PHYSICAL EXAMINATION OF REPTILES AND AMPHIBIANS

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Waiting Room Recommendations

All reptiles must be presented in appropriate escape proof containers. Snakes should be confined in snake bags or pillow cases that are secured with a knot. Large pythons may be transported in ice coolers that have a locking hinge; the drainage vent must be open to permit ventilation. During the colder months clients should be instructed to keep their reptiles warm (27°C/80°F) during transport to the hospital. Chelonians (turtles and tortoises) may be transported in appropriately sized boxes. Small and medium sized lizards should be brought in cloth bags or plastic containers. Large iguanas and monitor lizards may be placed in duffel bags and then put in cat carriers. Amphibians such as frogs, salamanders, etc., may be transported in appropriately sized plastic containers to which water or a moist substrate (sphagnum, peat moss) has been added. Reptile owners should be advised not to display their pets in the waiting room as other clients may find the experience disconcerting. If you know it will take more than 15 minutes before examining the reptile, a technician should place it on or near a heating source in a cage. Heating pads or heat lamps work well.

The waiting room should communicate to the owner that your practice is familiar with treating reptiles. Advanced Vivarium Systems, Lakeside, CA, 92040, publishes a series of booklets describing the captive husbandry of various reptiles and amphibians that are commonly maintained in captivity. Displaying these booklets in conjunction with photographs and posters of assorted reptiles enables the first time client to feel comfortable and learn about their pets while waiting to be examined. Poisonous reptiles should only be seen by veterinarians who are experienced in handling venomous species. Special precautions must be instituted for transporting and handling these dangerous reptiles. One should have an emergency hotline to the nearest human hospital that stores antivenom.

First time appointments should be given a minimum of 30 minutes. The veterinarian should be notified by the receptionist the type of reptiles being examined to be certain you are familiar with that particular species. It is strongly recommended that any veterinarian seeing reptiles should maintain some specimens as pets or for breeding purposes. Most problems with reptiles are due to poor husbandry practices.

Basic Examination Instrumentation

-gram scale
-rubber spatulas
-sexing probes
-spray bottle filled with alcohol
-rubber gloves
-ball-tipped needles
-heating pad

Anamnesis

Before the reptile or amphibian is examined, a thorough history must be obtained. Many times the problem can be accurately identified from the history alone. Anamnesis should include a discussion of captive conditions such as enclosure size, substrate type, temperature range during day and night, humidity levels, diet, frequency of feedings/defecation, color/consistency of feces, frequency of water bowl changes, presence of cagemates, hot rocks, hide boxes, etc. Clients should be encouraged to maintain cage cards noting date/source of acquisition, captive bred or wild caught, feeding/defecation frequency, shedding dates, periodic weight and any prior disease(s). One can see the importance of being familiar with the recommended husbandry conditions so as to identify associated problems. Maintaining history questionnaires in the waiting room for the client to complete will save time during the office visit.

Physical Examination

One should first observe the reptile before beginning the examination. Is it alert and responsive with a normal posture? Is the respiratory cycle normal? Are there any obvious swellings or injuries? Debilitated snakes are thin and have lose skin folds parallel to the spine. The ribs may be prominent. Malnourished lizards display bony protuberances especially around the pelvic girdle. Depleted fat deposits in the tail with prominent coccygeal vertebrae signify cachexia. Starving chelonians have sunken eyes and appear hollow in the axillary and inguinal pockets. They feel light in weight in comparison to their size. Dehydration is assessed by reduced skin turgor and sunken eyes.

Snakes should be initially grasped behind the head and lifted midbody with the other hand. Iguanas should be supported under the front and rear legs, stabilizing the head between the thumb and index finger. Handling large snakes requires the assistance of several people for adequate restraint. The marine toad (*Bufo marinus*) possesses cutaneous parotid glands that secrete bufotoxin which is readily absorbed through mucous membranes; accordingly, examination gloves should be worn when handling them. Intractable reptiles may need to be sedated to permit a thorough examination. Snake bites leave small puncture wounds which bleed freely. Large boids and pythons can inflict lacerations requiring stitches. Monitor lizards hold on with tenacity and have to be dislodged by spraying alcohol in their mouths. All reptiles should be weighed using the metric system. Some are naturally flighty

such as water dragons (*Physignathus cocincinus*) and basilisks (*Basiliscus plumifrons*). Others such as bearded dragons (*Pogona vitticeps*) and prehensile-tailed skinks (*Corucia zebrata*) tend to be more docile. Examples of nervous snakes are racers (*Coluber constrictor*) and tri-colored milksnakes (*Lampropeltis triangulum*). Tortoises tend to be shy and lethargic while water turtles such as red-eared sliders (*Chrysemys scripta elegans*) are very active.

