período aproximado de un año. El tratamiento consistió en una variedad de antibióticos de amplio espectro y debridadación superficial. El progreso de las lesiones, linfadenopatía, caquexia y el deterioramiento de la calidad de vida del animal eventualmente hicieron necesaria la eutanasia. El examen histopatológico postmortem del tejido perineal afectado reveló un carcinoma de las células escamosas. El segundo individuo, también fue presentado con lo que aparentaban ser heridas por mordedura infectadas de la piel perineal. El tratamiento inicial consistió en antibióticos de amplio espectro y debridadación superficial. Los resultados histopatológicos del primer animal y la aparición progresiva de las heridas del segundo animal incitó a someterlo a una biopsia. Se confirmó un carcinoma de las células escamosas. Varios intentos quirúrgicos para reducir el volumen de las áreas afectadas fallaron. La naturaleza invasiva del proceso neoplásico impidió la curación adecuada de los márgenes de excisión durante tres procedimientos quirúrgicos. El uso de radiación y quimioterapia inralesionales han probado ser poco prácticos en este caso particular, sin embargo estas opciones deben ser seriamente consideradas para su uso en cualquier caso adicional.

Las lesiones del carcinoma de las células escamosas varían en agresividad, sitio de predilección y factores de predisposición identificables dependiendo de la edad, especie y pigmentación del pelo/piel en el individuo afectado. Como resultado, el comportamiento biológico y el pronóstico de un carcinoma de células escamosas varía con la especie afectada y la localización del tumor. A pesar de que dos casos de carcinoma de células escamosas orales han sido recientemente observados en babuinos Hamadryas (Dr. Kathy Brasky, Southwest Foundation, comunicación personal), existe sólo un reporte de carcinoma de células escamosas afectando el perineo de un babuino Hamadryas. La influencia de factores ambientales diferente a la exposición a la luz ultravioleta de superficies de piel no pigmentadas no han sido identificados o cuantificados. Los posibles factores de predisposición en estos dos babuinos incluyen, edad, ciclos consecutivos sin concepción, irritación crónica de la piel perineal, el uso de desinfectantes químicos en materiales que hacen contacto directo con la piel isquiática y una exposición prolongada a la luz ultravioleta. A pesar de que las heridas en la piel perineal pueden encontrarse comunmente en babuinos mantenidos como grupo en cautiverio, la presencia de heridas crónicas que no sanan en la piel perineal pueden ser sugestivos de un proceso pre-neoplásico o neoplásico invasivo. Se ha asumido que los estados de inanición, promoción y progresión del carcinoma de células escamosas en pacientes de pequeñas especies al igual que en los humanos son influenciados por uno o más eventos genéticos o epigenéticos. La aparición confirmada de este tipo de neoplasia en babuinos hamadryas, pone en claro la necesidad de identificar los factores predisponentes, las circunstancias que rodean una intensificación no intencional de estos factores y opciones viables de tratamiento. Mas investigaciones de todos los casos confirmados son necesarias.

LITERATURE CITED

UNUSUAL BONE PATHOLOGY IN RED PANDAS

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Abstract

Red pandas (Ailurus fulgens fulgens) have been kept at Melbourne Zoo since 1982 and all animals (n=9) have entered the collection when adult, as breeding has never been successful in this institution. In April 1996 the collection consisted of a solitary pair of animals as four individuals have died and three transferred to other zoos. Losses in stock have all been due, in part, to bone-related problems. Severe spinal spondyloses resulting in generalized stiffness were found in pandas dying in 1982 and 1986. This report primarily deals with two pandas which presented in 1990 and 1991 with unusual and severe bone pathology which eventually necessitated their euthanasia in 1991 and 1995 respectively. The common features of each case was a severe periosteal new bone reaction involving the elbow joint and extensive remodeling of the distal humerus and proximal radius and ulna of each forelimb. Similar pathology in red pandas has been previously described in an adult male at the Helsinki Zoo in 1981 but no etiology of the condition was established.7

Resumen

Los Pandas rojos (Ailurus fulgens fulgens) han sido mantenidos en el Zoológico de Melbourne desde 1982, y todos los animales han formado parte de la colección siendo adultos, ya que la reproducción nunca ha sido exitosa en esta Institución. En abril de 1996 la colección consistía en una sola de estos animales, ya que 4 individuos murieron y 3 fueron transferidos a otros zoológicos. Las pérdidas de nuestros animales han sido debido, en parte a problemas relacionados con los huesos. Una espondilosis espinal severa que resultó en rigidez generalizada se encontró en los pandas que murieron en 1982 y 1986. Este reporte trata principalmente de dos pandas que presentaron este transtorno en 1990 y 1995 respectivamente. Las características comunes de cada caso fue una reacción severa periosteal de hueso nuevo que involucra la articulación del codo y una deformación extensiva del húmero distal y radio y una proximal de cada extremidad superior. Una patología similar en pandas rojos ha sido previamente descrita en un macho adulto en el zoológico de Helsinki en 1981, pero no se estableció ninguna etiología para esta condición.

Case Reports

Case 1: A 4-yr-old female (house name ‘Pitti Sing’), first presented with a left forelimb lameness in 1990 and was euthanatized in 1991 due to severe lameness, inappetence and loss of condition. Serial radiographs had shown a progressively more severe periosteal new bone reaction of the left elbow and periosteal bone reaction in the distal femur and proximal radius and ulna of this limb. This condition progressed from no detectable radiographic findings to massive bony changes over a period
of 3 mo. The right forelimb started to show identical radiographical changes 1 mo after changes were
detected in the left leg but lesions did not develop to the same severity and the animal was never
lame on this leg. Clinically, Pitti Sing’s left forelimb lameness resolved with cage rest but repeated
examinations revealed a decreasing range of movement in each elbow which paralleled the
increasing severity of radiographic lesions. Eleven months after initial presentation Pitti Sing became
lame again in her left foreleg and radiographs showed a slight increase in severity of the elbow
lesions and periosteal reaction in the right and left femoral necks. Euthanasia was performed and at
necropsy both elbow joints showed marked ankylosis, thickened joint capsules and severe erosions
of articular cartilage. Affected long bones had thickened cortices with a normal medullary cavity.
The liver was a pale yellow/brown color and was abnormally enlarged. Histopathological
examination of the elbow joints and long bones showed large amounts of sub-periosteal new bone
undergoing aggressive, orderly remodeling. There was also fissuring of articular cartilage with some
subarticular fibrous tissue proliferation. The liver sections showed marked cytoplasmic vacuolation
of hepatocytes. No renal lesions were noted.

Case 2: A 6-yr-old male (house name ‘Yum Sing’) was routinely examined in 1991 and found to
have a restricted range of movement in both elbow joints not associated with lameness. Radiographic
changes consisted of marked periosteal reaction in the distal femur and proximal radius and ulna
involving the elbow joints. In addition most of each forearm showed irregular thickening of the
cortices of radius and ulna and an obvious periosteal reaction in the distal epiphyses of these bones.
Radiographs taken in 1992, 1993 and 1994 showed a gradual reduction in periosteal reactivity
associated with the lesion but an obvious increase in the cortical thickness of the radius and ulna of
each forelimb, leading to obliteration of the medullary cavities of these bones. Yum Sing was
euthanatized in 1995 due to an acute lameness of the right forelimb which was caused by a
pathological fracture of the humerus. At necropsy bony changes corresponded with radiographical
findings but in addition bilateral thickening of the mandibular angles was evident as was the
deposition of osteophytes along the sagittal crest and zygomatic arches. Both kidneys appeared pale
and shrunken. On histopathological examination sections of bone appeared osteosclerotic with no
medullary differentiation and was comprised of dense lamellar bone with wide osteoid seams. There
was no periosteal bone reaction and no evidence of fibrous osteodystrophy. Multiple sections of
kidney revealed diffuse renal disease, characterized by contracted glomeruli with thickened basement
membranes and areas where tubules were dilated, atrophic and contained protein casts.

Although some radiographic and histopathologic differences exist between the two presented cases
it is assumed that they are representations of the same syndrome. This assumption is further
supported by the recent detection of identical radiographic changes in the right elbow of the
remaining female panda (house name, ‘Qantas’) at Melbourne Zoo.

Dietary Study

Red pandas at Melbourne Zoo are fed a mixture of bamboo, fruits, primate cake, commercial dog and
cat food supplemented with a multivitamin powder and the occasional egg. A five day trial was
conducted to estimate the daily consumption of each dietary item using the remaining pair of pandas
at the zoo. Leaves from the two species of bamboo fed (Phyllostachys aurea and Pseudosasa
*japonica* were analyzed for their Ca and P content while the amounts of these minerals and vitamin A in the remaining foodstuffs were derived from manufacturer information or published values. The results of this investigation are presented in Table 1. Melbourne Zoo pandas consume a diet containing 0.59% Ca and 0.32% P which are levels not greatly dissimilar to those recommended by the American Association of Zoological Parks and Aquariums in 1987 (0.75% Ca and 0.60% P). This report also recommends a minimum daily requirement of vitamin A (made up of free vitamin A contained in supplements and carotene found in plant matter) of 87 µg/kg body weight. The daily intake of vitamin A contained in all supplements fed at Melbourne Zoo was calculated to be 2043 µg/kg body weight. It is interesting to note that published minimum daily requirements of domestic animals varies from 12 µg/kg body weight in farm species to 600 µg/kg in a growing cat.

**Biochemical Values**

Table 2 presents serum values of Ca, P, retinol and 25 hydroxy vitamin D values of affected and unaffected animals compared to normal values where available. Ca and P values appear normal although these serum levels of these minerals are generally considered an insensitive indicator of their overall levels in the body. If an excess of dietary vitamin A was causing the described pathology then serum retinol levels would be expected to be obviously higher than normal values which was not the case in the single measurements taken in affected pandas. Hypervitaminosis D would be expected to be reflected by an increase in serum 25 hydroxy vitamin D levels and while there seems to be some variation in the concentrations measured in the four animals, these values do not greatly differ from levels deemed to be normal in other mammalian species (personal communication, Dr Chris Laing, University of Sydney, Australia).

**Discussion**

Dietary imbalances in the total intake, or ratio of intake of Ca and P could be expected to produce a range of metabolic bone disease in different species such as osteomalacia, fibrous osteodystrophy and osteosclerosis. However, it is unlikely that the pathology seen reflects an oversupply or imbalance in these minerals given that their dietary levels appear in order. Hypervitaminosis A has been primarily reported in cats and is characterized in mature animals by the formation of spinal exostoses and occasionally exostoses surrounding joints. The condition develops within 6-12 mo in young cats fed 15000-35000 µg/kg of the vitamin daily from the time of weaning. Although the pandas at Melbourne Zoo are fed far in excess of their minimum daily requirement of vitamin A, the amount is unlikely to be toxic and a diagnosis of hypervitaminosis A is not supported by measured serum retinol concentrations. Hypervitaminosis D in domestic animals is usually produces a characteristic calcification of soft tissues. This type of pathology was not seen in the affected pandas and serum calcium and 25 hydroxy vitamin D concentrations appeared normal.

The pathology seen in the presented cases does not conveniently match descriptions of similar processes seen in domestic animals. Hypertrophic pulmonary osteoperiostitis (HPO) in dogs is characterized by periosteal reaction in the distal phalanges, metacarpal and metatarsal bones which may extend to other bones. The vast majority of HPO cases are associated with a chest mass although occasionally the lesions have been seen with abdominal masses. Both pandas had some degree of periosteal reaction but it tended to be associated with the elbow joints, radius and ulna and neither case demonstrated a thoracic or abdominal mass.
Hypertrophic osteodystrophy is an idiopathic bone disease of young dogs that can result in the formation of periosteal and endosteal new bone particularly in the distal radius and ulna. Histopathologically it is characterized by necrosis and inflammation of trabecular bone, a thickened periosteum with subperiosteal fibrosis and inflammation. The cause of this disease is currently unknown although canine distemper virus may be involved as a recent study detected this virus in the bones of three affected dogs. There are frequent reports of canine distemper virus causing disease in red pandas and the sensitivity of this species to the virus is indicated by the production of clinical disease following the use of live attenuated vaccines. In red pandas canine distemper produces predominantly pulmonary signs. The examination of frozen bone sections from Case 2 for canine distemper virus is currently being performed.

ACKNOWLEDGMENTS

Thanks to Sheona Hollywood and the Melbourne Zoo Carnivore Department for their assistance with the dietary study.

LITERATURE CITED

Table 1. Daily calcium, phosphorous and vitamin A intake of red pandas at Melbourne Zoo.

<table>
<thead>
<tr>
<th></th>
<th>DRY MATTER (g/day)</th>
<th>Ca INTAKE (mg)</th>
<th>P INTAKE (mg)</th>
<th>VITAMIN A (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAMBOO</td>
<td>369</td>
<td>1456</td>
<td>785</td>
<td>0</td>
</tr>
<tr>
<td>FRUITS &amp; EGG</td>
<td>90</td>
<td>60</td>
<td>90</td>
<td>36</td>
</tr>
<tr>
<td>DOG/CAT FOOD</td>
<td>41</td>
<td>930</td>
<td>462</td>
<td>643</td>
</tr>
<tr>
<td>VITAMIN SUPP.</td>
<td>4</td>
<td>310</td>
<td>250</td>
<td>1140</td>
</tr>
<tr>
<td>PRIMATE CAKE</td>
<td>40</td>
<td>489</td>
<td>203</td>
<td>10440</td>
</tr>
<tr>
<td>TOTAL</td>
<td>544</td>
<td>3245</td>
<td>1790</td>
<td>12259</td>
</tr>
</tbody>
</table>

Table 2. Serum biochemical values for red pandas held at Melbourne Zoo.

<table>
<thead>
<tr>
<th></th>
<th>Ca (mmol/L)</th>
<th>P (mmol/L)</th>
<th>ALP (IU/L)</th>
<th>Retinol (mg/L)</th>
<th>25 OH vitamin D (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pitti Sing</td>
<td>2.19</td>
<td>1.29</td>
<td>57-992</td>
<td>0.34</td>
<td>10</td>
</tr>
<tr>
<td>Yum Sing</td>
<td>2.20 - 2.36</td>
<td>1.56 - 2.06</td>
<td>29-196</td>
<td>0.45</td>
<td>70</td>
</tr>
<tr>
<td>Qantas</td>
<td>2.31</td>
<td>1.87</td>
<td>6-15</td>
<td>0.37</td>
<td>19</td>
</tr>
<tr>
<td>Edwina (Unaffected)</td>
<td>2.20 - 2.80</td>
<td>1.45 - 2.50</td>
<td>8-35</td>
<td>0.57</td>
<td>59</td>
</tr>
<tr>
<td>NORMAL VALUE</td>
<td>2.35 ± 0.2*</td>
<td>1.68 ± 0.42*</td>
<td>36 ± 21*</td>
<td>0.20 - 0.80**</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

* ISIS normal values 24.4.96
** personal communication, Dr. Ellen Dierenfield, Wildlife Conservation Society, New York.
THE EFFECTS OF AN ALL FISH DIET ON URINARY METABOLITES AND CALCIUM OXALATE SUPERSATURATION OF ASIAN SMALL-CLAWED OTTERS (*Aonyx cinerea*)

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Abstract

At the Minnesota Zoological Garden, 17 of 19 Asian small-clawed (ASC) otters 2 yr of age or older (89%) have had urolithiasis. Of 11 young animals that were followed radiographically, nine developed calculi by 4 yr of age, one animal became affected between 4 ½ and 5 ½ yr, and one between 4 and 8 yr. Calculi were analyzed in six animals and all were composed of CaOx monohydrate with a few having CaOx dihydrate components as well.

In 1991 we analyzed urine from 24 hr collections in six otters with urolithiasis during periods of food consumption and fasting. We found no evidence of glucosuria, which had previously been reported in ASC otters with urolithiasis. The most significant finding was a profound hyperoxaluria when compared to humans, normal dogs, and dogs with CaOx urolithiasis (Tables 2 and 3). There was no significant difference in oxalate excretion between the fed and fasted states.

In 1994 we collected urine from six otters via cystocentesis approximately 4-5 hr after eating and again after a 24 hr fast. The results confirmed the hyperoxaluria noted in 1991 (Tables 2 and 3). Postprandial levels of urinary potassium, calcium, magnesium, phosphate, sulfate, and uric acid were all significantly higher than fasting levels. Calcium oxalate supersaturation (CaOxSS) levels were determined using a computer program called EQUIL-2. This system uses urine pH and the major ions in urine to calculate the potential for crystallization of common kidney stone components. CaOxSS values averaged over three meaning that the urine was elevated three times above the saturation point for calcium and oxalate.

In 1995, otters were divided into two groups of five each. One group was fed a low oxalate diet, ground herring with thiamin and vitamin E added (fish otters). The other group was kept on their original Nebraska Brand Feline diet (NBF otters). At the end of 1 mo, both diets were analyzed (Table 1) and urine and blood were collected as in 1994. Fish otters had a significantly lower CaOxSS than NBF otters during the postprandial period, although oxalate levels were surprisingly similar (Table 2). Fish otters also had significantly higher levels of urinary sulfate, phosphate, sodium, potassium, and chloride, and lower urinary pH in the postprandial period. Serum alkaline phosphatase (SAP) levels were higher in the fish otters (SAP mean=333) than the NBF otters (SAP mean=136). At the end of 6 mo SAP levels had risen further (mean=637) in the fish otters and some animals had elevations of the gamma glutamyltransferase (GGT) levels as well. Fatty liver change associated with an omega-6 fatty acid deficiency was suspected, and corn oil was added to the fish diet. One month later, SAP (mean=285) and GGT levels had decreased in all fish otters. An all fish diet may reduce CaOxSS but can lead to dietary imbalances and its long term effect on general health and the development of urolithiasis has yet to be determined.
Resumen

En el Jardín Zoológico de Minnesota 17 de las 19 nutrias de garras cortas asiáticas (NGC) de 2 años de edad o mayores han padecido urolitiasis (89%). De 11 animales jóvenes que se les siguió el caso radiográficamente, 9 desarrollaron cálculos a los 4 años de edad, un animal fue afectado entre los 4.5 y 5.5 años y uno entre los 4 y los 8 años. Los cálculos de seis animales fueron analizados y todos estaban compuestos de monohidrato de oxalato de calcio (CaOx) y algunos tenían también componentes de hidrato de oxalato de calcio.

En 1991 analizamos la orina recolectada cada 24 hr de seis nutrias con urolitiasis durante periodos de consumo de alimento y ayuno. No encontramos evidencia de glucosuria, la cual había sido previamente reportada en NGC con urolitiasis. El hallazgo más significativo fue una profunda hiperoxaluria cuando fue comparada con la de humanos, perros normales y perros con urolitiasis por CaOx (Tablas 2 y 3). No hubieron diferencias significativas en la excreción de oxalato entre los estados pre- y post prandiales.

En 1994 recolectamos la orina de seis nutrias mediante la cistocentesis aproximadamente 4 a 5 horas después de comer y de nuevo después de un ayuno de 24 horas. El hallazgo fue hiperoxalurio encontramos en 1991. Los niveles postprandiales de potasio, calcio, magnesio, fosfato, sulfato y ácido úrico urinarios fueron todos significativamente más altos que los niveles de las nutrias en ayuno. Los niveles de supersaturación del oxalato de calcio (CaOxSS) fueron determinados usando un programa de computadora llamado EQUIL-2. Este sistema usa el pH urinario y los iones mayores de la orina para calcular el potencial de la cristalización de los componentes comunes de las piedras de riñón. Los valores de CaOxSS fueron promediados sobre tres, sugiriendo que los niveles en orina eran tres veces superiores al punto de saturación para calcio y oxalato.

En 1995 las nutrias fueron separadas en dos grupos de 5 animales cada uno. A un grupo se le alimentó con una dieta baja en oxalato, arenque con tiamina y vitamina E adicionadas (Nutrias de Pescado). El otro grupo se mantuvo en su dieta original Nebraska Brand Feline Diet (Nutrias NBF). Al final del mes, ambas dietas fueron analizadas (Tabla 1) y la orina y sangre fue colectada como en 1994. Las nutrias de pescado tenían CaOxSS significativamente más bajo que las nutrias NBF durante el periodo postprandial, aunque los niveles de oxalato fueron sorprendentemente similares (Tabla 2). Las nutrias de pescado también tenían niveles significativamente más altos de sulfato, fosfato, sodio, potasio, y cloruro urinarios y pH urinario más bajo en el periodo posprandial. El nivel de fosfatasa alcalina sérica (FAS) fue más alto en las nutrias de pescado (promedio FAS = 333), que en las nutrias NBF (promedio FAS = 136). Al final de seis meses los niveles de FAS se incrementaron mas (promedio = 637) en las nutrias de pescado y algunos animales tuvieron también elevaciones de los niveles de gamma glutamiltransferasa (GGT). Se sospechó de hígado graso asociado con una deficiencia del ácido graso omega-6, y se le adicionó aceite de maíz a la dieta de pescado. Un mes después, los niveles de FAS (promedio = 285) y de GGT habían decrecido en todas las nutrias de pescado Una dieta a base sólo de pescado puede reducir la CaOxSS, pero puede llevarnos a desbalances en la dieta y su efecto a largo plazo sobre la salud en general y en el desarrollo de la urolitiasis tiene que ser determinado aún.
Introduction

The Asian small-clawed (ASC) otter (Aonyx cinerea) is a popular and commonly held species in captivity. Over 257 individuals are currently housed in 63 zoological parks and aquaria worldwide.\textsuperscript{11} The incidence of calcium oxalate (CaOx) urolithiasis in this species in captivity is reported as 66\%.\textsuperscript{1} Although the incidence of the disease in the wild is unknown, a parasitology survey done on wild ASC otters in Thailand reported only one renal calculus in gross pathologic examinations of 20 adult otters and it was associated with a renal parasite.\textsuperscript{4}

At the Minnesota Zoological Garden, 17 of 19 ASC otters 2 yr of age or older (89\%) have had urolithiasis. Of 11 young animals that were followed radiographically, nine developed calculi by 4 yr of age, one animal became affected between 4 ½ and 5 ½ yr, and one between 4 and 8 yr. Calculi were analyzed in six animals, and all were composed of CaOx monohydrate with a few having CaOx dihydrate components as well.

CaOx calculi form when urine is supersaturated with calcium and oxalate and precipitation occurs. The state of saturation is dependent upon not only the concentration of calcium and oxalate, but also the urine pH, ionic strength, and the potential for complexation within the urine. Also other substances in the urine can modify crystal formation by either inhibiting or promoting crystallization. This dynamic system is influenced by many factors, especially by changes in food and water consumption.\textsuperscript{18} Urinary calcium oxalate supersaturation (CaOxSS) can be determined using a computer program called EQUIL-2. This system uses urine pH and the major ions in urine to calculate the potential for crystallization of common kidney stone components.\textsuperscript{19}

CaOx uroliths are the most common calculi in humans, and multiple etiologies are described. Primary enzyme defects (primary hyperoxaluria), tubular defects (renal tubular acidosis), enteric problems (malabsorption), and hypercalcemia are relatively uncommon causes. Most patients are diagnosed with idiopathic CaOx renal lithiasis and may have hypercalciuria, hyperoxaluria, hyperuricosuria, and/or abnormalities of crystal modifiers.\textsuperscript{18} In humans, decreased urine volume is the single most significant risk factor, followed by hyperoxaluria.\textsuperscript{3,8,17,18} Hyperoxaluria can result from vitamin B\textsubscript{6} deficiency, excessive dietary intake of oxalate, increased endogenous production, or decreased calcium in the diet. Calcium in the distal intestine normally binds oxalate and keeps it from being absorbed. Hyperoxaluria can also result from a membrane defect which causes abnormal oxalate transport across renal tubular epithelial cells and erythrocytes.\textsuperscript{7} Risk factors associated with diet include: decreased fluid and potassium intake, excess consumption of protein, sodium chloride, vitamin C and D, and high oxalate foods, as well as deficiencies of vitamin B\textsubscript{6}, magnesium, phosphate, and calcium, and acidifying diets.\textsuperscript{3,8,18}

CaOx urolithiasis has also been studied in dogs.\textsuperscript{13,15} The most significant finding in affected schnauzer dogs is hypercalciuria when compared to normal beagles. Risk factors for dogs have been identified, and a flowchart for potential treatment has been developed.\textsuperscript{14}

Only one published report exists describing the composition of urinary metabolites in the ASC otter.\textsuperscript{2} This report suggests that glucosuria may be associated with urolithiasis in this species. A major obstacle to studying this disease in the ASC otter is the lack of information regarding normal renal function and urine metabolites of wild otters. These animals eat primarily crabs and fish in the wild.\textsuperscript{6}
At the Minnesota Zoo they are fed Nebraska Brand Feline (NBF) (Central Nebraska Packing Co, North Platte, Nebraska 69101, USA), containing soybean meal, a high oxalate food. Herbivores and carnivores have evolved to metabolize oxalate differently and it is possible that the ASC otter may not be able to metabolize even small amounts of dietary oxalate efficiently.

**Materials and Methods**

In 1991 we analyzed urine from 24 hr collections in six otters with urolithiasis during periods of food consumption and fasting. Urine was collected in metabolism cages into containers surrounded by dry ice after being filtered through several screens to reduce fecal contamination. Urine was analyzed for calcium, oxalate, phosphorous, and creatinine, and urinalyses were performed. Blood was collected for complete blood counts (CBC), serum chemistries, parathyroid hormone, and 25-hydroxyvitamin D levels.

In 1994 we collected and analyzed urine from six additional ASC otters. In order to analyze urine during periods of peak supersaturation and avoid the averaging effect of 24 hr collections, we collected urine via cystocentesis approximately 4-5 hr after eating and again after a 24 hr fast. This eliminated all possibility of fecal contamination and allowed us to process the urine promptly, avoiding changes in temperature and pH which can greatly effect urinary solute concentration. The animals were anesthetized approximately 3 hr after eating and kept sedated until urine was obtained. Blood was collected for CBC and chemistry analysis. Animals were then fasted for 24 hr and the procedure repeated. Urine samples were processed within 15 min of collection. Urine was cultured aerobically, pH was determined with a pH meter, and a urinalysis was performed. One third of the sample was acidified using 6 N hydrochloric acid in a ratio of 1 part acid to 15 parts urine. Urinary oxalate, calcium, and magnesium were determined on the acidified fraction. Urinary sodium, potassium, chloride, phosphate, sulfate, citrate, uric acid, glycolate, and glycerate were determined on a frozen aliquot. CaOxSS was calculated using the EQUIL-2 program.

In July 1995, otters were divided into two groups of five each. One group was fed a low oxalate diet, consisting of ground herring with thiamin and vitamin E added (fish otters). The other group was fed NBF (NBF otters). At the end of 1 mo, both diets were analyzed (Table 1), and urine and blood were collected and analyzed as in 1994.

The fish diet was altered in December 1995 to contain a variety of fish while attempting to keep dietary protein, fat, calcium, and phosphorous levels similar. Fish otters were gradually changed from ground fish to whole fish, and sheep bones were offered twice weekly to otters on both diets to promote dental health.

In January 1996, 6 mo after the diet change, animals were anesthetized for blood collection, general health assessment, and radiographs. Following this exam, corn oil was added to the diet of the fish otters to provide supplemental omega-6 fatty acids. Blood was collected from the fish otters again 1 mo after this addition.

**Results and Discussion**

The most significant finding of the 24 hr collections from 1991 was a profound hyperoxaluria when
compared to humans, normal dogs, and dogs with CaOx urolithiasis (Tables 2 and 3). There was no significant difference in oxalate excretion between the fed and fasted states. Fasting calcium excretion was similar to North American river otters\textsuperscript{10} and normal humans, but higher than in normal dogs. Calcium excretion was much higher during periods of food consumption than during fasting. Postprandial phosphorous excretion was considerably higher than in dogs and people. Urine volume and water consumption were significantly higher during periods of food consumption than during fasting. We found no evidence of glucosuria or hypercalcemia.

Results obtained in 1994 confirmed the hyperoxaluria noted in 1991 (Tables 2 and 3). Urinary calcium, oxalate, and phosphate levels were all similar to the previous 24 hr collections. There was no statistical difference in the urinary oxalate levels between the fed and fasting states, however, postprandial levels of potassium, calcium, magnesium, phosphate, sulfate, and uric acid were all significantly higher than fasting levels. CaOxSS values averaged over three, meaning that the urine was elevated three times above the saturation point for calcium and oxalate. Values of 1-2.1 are typical in normal people, whereas renal lithiasis patients usually have CaOxSS levels of 2-4 or above.

In 1995 the fish otters had a significantly lower CaOxSS than the NBF otters during the postprandial period and higher levels of urinary sulfate, phosphate, sodium, potassium, and chloride, but lower urinary pH (Table 2). No significant changes were seen between the two groups in the fasting state (Table 3).

After 1 mo, animals on the fish diet appeared healthy on physical exam and were in good body condition, although serum alkaline phosphatase (SAP) levels were higher in the fish otters (mean=333) than in the NBF otters (mean=136). Shortly after conversion to the fish diet, one otter died of acute thrombocytopenia and hemolytic anemia, but this did not appear to be related to the diet change.

After 6 mo on the all fish diet, SAP levels had risen further (mean=637) and some of the fish otters had elevations of gamma glutamyltransferase (GGT). Most of these otters had also developed a mild leukocytosis with a mild mature neutrophilia and mild monocytosis.

One month after the addition of corn oil to the fish diet, SAP levels had dropped in all otters (mean=285), although the levels were still above normal. GGT levels had returned to normal, but serum iron was slightly low in all of the fish otters (mean=73.4), with one animal having a mild reduction in the hematocrit (PCV=35). The remaining hematology parameters had returned to normal. There was no radiographic change in the uroliths in any of the otters except one fish otter whose calculi were slightly larger. This otter had been previously treated for a persistent urinary tract infection which is not typical of urolithiasis in this species.

The cause of the elevated SAP levels is still unknown, but since fish are known to be high in omega-3 fatty acids but relatively low in omega-6 fatty acids, an essential fatty acid deficiency was suspected. Fatty liver change has been found in some species due to fatty acid deficiency.\textsuperscript{16}

It was surprising that the postprandial urinary oxalate levels were so similar on the two diets, since the NBF diet contained 25 times more oxalate than the fish. This supports the theory that most of the urinary oxalate is due to endogenous production. Increased oxalate production might be due to the
high protein content of the fish diet, since certain amino acids such as glycine are one source of endogenous oxalate production. Despite the fact that the fish diet did not significantly alter urinary oxalate, it did reduce CaOxSS levels. The reason for this is unclear but probably is related to the increase in urinary excretion of potential crystal inhibitors such as phosphate and, perhaps, sulfate.

In conclusion, ASC otters have high levels of urinary oxalate compared to dogs and humans. Monitoring urinary metabolites and using EQUIL-2 to calculate CaOxSS levels may provide a way to evaluate the effect of diet change and medical therapy on urine supersaturation. An all fish diet may reduce CaOxSS but can lead to dietary imbalances. The result of long term feeding of a supplemented fish diet on general health, CaOxSS, and the development of urolithiasis has yet to be determined. Areas for future evaluation include the effect of various therapies that have been used successfully in certain cases of hyperoxaluria in people, such as potassium citrate, orthophosphate, vitamin B₆, and organic marine hydrocolloid. Also, increasing water consumption has the potential to reduce incidence of urolithiasis. ASC otters will spend more time in the water if it is warm and prefer water temperatures of approximately 29.4°C (85°F). By providing an optimum water temperature, animals might be encouraged to spend more time in the water, increase water consumption, and thereby reduce the potential for urolith formation by diluting crystal-forming solutes.

LITERATURE CITED

Table 1. Proximate analysis of two diets fed to Asian small-clawed otters (dry matter basis).

<table>
<thead>
<tr>
<th>NUTRIENT</th>
<th>NEBRASKA BRAND FELINE DIET Lot #51702</th>
<th>HERRING DIET Lot #015181</th>
<th>MINIMUM REQUIREMENTS OF THE DOMESTIC CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Dry Matter</td>
<td>39.3</td>
<td>24.8</td>
<td></td>
</tr>
<tr>
<td>% Crude Protein</td>
<td>47.8</td>
<td>68.1</td>
<td>20</td>
</tr>
<tr>
<td>% Fat</td>
<td>37.9</td>
<td>18.95</td>
<td></td>
</tr>
<tr>
<td>% Fiber</td>
<td>2.8</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>% Ash</td>
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<td></td>
</tr>
<tr>
<td>% Carbohydrates</td>
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<td></td>
</tr>
<tr>
<td>Calories per kilogram</td>
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</tr>
<tr>
<td>% Sodium</td>
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<td>0.789</td>
<td>0.2</td>
</tr>
<tr>
<td>% Phosphorous</td>
<td>0.776</td>
<td>1.67</td>
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</tr>
<tr>
<td>% Potassium</td>
<td>0.878</td>
<td>1.5</td>
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</tr>
<tr>
<td>% Calcium</td>
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<td>1.56</td>
<td>0.8-1.0</td>
</tr>
<tr>
<td>% Magnesium</td>
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</tr>
<tr>
<td>Zinc (ppm)</td>
<td>73.5</td>
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<td>50</td>
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<tr>
<td>Copper (ppm)</td>
<td>7.91</td>
<td>3.34</td>
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<tr>
<td>Manganese (ppm)</td>
<td>18.6</td>
<td>4.69</td>
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<td>Iron (ppm)</td>
<td>483</td>
<td>103.5</td>
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<td>Oxalate (ppm)</td>
<td>232</td>
<td>9.4</td>
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Table 2. Urinary metabolites of Asian small-clawed otters compared to dogs and humans during periods of food consumption.\(^a\)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MZG(^b) fish otters(^c) 1995 (n=4)</th>
<th>MZG(^b) NBF(^d) otters 1995 (n=5)</th>
<th>MZG otters 1994 (n=6)</th>
<th>MZG otters 1991 (24 hr(^e)) (n=6)</th>
<th>Calle’s otters(^2) (24 hr) (n=5)</th>
<th>Dog normal(^f) (24 hr) (n=33)</th>
<th>Dog CaOx(^g) calculi(^i) (24 hr) (n=14)</th>
<th>Humans normal(^h) (24 hr)</th>
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</thead>
<tbody>
<tr>
<td>Sodium (mEq/mg of cr(^j))</td>
<td>0.095</td>
<td>0.0192</td>
<td>0.0249</td>
<td>0.0155</td>
<td>0.0288</td>
<td>0.03-0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium (mEq/mg of cr)</td>
<td>0.174</td>
<td>0.073</td>
<td>0.0827</td>
<td>0.0328</td>
<td>0.0343</td>
<td>0.02-0.07</td>
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<tr>
<td>Calcium (mg/mg of cr)</td>
<td>0.281</td>
<td>0.196</td>
<td>0.197</td>
<td>0.136</td>
<td>0.184</td>
<td>0.0188</td>
<td>0.0928</td>
<td>0.02-0.25</td>
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<tr>
<td>Magnesium (mg/mg of cr)</td>
<td>0.145</td>
<td>0.182</td>
<td>0.159</td>
<td>0.0972</td>
<td>0.0763</td>
<td>0.06-0.125</td>
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<td></td>
</tr>
<tr>
<td>Chloride (mEq/mg of cr)</td>
<td>0.142</td>
<td>0.054</td>
<td>0.0537</td>
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<td>0.08-0.2</td>
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<tr>
<td>Phosphorous (mg/mg of cr)</td>
<td>5.63</td>
<td>3.44</td>
<td>3.28</td>
<td>2.26</td>
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<td>0.604</td>
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<td>Sulfate (mg/mg of cr)</td>
<td>7.75</td>
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<tr>
<td>Citrate (mg/mg of cr)</td>
<td>0.086</td>
<td>0.085</td>
<td>0.226</td>
<td>0.038</td>
<td>0.0946</td>
<td>0.244</td>
<td>0.007-0.033</td>
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<tr>
<td>Oxalate (mg/mg of cr)</td>
<td>0.114</td>
<td>0.131</td>
<td>0.129</td>
<td>0.14</td>
<td>0.083</td>
<td>0.064</td>
<td>0.0325</td>
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<tr>
<td>Uric Acid (mg/mg of cr)</td>
<td>0.2</td>
<td>0.122</td>
<td>0.126</td>
<td>0.412</td>
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<td>5.6</td>
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<td>Sp. gr.</td>
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</tr>
<tr>
<td>CaOxSS(^i)</td>
<td>2.2</td>
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<td>1.0-2.1</td>
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<td>Glycolate (mg/mg of cr)</td>
<td>0.153</td>
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<td></td>
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<td>Glycerate (mg/mg of cr)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02-.084</td>
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</tr>
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</table>

\(^a\)Values are expressed as means; \(^b\) MZG=Minnesota Zoological Garden; \(^c\) otters consuming fish; \(^d\) otters consuming Nebraska Brand Feline; \(^e\) Denotes 24 hr urine collections; \(^f\) CaOx=calcium oxalate; \(^g\) Mayo Medical Laboratories Bulletin, 1995, random samples; \(^h\) cr=creatinine; \(^i\) CaOxSS=calcium oxalate supersaturation
Table 3. Urinary metabolites of Asian small-clawed otters compared to other species during periods of fasting.\(^a\)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MZG(^b) Fish otters(^c) 1995 (n=4)</th>
<th>MZG NBF otters(^d) 1995 (n=5)</th>
<th>MZG otters 1994 (n=6)</th>
<th>MZG otters 1991 (24 hr) (n=6)</th>
<th>North American river otter(^e) (24 hr) (n=13)</th>
<th>Dog normal(^f) (24 hr) (n=33)</th>
<th>Dog CaOx(^g) calculi(^h) (24 hr) (n=6)</th>
<th>Humans normal(^i) (24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mEq/mg of cr)</td>
<td>0.123</td>
<td>0.026</td>
<td>0.011</td>
<td></td>
<td></td>
<td>0.0048</td>
<td>0.0099</td>
<td>.03-0.1</td>
</tr>
<tr>
<td>Potassium (mEq/mg of cr)</td>
<td>0.043</td>
<td>0.039</td>
<td>0.0245</td>
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<td></td>
<td>0.0123</td>
<td>0.0279</td>
<td>.02-.07</td>
</tr>
<tr>
<td>Calcium (mg/mg of cr)</td>
<td>0.139</td>
<td>0.0284</td>
<td>0.0456</td>
<td>0.028</td>
<td>0.0599</td>
<td>0.0135</td>
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<tr>
<td>Magnesium (mg/mg of cr)</td>
<td>0.162</td>
<td>0.121</td>
<td>0.0826</td>
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<td>0.0657</td>
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<td>Chloride (mEq/mg of cr)</td>
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<td></td>
<td>.08-.2</td>
</tr>
<tr>
<td>Phosphorous (mg/mg of cr)</td>
<td>1.51</td>
<td>2.53</td>
<td>1.15</td>
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<td>0.67</td>
<td>0.583</td>
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<tr>
<td>Sulfate (mg/mg of cr)</td>
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<td>0.806</td>
<td>0.894</td>
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<td>Citrate (mg/mg of cr)</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Oxalate (mg/mg of cr)</td>
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<td>1.023</td>
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<tr>
<td>CaOxSS(^i)</td>
<td>1.51</td>
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<td></td>
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<td>Glycolate (mg/mg of cr)</td>
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<td>Glycerate (mg/mg of cr)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>.022-.084</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Values are expressed as means; \(^b\) MZG=Minnesota Zoological Garden; \(^c\) otters consuming fish; \(^d\) otters consuming Nebraska Brand Feline; \(^e\) Denotes 24 hr urine collections; \(^f\) CaOx=calcium oxalate; \(^g\) Mayo Medical Laboratories Bulletin, 1995, random samples; \(^h\) cr=creatinine; \(^i\) CaOxSS=calcium oxalate supersaturation
CARFENTANIL CITRATE AS AN ORAL ANESTHETIC AGENT FOR BROWN BEARS
(*Ursus arctos*)

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Wildlife Safari, Winston, OR 97496, USA

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Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97339-0429, USA

Abstract

The delivery of oral opiate anesthetic agents is now being attempted in a wide variety of ruminant, primate, and carnivore species. Carfentanil citrate has been successfully used as an oral anesthetic agent in goats, gibbons and black bears. This report describes the oral application of carfentanil citrate in five brown bears (*Ursus arctos*), for a total of 10 inductions. Physiological parameters were recorded to determine appropriate carfentanil citrate dosages, and to describe the effects of anesthesia on each bear.

Five individuals (two males and three females) 6-7 yr of age were immobilized for physical examinations. Weights ranged from 195-393 kg, and each bear received a dosage of carfentanil citrate ranging from 6-15.2 µg/kg. All doses were mixed with 5-10 ml of honey, which the bears licked from a spoon.

Respiratory rate (0.2-8 bpm), heart rate (18-116 bpm), pulse oximetry percent (64-95%), temperature (34.0-36.7°C), electrocardiogram, blood-gases, and level of consciousness were monitored during the course of each anesthesia. At the end of each procedure, naltrexone was given as the reversal agent (75% i.v. and 25% s.c.).

After the start of ingestion, sternal recumbency occurred in an average of 7 min (range: 4-11 min), and full restraint and safe handling occurred in an average of 20 min (range: 8-33 min). Mean reversal time was 7 min after injection of naltrexone (range: 4-9 min). During recovery, panting was observed in both immobilizations for one of the female bears, and vomiting occurred in both anesthetic recoveries for another female.

The quick induction and recovery times, length of anesthesia, and ease of oral administration make carfentanil citrate a good immobilizing agent for use in brown bears.

Resumen

La liberación de agentes anestésicos narcóticos orales se está intentando ahora en una amplia variedad de rumiantes, primates y especies carnívoras. El citrato de carfentanil ha sido utilizado con éxito como un agente anestésico oral en cabras, gibones y osos negros. Este trabajo describe la aplicación oral de citrato de carfentanil en cinco osos pardos (*Ursus arctos*), para un total de 10 inducciones. Los parámetros fisiológicos fueron recopilados para determinar las dosis apropiadas...
Cinco individuos (dos machos y tres hembras) de 6 y 7 años de edad fueron inmovilizados para realizar exámenes físicos. Los pesos variaban de 195-393 kg, y cada oso recibió una dosis de citrato de carfentanil de 6-156.2 µg/kg. Todas las dosis fueron mezcladas con 5-10 ml de miel, la cual lamieron los osos de una cuchara.

Se monitoreó su frecuencia respiratoria (0.2-8 bpm), frecuencia cardíaca (18-116 bpm), porcentaje de oximetría de pulso (64-95%), temperatura (34.0-36.7°C), electrocardiogramas, gases sanguíneos y nivel de conciencia durante el curso de cada anestesia. Al final de cada procedimiento, se suministró naltrexona como agente regresivo (75% i.v. y 25% s.c.).

Después del comienzo de la ingestión, la recumbencia esternal ocurría en un promedio de 7 minutos (con un rango de 4-11 min.) y la sujeción completa con manejo seguro tuvo lugar en un promedio de 20 minutos (rango: 8-33 min). La media del tiempo de regresión fue de 7 minutos después de la inyección de naltrexona (rango: 4-9 min). Durante la recuperación, se observó jadeo de una de las hembras en ambas inmovilizaciones, y vómito en ambas recuperaciones de anestesia de las otras hembras.

La rapidez de inducción y tiempo de recuperación, la duración de la anestesia y la facilidad de la administración oral, hacen del citrato de carfentanil un buen agente inmovilizador para uso en osos pardos.