The general examination should commence with the head and proceed caudally. Rostral scales of snakes and lizards are often traumatized due to rough edges in the cages, persistent disturbances causing flight reactions, lack of a hide box or aggression with incompatible cage mates. Snakes can be seriously injured by live rodents that are left unattended in the cage. The nostrils should be patent and free of discharge. Green iguanas (*Iguana iguana*) have salt glands that communicate with the external nares; thus, occasional sneezing and subsequent build-up of dry, white secretions around the nostrils is a normal finding. Some tortoises excrete excessive sodium via ocular glands causing physiologic epiphora. The eyes should be bright, alert and free of exudate. Snakes have no eyelids; instead, their eyelids have fused to form a single scale called a spectacle which covers the cornea. Diurnal reptiles usually have large round pupils; nocturnal ones have vertical pupils. Unlike other reptiles, snakes shed their skin in one piece. Prior to shedding, their body takes on a milky hue which is due to lymph like fluid being produced between the old and new layers of skin. The eyes also become opaque at this time. In addition, check for discoloration, wrinkling, hemorrhage, or bulging of the eyes. Tree frogs which normally consume invertebrates develop corneal lipid dystrophy when fed a diet high in fat such as newborn mice (pinkies). Wrinkled or retained spectacles develop secondary to low ambient humidity. Exophthalmos is due to blockage of the harderian gland, abscessation or neoplasia. The snake mite (*Ophionyssus natricus*) congregates around the edges of the spectacles and gular fold. These are blood-sucking ectoparasites that transmit viruses, blood parasites and pathogenic bacteria. Dorsal and ventral surfaces of reptiles should be examined for evidence of dermatitis, injury, etc. Lizards shed their skin in patches. Chelonians shed their scutes individually. The plantar surfaces of the appendages should be checked for abrasions secondary to inappropriate substrate. The spine and extremities should be palpated for indentations, swellings, or softness due to fractures, abscesses or metabolic bone disease (MBD). Generalized tetany is observed with hypocalcemia, hypoglycemia or neurologic disease. A head tilt and/or strabismus is consistent with vestibulopathy. Snakes have no external ears; chelonians, lizards and amphibians possess a tympanum. Swelling of the tympanum is commonly seen in turtles and is secondary to middle ear abscesses. Pigmentation of the tongue is normal in some lizards such as the blue-tongued skink (*Tiliqua gigas*). Green iguanas have a reddish tip on their tongues while bearded dragons have yellow-tinged mucosa. Other reptiles have melanin deposits in the oral cavity to enhance absorption of heat such as the savannah monitor (*Varanus exanthematicus*). Some boids possess labial pits which are cavities located in or between the labial scales that are heat sensitive to assist in locating prey. The mouth parts of chelonians are susceptible to overgrowth due to the consumption of nonabrasive food in captivity. The mouth should be opened carefully to avoid damage to the delicate oral mucosa. Appropriately sized rubber spatulas work well. Iguanas, water dragons and basilisks may gape as a defensive display making the oral exam easier. The oral cavity should be free of any swellings, exudate,

petechiae or bubbles. These symptoms are consistent with stomatitis, septicemia or pneumonia. In snakes the glottis is located in the anterior part of the mandible while in lizards, chelonians, and amphibians it is in the larynx. Soft, pliable jaws (except in snakes) are secondary to MBD; however, the Florida soft-shelled turtle (*Trionx ferox*) normally has a soft carapace and plastron.

Auscultation can be challenging. A moistened gauze pad may be placed between the diaphragm of the stethoscope and skin to diminish background noise. In snakes, the heart is located one third down the length of the body. Its contractions may be visualized when the snake is placed on its back and viewed from the side. In lizards, the heart may be auscultated on the ventrum either between or posterior to, the brachial girdle. Chelonians are difficult to auscultate due to the presence of their shell; however, the respiratory cycle may be assessed by observing normal subtle movements of the forelimbs, the presence of open-mouth breathing or wheezing. Turtles suspected of having pneumonia may be observed for even-sided buoyancy by placing them in water.

Palpation of the abdomen is an important aspect of the physical exam and an understanding of general anatomy is essential. A snake's body may conveniently be divided into quarters. The first quarter contains the trachea, esophagus and heart. The second quarter contains the heart, lung, liver and stomach. The third quarter contains the spleen, pancreas, gonads, adrenal glands and small intestine. The fourth quarter contains the kidneys, colon, cloaca, cloacal opening (vent), musk glands and paired hemipenises in males. A healthy snake has firm abdominal musculature that resists digital palpation. Any visible abdominal swelling(s) should be correlated with the organs found in the corresponding regional quarter. Abnormalities associated with coelomic masses include abscesses, intestinal blockages, tumors, etc. In lizards and chelonians, the kidneys are recessed within the pelvic girdle and therefore are normally not palpable. The liver is variable in position depending on the genus; however, it usually extends from the brachial girdle to the edges of the ribs. In snakes, the gastrointestinal tract may be palpated for gas, swellings or foreign bodies. In chelonians, palpation is most rewarding when the coelomic cavity is examined through the inguinal pockets. These are the fossae anterior to the posterior limbs. Using the index finger, eggs, enlarged kidneys, cystic calculi and intestinal impactions can be palpated.

The cloacal opening is an essential area to examine in reptiles. Snakes have no urinary bladder and their normal waste products consist of feces and amorphous urates which are yellow in color. The accumulation of excreta around the cloacal opening (pasty vent) indicates enteritis. Lizards, chelonians and amphibians have a urinary bladder. The cloaca is divided into three compartments: a) copradeum--receives fecal material from the colon, b) urodeum--receives urates from the ureters and products of conception from the oviducts, and c) proctodeum--storage vat for feces/urates and absorption of water. Posterior to the cloacal opening are the musk glands and paired hemipenises in snakes and lizards. Chelonians have a single penis which is retracted anterior to the vent.

Sex determination is commonly requested by reptile owners. In general, it is more difficult to visibly sex hatchling and juvenile reptiles because sexual dimorphism becomes more

apparent with age. In snakes, sex determination is most accurately performed by the utilization of sexing probes which are available in various sizes depending on the size of the snake. They are made of stainless steel and blunted at the ends. Prior to insertion, the probe should be moistened with tap water. In snakes, each hemipenis is inverted into the tail. The lubricated probe is directed caudolaterally into the hemipenal sulcus. The paired musk glands have ducts that are located medially to the hemipenises. Probing these structures in a male snake will cause one to incorrectly identify the snake as a female. In females, the probe slides to a depth of two to three subcaudal scales and in males the probe reaches ten to fifteen scales. It is important to be gentle when performing this procedure as perforation of the musk glands or hemipenises can lead to infection. It is best to probe both hemipenal pockets to confirm male gender. In boids, males have larger pelvic spurs. These are claw like appendages that are vestiges of the pelvis used to stimulate the female prior to copulation. In lizards, such as bearded dragons, monitors and skinks, sex determination is most accurately accomplished by manually prolapsing the hemipenises under general anesthesia. Radiography may demonstrate calcified hemipenal spines in varanids (monitors). Sexual dimorphism in certain species may preclude these procedures. Mature male lizards such as iguanas, basilisks, anoles and chameleons have larger dewlaps, dorsal spines, head ornaments and change color during breeding season. Hemipenal bulges at the tail base may also be prominent in males. In turtles, males generally possess longer tails with the cloacal opening posterior to the edge of the supracaudal scutes. They also have a concave plastron to facilitate copulation. In box turtles, males commonly have red irises while females have brown irises. Male leopard (*Geochelone pardalis*) and red-footed tortoises (*Geochelone carbonaria*) have V-shaped anal plastrons. Some aquatic turtles have enlarged front toenails that are used in courtship. Many male anurans (frogs and toads) have nuptial pads present on the thumbs, chin and chest. These are areas of roughened pigmented epithelium that permit maintenance of a firm grasp on females during amplexus (copulation). Male frogs tend to be more vocal, utilizing mating calls during breeding season.