Introduction

The delivery of oral opiate anesthetic agents is now being attempted in a wide variety of ruminant, primate, and carnivore species. Carfentanil citrate has been successfully used as an oral anesthetic agent in goats at an average dosage of 30-90 µg/kg,20 in white-handed gibbons at a mean dosage of 381 µg/kg,14 and in black bears at dosages ranging from 6.8-18.8 µg/kg.18 This route of administration can potentially be of great value in zoological settings, with a more limited, as yet undefined role in wildlife populations. Remote delivery of anesthetic agents can result in darting injuries, improper dart placement, and can create apprehension and excitement, resulting in hyperthermia, particularly in bears. These complications can be avoided by administering drugs orally. Anesthetic agents used in recent years for adult bears include carfentanil, combinations of ketamine and xylazine, etorphine, fentanyl, and tiletamine HCl/zolazepam HCl, all typically given as intramuscular injections. This report describes the oral application of carfentanil citrate in five brown bears (Ursus arctos), for a total of 10 inductions. Physiological parameters were recorded to determine appropriate carfentanil citrate dosages, and to describe the effects of anesthesia on each bear.

Materials and Methods

Five captive brown bears (Ursus arctos) at the Wildlife Safari in Winston, Oregon were immobilized twice during the months of February and March, 1996. Animals ranged in age from
6-7 yr and included three females and two males. The bears were fed dog food, fruits and vegetables, breads, fish and skeletal meat. All bears were in good general health. Behavioral conditioning was done for 2 days prior to the immobilization procedures to improve each bear’s ability to lick honey from a spoon. The bears were confined to night quarters for this work, were not hibernating, and were fasted for 24 hr prior to induction. Carfentanil citrate (Wildnil, Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado) was mixed with approximately 5-10 ml honey, at dosages ranging from 6-15.2 µg/kg, and given p.o. from a spoon over a 2 min time period. Initial drug dosages were based on estimated body weights and extrapolated dosages from previously anesthetized black bears.\textsuperscript{18} Actual weights were obtained during the first anesthesia and were used to calculate dosages for the second immobilization. All bears were supplemented with oxygen insufflation (6 L/min) via unilateral nasal intubation during the entire period of anesthesia. Additional drugs were given as needed and included diazepam, atropine, ketamine and doxapram.

Data collected during the procedure included physical examination findings, along with CBC’s and serum chemistry panels. Continuous monitoring included temperature, pulse, respiration rate and pulse oximeter readings (Ohmeda Biox 3700, Ohmeda, Boulder, Colorado) taken every 1-5 min. Blood gases, ECG and heart sound recordings were taken when possible, starting approximately 10 min after animals were safe to handle. Behavioral observations for induction included time to sternal recumbency and time to safe handling from the start of honey ingestion, and were recorded every minute. Naltrexone (Wildlife Laboratories, Inc., Fort Collins, Colorado) was given as the reversal agent at a 100:1 ratio (antagonist:agonist), with 25% of the dose given i.v. or i.m. and 75% given s.c. Time from injection of naltrexone to sternal recumbency, to standing and to full recovery (bright, alert and walking steadily) were recorded, with observations taken each minute.

Blood samples were taken from the cephalic vein and immediately transferred into EDTA tubes for CBC analyses or allowed to clot for 1-2 hr prior to centrifugation and separation of serum for chemistry analyses. Serum samples were sent fresh to Oregon Medical Laboratories in Eugene, Oregon, for analysis the same day. Arterial blood (and occasionally venous) was obtained from either the dorsal pedal or sublingual vessels for the blood-gas analyses. Heparinized syringes were used to make collections between 31-89 min after ingestion of honey was started. Samples were immediately cleared of air bubbles, capped and stored on ice for 30-50 min prior to analysis on a Radiometer ABL 330 machine (Radiometer, Copenhagen, Denmark). All blood-gas values were adjusted for body temperature variations. Both arterial and venous blood-gas samples were analyzed for assessment of acid-base status, and a comparison of oxygen saturation values obtained from the pulse oximeter versus blood-gas analysis was made. Heart sound recordings and ECG tracings were obtained using a Simulscope (Cardionics, Houston, TX) and later evaluated through a computer link. For statistical analyses, standard errors were calculated based on average parameter values for each bear such that n=5 bears for each statistic.

Results

Mean serum chemistries and CBC values (Table 1) were within normal ranges based on reported values in the literature,\textsuperscript{4,5,13,21} except two CBC estimates. Albumin was at 4.9 g/dl with a reported normal range of 1.4-3.6 g/dl,\textsuperscript{13} and neutrophils were at 45% with a reported normal range of 50-90%.\textsuperscript{13}
Table 2 summarizes the anesthetic records for the five bears. Elapsed time for honey consumption was 2 min in all cases except one, in which the honey was ingested in 1 min. Induction was smooth, with no apparent anxiety or excitement observed in any of the bears. Sternal recumbency occurred an average of 7 ± 1 min (mean ± SE hereafter unless otherwise noted) after ingestion was started (range: 4-11 min), and full restraint and safe handling occurred between 8-33 min (mean: 20 ± 6 min). A deeper plane of anesthesia was reached 10-15 min after bears were safe to handle, as evidenced by diminished reactions to external stimuli.

Heart rates during anesthesia, before and after atropine administration, averaged 70 ± 9 beats per min (bpm; range: 18-116), respiratory rates averaged 1.6 ± 0.7 breaths per min (bpm; range: 0.2-8), pulse oximeter oxygen saturation values ranged between 64-95% (average: 83 ± 1), and body temperatures fluctuated between 34.0-36.7°C. (mean: 35.7 ± 0.2°C.). All ECG and heart sound recordings appeared normal with the exception of a split first heart sound in one bear. A third heart sound could be heard in one bear.

Rapid, shallow breathing was observed in all the bears, but it was not possible to quantify this, so only deep breaths were recorded. All blood-gas analyses had low pH values of 7.2 or 7.3 (Table 3). The PO2 values for bears with temperatures below 36.1°C. were adjusted using a nomogram based on the standard dissociation curve according to Severinghaus (1965). Arterial PCO2 levels were elevated (54.3 ± 6.2 mm Hg; 46-68; mean ± SD: range; n=10), and PO2 levels were decreased despite oxygen supplementation (63.5 ± 9.8 mm Hg; 51-86; mean ± SD: range; n=10). Surprisingly, venous PO2 levels were higher than expected, ranging between 41-65 mm Hg (48.2 ± 7.9 mm Hg; mean ± SD; n=5). Venous PCO2 levels fell within the range for arterial PCO2 levels, averaging 56.4 ± 1.9 mmHg (range: 54-59; n=5). In a few cases, there was little difference between arterial and venous blood-gas values. Bicarbonate levels were within normal ranges.

Drugs given during the procedures included diazepam, ketamine, atropine and doxapram. Diazepam (Elkins-Sinn, Inc., Cherry Hill, New Jersey), given i.v. at 0.05 mg/kg to one bear, was effective in eliminating muscle rigidity early in the course of the anesthesia. The other nine immobilizations produced generalized muscle relaxation without the use of a sedative; however, two bears needed supplemental ketamine (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) at 1.1 µg/kg i.m. late in the induction phase. This was most likely due to an inadequate dosage of carfentanil (6 µg/kg) in one case. Atropine (Anthony Products, Arcadia, California), dosed at 0.03-0.04 mg/kg, was used in nine of the 10 anesthesias to improve heart rate. In general, a doubling of the heart rate was noted 5-10 min following i.m. administration of atropine. Doxapram HCl (Dopram V, Aveco Inc., Fort Dodge, Iowa) was used to stimulate breathing if less than 5 bpm were observed. When used at 0.2-0.4 mg/kg i.v. in six anesthesias to stimulate respiration, no apparent respiratory rate increases were observed. Because pulse oximetry readings usually remained above 85% SaO2, no attempts were made to give higher dosages (1-10 µg/kg) of doxapram to determine an effective dose.

After administration of the reversal agent, all bears woke up quietly and without apparent apprehension or disorientation. The 25% dose of naltrexone was given i.v. in seven of the 10 bears, and given i.m. in the remaining three. The average time interval and range from injection of reversal to sternal recumbency was 5 min (range: 3-8 min) and 7 min (range: 6-8 min) for i.v. and i.m. injections, respectively. The average time interval from injection of reversal to standing was 7 min (range: 4-9 min) and 8 min (range: 7-9 min) for i.v. and i.m. injections, respectively. The average
time interval from injection of reversal to full recovery was 10 min (range: 8-14 min) and 11 min (range: 8-15 min) for i.v. and i.m. injections, respectively. During recovery, panting was observed in both immobilizations for one of the female bears, and vomiting occurred in both anesthesias for another female.

Discussion

Abnormalities noted in the serum chemistries and CBC values included an elevated albumin and decreased neutrophil percentage. These deviations in values are not readily explained, and are most likely normal for these bears. It has been reported that blood urea nitrogen (BUN):creatinine (Cr) ratios in Alaskan brown and black bears are less than 10 during the denning and hibernation season, and over 30 during the summer.6,16 The bears in this study had a BUN:Cr ratio of 5.5, based on the mean serum chemistry values. The brown bears used in this study were not hibernating; however, a reduced metabolic rate was suspected because of decreased activity and appetite levels.

Anesthetic agents used in recent years for adult bears include: (1) i.m. injections of carfentanil,8 (2) combinations of ketamine and xylazine,2,12 and (3) etorphine HCl,3,9 both also given i.m. The average induction time for ketamine:xylazine combinations is 13 min.2,12 Etorphine HCl induction times average 9 min (range: 3-30 min), with an average recovery time of 19 min (range: 0.5-120 min) after injection of reversal.3,9 In comparison, oral carfentanil, as utilized in this study at 8 µg/kg dosages, had mean induction (to safe handling) and recovery times of 20 ± 6 min (range: 8-33 min) and 11 ± 2 min (range: 8-15 min), respectively. Although anesthesia was not taken beyond 108 min from time of carfentanil ingestion to naltrexone injection, based on duration of anesthesia for other synthetic opioids, it is anticipated that length of immobilization could be extended for several hours prior to reversal.

Ensuring transmucosal absorption of carfentanil by limiting access to the honey/carfentanil spoon was important for proper delivery of the calculated carfentanil dose. In a previous study, times to sternal recumbency were shorter for black bears ingesting a honey-carfentanil mixture in >2 min than bears which ingested the mixture in <1 min.18 Induction took 40 min longer in a wapiti given carfentanil via an orogastric tube versus direct oral administration.15 Dosages required for immobilizations may increase with rapid ingestion, presumably because the drug is degraded to some extent in the gastrointestinal tract. Based on carfentanil dosages ranging from 6-15.2 µg/kg, 8 µg/kg was the minimum dosage which resulted in a good induction, not requiring further supplementation. In comparison, a mean dose of 10.8 µg/kg was recommended for black bears.18 This difference is most likely explained by altered metabolic rates. As stated previously, these bears were not hibernating, but appeared to be in a reduced metabolic state. This condition probably reduced the anesthetic dosage required at this time of year. A similar situation has been observed with Telazol (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa), given to denning black bears during the winter season in the wild.11

Heart rates were low without atropine administration, averaging between 30-40 bpm. This is a common side effect of opioid anesthesia.7 Heart rates for non-anesthetized polar bears are reported to range between 40-65 bpm,8 and are 60-90 bpm for active bears, in general.5 Generally, pulse strength was weak with heart rates in the 35-50 bpm range. By administering atropine, heart rates were increased and arterial catheters were easier to place because of improved pulse quality.
However, since CNS effects of atropine may include respiratory depression, a lower dosage of atropine may be sufficient for brown bears, since they responded very well to 0.03-0.04 mg/kg doses in this study, and respiratory depression was a problem. Splitting of the 1st heart sound in one bear is caused by a slight delay in the closing between the two A-V valves, and may be associated with the tachycardia induced by the atropine injection.

Respiratory rates were low (averaging between <1-5 bpm) through all 10 anesthesias. Dosages for the second set of immobilizations were approximately half that of the first set of immobilizations, yet respiratory rates were consistently lower when the lower dosages were given. We cannot explain this result. The lack of a response to doxapram may be related to reduced metabolic states, which could make the central nervous system less sensitive to stimulants. All blood-gas analyses indicated respiratory acidosis, which we attributed to decreased respiratory rate. Bicarbonate levels were within the normal range as anticipated, since renal compensatory resorption of HCO₃ is not expected to occur until 24-48 hr after the onset of respiratory acidosis. Several analyses from the same animals had very similar venous and arterial blood-gas values, which can occasionally occur with severe respiratory depression. On the other hand, if bears have arteriovenous anastomoses underneath the lingual epithelium, as some species do, this may have resulted in collection of mixed venous-arterial samples which we recorded as arterial samples. Fluctuations in PCO₂ levels may partially be due to changes in blood flow, brought about by atropine-induced increases in heart rate. Low PO₂ levels may result from reduced body temperatures and factors affecting the oxygen-hemoglobin dissociation curve, causing a shift to the right. Increased concentrations of CO₂, low pH and increased concentrations of 2,3-diphosphoglycerate (DPG) shift this curve to the right. Elevated concentrations of DPG allow oxygen to dissociate more readily from hemoglobin into the tissues, but also make it more difficult for hemoglobin to combine with O₂ in the lungs when the alveolar PO₂ is reduced. One of our arterial blood-gas analysis with a PO₂ of 59 and PCO₂ of 68 may reflect the effects of mixed-blood samples due to sub-lingual arteriovenous anastomoses and DPG.

Body temperatures were low and most likely due to the cold, concrete floors upon which the bears were initially immobilized and possibly their winter metabolic rates. Normal temperatures range between 37.5-38.3°C, and hibernating bears’ temperatures are between 31-36°C. Once the bears were safe to handle, they were placed on stretchers, elevated from the floor by 3-4 inches. No other attempts to increase body temperature were made.

Reversal of carfentanil with naltrexone was quick and complete with no episodes of renarcotization occurring. Fat animals, like bears, are more likely to recycle carfentanil because of the drug’s lipophilic nature, especially when dosages exceed 10 µg/kg. Problems with renarcotization have been reported in polar bears immobilized with i.m. injections of carfentanil and reversed with naloxone. There may be a difference in the tendency for recycling based on the route of administration, and type of antagonist and dosage used. The panting observed in one bear during both recoveries could be due to a natural physiological response to the build-up in CO₂ levels, a reaction to naltrexone, or another unknown cause. Increased respiratory rates were also observed upon recovery of polar bears immobilized by darting with carfentanil. Vomiting in one bear occurred nearly 1 hr after both reversals, and has been reported as an occasional side-effect of opioid anesthesia.
Carfentanil dosage requirements may be different between seasons for bears not hibernating, but experiencing decreased metabolic rates as evidenced by lower appetites and activity levels. Oxygen supplementation should be given with carfentanil anesthesia because of the decreased respiratory rates. Doxapram, while not effective when given at 0.2 and 0.4 mg/kg, may stimulate respiration at higher dosages or metabolic rates. The quick induction and recovery times, length of anesthesia, and ease of oral administration makes carfentanil a very good immobilizing agent for use in brown bears.

ACKNOWLEDGMENTS

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Table 1. Results of serum chemistry and CBC.

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<td>Cr (mg/dl)</td>
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<td>Hct (%)</td>
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Table 2. Anesthetic record summary.

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<th>BEAR</th>
<th>WEIGHT (kg)</th>
<th>DOSE (mg)</th>
<th>DOSAGE (µg/kg)</th>
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<th>sternal</th>
<th>standing</th>
<th>complete</th>
<th>TEMP (°C)*</th>
<th>PULSE (bpm)</th>
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<td>13</td>
<td>6</td>
<td>20</td>
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<td>11</td>
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<td>8</td>
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<td>35.7±0.2</td>
<td>69.6±9.4</td>
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Data presented as 1st and 2nd anesthesias for each bear, respectively. Standard errors are based on averages for each bear (n=5).
NA: not available (animal was sternal at beginning of induction), F: female, M: male
*TPR values are averages for each bear based on observations taken from time the bear was tractable to time of reversal.
Table 3. Blood gas analyses.

<table>
<thead>
<tr>
<th>BEAR</th>
<th>DATE</th>
<th>TIME*</th>
<th>BLOOD**</th>
<th>TEMP (°C)</th>
<th>pH</th>
<th>pCO₂</th>
<th>pO₂</th>
<th>HCO₃ calc.</th>
<th>calc. SaO₂ (%)</th>
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*TIME: mins. after start of anesthetic ingestion
**BLOOD: A (arterial), V (venous)
F: female, M: male
Abstract

Three geriatric tigers (*Panthera tigris*), one geriatric Asian lion (*Panthera leo*), and one geriatric Chinese leopard (*Panthera pardus*), were diagnosed with degenerative spinal column disease. All were long-term, captive-born animals held at the Knoxville Zoo, Tennessee, and diagnosis was confirmed at necropsy. Age and weight at euthanasia ranged from 14-19 yr and 50-175 kg, respectively. All animals were euthanatized due to an assessment of poor quality of life based on the presence of progressive lameness, weight loss, muscle atrophy, ataxia, and/or rear limb paresis. Prior to euthanasia, four animals exhibited progressive deterioration over a range of 2-4 mo, and one animal exhibited clinical signs attributed to spinal disease sporadically over several years. Two animals exhibited acute signs of ataxia and severe rear limb weakness 24-48 hr prior to euthanasia.

Radiographic data were available on four animals and were clinically useful in assessing the severity of degenerative spinal disease. Radiographs were taken at the time of euthanasia and/or from 6 mo to 6 yr prior to euthanasia. Radiographic signs included cervical, thoracic, and/or lumbar intervertebral disc mineralization or herniation and discospondylosis. In all four cases, radiographic evidence was suggestive of degenerative disc disease and secondary spondylosis. In addition, one case also revealed radiographic signs of possible spinal column trauma.

Only one animal received treatment specifically for spinal disease. Meclofenamic acid therapy at a dose of 1 mg/kg p.o. every other day was attempted for 4 wk, however, no significant changes were seen clinically.

Necropsy and histopathology results verified clinical and radiographic signs. Chronic, multifocal degenerative disc disease with bridging ankylosing spondylosis was diagnosed on necropsy. Degree of involvement of cervical, thoracic and/or lumbar vertebrae varied among animals. In the animals in which the spinal cord was examined histologically, there was evidence of acute or chronic traumatic damage from the protrusion of disc material into the spinal canal. Age-related degenerative lesions in other organs and tissues were also reported, however, the spinal disease represented the most significant and clinically relevant finding.

Degenerative spinal disease is common in certain breeds of domestic dogs but is seldom recognized clinically in domestic cats. Based on our experience, crippling spinal arthritis can be a significant problem in large breed exotic cats. Although the etiology of this disease remains uncertain, it is
presumably related to old age abnormalities and activity levels of these animals. A syndrome of deforming cervical spondylosis occurs in cats with chronic vitamin A excess, most frequently caused by feeding beef livers or other diets high in vitamin A. However, the location of ankylosis in the thoracolumbar spine and association of ankylosis with degenerative disc disease in the large cats is inconsistent with vitamin A toxicity.

Resumen

Se diagnosticaron tres tigres geriátricos (Panthera tigris), un león asiático geriátrico (Panthera leo), y un leopardo chino geriátrico (Pantera pardus) con enfermedad degenerativa de la columna espinal. Todos eran animales mayores y nacidos en cautiverio, mantenidos en el Zoológico de Knoxville, en Tennessee, y el diagnóstico fue confirmado en la necropsia. La edad y peso a la hora de la eutanasia variaba de 14 a 19 años y 50-175 kg, respectivamente. Todos los animales fueron sacrificados debido a una evaluación de su pobre calidad de vida basada en la presencia de cojera progresiva, pérdida de peso, atrofia muscular, ataxia y/o paresia de las extremidades posteriores. Antes de la eutanasia, cuatro animales mostraron deterioro progresivo en un rango de 2 a 4 meses y un animal mostró signos clínicos atribuidos a la enfermedad espinal esporádicamente durante varios años. Dos animales mostraron signos agudos de ataxia y debilidad severa de las extremidades posteriores. De 24 a 48 horas previas a la eutanasia.

Los datos radiográficos estuvieron disponibles en cuatro animales y fueron clínicamente útiles para asesoraros sobre la severidad de la enfermedad espinal degenerativa. Las radiografías fueron tomadas al tiempo de la eutanasia y/o de seis meses a seis años previos. Los signos radiográficos incluyeron mineralización o hernia de discos intervertebrales cervicales, torácicos y/o lumbares y discospondilosis. En los cuatro casos, la evidencia radiográfica sugería una enfermedad degenerativa de disco y espondilosis secundaria. Además, un caso tambien reveló signos radiográficos de un posible trauma de la columna vertebral.

Solo un animal recibió tratamiento específico para enfermedad espinal. Se intentó una terapia con ácido meclofenámico a una dosis de 1 mg/kg vía oral cada tercer día por cuatro semanas, sin embargo no se observaron cambios clínicos significativos.

Los resultados de la necropsia e histopatología confirmaron los signos clínicos y radiográficos. En la necropsia se diagnosticó una enfermedad degenerativa de disco crónica y multifocal con puentes de espondilosis anquilosante. El grado de involucramiento de las vértebras cervicales torácicas y/o lumbares varió entre animales. En los animales en los cuales la médula espinal fue examinada histológicamente, existió evidencia de daño traumático agudo o crónico por la protrusión del material del disco dentro del canal espinal. Las lesiones degenerativas relacionadas con la edad en otros órganos y tejidos también fueron reportadas, sin embargo, la enfermedad espinal representó el hallazgo clínicamente más relevante y significativo.