Although captive bred reptiles are becoming more available through private breeders there are still many wild caught imports. The two most common types are ball pythons (*Python regius*) and juvenile iguanas. The hatchling iguanas are farm raised imports from Central America that are hatched from gravid females captured in the wild and later released after ovipositing. All ball pythons and iguanas are heavily parasitized. These species should be empirically dewormed during the first office visit. Ball tipped needles work well for this purpose, especially in hatchlings.

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COMMON INFECTIOUS DISEASES OF REPTILES AND AMPHIBIANS: AN ETIOLOGIC REVIEW, DIAGNOSTICS AND TREATMENT RECOMMENDATIONS

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Introduction

The most commonly encountered and well-documented infectious agents are presented here as a condensed overview to serve as a quick reference. Specific diagnostic considerations with herptiles in mind may aid in a final diagnosis and general treatment recommendations are intended to aid successful resolution. A reference list of selected sources is included and can be used to further investigate specific etiologic agents.

Viral diseases

Although herptile virology research is less advanced that of higher vertebrates, numerous associated viral species have been identified. A causal relationship for many is unproven. The following viruses are well documented to be associated with disease. Often disease presentation will parallel the mammalian or avian counterpart. For example, ophidian paramyxovirus may exhibit purely CNS signs, pneumonic, or a combination thereof, just as other paramyxoviruses, such as canine distemper and newcastles disease may be neurotropic, upper respiratory or both.

Adenovirus, hepatic necrosis of crocodilians/lizards/snakes
Poxvirus, dermal and buccal lesions in crocodilians/lizards
Herpesvirus, dermal papules/papillomas, hepatic necrosis, oropharyngeal ulceration/abscessation in chelonians/snakes, associated with renal adenocarcinomas of frogs
Paramyxovirus, neurologic, respiratory or chronic disease of snakes, responsible for epizootics in serpentine collections
Iridovirus, hepatic necrosis in chelonians, erythrocytic disease of lizards/frogs, tadpole edema and disseminated visceral necrosis
Togavirus, subclinical viremia of tortoises, may be nonpathologic for tortoises but they may act as reservoir for equine encephalitis
Boid inclusion body disease virus, as yet incompletely identified, encephalitis/CNS disease, recurrent regurgitation in pythons and boas

Viral Diagnostics

Few herpetologic viral pathogens have well developed diagnostic tests. Ophidian paramyxovirus serologic tests are available through the University of Tennessee Vet School and an experimental vaccine for that disease is currently under development at another lab. Most other viral agents are identified by characteristic histologic lesions and cell associated

intralesional viral particles. Isolation may require reptile or amphibian cell line tissue culture which are now available commercially and some labs currently use. The primary pitfall of diagnosis is the presence of secondary invaders which may be more readily detected.

Viral Treatment

With few exceptions, there are sparse specific antiviral medications. Treatment usually involves supportive care, treatment of secondary pathogens, good husbandry and most importantly, quarantine of the infected specimen(s) to prevent further losses. Two possible exceptions are as follows. Acyclovir for herpesvirus treatment is available and may be useful in treating some cases. Amantadine, an experimental drug used in humans against Influenza A (myxovirus) may have some efficacy against paramyxoviruses as well. In an epizootic outbreak the losses can be so severe, these experimental drugs may be worth trying.

Bacterial diseases

Primarily gram negative pathogens: most are associated with poor sanitation/husbandry or occur secondary to trauma. Many can be cultured from clinically healthy specimens. Once established in a collection however, these pathogens may become epizootic. Common presentations in reptiles include stomatitis, glossitis, osteomyelitis, periauricular/cutaneous/digital abscesses, intermandibular/pharyngeal cellulitis. In amphibians presentations include red leg, ulcerative dermatitis, anasarca/edema, cutaneous emphysema and corneal ulceration. Commonly:

Pseudomonas, Aeromonas, Proteus, Acinetobacter, Klebsiella, all may cause similar presentations as listed above
Citrobacter, primary etiologic agent of septicemic cutaneous ulcerative disease of soft shelled turtles

Occasionally:
Staphylococcus, Streptococcus
Mycoplasma, upper respiratory tract disease of tortoises

Others, rarely:
Salmonella, more often commensal
Pasteurella, secondary with chelonian mycoplasma upper respiratory disease.
Chlamydia, visceral organ necrosis, red leg
Mycobacterium, numerous species may exhibit dermatologic, respiratory or gastrointestinal tuberculosis.
Rickettsia: Haemobartonella, Aegyptianella, pathogenicity is unclear but are associated with erythrocytic surface and cytoplasmic inclusions
Coxiella, also undocumented pathogenicity but reptilia may serve as reservoirs for such diseases as Q Fever

Bacterial Diagnostics

Incubation of some herptile bacterial pathogens may require lower than 37 C, the temperature many labs routinely use. Many labs will lower the temperature to ~25-30 C upon request. Culture of lymphatic fluid from frogs from the femoral or dorsal paralumbar lymphatic sacs may minimize surface contaminants and pinpoint a septicemic etiology.

Bacterial Treatment

Localized lesions often heal well with debridement +/- topical antimicrobials even without systemic antibiotic therapy, however many cases develop insidious disseminated disease or hepatitis months later. Systemic antibiotic therapy continued beyond clinical signs of disease may minimize reoccurrence.

Systemic injectable antibiotics can be used topically for localized lesions (ex: stomatitis, cutaneous abscesses) but the total amount should not exceed the dose for systemic treatment.

Although some lipophilic antibiotics such as quinolones may be partially absorbed by amphibians when applied cutaneously, systemic therapeutic blood levels are generally not obtained by this method. Treatment of superficial infections such as red leg with aminoglycosides in the water as once recommended, is poorly effective for prevention of septicemia but may decrease environmental contamination.

In addition to intramuscular injection sites, amphibians may be treated via lymphatics. Because of the presence of lymph "hearts" within the amphibian lymphatic system, absorption using this mode of administration is nearly as rapid as intravenous. The lymphatics are readily accessible by injection into the paralumbar lymphatic sacs, found essentially subcutaneously on dorsum of frogs.

Bacterial enteritis can be sometimes be treated with aminoglycosides orally in spite of their poor absorption. By using this mode nephrotoxicity can be minimized and the antibiotics can act locally where they are needed the most.

Nephrotoxic and water soluble drugs generally should be given in the cranial 1/2 of the body to avoid the portonephric blood vascular system. It can be used to one's advantage however if rapid renal excretion is desired such as treatment of urogenital disease or for renal contrast media radiography.

Fungal diseases

Fungal etiologic agents are often opportunists, but are occasionally primary pathogens. Cutaneous, respiratory or visceral granulomatous lesions must be differentiated from mycobacteriosis. Commonly:

Phycomycosis, Chromomycosis, dermal/cutaneous ulcerative disease or disseminated in amphibians
Dermatophyton, Tricophyton, Saprolegnia, superficial dermal and shell mycosis, severe cases may invade deeper tissues
Fusarium, chelonian scute infections
Aspergillus, Penicillium, Candida, reported as primary respiratory pathogens
Schizangeilla, previously classified as an algae, may be exhibited as an encapsulated granulomatous lesion of snakes and lizards

Fungal Diagnostics

Histopathologic examination of impression smears, biopsies or necropsy specimens are most commonly used to identify fungal elements. Culture may require some specialization and laboratories should be contacted to obtain instructions for specific sampling techniques to minimize bacterial overgrowth of cultures.

Fungal Treatment

Because of the encapsulation of many fungal lesions, excision of the lesion where possible may increase treatment efficacy. Topical or systemic antifungals are recommended along with surgical intervention to minimize reoccurrence. With major organ involvement or disseminated disease the prognosis is poor to guarded. Ketoconazole and griseofulvin are reported to be useful as systemic treatments.

Algal diseases

Aquatic, semiaquatic and species kept under high humidity are only sporadically diagnosed with algal infections. The most common algae involved are:

Basicladia, superficial proliferative aquatic chelonian pathogen or commensal organism, may cause pitting of the carapace over long periods of time but systemic illness not documented
Prototheca, reports of granulomatous encapsulated masses in snakes and lizards, confusion exists over previous reports due to the recent reclassification of some cases as a fungal organism (see *Schizangeilla* above)

Algal Diagnostics

Superficially resembling fungal granulomatous or proliferative lesions definitive diagnosis must be on histopathologic appearance.

Algal Treatment

Encapsulated granulomatous lesions should be widely excised including the entire capsule prior to topical treatments. Medications reported to efficacious include 0.125-0.25% sodium

hypochlorite, ketoconizole, povidone iodine, chlorhexadine, and silver sulfadiazine. The necessity of treating superficial carapace infections of Basicladia is questionable.