La enfermedad espinal degenerativa es común en ciertas razas de perros domésticos pero es raramente reconocida clínicamente en gatos domésticos. Aunque la etiología de dicha enfermedad permanece como incierta, está presumiblemente relacionada a las anormalidades de la vejez y a los
niveles de actividad de estos animales. En gatos ocurre un síndrome de espondilosis cervical deformante cuando existe exceso crónico de vitamina A, más frecuentemente causado por la alimentación con hígado de res u otras dietas altas en vitamina A. Sin embargo, la localización de la anquilosis en la columna toraco-lumbar y la asociación de la anquilosis con una enfermedad de disco degenerativa en felinos grandes es inconstante con la toxicidad de vitamina A.
INTERNATIONAL COOPERATION WITH RANGE COUNTRY EFFORTS TO CONSERVE TIGERS (*Panthera tigris*) AND THE VETERINARIAN'S ROLE

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Abstract

The five tiger (*Panthera tigris*) subspecies are among the many species of felids facing severe threats of extinction. Range country and regional conservation programs offer the greatest probability of preventing the loss of these species. International multidisciplinary teams of professionals, primarily from zoos, with expertise in various aspects of tiger management have worked collaboratively with conservation programs in China, Indonesia and Thailand to assist in accelerated development of their comprehensive conservation programs for tigers. The specific activities of the teams vary according to the needs that each program determines it has. Activities include technology transfer and training, assessments of facilities and programs and providing recommendations concerning animal husbandry, veterinary care, and program management and structure. Technology transfer has been a significant component in these cooperative projects with hands-on animal work and training programs provided as appropriate and as requested by each program according to their own evaluation of their needs. The structure of the tiger programs and the role of veterinarians working with them may provide a basis from which conservation programs for other species may derive useful information for the design of similar internationally cooperative programs.

Unusual results have been noted during work with two of the tiger programs. Captive Sumatran tigers (*P. t. sumatrae*) in Indonesia demonstrated a particular sensitivity to xylazine with doses greater than 0.2 mg/kg producing significant respiratory depression in a number of cats. In this same population of tigers serum chemistry results were obtained from 30 of the 51 tigers examined. All of these tigers were found to have elevated blood urea nitrogen (BUN) levels in comparison to tigers in the International Species Inventory (ISIS) population. The range of BUN values for the Indonesian tigers was 41.4 to 117.3 mg/dl. Serum creatine levels in these same animals fell within normal ISIS ranges. During work with the South China tiger (*P. t. amoyensis*) in China an apparent difference in ketamine sensitivity between male and female tigers was observed. Both sexes received an average dose of xylazine of 0.47 mg/kg. Males subsequently required an average ketamine dose of 7.73 mg/kg to achieve adequate immobilization while females consistently required a higher average ketamine dose of 11.93 mg/kg.
**Resumen**

Las cinco subespecies de tigre (*Panthera tigris*) se encuentran entre las muchas especies de felinos que enfrentan la severa amenaza de la extinción. Y los programas de conservación regionales ofrecen la mejor probabilidad de prevención de la pérdida de estas especies. Los equipos internacionales multidisciplinarios, principalmente de zoológicos, con expertos en varios aspectos de manejo de tigres han trabajado en conjunto con programas de conservación en China, Indonesia y Tailandia para asistirlos en un desarrollo acelerado de sus comprensibles programas de conservación para tigres. Las actividades específicas de los equipos varía de acuerdo a las necesidades que cada programa determine que posee. Las actividades incluyen transferencia de tecnología y entrenamiento, evaluación de instalaciones y programas y recomendaciones concernientes a la crianza del animal, cuidados veterinarios, y programas de manejo y estructura. La transferencia de tecnología ha sido un componente significativo en estos proyectos de cooperación con trabajo directo en animales y programas de entrenamiento proporcionados según sea apropiado y requerido por cada proyecto de acuerdo a la evaluación de sus necesidades. La estructura de los programas del tigre y el papel de los veterinarios que trabajan con ellos puede proporcionar una base de la cual los programas de conservación para otras especies pueden obtener información útil para el diseño de planes de trabajo en programas de cooperación internacionales similares.

Se han notado resultados inusuales durante el trabajo con dos de los programas de tigres. Los tigres de Sumatra cautivos (*Panthera tigris sumatrae*) en Indonesia demostraron una sensibilidad particular a la xilazina con dosis mayores de 0.2 mg/kg produciendo una depresión respiratoria significativa en un número de gatos. En esta misma población de tigres, los resultados de la química sérica se obtuvieron de 30 de los 51 tigres examinados. A todos ellos se les encontró un nitrógeno úrico sanguíneo (BUN) elevado en comparación con los tigres de la población del inventario Internacional de Especies (ISIS). El promedio de los valores del BUN para los tigres de Indonesia fue de 41.4 a 117.3 mg/dl. Los niveles de creatinina sérica en estos mismos animales cayeron dentro de los rangos normales del ISIS. Durante el trabajo con los tigres del sur de China (*Panthera tigris amoyensis*) en China, se observó una diferencia aparente en la sensibilidad a la Ketamina entre machos y hembras. Ambos sexos recibieron una dosis promedio de Xilazina de 9.47 mg/kg. Los machos subsecuentemente requirieron un promedio de dosis de Ketamina de 7.73 mg/kg para lograr una inmovilización adecuada mientras que las hembras consistentemente requirieron un promedio de dosis de Ketamina más alto, de 11.93 mg/kg.

**Introduction**

The five surviving subspecies of tigers are threatened with extinction throughout their range. The primary threats facing tigers include poaching to provide tiger parts for the traditional medicine market, habitat loss secondary to human population growth and agricultural development, and predator control in populated areas adjacent to tiger ranges. Recent global population estimates for wild and captive tiger populations of the five subspecies are summarized below.
<table>
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<th>Taxon</th>
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<th>Captive Population</th>
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<td>South China tiger <em>Panthera tigris amoyensis</em></td>
<td>30-80 also “fewer than 20”</td>
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<td>Bengal tiger <em>Panthera tigris tigris</em></td>
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According to the Mace-Lande categories and criteria of risk the South China, Sumatran and Siberian tiger populations are considered “critical” and the Indochinese and Bengal populations are “endangered”.6

**Global Animal Survival Plan**

The severity of the crisis facing tigers provided the impetus for development of one of the few Global Animal Survival Plans (GASP) currently in place. The GASP is a strategy for the management of tigers at the international level that links in situ and ex situ conservation activities for the recovery and/or long-term maintenance of captive and wild populations.6 The Global Animal Survival Plan for tigers was initially developed under the aegis of the Conservation Breeding Specialist Group (CBSG), Species Survival Commission (SSC) of the World Conservation Union (IUCN) in 1992 and has been subsequently reviewed. The GASP provides a strategic framework for zoos to participate effectively in international tiger conservation. This framework has evolved into a true multinational effort to conserve tigers that involves conservation organizations, zoological parks, universities, governments, wildlife agencies and corporations working collaboratively for the benefit of the species. The programs that are developing from this framework provide an opportunity for zoos and other organizations to apply their diverse assets and expertise in a coordinated, collaborative program that greatly magnifies the benefit for the species over anything that isolated programs could accomplish working noncooperatively. This cooperative conservation effort focuses on technology transfer and capacity building in the range countries of tigers in support of their development of their tiger conservation programs.

Comprehensive conservation programs are in development throughout the tiger range countries. The Tiger GASP has provided the framework and contacts through which appropriate multi-disciplinary teams have been assembled from zoos and conservation organizations in Asia, Australia, Europe and North America to work with range country and regional tiger conservation programs. Various types of expertise and support are provided to these programs at their request and according to their own determination of their needs and goals. Teams may include conservation biologists, population
biologists, animal managers, geneticists, reproductive physiologists, zoo directors, nutritionists and, of course, veterinarians from within and from outside the range country working together in a logical sequence of steps to help build an effective conservation program. Teams provide a wide variety of services to these programs including assessment of current program status, evaluation and advice concerning both population and individual animal management programs, genome resource bank development and hands on animal work for identification, health assessment and reproductive evaluation purposes. The primary focus of the remainder of this paper will be on veterinary aspects of these programs. A comprehensive review of the cooperative development of a regional conservation program is available in the 1996 International Zoo Yearbook.7

Veterinary Role

The veterinarian serves multiple functions in these programs: 1) to provide advice concerning specific health issues with which he or she is familiar with regard to both population management and individual animal management, 2) to collect and collate information about regional tiger health issues that may be new to the team’s veterinarian or outside of their experience but significant for tigers in the area, 3) to interact with other zoo professionals in areas where their expertise overlaps such as animal management, genetics or reproductive physiology (This includes soliciting input from other team members regarding health management), 4) to assess the current medical management programs of institutions and organizations managing tigers, 5) to provide training as required in specific health related procedures, 6) to provide hands-on animal immobilization, examination and medical procedures if needed to support the goals of the particular program, 7) to develop and maintain professional rapport with the veterinary peers that work within the regional programs and 8) to facilitate formation of professional networks within programs and between regional programs in order to establish systems for information dispersal, professional collaboration, data collection and establishment of baseline values for physiological parameters such as serum chemistries.

One of the significant roles the veterinarian can play is as a catalyst for development of a network of veterinarians within the regional program and between programs. These networks can serve as a basis for the exchange of information and for the sharing of expertise, skills and even equipment between institutions in order to improve the overall medical programs in the population. These professional associations may also serve to establish systems for centralized collection and dispersal of data such as hematology or serum chemistry information from animals within the regional programs. The other functions of the veterinarian vary according to the requirements of each regional program and are determined by their requests and their goals.

The most consistent function required of the veterinary participants in this program so far has been to provide demonstrations of or training in techniques that we utilize for immobilization, examination and medical procedures. Training generally consists of collaborative hands-on animal procedures with the veterinarian from the local regional program and the veterinarian from the advisory program working together. The program veterinarian initially demonstrates procedures and then turns the work completely over to the regional veterinarian as quickly as possible. It is essential that all animal work be collaborative and with the intent of turning the procedures over to the regional veterinarians. Lecture material is not emphasized. There is a very high level of professional expertise among the veterinarians working in the regional programs and they are
generally academically well-trained. The application of their expertise is often limited only by the restricted availability of equipment and supplies in their institutions and in some instances by limited opportunity to acquire experience. The technology for some of the medical procedures is simply unavailable in some areas. The hands-on animal work with the program veterinarian and the equipment they bring with them provides an opportunity for regional veterinarians to apply their expertise and acquire additional experience in a controlled situation. It is essential that the regional veterinarians at each institution perform the medical procedures and not just observe. Collaborative work on the medical procedures increases the confidence level of both the veterinarians and of associated professionals such as animal managers, zoo directors and regional program managers that rely on the veterinarians expertise.

These medical procedures also provide support for other professionals involved in the program such as the permanent identification of animals for population management, the collection of skin biopsies for genetic evaluation or electroejaculation for genome resource banking. In order to facilitate these immobilizations in a variety of institutions and programs the veterinarians involved in the tiger program have developed a standardized protocol for these procedures. This protocol is an evolving document and the most recent revision is included as an addendum to this paper.

Reports

Reports produced by the advisory team constitute the most significant function they perform in many respects. The reports disseminate information acquired during the project, form a foundation for planning and action in the program and help build the professional network that is essential to the success of conservation programs. Individual animal immobilization reports are provided from the program veterinarian to fellow professionals at each institution concerning the procedures performed on animals at their institution. A summary assessment of all examination and medical procedures is provided to the coordinator of the program for their final report to the regional program. Examples of the summary report and an individual animal report from the South China tiger program are provided as addenda following this paper. The veterinarian is also responsible for evaluating the current status of medical programs at each institution visited during the project. An example of an institutional report is also provided as an addendum.

Finally the veterinarian is primarily responsible for determining medical recommendations to be combined with recommendations from other professionals involved in the team. These final recommendations suggest which issues may be of the greatest concern for the program and may suggest possible solutions to problems experienced by the program. These recommendations are collated with those of other team members and provided to the regional program for their consideration in the continuing development of their conservation program. An example of the medical recommendations that were made during the initial work with the South China tiger program is also attached as an addendum. Complete medical reports from each of the regional programs that the team has worked with in Indonesia, Thailand and China are available from the author or from the Conservation Office at the Minnesota Zoological Garden, Apple Valley, Minnesota.

One of the hallmarks of the tiger projects is that reports are widely distributed and all information is available to everyone involved in the project. This approach to information distribution is essential...
to development of an effective conservation program.

Regional Programs

Each regional program is unique in its objectives, challenges and requirements. Training is provided as requested by the program and according to their perceived needs. In general training is focused on hands-on training. The knowledge base and academic training level is generally high among regional veterinarians involved in the tiger programs. However the opportunity to acquire experience has often been limited. Consequently training is directed at reinforcement of existing knowledge, skills and confidence levels and at transfer of technology that regional professionals may have less experience with. Recommendations that are made are also structured to each regional program and are based on a realistic assessment of the resources and goals of each program. These recommendations must be sensitive to cultural, economic and political realities without compromising animal care. The emphasis is generally on establishing fundamental management protocols and a structure that provides consistency in medical care for the population of animals.

Sumatran tiger program - The Sumatran tiger conservation program in Indonesia is an international cooperative program in which in situ and ex situ programs work together to conserve the species. A Population and Habitat Viability Analysis (PHVA) for the wild tiger population was held in 1992. The PHVA publication included a draft master plan for the captive tiger population in Indonesia. A captive breeding workshop was also held in 1992. Five tigers were immobilized, examined and permanently identified with tattoos and transponders during this workshop. During these procedures semen was collected from males to establish a Genome Resource Bank (GRB) that is part of this regional program. Initial recommendations made at the captive breeding workshop in 1992 addressed facility evaluation and recommended changes, cleaning and disinfection protocols, and recommended a nutritional assessment. Between 1992 and 1995 the tiger teams visited nine different zoos in Java and Sumatra and worked with professional staff at these zoos to perform examinations and procedures on 51 out of 57 tigers managed in the program. All tigers received complete physical examinations including blood cell counts and serum chemistry evaluations. In addition tissue biopsies were collected for genetic evaluations, semen was collected from males for the GRB, all animals were permanently identified with tattoos and transponders, and prophylactic medical procedures were performed such as dental calculus removal. Complete endodontic procedures were performed on fourteen broken or worn canine teeth in six genetically valuable tigers. Medical recommendations were made at the master plan meeting in 1994 to address the dental pathology found in several tigers, establish a serum bank and consider disease testing, and to develop a health management system for tigers in the program. Recommendations made in 1995 concerned development of centralized data collection in a manner similar to ISIS and specific recommendations for viral disease screening, vaccination programs and medical procedures.

Two items concerning tigers in this program were particularly noteworthy. 1) The tigers in Indonesia seemed to be particularly sensitive to xylazine (Rompun, Miles Inc., Agriculture Division, Animal Health Products, Shawnee Mission, Kansas, 66201 USA). Doses of greater than 0.2 mg/kg produced significant respiratory depression in these tigers. 2) Serum chemistry results were evaluated from 30 of the 51 tigers examined. All animals had blood urea nitrogen (BUN) values that would be considered elevated. The normal ISIS range for BUN values in tigers is 20.7-35.1 mg/dl. The tigers in the Indonesian population had BUN values of 41.4-117.3 mg/dl. The serum creatinine levels for
this same group of tigers was 1.76-3.9 mg/dl. The significance of these elevated BUN values is not clear but they are possibly related to a very high protein diet in most of these cats. This is being investigated further.

South China tiger program- During 1995 twenty-two of the fifty tigers in captivity were immobilized at four institutions. They received complete examinations as described above. Sixteen fractured or worn canines with exposed root canals were found in nine tigers. Two items of particular interest were noted in this group of animals. First, sensitivity to ketamine (Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501 USA) varied between the sexes during immobilization procedures. Both male and female groups received similar mean xylazine doses of 0.47 mg/kg. At this xylazine dose males were satisfactorily immobilized with an average dose of 7.73 mg/kg. Females however required ketamine doses of 11.93 mg/kg to achieve adequate immobilization. Second, semen was collected from 11 male tigers; overall the semen quality of this group of tigers was relatively low when compared with semen collected from Sumatran males in Indonesian zoos. This initially appears to be related to the relatively high level of inbreeding in the South China tiger population although other factors such as nutrition are also being investigated.1

Semen quality as it relates to inbreeding in this population is crucial considering the small population size. Natural breeding in this population has been variably successful. Medical recommendations made for this program focused primarily on development of an aggressive data and information collection and distribution program and on maximizing the protection of this population from disease through testing and vaccination.

Indochinese tiger program in Thailand- During 1995 a master plan workshop was held to initiate development of a management program for the captive Indochinese tiger population in Thailand. Medical recommendations developed by the veterinary participants in this workshop focused on establishing population management criteria including minimum animal transfer requirements, quarantine requirements, collection of disease information, development of a tiger husbandry manual similar to the manual published for the tiger Species Survival Plan5 and establishment of minimum medical care standards for the population.

Summary

The collaborative, team approach in support of regional conservation programs is one of the most effective ways for veterinarians from zoological parks in North America to participate in conservation programs. This approach provides the veterinarian with the opportunity to learn from colleagues in range countries and to apply a wide range of their medical and management expertise in the preservation of endangered species.

LITERATURE CITED


Addendum 1. Immobilization and examination protocol for tigers; Henry Doorly Zoo, August 1, 1995.

This protocol is based on a team approach to animal immobilization, examination and procedures. Immobilizations are carried out most safely and efficiently when this team approach is utilized. In the case of a tiger immobilization, the team would consist of a veterinarian, a veterinary technician and at least one animal management staff member. Although the veterinarian has primary immobilization responsibility, safe and successful monitoring of the animals vital signs and depth of anesthesia requires the cooperation of all team members. Safe immobilization also depends on constant communication of information such as respiratory rate between team members.

Pre-Anesthetic considerations -

Animal should be off food for 24 hr prior to immobilization. Water may be provided before anesthesia but not for several hours after anesthesia due to the risk of drowning.

If the ambient temperature is high or if cats are excited, then 15 gallons of water must be available before the procedure starts for cooling the animal during procedures.

Animal should be confined alone in smallest area possible.

Anesthetic Procedure

A complete immobilization record should be maintained during the procedure.

1° drug administration - blowdart
2° drug administration - pole syringe, hand injection

Sedation - 1 dart - xylazine 0.4 mg/kg i.m. 10 min interval. Vomiting can be expected. (Reduce dose for Sumatran tigers in Indonesia to 0.2 mg/kg).

Immobilization - 2-3 darts - ketamine 4-6 mg/kg i.m. Surgical anesthesia in 5-10 min. (South China tigers in China require higher doses of ketamine with the above xylazine dose. 7.7 mg/kg for males and 11.93 mg/kg for females.)

Supplements - ketamine 1.0 mg/kg i.m. or diazepam 0.01-0.05 mg/kg administered slowly i.v., (midazolam 0.01 - 0.03 mg/kg i.v. or i.m. is an alternative to diazepam). To control seizure activity use only diazepam or midazolam.

Reversal (if needed) - yohimbine 0.05 mg/kg. In emergencies the benzodiazepine antagonist, flumazenil (0.1 mg/kg i.v.) can be used.

(Yohimbine reverses xylazine. At Omaha xylazine is not routinely reversed in all immobilizations. Yohimbine is used only when indicated by depressed respiration or cardiac arrhythmias or depression. In some institutions animals are routinely reversed with yohimbine even when no physiologic problems occur.)
Seizures - It is not uncommon for tigers to have seizures of 30-60 sec duration during immobilization procedures. The seizuring cat may have severe muscle contractions in all limbs, arch the back and may snap its jaws. The seizures generally do not cause the animal any residual harm, however, it is important to control the seizures if they occur so that they do not progress into more serious continuous grand mal seizures. The seizure activity is normally controllable with the administration of diazepam (0.01-0.05 mg/kg i.v.) or midazolam (0.01-0.03 mg/kg i.v. or i.m.).

Emergency Drugs - should be available at the immobilization site.

- Doxapram - for respiratory depression or arrest - high probability of being used. Use implies cautious continuation of procedure. Range 0.2-0.5 mg/kg i.v. or i.m.

- Yohimbine - for respiratory depression or arrest, cardiac arrhythmia or arrest - medium probability of use. Use requires ending procedures. Dose range 0.05 mg/kg i.v. or i.m. The cat should become alert within 2 to 10 min.

- Epinephrine - for cardiac arrest - low probability of being used. Use requires ending procedure. Dose range 0.5 - 1.0 ml of 1:1000 solution intracardiac.

- Prednisolone - for circulatory collapse - low probability of being used. Use requires ending procedure. Dose range 10 mg/kg i.v.

- Dexamethasone - for circulatory collapse - low probability of being used. Use requires ending procedure. Dose 1 mg/kg i.v.

Emergency Equipment - available at immobilization site.

- Endotracheal tubes with inflatable cuffs - sizes 14, 16, 18 mm for adult tigers.

- Ambu bag to attach to tube to move air.

- Stomach tubes and funnel - for cold water enemas in cases of hyperthermia.

Examination - estimate 5-10 min for examination.

1) Continuously monitor respiration rate and depth from first dart. (8-24/min. normal). One person’s primary job should be respiratory monitoring throughout the procedure.

2) Determine depth of anesthesia to be adequate by stimulation of head with pole syringe or stick.

Hands on evaluation - response to stimulation of body, feet, cornea, ears and tongue. A well-anesthetized adult tiger should maintain a respiratory rate of 8-24 breaths per minute and a heart rate of 60-120 beats per minute. The jaws can be opened and the tongue exteriorized with little or no resistance.

3) Assign one person with experience to control the head of the animal throughout the
procedure. This person also monitors respiration rate.

4) Auscult heart rate (60-120 beats per minute is normal) and rhythm. Auscult lung sounds.

5) Examine oral cavity - capillary refill rate, color of mucous membranes and condition of pharynx, gingiva and teeth.

6) Rectal temperature - (100°-102°F normal). A temperature of 102°-104°F requires re-check in 5 min. Temperatures over 104°F require immediate cold water enema.

7) Examine externally - hair coat, skin, claws (evert each claw), pads.

8) Palpate - retropharyngeal area, prescapular lymph nodes, abdomen, inguinal region and testicles. Exteriorize penis.