General treatment considerations

In nature or with proper husbandry, both reptiles and amphibians are quite resistant to infectious diseases, as demonstrated by long term captive amphibians (20 yr+) and elderly giant tortoises (100 yr+). Most commonly one or more debilitating factors are the primary or inciting cause of disease. An integral part of any useful treatment is investigation of potential predisposing factors. Elimination of these factors is essential for an effective cure. Simple measures such as providing a thermal gradient allows poikilotherms to create the optimal self defence body temperature. For example, a small group of prairie rattlesnakes (*Crotalus viridis*) without access to a thermal gradient died at 10 weeks post exposure to ophidian paramyxovirus without mounting a detectable antibody titer. Others in the same room were also experimentally infected but were given localized substrate heat. These mounted a detectable immunologic response, and some survived in excess of one year before they succumbed. Furthermore, togavirus viremia duration in chelonians was demonstrably shortened when the ambient temperature was increased to 30 C. Numerous other environmental factors beyond the scope of this manuscript may inhibit or enhance recovery as well.

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COMMON PARASITIC DISEASES OF REPTILES AND AMPHIBIANS

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Any reptile or amphibian new to a collection, especially a collection that maintains species from different parts of the world or different taxa, should include a fecal and hematological examination as part of the initial physical examination. Thin smears can be prepared from blood collected by various methods and stained with Wright's, Diff-Quik® or Giemsa. Fecal examinations can consist of a floatation technique but should also utilize a direct smear, especially when screening for pathogenic protozoa; fresh feces are best. If a patient is presented and no stool sample is available, feces can frequently be expressed with gentle palpation or a bolus of saline solution can be washed into the colon and withdrawn by the use of a red rubber nasogastric tube or urinary catheter. Be certain to provide adequate lubrication (water soluble and non-spermicidal if the animal is intended for breeding purposes) and select a tube of appropriate diameter. Samples collected from the cloaca are often urate contaminated and may not be diagnostic. In male snakes, sperm may also comprise a fraction of the sample. In certain instances where lung infestation is suspected, swabs of the oropharyngeal mucosa or a tracheal wash may be indicated. In the case of a tracheal or lung wash, a sterile, lubricated catheter is passed through the epiglottis into the trachea and advanced to the lungs. A volume of sterile saline (1 cc or greater depending on size) is flushed and withdrawn. I will frequently "roll" the patient back and forth to get a specimen. The sample obtained is then used for diagnosis by direct smear or floatation (and is also useful for culture and sensitivity if indicated).

Signs of parasitism may range from none at all to acute death. Clients frequently ask why parasites that have been in the animal in the wild do not cause sickness until that animal is placed in a captive environment. They don't realize it, but they have answered their own question. We are unable to duplicate the natural environment of these animals and therefore induce stress, which predisposes these animals to parasitic diseases. In captivity, parasites with direct life cycles are favored over those that require an intermediate host. Feeding wild caught amphibians, reptiles or rodents to captive amphibians or reptiles can allow even these latter parasites to become problematic in a captive situation. Another common problem is the introduction of species from different geographical areas into the same cage. We certainly refrain from housing snakes and lizards together, or should, so why not old world and new world boids?! Species may have different tolerances for parasites based on their country of origin.

Proper housing is as important to parasite control as adequate medication. If at all possible, animals should be kept on paper or Astroturf®. Mulches, sand and soil all have the capacity to harbor eggs or cysts, thus providing the opportunity for reinfection. Cages should be cleaned as soon as they are soiled and care taken to wash hands between cages.

Blood parasites

Only the most common hemoparasites will be discussed, as the number of described species is extensive and the literature often not helpful in determining the pathogenicity of certain species. Hemoparasites are found within cells or free in the plasma. Their development may involve other organ systems. Often, there is little or no clinical disease. Where hemoparasitism does result in anemia, thrombocytopenia or purpura may occur. There is no data on the efficacy of therapy, but hemoprotozoa are often treated with tetracycline and/or chloroquine phosphate-primaquine phosphate.

Pirhemocyton - I have included this organism here because when first described it was believed to be a protozoan parasite of erythrocytes. We now know that it is probably an iridiovirus. It is responsible for RBC destruction and anemia. If the affected animal also has a concomitant infection with *Plasmodium*, death often results.