9) Ophthalmic examination - followed by ophthalmic antibiotic ointment in eyes to keep corneas moist.

10) Otic examination.

Sample Collection - 3-10 min

1) Blood - 30-60 ml of blood collected from medial saphenous vein. Alternative blood collection sites are cephalic, tail and jugular veins. A 60 ml syringe is used with an 18-ga 1½" needle. Blood sample will be placed in EDTA tube for complete blood count first. The remainder of sample will go into serum tubes unless heparin or other types of samples are required.

2) Biopsy samples (genetic analysis) - 6 mm punch biopsies of full skin thickness on medial inner rear leg from surgically prepared site. Close biopsy with suture material. Apply fly repellent around sites.

3) Fecal samples - if necessary, directly from rectum.

4) Ear swab - if indicated by examination.

5) Aspiration from any abscesses or masses if noted on exam and facilities are available for culture or histology.

6) Semen collection - by electroejaculation. Commonly an electroejaculation procedure for tigers consists of the following series of stimulations.

   10 stimulations at 2 volts
   10 stimulations at 3 volts
   10 stimulations at 4 volts
   Break for 5 min
10 stimulations at 3 volts
10 stimulations at 4 volts
10 stimulations at 5 volts
Break for 5 min
10 stimulations at 4 volts
10 stimulations at 5 volts

**Procedures - 10-15 min**

1) Clean teeth - if required.

2) Trim excess claw sheaths if required.

3) Confirm tatoos, transponders, identification.
   a) Studbook number tattoo in medial upper rear leg.
   b) Trovan transponder may be interscapular or at base of left ear.

**Identification Procedures -**

Tattoo - Permanent tattoos of either permanent or temporary studbook numbers should be placed in the medial surface of the upper rear leg. If the studbook number is temporary it should be placed on the left leg and if the number is the permanent studbook number it should be placed on the right leg. The tattoo site should be clipped to remove hair and surgically prepped before tattooing.

Transponder - A “Trovan” system identification transponder should be placed at the base of the left ear at a surgically prepared site. Other transponder systems may be used. Transponders are placed interscapularly in some tigers.

**Treatments -**

Each cat receives a single dose of injectable antibiotic. No follow-up planned. Antibiotic choices depend on stability at room temperature.

   - Tribrissen - trimethoprim-sulfa - 20 mg/kg
   - Naxcel - cephalosporin - 0.5 mg/kg (usually requires refrigeration)
   - Amikacin - aminoglycoside - 10 mg/kg
   - Benzathine penicillin - usually requires refrigeration 20,000 IU/kg

**Vaccinations -**

Routine annual vaccination using killed vaccines for rabies, feline rhinotracheitis, calicivirus and panleukopenia may be given at this time.

**Recovery -**
Cats should be recovered in areas that do not have hazards in them such as pools of water, not even shallow drinking bowls. Cats should be directly observed throughout recovery until they can maintain sternal recumbency. A routine immobilization which is 1 hr or less in duration and not reversed usually requires 1-3 hr for the animals to recover sufficiently to be able to maintain sternal recumbency. Normal coordination and behavior has fully returned within 24 hr. If yohimbine is used to reverse the xylazine then cats are usually in sternal recumbency in 15 min. However, these cats are often severely ataxic and may hallucinate due to residual ketamine in their systems. Reversed animals have a slightly higher probability of injuring themselves during recovery.

**Routine whole blood sample tests** -

- White blood cell count
- Red blood cell count
- Hemoglobin
- Hematocrit
- Mean corpuscular volume
- Platelet count
- Segmented Neutrophils - absolute and %
- Band Neutrophils - absolute and %
- Lymphocyte - absolute and %
- Monocytes - absolute and %
- Eosinophils - absolute and %
- Basophils - absolute and %

**Routine serum chemistry tests** -

- Total protein
- Albumin
- Cholesterol
- Uric acid
- Creatinine
- Bilirubin
- Blood urea nitrogen
- Glucose
- BUN/CR
- Alkaline phosphatase
- Alanine transferase (SGPT)
- Aspartate transferase (SGOT)
- Lactic dehydrogenase
- Triglycerides
- Sodium
- Potassium
- Chloride
- Calcium
- Phosphorous
- Total CO₂
- Anion Gap
- Calculated osmolality

For further information or to discuss this protocol please contact:
Addendum 2. South China tiger project, November 6-20, 1995.

Tiger Physical Examination Report

Immobilization Date: 09-11-95
Studbook Number: 111
Species: *Panthera tigris amoyensis*
Location: Shanghai Zoo, Shanghai, China
Tattoo: 111 located in medial upper right rear leg
Transponder: Trovan 0000F78FA3 located interscapular
Body Weight: 113 kg (actual)
Sex: Female
Age: 15 yr, 6 mo
Birth Date: 08-04-80
Origin: Captive born - Sire 27, Dam 26

Summary

The tiger was immobilized with 50 mg (0.44 mg/kg) xylazine and 900 mg (7.96 mg/kg) ketamine administered intramuscularly by blowdart and pole syringe. Anesthesia was maintained for 40 min of working time with two supplements of ketamine (500 and 300 mg) given intramuscularly and one supplement of midazolam (1 mg) given intravenously. The first supplement of ketamine was given 30 min after the last immobilizing dose. Anesthesia would have been improved with a supplemental dose of 200-300 mg ketamine given intramuscularly at approximately 20 min after the last immobilizing drug dose. Trimethoprim and sulfadiazine (2400 mg) were given to prevent secondary infections. This cat was in good body condition. The teeth had minimal calculus which was removed by hand scaling. The lower right canine was fractured with an exposed root canal. The claws on all four feet were overgrown and were trimmed. Abrasions were present on the ventral surface of all four feet. A 5 cm diameter subcutaneous boney mass was present on the right maxilla. The tiger was tattooed with studbook number “111” in the upper medial right rear leg. Trovan transponder “0000F78FA3” was placed subcutaneously on the dorsal midline interscapularly. Whole blood and serum were collected for complete blood cell count, serum chemistries and serum banking. Two full thickness, 6 mm diameter skin biopsies and a plucked hair sample were collected.
Addendum 3. Institutional report.

INSTITUTION: Chongqing Zoo
DATE VISITED: November 16, 1995
VETERINARIANS: Dr. Zhao Guanh (Chief vet)
Dr. Wu Den Hu
Dr. Xie You Xing

FACILITY

General: The veterinary hospital is a two story facility that appears to provide adequate space, but lacks a well-stocked equipment inventory. An attached quarantine area exists and at the time of the visit, housed newly arrived waterfowl.

Surgery: A separate surgery/treatment room exists, but its level of cleanliness should be addressed. The veterinarian said that they have an autoclave. Further training in sterile techniques (including any procedures that involve the reproductive tract) is advisable.

Post-Mortem: A separate necropsy room is present and appears adequate for all but the largest animals.

MEDICAL RECORDS

Animal Records: Each veterinarian keeps individual records of the procedures that he has performed. As such, there are apparently no individual animal records without performing a compilation of those of each clinical veterinarian.

Post-Mortem Records: Necropsy records exist for at least the past 10 yr, but they were not examined.

SUPPLIES

Anesthetic Equipment and Drugs: The veterinarian noted that the Zoo is need of a blowpipe as the one that they have is crooked. Ketamine is available (in China it is 50 mg/ml and approximately 10 yuan per bottle). Xylazine is not readily obtainable.

Other Drugs: In general, the pharmacy was not well-stocked and many of the drugs appeared quite old. Interpretation of the drugs available was limited by the Chinese only labels on most medications.

Other Restraint Equipment: A squeeze cage was present and per the veterinarian had been successfully used for tigers.

Medical Summary

Physical examination and routine medical procedures were carried out on 11.11 South China Tigers at four institutions. Medical management programs, facilities and equipment were also assessed. The primary clinical abnormality found in a significant number of tigers was fractured canine teeth with exposed root canals. A total of 16 fractured canines were found in 9 animals. A number of other minor problems were identified in individual animals. Most of the tigers with the exception of four animals had remarkably little calculus on their teeth. This is a common problem in captive animals.

The tigers were all immobilized with a combination of xylazine and ketamine delivered by blowdart. Supplements of ketamine and/or midazolam were utilized to maintain anesthesia and to control seizure activity. A notable difference in the anesthetic dose required emerged between the males of this population and the females. The effective xylazine dose required was similar for males and females at about 0.47 mg/kg. The ketamine dose required to produce good anesthesia was quite different between the sexes with males satisfactorily immobilized with 7.73 mg/kg of ketamine on average while females required an average ketamine dose of 11.93 mg/kg to produce adequate anesthesia. This difference in dose requirement is not easily explained and warrants further investigation, initially through review of records.

Each tiger was given a complete physical examination including examinations of the eyes, ears, teeth and claws as well as general physical condition. Each animal was permanently identified with a tattoo of the tigers permanent studbook number placed in the inside upper right rear leg and with a “Trovan” transponder placed under the skin between the shoulder blades on each tigers back.

A blood sample was collected from each animal for hematology, serum chemistries and for serum banking. Two 6 mm full thickness skin biopsies were collected from each tiger from the inside upper right rear leg. These samples were processed for cryopreservation and placed in long term cryostorage in liquid nitrogen for possible genetic work in the future. Hair samples were also collected and stored from each tiger. Semen was collected by electroejaculation from male tigers, evaluated and processed for cryopreservation where appropriate.

Each zoo made arrangements for a standard battery of hematology and serum chemistry tests to be performed on blood samples from each of the animals at their institution. Results of these tests were not available to the project team at the time of this report. It is extremely important that the results of these tests from all the animals in all of the zoos be collected and reviewed as a pooled set of data. Each tiger’s serum chemistry and hematology tests are very important measurements of the health of that individual animal. The pooled information provides a crucial measure of the health of the population and is indispensable in identifying significant problems in this endangered population of tigers. Pooling this information also permits the initial establishment of normal values for these tests in this population of tigers. The previous experience of this team with the Sumatran tiger population in Indonesia demonstrated the value of central collection and evaluation of this information (see Kidney Pathology Summary in 1995 Sumatran tiger project report).
Kidney Pathology Summary

Blood sample values were received from 30 tigers processed in 1992 and 1994. One notable abnormality was detected in all of these samples. The Blood Urea Nitrogen (BUN) value on all samples for which results were available from tigers processed in 1992 and 1994 were all outside of the normal range for tigers. This normal range for tigers is established by the International Species Inventory System (ISIS) based on 578 samples collected from tigers in North America and Europe primarily. The normal ISIS range for BUN values in tigers is 20.7 to 35.1 mg/dl. Samples from tigers in Indonesia had values ranging from 41.4 to 117.3 mg/dl. The average value for Indonesian tigers was 73.7 mg/dl and five tigers had values over 100 mg/dl.

The significance of these measurements is that in the ISIS population of tigers BUN values such as those found in Indonesian tigers would indicate compromised kidney function and shortened life expectancy with BUN values over 100 mg/dl indicating a life expectancy of less than a year without extraordinary medical intervention measures. Tigers with BUN values greater than normal but less than 100 mg/dl would have a longer life expectancy but reduced normal biological function, possibly including reduced reproductive ability.

This is not a clearly defined issue however. A population of animals who all have similar values, such as the Indonesian tigers, indicates that this range of values could be normal for this population. This difference in the normal range between this population and other populations would most probably be due to a factor all animals in the population share such as a high protein diet. It is also noteworthy that another serum test of kidney function done on this same group of tigers was normal. The serum creatinine levels on these same animals generally all fell within the normal range for ISIS. (ISIS creatinine normal range = 1.5 - 3.1 mg/dl, Indonesian tigers range = 1.76 - 3.9 mg/dl). In addition, one tiger that was found in 1992 to have a BUN over 100 mg/dl has continued in apparent good health since that time although she has not reproduced in spite of breeding attempts. This cat's survival for 2.5 yr would not have been predicted based on past experience.

The elevated BUN values in the population of tigers in Indonesia warrants further investigation. It is possible that this is normal for these tigers. However, it is conceivable that although these BUN values may be normal based on factors such as very high protein diets, long term exposure to these diets and resulting long term elevated BUN values might damage the kidneys, reduce life expectancy of the animals and reduce reproductive ability.

Results

Generally this population of tigers appeared to be in good health and to be well-cared for. A few
items of concern were identified by the team and warrant further consideration.

1. Sanitation and cleaning procedures at the zoos seemed to be conscientiously carried out. However in most of the tiger cages there did seem to be a film on the concrete and a greasy feel. It is conceivable that more detergent needs to be used to scrub cages down and remove all organic material from the surface prior to the application of disinfectants each time the cage is cleaned. It may also be of value to seal all concrete surfaces. Even concrete that appears smooth has microscopic pores in it that collect organic debris and can be a source of disease.

2. Nutrition will be more thoroughly reviewed in another portion of this report. Two items were of particular concern from a medical perspective. The first was that the use of vitamins and the pattern of feeding them to the tigers varied a great deal between institutions and within institutions with no clear rationale that was apparent. Vitamins can be crucial to an animal's health, reproduction and longevity but they can also be hazardous when provided inappropriately. The second item was that a clear benefit seemed to occur in zoos that provided bones to their tigers. Tigers who were routinely given large bones to gnaw on had far less calculus or tartar on their teeth.

3. This population of tigers is vulnerable to significant outbreaks of disease which may result in the loss of animals. Most tigers are not vaccinated for any preventable disease and where they are vaccinated the vaccines used were probably expired or out of date and did not provide protection. Although infectious disease has not apparently been seen in this population of tigers we feel that it poses a substantial risk in a population of animals that cannot afford to lose any members for preventable reasons. It is also very likely that genetic management of this population will require a substantial number of animal moves. These moves increase the probability of moving undetected disease problems throughout the population of tigers.

4. Overgrown claws were found in some animals. These claws can grow around back into foot pads and become infected. This problem can be prevented by providing scratching logs for the tigers and by performing annual physical exams.

5. There is a great deal of variation between institutions with regard to equipment and supplies to provide medical care. All institutions need at least some equipment and some institutions need a great deal of equipment and supplies in order to be able to provide basic medical care. This is a significant concern which needs to be addressed in all institutions in the program. Adequate medical supplies and equipment must be available to provide basic medical care for these tigers.

6. It appeared that it was a policy at most institutions for veterinarians to keep their own medical records. Centralized medical records for individual animals in each institution were not maintained with the exception of one zoo and there was no sharing or pooling of information in a structured manner between institutions. The centralized collection and maintenance of medical records and related information within institutions and between institutions is important for several reasons. The most significant for this population is that the most dependable method to identify disease problems that are affecting the overall population is through centralized data collection. The sharing of information also allows the establishment of normal ranges for data such as serum chemistry values.
and provides crucial information for everyone about effective treatments for disease problems.
Addendum 5. South China tiger project final recommendations (27-11-95).

1. Complete copies of all medical records of all South China tigers in captivity at any time within the last 10 yr should be submitted by the chief veterinarian at each zoo to the species coordinator within 3 mo. The species coordinator should submit complete translated copies to the global tiger coordinator within 6 mo.

These records should include:
   a. all clinical disease problems observed
   b. all treatments used and their outcomes as well as adverse reactions
   c. laboratory data including parasitology, hematology, and serum chemistries
   d. complete necropsy reports from all animals that have died including gross necropsy results, histopathology reports, and bacterial culture or virus isolation results

2. The collected medical information should be evaluated by veterinarians within the South China tiger program and the global tiger program in order to identify disease threats to the population of tigers and to establish baseline data for laboratory tests. The species coordinator and global tiger coordinator are responsible for identifying appropriate veterinarians to evaluate the data. All available information should be supplied to the evaluators within a week of the deadlines in recommendation 1 and a report submitted from the evaluator to the coordinator within 1 mo after the information is provided. This report should then be directly distributed to all veterinarians involved in the program for their consideration.

3. All South China tigers currently in captivity should be vaccinated with two injections 3 wk apart of killed vaccine for feline rhinotracheitis, calicivirus, and panleukopenia and with killed rabies vaccine. Only killed vaccines should be used. All tigers should be revaccinated twice yearly for these disease threats. Initial vaccinations of all tigers in the program should be completed within 3 mo. The vaccine produced by Fort Dodge Laboratories for rhinotracheitis, calicivirus and panleukopenia has a history of safe and efficacious use in tigers in the North American tiger population and is the recommended choice. Care must be taken to insure that the vaccine used is not outdated.

4. Animals of other species which are also vulnerable to some of the same diseases as tigers and which are housed in proximity to tigers should also be vaccinated in order to help protect the tigers from exposure to disease. This includes domestic cats on display or housed nearby, other nondomestic felid species such as lions, clouded leopards and others, canid species including domestic dogs in the vicinity of tigers and may include other species such as procyonids.

5. Complete endodontic repair procedures should be performed on all fractured teeth in tigers within the managed population within 12 mo.

6. All South China tigers currently in captivity should be screened for exposure to potential disease threats through serum antibody testing for Feline Leukemia Virus, Feline Infectious Peritonitis, Feline Immunodeficiency Virus and Canine Distemper Virus. Due to variability in testing results between laboratories, it is strongly recommended that duplicate samples be submitted to at least one
laboratory which has a data base of virus testing in tigers for comparison purposes to assess risk to the population.

7. The CAZG or the South China tiger management program should establish minimum medical management criteria for tigers in the program. These criteria could be formulated by a veterinary advisor or advisory group from zoos participating in the program. These minimum criteria should include fecal examinations twice per year for parasites, a vaccination program, an annual physical examination, and annual blood and serum chemistry tests. A significant advantage would be gained by running serum chemistry tests for all tigers performed at a central laboratory which has rigid quality control standards. Alternatively duplicates of samples could be run at a central laboratory as a quality control check.

8. The CAZG should consider hosting a workshop to provide training in medical procedures and working groups to formulate criteria and plans for items mentioned above.
A REVIEW OF BLASTOMYCOSIS IN LARGE ZOO CARNIVORES

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Abstract

Seven cases of blastomycosis have occurred in large zoo carnivores after residing in eastern Tennessee. Two cases have been previously reported in a male polar bear (Ursus maritimus) and an African lion (Panthera leo).1,2 Five additional cases have been observed at one zoological park in another polar bear, two Asian lions (Panthera leo persicus), a Siberian tiger (Panthera tigris), and a cheetah (Acinonyx jubatus). The findings of these seven cases of blastomycosis are reviewed here.

Overall, blastomycosis in large zoo carnivores presents a diagnostic challenge. A history of increasing lethargy, anorexia, and weight loss, possibly accompanied by subtle respiratory signs is typical. Abnormalities on routine bloodwork are few and inconsistent. Radiographic changes in the thorax, coupled with a history of geographical exposure represent the findings most suggestive of blastomycosis. Confirmation of blastomycosis requires AGID serology, coupled with cytology and culture of the respiratory tract or lesions. Treatment with itraconazole can be effective, and oral doses of 2 mg/kg twice daily and 5 mg/kg once daily provide high concentrations of drug in serum, pleural effusion and cerebral spinal fluid.1 Initial delivery of doses by gavage or supplementation with intravenous amphotericin B can facilitate the induction of treatment. Spread of the disease to the central nervous system may be evident on necropsy.

Resumen

Han ocurrido 7 casos de blastomicosis en carnívoros grandes de zoológico después de residir en el este de Tennesse. Dos casos se habían reportado previamente en un macho de oso polar (Ursus maritimus) y un león africano (Panthera leo).1,2 Cinco casos adicionales han sido observados en un parque zoológico, en otro oso polar, dos leones asiáticos (Panthera leo persicus), un tigre siberiano (Panthera tigris) y en un cheetah (Acinonyx jubatus). En este estudio se revisan los hallazgos de estos siete casos de blastomycosis.

La blastomicosis en grandes carnívoros de zoológicos, presenta sobre todo un reto de diagnóstico. Es típica una historia clínica de letargia que se va incrementando, existe anorexia y pérdida de peso, posiblemente acompañada por signos respiratorios leves. Las anormalidades en los exámenes sanguíneos de rutina son pocas e inconstantes. Los cambios radiográficos en el tórax, a la par con una historia de exposición geográfica representa los hallazgos más sugestivos de blastomycosis. La confirmación de blastomicosis requiere serología AGID, en conjunto con citología y cultivo del tracto respiratorio y lesiones. El tratamiento con Itraconazol puede ser efectivo y dosis orales de 2 mg/kg 2 veces al día y 5 mg/kg una vez al día proporcionan altas concentraciones séricas de la
Introduction

Seven cases of blastomycosis have occurred in large zoo carnivores after residing in eastern Tennessee. Two cases have been previously reported in a male polar bear (Ursus maritimus) and an African lion (Panthera leo).\textsuperscript{1,2} Five additional cases have been observed at one zoological park in another polar bear, two Asian lions (Panthera leo persicus), a Siberian tiger (Panthera tigris), and a cheetah (Acinonyx jubatus). The findings of these seven cases of blastomycosis are reviewed here.

Clinical Signs and Initial Diagnostic Tests

The affected animals were all adults and lived an average of 5.5 yr in Tennessee before developing lethargy and weight loss. The lions and tiger showed respiratory signs, including dyspnea and sneezing. Data from a clinical workup was available in four animals. Initial physical examination was unrewarding, and complete blood counts showed few abnormalities. One lion had an elevated white blood cell count, two lions had band cells, and one lion and one polar bear had a slight monocytosis. Serum chemistry values were remarkably normal, although both lions had slightly elevated serum calcium. Radiographs were taken on four animals. Thoracic abnormalities were noted in each animal examined, including pulmonary infiltrates, bullous formation, lung lobe collapse, lung masses, and pleural effusion. Cytological examination of the respiratory tract revealed budding organisms consistent with \textit{Blastomyces} spp. in only one of four animals. Culture of the respiratory tract grew \textit{Blastomyces dermatitidis} in one of two animals.

Blastomycosis Serology

Agar gel immunodiffusion (AGID) blastomycosis titers were performed antemortem on three animals. The test was positive in two of three animals at initial testing. The third animal was equivocal on initial testing, but was positive 1 mo later. Cerebral spinal fluid was also positive in the one animal sampled. Retrospective postmortem testing of frozen serum was performed on another animal, in which serum banked 3 yr previously was positive.

Antifungal Treatment

Itraconazole therapy for treatment of blastomycosis was attempted in three animals. One Asian lion ate poorly, refused medication, and was euthanatized due to a rapidly declining condition 2 wk after diagnosis. A polar bear was treated with 2 mg/kg p.o. b.i.d. and clinical improvement was noted within 1 wk. Treatment was continued for 90 days with no adverse side effects.\textsuperscript{1} A Siberian tiger refused to eat the day following diagnosis, so he was reimmobilized and given 40 mg of amphotericin B i.v. and was gavaged with 5 mg/kg itraconazole. He resumed feeding the following day and was given 5 mg/kg itraconazole p.o. s.i.d. for 30 days. After initial improvement, the tiger...
became quadriparetic and was euthanatized. The itraconazole level in the cerebral spinal fluid was 10.1 μg/ml.

**Pathology**

Six of seven affected animals had gross lesions of blastomycosis. The treated polar bear recovered, and did not have lesions at the time of death 8 yr later. The character of lesions was similar in all cases. Fungal organisms compatible with *Blastomyces* spp. were found within most lesions, however, the distribution of lesions differed among species. Five of the six cases with gross lesions had nodular to diffuse consolidation of the lungs due to coalescing granulomas with varying degrees of central necrosis or calcification. In addition, both Asian lions had histiocytic lymphadenitis of the bronchial lymph nodes, and one polar bear had extensive granulomas on all pleural and peritoneal surfaces. Two of three felids in which the brain was examined had central nervous system involvement. The treated tiger had extensive granulomatous encephalomyelitis, but no lung lesions. Extension to the central nervous system was also noted in the African lion. The cheetah had a single pulmonary granuloma which was an incidental finding at the time of necropsy.

**Discussion**

Blastomycosis in large zoo carnivores presents a diagnostic challenge. A history of increasing lethargy, anorexia, and weight loss, possibly accompanied by subtle respiratory signs is typical. Abnormalities on routine bloodwork are few and inconsistent. Radiographic changes in the thorax, coupled with a history of geographical exposure represent the findings most suggestive of blastomycosis. Confirmation of blastomycosis requires AGID serology, coupled with cytology and culture of the respiratory tract or lesions. Treatment with itraconazole can be effective, and oral doses of 2 mg/kg b.i.d. and 5 mg/kg s.i.d. provide high concentrations of drug in serum, pleural effusion and cerebral spinal fluid. Initial delivery of doses by gavage or supplementation with intravenous amphotericin B can facilitate the induction of treatment. Spread of the disease to the central nervous system was evident on necropsy in two of three felids examined, and not apparent in either polar bear.

**LITERATURE CITED**

POSSIBLE VACCINE-INDUCED VIRAL DISEASE IN CHEETAHS

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Abstract

Six 8-wk-old, mother-reared cheetah cubs (Acinonyx jubatus) developed progressive upper respiratory infection within 24 hr of receiving a multivalent, modified-live virus feline vaccine, which had been used safely in this species previously. The cubs were treated with acyclovir in an attempt to avert herpessviral dermatitis and conjunctivitis that had been seen in other young cheetahs. All cubs showed anemia and leukopenia. One cub developed pneumonia and died 12 days later despite treatment. A second cub developed oral ulcerations, pneumonia and gastroenteritis, and died 19 days after the initial infection. A third cub with similar depression, anorexia, and oral and glossal ulcers responded to maternal blood transfusion. Feline calicivirus was isolated from the dead cubs; one also had histological lesions typical of feline parvovirus (panleukopenia). The timing of the infection and the involvement of more than one virus suggested that the disease was vaccine-induced, although the possibility of natural infection could not be discounted. The use of acyclovir may have rendered the cubs more susceptible to the effects of resistant viruses.

Resumen

Una camada de cachorros de cheetah (Acinonyx jubatus) de 8 semanas de edad criados por su madre, desarrollaron un infección de las vías respiratorias superiores dentro de 24 hr. de haber recibido una vacuna multivalente de virus felino vivo modificado, la cual ha sido usada de manera segura en esta especie previamente. Los cachorros fueron tratados con Aciclovir en un intento de revertir la conjuntivitis y dermatitis por herpes viral que han sido observadas en otros cheetahs jóvenes. Todos los cachorros mostraron anemia y leucopenia. Un cachorro desarrolló neumonía y murió 12 días después a pesar del tratamiento. Un segundo cachorro desarrolló ulceraciones orales, neumonía y gastroenteritis y murió 19 días después de la infección inicial. Un tercer cachorro con depresión similar, anorexia y úlceras orales respondió a una transfusión de sangre materna. Se aisló calicivirus felino de los cachorros muertos; uno también tenía lesiones histológicas típicas de parvovirus felino (panleukopenia). El tiempo de la infección y de la complicación por más de un virus sugirió que la enfermedad fue inducida por la vacunación, aunque la posibilidad de una infección natural no puede ser descartada. El uso de Aciclovir pudo haber hecho a los cachorros más susceptibles a los efectos de virus resistentes.

Introduction

A report on the use of feline viral vaccines in cheetahs has shown that serological response to some components of multivalent inactivated vaccines is quite poor. A schedule requiring a vaccination
Modified live-virus vaccines promote a better immune response, especially if given after 12 wk of age, and have been used safely in many non-domestic felines, including cheetahs.3,4

Case Report

Six 9-wk-old, mother-reared cheetah cubs (Acinonyx jubatus) developed progressive upper respiratory infection within 24 hr of receiving a multivalent, modified-live virus feline vaccine (Felocell CVR, Pfizer Animal Health [formerly Smith Kline Beecham], Ville St. Laurent, Quebec). Feline viral rhinotracheitis was suspected initially and the cubs were treated with the antiherpetic agent acyclovir (Zovirax, Burroughs Wellcome, Kirkland, Quebec) in an attempt to avert herpesviral dermatitis and conjunctivitis that had been seen in young vaccinated cheetahs in previous years. The severity of the respiratory infection increased despite antiviral and antibacterial therapy. All the cubs developed anemia and leukopenia within the following 2 wk period. One (#30802) died 12 days after vaccination from pneumonia and gastroenteritis with pathological lesions compatible with concurrent calicivirus and feline panleukopenia virus infections, and secondary bacterial invasion.

A second cub (#30803) developed oral ulcerations, pneumonia and gastroenteritis, and died 19 days after the initial infection despite intensive therapy. A third cub (#30805) with similar depression, anorexia, and oral and lingual ulcers responded very rapidly to a single maternal blood transfusion. The remaining cubs showed oral ulcers but recovered without further treatment. Initial attempts to isolate viruses from the clinically affected cubs were unsuccessful. Feline calicivirus was cultured from the dead cubs. The disease was likely due to concurrent feline calicivirus infection and panleukopenia.

Blood samples taken on the second day after the initial signs and 2 wk and 4 wk later showed significant increases in HI titer for panleukopenia virus (FPV) in all 6 cubs, and in neutralizing antibody against calicivirus (FCV) in three of the four survivors, (a response to either natural or vaccine-induced infection), but no increase in neutralizing titer against feline herpesvirus 1 (FVR) (Table 1).

Discussion

There were three possibilities for the source of the infection--a feral domestic cat, excretion of the virus by the dam, and vaccine-induced disease, or some combination of these events. Although the cubs first showed clinical signs almost immediately after vaccination, the timing of the infection and the involvement of all the litter and of more than one virus suggested that the disease was vaccine-induced. However, the possibility of natural infection cannot be discounted, and there was some evidence that the cubs had first shown signs the day prior to vaccination. Attempts were made to compare the isolated virus with vaccine virus.

Wild felines are susceptible to many of the virus diseases affecting domestic cats. The cheetah was the first non-domestic cat reported with feline calicivirus infection.2 Routine vaccination has reduced the incidence of infection in zoo animals. However, we had previously experienced severe
feline herpesviral disease in cheetahs given inactivated products, and this condition necessitated treatment by hypervaccination using the same modified live virus vaccine, and the use of long-term antiviral drugs. We have also used the vaccine previously in young cubs. It is not known why these cubs developed viral infections of such severity, although they were infected at a time when antibody levels are at their lowest.4,5 The use of acyclovir may have rendered them more susceptible to the effects of the resistant (non-herpes) vaccine viruses, or may have even caused immunosuppression directly. Acyclovir is converted intracellularly into acyclovir triphosphate which selectively inhibits herpesvirus DNA polymerase. Acyclovir is remarkably non-toxic and has a very limited effect on bone-marrow or immune function in man and domestic animals.1 In fact, its principal use is in the prevention and treatment of viral infections in immunosuppressed patients. However, in this case its use may have exacerbated the myelosuppressive effects of the panleukopenia virus. Ganciclovir, a related antiviral drug with a similar mode of action, is a powerful bone-marrow depressant inducing leukopenia, neutropenia and thrombocytopenia. We have used acyclovir for periods of up to 8 mo in juvenile (6 mo to 1 yr) cheetahs without evidence of side-effects.

Booster doses of the same vaccine were given at 12 and 16 wk of age. The vaccine was used in a single cub from another litter subsequently without adverse effects, and will be used in future litters.

LITERATURE CITED
Table 1. Serum antibody titres against feline panleukopenia virus (FPV), feline calicivirus (FCV), and feline rhinotracheitis virus (FVR) in 6 cheetah (*Acinonyx jubatus*) cubs.\(^a\)

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\(^a\)Vaccination date: 95-08-17
\(^b\)Hemagglutination inhibition
\(^c\)Virus neutralization
TESTICULAR AND OVARIAN FUNCTION IN SOUTH AMERICAN SMALL FELIDS ASSESSED BY FECAL STEROIDS

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Abstract

In 1994, ocelots (*Felis pardalis*), margays (*F. wiedii*) and tigrinas (*F. tigrina*) were among the 23 taxa assigned to the endangered category by Mace-Lande criteria at the South American Felid Conservation Assessment and Management Plan (CAMP) workshop held in Brazil. These South American felid species are threatened by a host of factors with loss of habitat being the most pervasive. In captivity, the breeding success of Latin American felids also is very low. Without improvement in captive breeding efficiency or future imports from the wild, it will be difficult to sustain effective population sizes for these species and meet target levels of genetic diversity. One important component of the CAMP process involves developing captive propagation recommendations for most of the reviewed taxa. However, for the small cats especially, efforts to implement these strategies are hampered by a lack of adequate baseline reproductive information. In this study, non-invasive fecal steroid metabolite analyses were used to begin generating a reproductive database for the ocelot, margay and tigrina, including evaluations of: 1) estrous cyclicity in females; 2) testicular function (steroidogenic and spermatogenic) in males; and 3) the influence of season on reproductive activity in males and females.

Data presented in this paper demonstrate the utility of fecal steroid analyses for non-invasively monitoring ovarian and testicular function in ocelots, margays and tigrinas. In each species, there was an influence of season on reproductive activity, although for some parameters the effect was modest. This information is important for defining normal reproductive processes which might prove valuable for aiding captive breeding strategies to improve species conservation. For species like the tigrina and margay that rarely reproduce in captivity, reliable use of assisted reproductive technologies based on information generated by non-invasive hormone monitoring could contribute to improved breeding success.
Resumen

En 1994 los ocelotes (Felis pardalis), tigrillo-margays (Felis wiedii) y tigrillos (Felis tigrina) estaban entre los 23 órdenes taxonómicos asignados a la categoría en peligro de extinción por el criterio Mace-Lande del plan de manejo y evaluación para la Conservación de Felinos Sudamericanos (CAMP) con residencia en Brasil. Estas especies felinas Sudamericanas están amenazadas por un sinnúmero de factores, siendo la pérdida del hábitat la de más peso. En cautiverio, el éxito de reproducción de los felinos de América Latina también es muy bajo. Sin la mejora de la eficacia en crianza en cautiverio o futuras introducciones al medio silvestre será difícil mantener números importantes de poblaciones para estas especies y encontrar niveles deseables para la diversidad genética. Un componente importante del proceso CAMP involucra el desarrollo de recomendaciones para la propagación en cautiverio de la mayoría de las especies revisadas. Sin embargo especialmente para los gatos pequeños, los esfuerzos para implementar estas estrategias se ven entorpecidos por una deficiencia de información reproductiva adecuada. En este estudio se utilizaron sistemas de análisis no invasivos de metabolitos esteroides fecales para comenzar a generar una base de datos reproductivos para el ocelote y tigrillos, incluyendo evaluaciones de 1) cíclicidad del estro en hembras; 2) función testicular (esteroidogénico y espermatogénico) en machos; y 3) la influencia de las estaciones en la actividad reproductiva de machos y hembras.

Introduction

In 1994, ocelots (Felis pardalis), margays (F. wiedii) and tigrinas (F. tigrina) were among the 23 taxa assigned to the endangered category by Mace-Lande criteria at the South American Felid Conservation Assessment and Management Plan (CAMP) workshop held in Brazil. These South American felid species are threatened by a host of factors with loss of habitat being the most pervasive. In captivity, the breeding success of Latin American felids also is very low. Without improvement in captive breeding efficiency or future imports from the wild, it will be difficult to sustain effective population sizes for these species and meet target levels of genetic diversity. One important component of the CAMP process involves developing captive propagation recommendations for most of the reviewed taxa. However, for the small cats especially, efforts to implement these strategies are hampered by a lack of adequate baseline reproductive information. In this study, non-invasive fecal steroid metabolite analyses were used to begin generating a
reproductive database for the ocelot, margay and tigrina, including evaluations of: 1) estrous cyclicity in females; 2) testicular function (steroidogenic and spermatogenic) in males; and 3) the influence of season on reproductive activity in males and females.

**Materials and Methods**

Adult ocelots (n = 3 males, 4 females), margays (n = 3 males, 2 females) and tigrinas (n = 3 males, 5 females), housed as singletons at either the Zoológico de Curitiba or Itaipú Wildlife Breeding Center in Brazil, were evaluated for 14 consecutive months. Estrus behaviors observed during routine caretaking procedures were recorded daily. Fecal samples were collected 5 times per week for characterizing longitudinal gonadal steroidogenic activity. All fecal samples were stored at -20°C until processed and analyzed as described previously. Briefly, to extract fecal steroid metabolites, samples were thawed and ~0.5 g of well-mixed feces were boiled in 5 ml of aqueous ethanol (90%) for 20 min. After centrifuging at 500 x g for 10 min, supernatant was recovered and the pellet resuspended in 5 ml of 90% ethanol, vortexed for 1 min and re-centrifuged. Both ethanol supernatants were combined, dried completely, redissolved in 1 ml methanol and diluted in phosphate-buffered saline (0.01 M PO₄, 0.14 M NaCl, 0.5% BSA, 0.01% NaN₃) before analysis. Fecal estradiol and androgen metabolites were quantified by radioimmunoassay. Assays were validated for each species by demonstrating: 1) parallelism between binding inhibition curves of fecal extract dilutions and the appropriate standards; and 2) significant recovery (>90%) of exogenous estradiol (5-240 pg) or testosterone (5-625 pg) added to fecal extracts. Assay sensitivities, based on 90% of maximum binding, were 5 and 10 pg per tube for estradiol and testosterone, respectively. Intra- and interassay coefficients of variation were <10%. Data are expressed on a per g wet fecal weight basis.

Testicular volume and seminal characteristics were analyzed monthly in all males using standardized electroejaculation and semen evaluation protocols. Anesthesia was induced in margays and tigrinas using a combination of ketamine HCl (20 mg/kg, i.m.; Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, IA 50501) mixed with xylazine (1 mg/kg, i.m.; Rompun, Mobay Corp., Shawnee, KS 66205), and in ocelots using zolazepam/tiletamine (10 mg/kg, i.m.; Telazol, Fort Dodge Laboratories, Inc., Fort Dodge, IA 50501). Semen was collected using an AC, 60 Hz sine-wave electroejaculator and rectal probe containing 3 longitudinally-positioned electrodes. A regimented sequence of electrical stimuli was given in an on-off pattern in 3 series (Series 1 and 2 = 30 stimuli/series; Series 3 = 20 stimuli) over an ~10 min interval. Each series consisted of repeating sets of 10 stimulations applied at increasing voltages (2-6 volts). Total ejaculate volume and sperm progressive motility were evaluated after each ejaculation series. Sperm concentration was estimated using a hemocytometer.

**Data Analyses**

Significant increases in fecal estradiol concentrations were determined by an iterative process in which high values were excluded if they exceeded the mean plus 2 standard deviations. The highest concentration within a group of elevated samples was considered the peak. Baseline values were those remaining after all high values were excluded. For seasonal analyses, winter (June-August),
spring (September-November), summer (December-February) and autumn (March-May) were defined in successive 3-mo intervals. Differences among species and seasons in endocrine and seminal data were determined by a one-way analysis of variance (ANOVA) followed by Duncan’s New Multiple Range tests.

Results and Discussion

Concentrations of fecal androgen metabolites varied among species (p < 0.05), with overall yearly means being highest in the ocelot (592.2 ± 97.1 ng/g), intermediate in the tigrina (195.7 ± 25.7 ng/g) and lowest in the margay (112.8 ± 15.0 ng/g). Spermic ejaculates were collected throughout the year, although seasonal trends in seminal characteristics and fecal androgen profiles were exhibited in all three species (p < 0.05). In ocelots and margays, testicular and ejaculate volumes were greater (p < 0.05) during the spring and summer with parallel, though non-significant (p > 0.05), increases in total sperm per ejaculate, motile sperm per ejaculate and fecal androgen concentrations also observed. For tigrinas, fecal androgen concentrations increased from mid-winter through spring (p < 0.05), followed by increases in testicular and ejaculate volumes and motile sperm per ejaculate (p < 0.05). For all species combined, androgen metabolite concentrations were positively correlated with ejaculate volume (r = 0.38; p < 0.05), total sperm per ejaculate (r = 0.39; p < 0.05), and motile sperm per ejaculate (r = 0.36; non-significant, p = 0.08).

In females, baseline fecal estradiol concentrations were highest in the ocelot (131.6 ± 4.5 ng/g), intermediate in the margay (101.7 ± 3.7 ng/g) and lowest in the tigrina (59.0 ± 2.0 ng/g) (p < 0.05). Similarly, lower mean peak estradiol concentrations were observed in the tigrina (406.5 ± 43.8 ng/g; p < 0.05) compared with ocelots (731.7 ± 52.3 ng/g) and margays (646.6 ± 50.0 ng/g) which had similar concentrations (p > 0.05). Based on the interval between estradiol peaks, estrous cycle length did not differ among species (margay, 19.5 ± 2.1 days; ocelot, 16.5 ± 1.5 days; tigrina, 15.8 ± 1.5 days). Two ocelots did exhibit regular signs of behavioral estrus, primarily vocalizing and rubbing. For those two females, mean estrous cycle length was 13.9 ± 1.7 days on the basis of behavioral observations, which was comparable to that determined by fecal estradiol analysis (14.9 ± 0.8 days). Overall fecal estradiol concentrations fluctuated throughout the year, following the same patterns as those observed for fecal androgens within species. Female ocelots exhibited an increase (p < 0.05) in fecal estradiol during the spring and summer. A similar trend was observed in margays where fecal estradiol concentrations were lower during the fall (p = 0.06) and winter (p = 0.14). In the tigrina, the highest fecal estradiol concentrations occurred in the winter and spring (p < 0.05).

In conclusion, these data demonstrate the utility of fecal steroid analyses for non-invasively monitoring ovarian and testicular function in ocelots, margays and tigrinas. In each species, there was an influence of season on reproductive activity, although for some parameters the effect was modest. This information is important for defining normal reproductive processes which might prove valuable for aiding captive breeding strategies to improve species conservation. For species like the tigrina and margay that rarely reproduce in captivity, reliable use of assisted reproductive technologies based on information generated by non-invasive hormone monitoring could contribute to improved breeding success.
ACKNOWLEDGMENTS

The authors thank the staff at the Zoológico de Curitiba and the Itaipú Binacional Wildlife Breeding Center for their cooperation and for collecting samples. We extend thanks to Nuvital Nutrientes Ltd. for providing vitamin and mineral supplements for the cats. This research was funded, in part, by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Fundação da Universidade Federal do Paraná (FUNPAR), Conselho Nacional de Ciência e Tecnologia (CNPq), the New Opportunities in Animal Health Sciences (NOAHS) Center, the Philip Reed Foundation, and the Friends of the National Zoo (FONZ).

LITERATURE CITED

ISOLATION AND DETECTION OF ASIAN ELEPHANT IgG

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Abstract

Serological tests of elephant serum have been limited due to a lack of suitable detecting reagents. This has restricted the repertoire of assays for diagnosis as well as determination of species relatedness. To address this problem we isolated Asian elephant IgG, prepared antisera reactive with it, and coupled an IgG fraction of anti-elephant IgG to fluorescein-5-isothiocyanate (FITC) and to biotin. The cross-reactivity of anti-Asian elephant IgG with IgG of other species was determined. Conversely, the reactivity of antisera prepared against IgG of other species was examined to determine their usefulness as diagnostic reagents for Asian elephant studies.

To purify Asian elephant IgG, serum was applied to immobilized recombinant protein A/G. Bound IgG was eluted with low pH buffer. The IgG was further purified by column chromatography on diethylaminoethyl and cibacron blue dye equilibrated with 0.02 M Tris-HCl, pH 8.0 containing 0.028 M sodium chloride. The flow-through (containing IgG) was concentrated using an ultrafilter with a 100 kDa cut-off. The purity of the preparation was established by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a 7.5% resolving gel. Antisera reactive with Asian elephant IgG was produced in rabbits. Its specificity was confirmed with western blots prepared by SDS-PAGE of Asian elephant serum. The IgG fraction was isolated from rabbit antisera using immobilized recombinant protein A/G. The IgG fraction (anti-eIgG) was labeled with FITC using a Flurotag FITC conjugation kit (Sigma, St. Louis, MO). Anti-eIgG was also coupled to biotin (succinimidyl-6-[biotinamido] hexanoate) in carbonate-bicarbonate buffer, pH 8.0. The FITC conjugate has been useful for indirect fluorescence assays (IFA) of elephant IgG bound to antigens on microscope slides. To determine the reactivity of antibody prepared against elephant IgG with IgG of other species we developed a capture enzyme linked immunosorbent assay (ELISA). Plates were coated with anti-eIgG and then received sera or purified IgG from various species. Bound antibody was detected with biotin labeled anti-Asian elephant IgG followed by peroxidase labeled avidin and 2,2'-Azino-bis(3-ethylbenzthiazoline 6-sulfonic acid) dianionmum (ABTS) substrate. The reactivity of commercially available conjugated antisera with elephant IgG was determined by coating ELISA plates with purified IgG followed by peroxidase conjugated antisera and substrate.

The reactivity of sera diluted 1/320,000 was determined in the capture ELISA with anti-eIgG and biotin anti-eIgG (Fig. 1). Relative to Asian elephant sera the reactivity of African elephant was 28.2%, Sri Lankan elephant 116.3%, and horse 9.2%. In the IgG capture system (Fig. 2), when purified IgG was added to the plates, the reactivity to bovine IgG was 40.7%, feline IgG 5.2%, and
canine IgG 18.5%, relative to Asian elephant IgG. When purified IgG was coated on ELISA plates and detected with conjugated anti-IgG prepared against various IgGs (Fig. 3) the reactivity to elephant IgG of anti-bovine IgG was 65.3%, anti-deer IgG 26.9%, and anti-rabbit IgG 17.8% compared to the homologous Asian elephant IgG reaction. The reactivity of anti-bovine IgG with elephant IgG was only about one third as intense, however, as the homologous bovine reaction (anti-bovine IgG with bovine IgG).

We conclude that anti-Asian elephant conjugates will be useful for diagnosis in Asian elephant species and subspecies. The apparent weak cross-reactivity between anti-Asian elephant IgG and African IgG in serum is somewhat surprising. The development of African elephant reagents will permit us to more thoroughly examine the cross-reactivity of African and Asian elephant IgGs.

Resumen

Las pruebas serológicas en elefantes han sido limitadas debido a la escasez de reactivos para una detección adecuada. Esto ha restringido el repertorio de ensayos para el diagnóstico, así como la determinación de parentescos entre especies. Para encarar dicho problema, aislamos la IgG de elefante asiático, preparamos con el un reactivo antisuero y unimos una fracción de la IgG y de la IgG anti-elefante a fluoresceína-5-isoctiocianato (FITC) y a biotina. Se determinó la reacción cruzada de la IgG anti-elefante asiático con la IgG de otras especies. Recíprocamente, la reacción del antisuero preparado contra la IgG de otras especies se examinó para determinar su utilidad como reactivo diagnóstico en estudios de elefante asiático.

Para purificar la IgG de elefante asiático, se aplicó suero a una proteína recombinante inmovilizada A/G. La IgG ligada fue extraída con una solución tampón de pH bajo. La IgG fue purificada más adelante por una cromatografía en columna en dietilaminoetil y azul de cibacrom equilibrada con TRIS-HCl 0.02 M, pH 8.0 conteniendo cloruro de sodio 0.028 M. El resultante (contenido de IgG), fue concentrado utilizando un ultrafiltro con un corte de 100 kDa. La pureza de la preparación se estableció por electroforesis en gel de sulfato duodecil sódico poliacridamida (SDS-PAGE), usando un gel resolvente al 7.5%. La reacción del antisuero con la IgG de elefante asiático fue producida en conejos. Su especificidad fue confirmada con Western-blot preparados por SDS-PAGE de suero de elefante asiático. La fracción de IgG fue aislada de antisuero de conejo usando proteína recombinante inmovilizada A/G. La fracción IgG (anti-IgG) fue nivelada con FITC utilizando un equipo de conjugación Flurotag FITC (Sigma, St. Louis, MO). El anti-IgG fue también combinado con biotina (succinimidil-6-[biotinamil]hexanato) en solución tampón carbonada-biocarbonada pH 8.0. El conjugado de FITC ha sido útil para ensayos de fluorescencia indirecta (IFA) de IgG de elefante ligada a antígenos en portaobjetos. Para determinar la reactividad de preparados contra la IgG de elefante con IgG de otras especies desarrollamos un ELISA de captura. Las placas fueron cubiertas con anti-IgG y luego recibieron suero o IgG purificada de varias especies. El cuerpo ligado fue detectado con anti-IgG de elefante asiático marcado con biotina seguido por avidin marcado con peroxidasa y sustrato 2,2'-Azino-bis(3-ethylbenzotiazolona 6-ácido sulfonico) diamonio (ABTS). La reactividad del antisuero conjugado disponible comercialmente, con la IgG de elefante fue determinada cubriendo las placas de ELISA con IgG purificada seguida de antisuero conjugado con
peroxidasa y sustrato.

La reactividad del suero diluido 1/320,000 fue determinada en la prueba de ELISA contra anti-IgG y biotina anti-IgG (Fig. 1). En relación al suero de elefante asiático, la reactividad de el elefante africano fue de 28.2%, de el elefante de Sri Lanka 116.3%, de el caballo 9.2%. En el sistema de captura de IgG (Fig. 2), cuando la IgG fue adicionada, la reactividad de la IgG de bovino fue de 40.7%, de felino 5.2% y de canino 18.5%, cuando la IgG purificada fue cubierta en las placas de ELISA y detectada con un conjugado anti-IgG preparadas contra varios IgG’s (Fig.3), la reactividad hacia IgG de elefante del IgG anti-bovino fue de 65.3%, de la IgG anti-venado de 26.9% y la anti-conejo de 17.8% comparado contra el homólogo de reacción de la IgG de elefante asiático. La reactividad de la IgG anti-bovino con la IgG anti-elefante fue solo cerca de una tercera parte más intenso como el homólogo de reacción de bovino (IgG anti-bovino con IgG bovino).

Concluimos que los conjugados anti-elefante asiático pueden ser útiles para diagnóstico en especies y subespecies de elefante asiático. La reactividad cruzada, aparentemente débil entre la IgG anti-elefante asiático y la IgG africana en suero es algo sorpresivo. El desarrollo de reactivos de elefantes africanos nos puede permitir examinar más profundamente la reacción cruzada de IgG’s de elefantes asiáticos y africanos.
Figure 1. Capture ELISA with Sera

405 nm O.D.

Sera Diluted 1/320,000
Figure 2. Capture ELISA with Purified IgG Antigen

405 nm O.D.

Source of IgG

- Asian Elephant
- Bovine
- Cat
- Dog

IgG ng/ml

Source of IgG

- Asian Elephant
- Bovine
- Cat
- Dog

IgG ng/ml
Figure 3. Direct ELISA with Purified IgG Antigen

Source of IgG
- Elephant
- Cat
- Dog
- Bovine

405 nm O.D.
TOTAL RECONSTRUCTION OF CHAPULTEPEC ZOO IN MEXICO CITY: A VETERINARIAN'S POINT OF VIEW

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Abstract

Located in the middle of Mexico City, Chapultepec Zoo is known to the world because its giant pandas have successfully bred. This zoo was totally remodeled between June 1992 and August 1994. Together with architects, designers, administrators, engineers, biologists, etc., veterinarians were involved in most aspects of this project. New immobilization protocols and serological surveys were performed in most species; the construction of more effective facilities in order to improve preventative medicine was supervised by veterinarians. The ideas of the modern zoo, conservation and education, were considered during the entire process.

Resumen

El Zoologico de Chapultepec se encuentra en medio de la Ciudad de Mexico y es conocido en todo el mundo debido a la reproduccion exitosa de los pandas gigantes. Este zoologico fue remodelado totalmente entre junio de 1992 y agosto de 1994. Colaborando con arquitectos, diseñadores, administrativos, ingenieros, biologos, etc., los veterinarios estuvieron involucrados la mayor parte del proyecto. Nuevos protocolos de inmovilizacion y estudios serologicos se llevaron a cabo en muchas especies la construccion de instalaciones en las que se llevara a cabo una medicina preventiva mas efectiva fue supervizada por veterinarios. La idea de un zoologico moderno, de la conservacion y de la educacion fue considerada durante todo este proceso.
VIDEO-ASSISTED THORACOSCOPIC LUNG BIOPSY IN AN ORANGUTAN (*Pongo pygmaeus*)

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Abstract

A 19-yr-old, male orangutan (*Pongo pygmaeus*) was successfully treated for chronic air sacculitis by permanent marsupialization of the sac and appropriate antimicrobial therapy. Thoracic radiographs taken during the course of his treatment revealed chronic pneumonia. Very resistant strains of *Pseudomonas aeruginosa* were consistently isolated from bronchial aspirates and despite the use of bromhexine hydrochloride (Bisolvon tablets, Boehringer Ingelheim, 50 Broughton Road, Artarmon, 2064, Australia) (0.2 mg/kg p.o. b.i.d.) and salbutamol (Ventolin sugar-free syrup, Glaxo Australia, 1061 Mountain Highway, Boronia, 3155, Australia) (0.1 mg/kg p.o. b.i.d.), and appropriate antibiotics, the lung changes persisted. Clinically, intermittent coughing and a nasal discharge also persisted.

In order to further define the lung pathology with a view to instituting more appropriate therapy, a decision was made to perform a lung biopsy. A technique using video-assisted thoracoscopy and surgical stapling equipment was chosen to avoid the risks and post surgical complications usually associated with open thoracotomy techniques.

Anesthesia was induced using tiletamine and zolazepam (Zoletil 100, Virbac Australia, 15 Pritchard Place, Peakhurst, 2210, Australia) (8 mg/kg i.m. via a blowdart) and maintained with isoflurane (Forthane, Abbott Australia, Captain Cook Drive, Kurnell, 2231, Australia) and oxygen administered via a 41 Fr double-lumen, cuffed endotracheal tube. Neuromuscular blockade was achieved using vecuronium bromide (Norcuron, Organon Teknika, Akzo Pharma, 5 Hudson Avenue, Castle Hill, 2154, Australia) (0.07 mg/kg i.v.). A ventilator was used for intermittent positive pressure ventilation.

The animal was placed in left lateral recumbency. After deflation of the right lung, the thoracic cavity was entered through a small incision in the eighth intercostal space on the anterior thoracic wall. The lung was inspected using a 10 mm rigid endoscope attached to a camera and television monitor. The lung had completely collapsed and the surface appeared normal. The caudal lobe was chosen as the biopsy site. A 20 mm flexible thoracic trocar (Flexipath Trocar, Ethicon Endo-Surgery, 1-5 Khartoum Road, North Ryde, 2113, Australia) was inserted into the thorax through a stab incision in the posterior thoracic wall in the same intercostal space. Through this port a 45 mm Thoracic Endo Linear Cutter (Endopath EZ45, Ethicon Endo-Surgery) was introduced and the biopsy performed, by visualization of the area on the television monitor. A triangular piece of lung tissue was removed. After inspection for leaks and hemorrhage the lung was gently inflated and free...
air aspirated through an intercostal catheter during closure, which was removed once the wound was completely closed.

Neuromuscular blockage was reversed using neostigmine methylsulfate (Prostigmin Injection, Roche Products, 4-10 Inman Road, Dee Why, 2099, Australia) (0.05 mg/kg i.v.) and atropine sulfate (Atrosine Mitis, Parnell Laboratories, 6/476 Gardeners Road, Alexandria, 2015, Australia) (0.02 mg/kg i.v.), and post-operative pain relief achieved with morphine sulfate (Morphine Sulfate Injection, Astra Pharmaceuticals, 66-78 Talavera Road, North Ryde, 2113, Australia) (0.2 mg/kg i.m.) and recovery was uneventful. The biopsy revealed bronchiectasis and emphysema with fibrin and mucous plugging of the alveoli. *P. aeruginosa* was cultured, sensitive to ciprofloxacin.

A 6 wk course of ciprofloxacin hydrochloride (Ciproxin 500, Bayer Australia, 875-893 Pacific Highway, Pymble, 2073, Australia) (6 mg/kg p.o. b.i.d.), bromhexine hydrochloride (Bisolvon tablets, Boehringer Ingelheim) and salbutamol (Ventolin sugar-free syrup, Glaxo Australia) resulted in a significant clinical improvement. Thoracic radiographs taken 2 mo post treatment were normal.

**Resumen**

Un macho de orangután de 19 años, fue tratado satisfactoriamente de una aerosaculitis crónica por medio de una “marsupialización” permanente del saco y una adecuada terapia antimicrobiana. Radiografías torácicas tomadas durante el curso del tratamiento revelaron un neumonía crónica. Varias cepas resistentes de *Pseudomonas aeruginosa* se aislaron consistentemente de las aspiraciones bronquiales y a pesar del uso de clorhidrato de bromhexina (Bisolvon Tablets, Boehringer Ingelheim, 50 Broughton Road Artarmon 2064, Australia, 1061 Mountain Highway, Boronia, 3155, Australia) (0.2 mg/kg oral dos veces al día.) y salbutamol (Ventolin, Glaxo Australia, 1061 Mountain Highway, Boronia, 3155, Australia (0.1 mg/kg oral dos veces al día) y antibióticos apropiados los cambios en el pulmón persistían. Así como tos intermitente y descargas nasales.

A fin de definir la patología pulmonar con mayor precisión y establecer una terapia más adecuada se decidió tomar una biopsia pulmonar. Se eligió un equipo con cámara de video y equipo quirúrgico de grapas para evitar los riesgos y complicaciones postquirúrgicas usualmente asociadas con las técnicas abiertas de toracotomía.

La anestesia fue Inducida usando Tiletemina y Zolazepam (Zoletil 100, Virbac Australia, 15 Pritchard Place, Peak hurst 2210 Australia) (8 mg/kg intramuscular con cerbatana) y mantenido con isofluorano (Forthane, Abbott Australia, Captain Cook Drive, Kurnell, 2231, Australia) y oxígeno administrado a través de una sonda francesa de dos vías # 41. El bloqueo neuromuscular fue logrado usando bromuro de vecuronium (Norcuron, Organon Teknika, Akzo Pharma, 5 Hudson Avenue, Castle Hill, 2154, Australia) (0.07 mg/kg i.v.). Se utilizó un ventilador para crear un presión intermitente positiva.

El animal fue puesto en recumbencia lateral izquierda. Después de la deflación del pulmón derecho,
la cavidad torácica fue sometida a una incisión pequeña en el 8° espacio intercostal en la pared torácica anterior. El pulmón fue inspeccionado usando un endoscopio rígido de 10 mm unido a una cámara de monitor de televisión. El pulmón estaba completamente colapsado y la superficie parecía normal. El lóbulo caudal fue elegido para ser el sitio de la biopsia. Un trócar torácico flexible de 20 mm (Flexipath Trocar, Ethicon Endo-Surgery, 1-5 Khartoum Road, North Ryde, 2113, Australia) fue introducido en el tórax a través de la incisión en la pared torácica posterior en el mismo espacio intercostal. A través de este punto un cutter torácico endolineal de 45 mm (Endopath EZ45, Ethicon Endo-Surgery) fue introducido y la biopsia fue realizada, por visualización del área en el monitor de TV. Se extrajo un pedazo triangular de tejido pulmonar. Después de la inspección para corroborar que no existieran hemorragias y perforaciones, el pulmón fue inflado suavemente y el aire libre aspirado a través de un cateter intercostal durante la sutura; una vez retirado el cateter se cerró por completo el orificio.

El bloqueo neuromuscular fue revertido usando metilsulfato de neostigmina (Prostigmin Injection, Roche. Products 4-10 Inman Road, Dee Why, 2099, Australia) (0.05 mg/kg i.v.) y sulfato de atropina (Atrosine Mitis, Parnell Laboratories, 6/476 Gardeners Road, Alexandria, 2015, Australia) (0.02 mg/kg i.v.), para aliviar el dolor post operatorio se aplicó sulfato de morfina (Morphine Sulfate Injection Astrapharmaceuticals 66-78, Talavera Road North Ryde, 2113, Australia) (0.2 mg/kg i.m.). La recuperación fue satisfactoria. La biopsia reveló bronquiectasia y enfisema con fibrina y moco adheridos al alvéolo. El cultivo fue positivo a *P. aeruginosa* sensible a ciprofloxacina.

Un tratamiento de 6 semanas de hidrocloruro de ciprofloxacina (Ciproxin 500 Bayer, Australia 875-893 Pacific Highway Pymble 2073 Australia) (6 mg/kg oral 2 veces al día), Hidrocloruro de Bromhexina (Bisolvon tablets, Boehringer Ingelheim) y salbutamol (Ventolin jarabe sin azcar, Glaxo Australia) fue muy satisfactorio. Se tomaron placas radiográficas 2 meses después del tratamiento las cuales se apreciaron como normales.

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LITERATURE CITED

IMMOBILIZATION OF A PYGMY HIPPOPOTAMUS (*Choeropsis liberiensis*) FOR THE REMOVAL OF AN ORAL MASS

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**Abstract**

A 25-yr-old male pygmy hippopotamus (*Choeropsis liberiensis*) was placed under general anesthesia for the removal of a 4.5 cm by 7 cm oral mass, and a tusk trim in February of 1996 at the Franklin Park Zoo, Boston, MA 02121, USA.

The anesthetic protocol decided upon was induction via oral and injectable medications, followed by endotracheal intubation and maintenance of anesthesia with isoflurane (IsoFlo, SOLVAY Animal Health, Inc., Mendota Heights, MN 55120, USA) in oxygen. The preanesthetic consisted of an oral dose of injectable detomidine hydrochloride (Dormosedan, Pfizer Animal Health, Pfizer Inc., West Chester, PA 19380) at a dosage of 44 µg/kg mixed with oral diazepam (Valium, Zenith Laboratories, Inc., Northvale, NJ 07647 USA) at a dosage of 0.514 mg/kg. Anesthesia was induced with a combination of ketamine hydrochloride (Ketaset, Fort Dodge Laboratories Inc., Fort Dodge, Iowa 50501 USA) at a dosage of 1.25 mg/kg mixed with hyaluronidase 30 USP (Wydase, Wyeth Laboratories Inc., A Wyeth - Ayerst Company, Philadelphia, PA 19101 USA) and butorphanol tartrate (Torbutrol, Fort Dodge Laboratories, INC., Fort Dodge, Iowa 50501 USA) at a dosage of 18.4 µg/kg given i.m. Supplemental dosages of ketamine (0.73 mg/kg), detomidine (18.36 µg/kg, and butorphanol (18.4 µg/kg) were given i.m. at 10-30 min intervals until a 10 french endotracheal tube could be inserted into the trachea and the animal was then maintained on isoflurane in oxygen throughout the rest of the procedure. The mass was removed and the hole in the gingiva was sutured with absorbable suture (0- Dexon, Davis & Heck, Inc., Manati, P.R. 00701 USA). The maloccluded tusk was trimmed with an electric saw.

The hippo was placed on oral antibiotics (Sulfamethoxazole and Trimethoprim 800 mg/160 mg, Carlisle - EON Laboratories Manufacturing Inc., Laurelton, NY 11413 USA) at a dosage of 20 mg/kg twice daily and non-steroidal anti-inflammatories (Phenylbutazone, Vetus Animal Health, Burns Veterinary Supply, Rockville Centre, NY 11570 USA) at a dosage of 1.5 mg/kg twice daily. Histopathology on the mass revealed a well-differentiated, proliferative, benign osteoma. Approximately 2 mo postoperatively, the mass began to regrow.
Resumen

Un macho de hipopótamo pigmeo (*Choeropsis liberiensis*) de 25 años de edad, fue puesto bajo anestesia general para extraerle una masa oral de 4.5 cm x 1 cm., así como rebajarle un colmillo, en febrero de 1996 en el Parque Zoológico Franklin en Boston.

El protocolo de anestesia elegido fue una inducción vía oral, además de medicamentos inyectables, seguidos de intubación endotraqueal y mantenimiento de la anestesia con isofluorano (IsoFlo, SOLVAY, Animal Health, Inc., Mendota Heights, MN 55120, USA) en oxígeno. La pre-anestesia consistió en hidrocloruro de detomidina inyectable (Dormosedan Pfizer Animal Health, Pfizer Inc. West Chester, PA 19380) administrado vía oral a dosis de 44 µg/kg mezclado con diazepam oral (Valium, Zenith Laboratories, Inc., Northvale, NJ 07647 USA) a dosis de 0.514 mg/kg. La anestesia fue inducida con una combinación de hidrocloruro de ketamina (Ketaset, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501, USA) a una dosis de 1.25 mg/kg mezclado con hialuronidasa 30 USP (Wydase, Wyeth Laboratories Inc., A Wyeth - Ayerst Company, Philadelphia, PA 19101 USA) y tartrato de butorfenol (Torbutrol, Fort Dodge Laboratories, Inc., Fort Dodge Laboratories Inc., Fort Dodge Iowa 50501 USA) a una dosis de 18.4 g/kg vía intramuscular. Dosis suplementarias de ketamina (0.73 mg/kg), detomidina (18.36 µg/kg) y butorfenol (18.4 µg/kg) fueron utilizadas vía intramuscular en intervalos de 10 a 30 minutos hasta que una sonda francesa endotraqueal # 10 pudo ser insertada en la tráquea y el animal pudo ser mantenido con isofluorano y oxígeno hasta el final del procedimiento. La masa fue retirada y la cavidad en la gingiva fue suturada con material absorbible (O-Dexon, Davis & Geck, Inc., Manatí, P.R. 00701 USA). El colmillo destapado fue cortado con una sierra eléctrica.

El hipopótamo fue mantenido con antibióticos orales (Sulfamethoxazole y Trimethoprim 800 mg/160 mg, Carlisle- EON Laboratories Manufacturing Inc., Laurelton, NY 11413 , USA) a una dosis de 20 mg/kg dos veces al día y un antinflamatorio no esteroide (Phenylbutazone, Vetus Animal Health, Burns Veterinary Supply, Rockville Centre, Ny 11570 USA) a una dosis de 1.5 mg/kg dos veces al día. El histopatológico de la masa reveló un osteoma benigno proliferativo bien diferenciado. Aproximadamente después de 2 meses la masa empezó a crecer nuevamente.

Introduction

Pygmy hippopotami (*Choeropsis liberiensis*) have historically been hard animals to safely and effectively anesthetize. Their body fat ratio, thick skin, body size and shape, and lack of peripheral vasculature makes it very difficult to induce, maintain and monitor anesthesia. There are few published reports of anesthesia in pygmy hippopotami. The most frequently reported reason for anesthesia in pygmy hippopotami in zoological collections is oral work, mainly tusk trimming.1

In the spring of 1995, a rapidly enlarging oral mass was noted on the left gingival surface of the mandible in a 25-yr-old pygmy hippopotamus at the Franklin Park Zoo, Boston, Massachusetts USA. Because the mass was rapidly enlarging and appeared to interfere with mastication, the decision was made to immobilize the hippopotamus to remove the mass and perform a tusk trim on his maloccluded tusks. Several anesthetic protocols that had been used at other institutions to immobilize pygmy hippopotami were reviewed, and a protocol using several types of inducing
agents, and isoflurane inhalation as the maintenance drug was decided upon. A temporary squeeze chute was built in order to facilitate hand injections of the animal. A team of veterinarians, a dentist, an oral surgeon, and an oncologist were assembled to assist in the procedure.

**Case Report**

“Clarence,” a 25-yr-old, male, pygmy hippopotamus, weighing approximately 600 lbs, had been housed at the Commonwealth Zoological Corporation for 23 yr. He was housed with a female pygmy hippopotamus and had no obvious signs of disease prior to the growth of the oral mass, which was noticed in the winter of 1995. The mass had been rapidly increasing in size since the spring of 1995 and had become ulcerated, developed superficial vascularization, and had started to interfere with mastication. There was also a tusk malocclusion that had progressively gotten worse over the last few years.

The hippopotamus was taken off all food 5 days preoperatively and kept out of the pool for 2 days preoperatively. His skin was kept moist during that time by applying water based skin softeners delivered by a spray bottle. A temporary squeeze chute was built by forming a narrow triangle out of a swing gate, a wall and a rear guillotine door in the animals off exhibit holding area. The hippo was walked through this passageway several times before the day of the procedure in order to familiarize the animal with it.

Preanesthetic tranquilizers consisted of a combination of detomidine (44 µg/kg) and diazepam (0.5 mg/kg) given orally. The diazepam was in tablet form that was crushed up and mixed with injectable detomidine and water in an oral dosing syringe. This solution was then squirted into the animals mouth in his holding stall. The animal appeared slightly off balance approximately 30 min later and he was herded into the squeeze chute at that time.

A combination of drugs was used to induce anesthesia and provide enough muscle relaxation to allow endotracheal intubation. Atropine sulfate (Atroject - Vetus Animal Health, Burns Veterinary Supply, Rockville Centre, NY 11570 USA) at a dosage of 20 µg/kg and ketamine (0.2 mg/kg) were given by hand injection intramuscularly. The intramuscular drugs were delivered via a 2” 10-ga metal trocar needle with a .72 mm x 12.70 cm 22G5 spinal needle (Becton Dickinson and Company, Franklin Lakes, NJ 07417 USA) inserted through the bevel of the trocar. The trocar needle was the only needle that could penetrate the skin without bending and breaking. The spinal needle was used to deliver the drug into the deeper layers of muscle while avoiding deposition into the subcutaneous fat. The injections were made in the neck and shoulder region.

There was only mild sedation produced by the first set of injections and a combination of butorphanol (18 µg/kg), ketamine (0.7 mg/kg) and hyaluronidase (30 USP) were then given. At this point, 91 min had elapsed from the time the first oral dose of tranquilizer was given and 60 min had elapsed from the time of the first intramuscular injection of ketamine. The animal still appeared only slightly tranquilized and 36 µg/kg of detomidine was given intramuscularly.

Another 30 min elapsed without any further sedation being observed. At this time, the animal was unsteady on his feet but refused to stay recumbent and was aggressive when approached. Because of the fear of overheating in the squeeze chute, he had been allowed to go back into a small holding stall that opened into the chute.
One hundred and forty one minutes had elapsed since the initial oral dose of detomidine and diazepam and 120 min had elapsed since the first injectable drugs were given, and one last attempt was made in order to facilitate intubation. Detomidine (18 µg/kg) and butorphanol (18 µg/kg) were combined with hyaluronidase (30 USP) and given intramuscularly. Seven minutes later, another two doses of ketamine (0.4 mg/kg) were given intramuscularly and the animal was now tractable enough to approach for an intravenous injection of ketamine (0.4 mg/kg) given in the lateral tail vein.

Following the intravenous ketamine dose, the animal went into sternal recumbency and nasal insufflation was begun using a long, nasogastric tube connected to a large animal anesthesia machine. Isoflurane in oxygen was delivered at a concentration of 5% at 10 L/min while endotracheal intubation was performed.

Intubation was difficult due to the narrowness of the caudal pharynx and the inability to visualize the epiglottis. A large, wooden mouth gag, with a hole drilled into the center of it, was placed behind the upper and lower tusks to span the width of the oral opening. The intubator’s arm was introduced into the oral cavity through the hole in the mouth gag, and the tip of the epiglottis was palpated. The endotracheal tube was passed after some difficulty due to the long distance between the mouth and the larynx.

A large animal anesthetic machine, with an isoflurane vaporizer, a circle breathing system, and a 30-L rebreathing bag was used to deliver 2-3% isoflurane in 100% oxygen at 10 L/min. A pulse oximeter probe (Nellcor Inc., Hayward, CA USA) was attached to the tongue and arterial oxyhemoglobin saturation (SaO2) was maintained between 95-99% throughout the procedure.

Venipuncture was unsuccessfully attempted for blood samples. The lateral tail veins could be punctured but collapsed when blood was withdrawn. There were two small lingual vessels on the lateral side of the ventral surface of the tongue that were used as injection sites, but blood could not be withdrawn from them. Attempts at venipuncture of the auricular vessels were also unsuccessful.

An attempt was made to remove the mass with a scalpel blade but the mass was very hard and could only be removed with bone chisels and an electrical saw. The mass was removed in two pieces and the maloccluded tusk was trimmed to approximately 1 inch above the gingival margin. Histopathology done on the mass revealed a benign osteoma.

The surgical procedure took 37 min. The tumor was removed and the site was sutured with 0-dexon after being flushed with sterile physiological saline. During this time the animal’s SaO2 remained stable above 95%. The animal was intermittently ventilated using positive pressure ventilation to assist in respiratory efforts. He remained in sternal recumbency throughout the procedure. Subcutaneous fluid administration was attempted but because of the thickness of the skin, was unsuccessful. The animals’ body temperature ranged from 33.6°C (92.5°F) to 35.3°C (95.5°F). Respiratory rate and heart rate were difficult to monitor due to movement around the animal but averaged about 60 beats per min for heart rate and 4 breaths per min.

Isoflurane administration was discontinued and 100% oxygen was administered via the endotracheal tube for approximately 2 min. The animal was given doxapram hydrochloride (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501 USA) at a dosage of 1 mg/kg and yohimbine (Yobine, Lloyd Laboratories, Shenandoah, Iowa 51601 USA) at a dosage of 0.11 mg/kg i.m. and was standing.
2 min after the injections were given. Post-operative antibiotics consisted of ceftiofur sodium (Naxcel, Upjohn Company, Kalamazoo, MI 49001) at a dosage of 1 mg/kg i.m. during recovery and sulfamethoxazole and trimethoprim tablets at a dosage of 20 mg/kg p.o. b.i.d. for 7 days. Post-operative non-steroidal anti-inflammatories consisted of phenylbutazone at a dosage of 1.5 mg/kg p.o. b.i.d. for 3 days.

Discussion

There are limited reviews of hippopotamus anesthesia in the medical literature. These animals are notoriously hard to safely and effectively anesthetize. Not only are they aggressive by nature, but their body size, shape, lack of peripheral vasculature and thick subcutaneous fat layer makes delivery and monitoring of anesthesia very difficult.

The use of oral tranquilizers at the start of the procedure did seem to have a calming affect on the animal and facilitated moving him to the squeeze chute.

It is hard to estimate the proper timing between injections of anesthetic inducing agents. In this case, the anesthetic induction period was excessively prolonged (157 min) but the animal did remain stable throughout the procedure. Because of the deposition of thick subcutaneous fat, anesthetic induction can be tricky. Not only is it hard to assure deliverance of the drug into the muscle layers, but absorption of the drug by the fat can cause a seemingly lightly anesthetized animal to then become deeply anesthetized for prolonged periods of time. Caution should be used when re-dosing the animal due to this phenomenon. However, too long a time period between injections, combined with low drug dosages, probably caused the prolonged induction time in this case.

Because of the location and size of the tumor, a prolonged plane of surgical anesthesia was needed and endotracheal intubation and inhalant anesthesia was ideal. It was extremely difficult to intubate the hippo due to the elongated palate and the small tracheal opening. A fibroscopic endoscope was initially used to try to aid in visualization of the epiglottis and laryngeal opening but was unsuccessful. Blind intubation was the method used. Once the endotracheal tube was in place, the animal was maintained at a surgical plane of anesthesia without complications. Blood gas monitoring would have been ideal, but because of the lack of peripheral vasculature, blood samples could not be attained.

A total of 1050 mg of ketamine, 10 mg of butorphanol and 20 mg of detomidine were used on this animal. Some of the drugs were combined with hyaluronidase to increase their distribution. The total time of the procedure, from oral tranquilization to recovery, was 216 min. The hippo was in sternal recumbency for 64 min without any noticeable adverse affects.

Even though the mass was diagnosed as a benign osteoma with a good prognosis, the tumor margins could not be completely excised and the mass regrew to almost its’ former size within 3 mo of the surgery.

LITERATURE CITED

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ASSESSMENT OF A COMMERCIALLY AVAILABLE RADIOIMMUNOASSAY FOR THE DETECTION OF FECAL CORTISOL METABOLITES IN SEVERAL NON-DOMESTIC FELID SPECIES

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Abstract

The objective of this study was to assess the ability of a commercially available radioimmunoassay kit to detect cortisol metabolites in the feces of several non-domestic cat species. To determine if the cortisol metabolites measured in the feces reflected circulating concentrations of cortisol, a clouded leopard was anesthetized and injected i.m. with 250 IU of synthetic ACTH once every hour for 3 hr. Blood samples were collected before ACTH administration and at 0.5 hr and 1 hr following injection. Fecal samples were collected daily for 1 wk before and after the procedure. Fecal samples were analyzed for cortisol metabolite concentrations using a double antibody 125I corticosterone kit from ICN Biomedicals (Costa Mesa, CA), and serum samples were analyzed for cortisol with a solid phase 125I cortisol kit. A significant difference was observed between baseline fecal cortisol metabolite concentrations (141.41±16.77 µg/g dry feces, mean±SEM) and the peak value (8698.96 µg/g), observed approximately 24 hr after the final injection. Serum cortisol concentration also increased from 567.75 ng/ml pre-ACTH to 725.25 ng/ml post-ACTH (n.s.). To determine if fecal corticoid monitoring could be used to detect changes in cortisol secretion after a presumed “stress,” fecal samples from clouded leopards (n=6) and tigers (n=3) that underwent artificial insemination (AI) were analyzed. Fecal cortisol metabolite concentrations were significantly elevated over baseline for 2 days following the procedure in six of nine cats. Finally, to determine if species differences exist in baseline fecal cortisol metabolite concentration, longitudinal samples (12 to 30 samples/animal) from several different species were analyzed. The mean basal values for cheetahs (n=5 individuals), clouded leopards (n=10), snow leopards (n=3), ocelots (n=3), tigers (n=5), and pallas cats (n=3) were 77.39±12.19 µg/g, 466.44±42.79 µg/g, 302.37±34.41 µg/g, 1920.71±275.06 µg/g, 617.98±131.08 µg/g and 185.19±19.60 µg/g dry feces, respectively. Ocelots had a significantly higher mean baseline metabolite concentration compared to all other species. These data indicate that a commercially available radioimmunoassay can be used for non-invasive monitoring of adrenocortical activity in several non-domestic felids.

Resumen

El objetivo de este estudio fue evaluar la eficacia de un kit de radioinmunoensayo comercialmente disponible, para detectar metabolitos de cortisol en las heces de varios felinos no domésticos. Para determinar si los metabolitos de cortisol medidos en las heces reflejaban concentraciones circulantes de cortisol, un leopardo fue anestesiado e inyectado i.m con 250 u.i. de ACTH sintética cada hora durante 3 horas. Muestras de sangre fueron colectadas antes de la administración de ACTH a la media hora y 1 hr. posteriores a la inyección. Se colectaron muestras de heces diariamente desde una semana antes hasta una semana después del tratamiento. Las muestras de heces fueron...
analizadas para checar concentraciones de metabolitos de cortisol, usando un kit de anticuerpos dobles $^{125}$I de corsticosterona de ICN Biomedicals (Costa Mesa, CA) y se determinaron niveles de cortisol en muestras de suero con un kit de fase sólida de cortisol 1251. Se observó una diferencia significativa entre los metabolitos basales de cortisol en heces (141.41±16.77 g/g de heces secas, media±SEM) y el pico de evaluación (8698.96 µg/g) fue observado 24 hrs aproximadamente después de la última inyección. La concentración de cortisol en el suero también se incrementó de 567.75 ng/ml pre-ACTH a 725.25 ng/ml después ACTH (n.s). Para determinar si el monitoreo de corticoides fecales puede ser útil en la detección de variantes en la secreción de cortisol después de un estres, se analizaron heces de Leopardos (n=6) y tigres (n=3) que fueron inseminados artificialmente (A.I). Las concentraciones de metabolitos de cortisol en las heces fueron significativamente más elevadas sobre las concentraciones basales durante 2 días después del procedimiento en 6 de los 9 felinos. Por último, se determinó si existían diferencias entre especies en las concentraciones de metabolitos basales de cortisol en heces. Para ello se analizaron muestras longitudinales de heces (12 a 30 muestras/animal) en diferentes especies. Los valores de cheetahs (n=5 individuos), leopardos (n=10) leopardos de las nieves (n=3), ocelotes (n=23), tigres (n=25) y gatos de pallas (n=3) fueron: 77.39 ±12.19 µg/g, 466.44±42.79 µg/g, 302.37±63.41 µg/g, 1920.71±275.06 µg/g, 617.98±131.08 µg/g y 185.19±19.60 µg/g de heces secas respectivamente. Las heces de ocelotes fueron significativamente más altas en metabolitos basales comparados con otras especies. Esto indica que el Kit Comercial disponible para radioinmunoensayo puede ser usado como método de análisis no invasivo de actividad adrenocortical en varias especies de felinos no domésticos.
DEVELOPMENT OF AN INDIRECT IMMUNOFLUORESCENT TEST FOR THE DETECTION OF MALARIA ANTIBODIES IN PENGUINS (SPHENISCIFORMES)

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Abstract

Malaria in penguins is a major problem in captive outdoor populations, causing high mortality specially in juveniles lacking maternal antibodies with no previous exposure to the parasite. Penguins have evolved in mosquito free areas, and infrequently have had contact with Plasmodium protozoan. The lack of an accurate diagnosis method is a limiting factor for the epidemiology and effective control of this disease. Antemortem diagnosis based on clinical signs, hematology and isodiagnostic methods, is often unrewarding.

Age, weight, complete hematology and antibody titers to malaria antigen were assessed in 53 birds in 3 zoos. An Indirect Fluorescent Antibody Test (IFAT) using Plasmodium falciparum as the antigen was developed to detect malaria antibodies induced by Plasmodium spp. Stained blood smears were used for the detection of schizonts. This test was validated with a sample group consisting of 53 individuals (16 Spheniscus demersus from London Zoo, 24 Pygoscelis papua from Edinburgh Zoo, three Eudyptes crestatus and ten S. humboldti from Whipsnade Animal Park in England).

The birds had different age distributions. Results were analyzed with Kruskal-Wallis one-way ANOVA and Mann-Whitney U-tests. The penguins showed age dependent variations in antibody titers. IFAT was higher in adult penguins (p<0.05) followed by juveniles and later by chicks. The other variables (leukocyte counts, weight, location and species) were not statistically different among the three age categories or for any one age category. London and Whipsnade Zoo penguins had higher antibody titers against malaria, probably due to higher exposure to the vector. This test allows us to identify part of the population of penguins at higher risk. It permits to accurately target prophylactic treatment, and identifies sites which need vector control.

Resumen

Tradicionalmente los pingüinos han evolucionado en áreas libres de mosquitos, aquellos mantenidos en cautiverio en exhibiciones al aire libre presentan esta enfermedad cuando son expuestos al vector. Aves juveniles en su primer año de vida son especialmente susceptibles. Anteriormente los diagnósticos antemortem se basaban en signos clínicos, valores hemáticos y métodos isodiagnósticos, siendo estos poco efectivos. Un factor limitante dentro del estudio de esta enfermedad ha sido la falta de un método diagnóstico específico.

La edad, el peso, valores hemáticos y niveles de títulos de anticuerpos contra la malaria fueron

Las aves presentaron diferentes edades. Los resultados fueron analizados a través de una prueba de Kruskal-Wallis, análisis de varianza de una sola vía y la prueba U de Mann-Whitney. Los títulos de anticuerpos presentaron variaciones significativas dependiendo de la edad. DII obtuvo valores más altos en animales adultos que en juveniles y que en pichones (p<0.05). Las otras variables (contaje de leucocitos, peso, localidad y especie) no presentaron diferencias significativas entre las tres diferentes categorías de edad o dentro de la misma categoría. Los pingüinos de los zoológicos de Londres y Whipsnade presentaron títulos de anticuerpos más altos contra la malaria, probablemente estas poblaciones están más expuestas al vector que la población de Edimburgo. Esta prueba diagnóstica nos permite identificar la parte de la población que se encuentra en mayor riesgo, nos permite determinar a que parte de la población se le debe de aplicar tratamiento profiláctico, e identifica las localidades en las que sería conveniente implementar control de vectores.
REDUCING DENTAL CALCULUS FORMATION IN LEMURS

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Abstract

Many species of exotic animals inherently accumulate dental calculus, commonly called tartar. Since calculus deposits interfere with normal plaque removal during mastication and are known to facilitate the development of periodontal disease, practical measures for the prevention of calculus formation are needed. Prior research in our laboratories has shown that the use of crystal growth inhibitors and sequestrants significantly decreases the rate of calculus formation in animals. To assess the impact of such a measure in exotic animals we initiated a longitudinal, two-way crossover study in a colony of ring-tailed and collared lemurs. At initiation, the animals were given a thorough dental prophylaxis and provided either the experimental or placebo dietary regimen. After approximately 6 months the lemurs (N=14) were examined for dental calculus, given another prophylaxis and provided the alternative regimen for a similar test period followed by another clinical calculus examination. Calculus evaluations were performed independently by two examiners. The experimental regimen was dry chow coated with 0.6% sodium hexametaphosphate; the control regimen was a similar dry chow. Both diets had similar levels of calcium and phosphorus as well as all other nutrients. Mean calculus scores for the control and experimental regimens were 2.65 and 1.01 for Examiner #1 and 2.64 and 1.02 for Examiner #2. These values represent statistically significant (p<0.001) reductions in calculus formation of 62% and 61% for the two examiners, respectively. No clinically significant changes were observed in body weights or in blood chemistry values. From these data it is apparent that coating dry chow diets with 0.6% sodium hexametaphosphate results in significantly less calculus formation in lemurs.

Resumen

Muchas especies de animales exóticos acumulan calcio dental, comúnmente llamado sarro, de manera inherente. En vista de que los depósitos interfieren con la remoción de placa dental durante la masticación, y que se sabe que facilitan el desarrollo de enfermedad periodontal, se necesitan medidas practicas para la prevención de la formación de sarro. Investigaciones previas en nuestros laboratorios han demostrado que el uso de inhibidores de crecimiento de cristales y agentes sequestrantes disminuyen de manera significante la tasa de formación de calcio dental en animales. Para evaluar el impacto de tal medida en animales exóticos, iniciamos un estudio longitudinal doble cruzado en una colonia de lemures de cola anillada y de collar. Los animales recibieron un tratamiento de profilaxis dental y se les sometió ya sea al tratamiento experimental o recibieron un
regimen de placebo dietético. Después de aproximadamente seis meses, los lemures (N=14) fueron
examinados para buscar evidencia de calcio dental, se les sometió a una segunda profilaxis, y se
les dio el régimen alternativo durante un periodo similar, seguido por un otro examen oral para buscar
evidencia de calcio dental. Las evaluaciones de calcio se llevaron a cabo de manera independiente
por dos examinadores diferentes. El régimen experimental consistió en alimento comercial seco
cubierto con 0.6% de hexametofosfato de sodio. El régimen de control fue un alimento comercial
similar. Ambas dietas tenían niveles similares de calcio y fósforo, así como de otros nutrientes. Las
medias de la clasificación de calcio de los regímenes de control y experimental fueron de 2.65 y
1.01 para el Examinador #1 y de 2.64 and 1.02 para el Examinador #2. Estos valores representan
reducciones estadísticamente significativas (p<0.001) en la formación de calcio de 62% and 61%
para ambos examinadores respectivamente. No hubieron cambios clínicamente significativos en los
pesos corporeos ni en los valores de química sanguínea. A partir de estos datos, resulta aparente que
el recubrir la dieta con 0.6% de hexametofosfato de sodio resulta en la formación significativamente
reducida en lemures.

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