Hemogregarines - A single schizont typically attacks a RBC, where replication occurs intracytoplasmically. Hemogregarines are the dominant and characteristic hemoparasites of snakes but affect all classes of reptiles including the Tuatara and sea snakes. *Hemogregarina* is found in aquatic reptiles and relies on leeches for sexual reproduction. *Hepatozoa* is found in terrestrial arthropods or leeches. There is little pathology.

Hemoproteus - These are also found within the erythrocyte cytoplasm and vary from one to several per cell. Turtles and lizards are the usual hosts. There is little pathology.

Leishmania - Only the pro- and amastigote stages are observed. Promastigotes are found in the blood, amastigotes are intracellular. Transmission is probably by the sandfly. This is a benign infection. Lizards are the hosts.

Plasmodium - There are 68 species known from turtles, lizards and snakes. Depending on the species, different stages of the organism may be found within the cytoplasm of RBC's, mononuclear leukocytes or endothelial cells of visceral organs. Some species may cause anemia or thrombocytopenia. An insect vector is required for transmission.

Lankesterella - This protozoan may be transmitted by leeches. They penetrate and destroy RBC's, possibly resulting in anemia.

Trypanosomes - These affect crocodilians, turtles, lizards and snakes, frogs and newts. Biting flies and leeches are responsible for transmission. Animals may become listless, refuse food and die due to heavy parasitism, but pathology is usually rare.

Microfilaria - The presence of microfilaria suggests the presence of a pair of adults in the eye, subcutis or coelomic cavity. With the exception of adults in the eye, pathology is rare.

Flukes - *Spirorchis* is found in the blood of semiaquatic turtles.

Enteric parasites

Protozoa

The numbers and genera of protozoa present in a reptile or amphibian are probably influenced by individual animal differences in the physiological parameters that affect the intestinal tract. Differences, especially in the pH and passage of digesta can have profound effects on the make up of the protozoal community. Other factors can include natural antagonism between the different species and predation. Protozoa can also affect the bacterial flora due to substrate competition and predation.

I do not believe I have ever performed a fecal examination on a reptile or amphibian without encountering some protozoa, except in cases of prolonged anorexia and debilitation. Even some of these animals frequently have thriving communities.

Flagellates - At least 6 genera of flagellates have been identified, probably transmitted by infective cysts or by copulation. The organism most likely to cause problems in captivity is *Hexamita*, which is known to affect the urinary bladder and kidneys of aquatic turtles (renal hexamitosis), an often fatal infection. *Chilomastix* is frequently found in the intestine of frogs, toads and salamanders. *Trichomonas* and *Tritrichomonas* are widely distributed among amphibians and reptiles. They can be identified by the well developed undulating membrane. *Giardia* are frequently encountered in frog and toad tadpoles. No pathology has been attributed to them. Retortamonad and diplomonad flagellates have recently been reported from poison dart frogs.

Opalinids - Resembling flagellates and ciliates, opalinids lack a cell mouth and have only one type of nuclei. *Zelleriella* were observed in dendrobatid frogs.

Ciliates - *Balantidium* species are common in herbivorous turtles and lizards. Unless this ciliate is present in large numbers, it is unlikely to be causing any pathogenic effect. Another ciliate, this species very large, is *Nyctotherus*. It can be found in turtles and the green iguana. The cyst of this "commensal" can easily be mistaken for a trematode egg. *Nyctotheroides* has been observed in anurans.

Amoeba - Clinical signs of anorexia, weight loss, blood or mucus in the stool, vomiting, green discoloration of urates, or midbody to caudal swellings of the body may be suggestive of infection with *Entamoeba invadans*. This is a highly pathogenic parasite in lizards and snakes. Trophozoites may be observed crawling across the field of view from a direct smear. Amoeba may be commensal symbionts in turtles and crocodilians, frequently making them responsible for infection of exposed lizards and snakes. I treat for amoebiasis routinely in monitors, turtles and crocodilians, and native species of all other reptiles. Several other species of pathogenic amoeba exist, of which *Acanthamoeba* has been implicated in fatal infection. Star gazing is a sign of central nervous system involvement with this organism. My treatment protocol for amoeba, flagellates and ciliates is with metronidazole 275 mg/kg PO repeated in 2 weeks. A dose of 125 mg/kg is also reportedly effective.

Coccidia - *Sarcocystis* and *Toxoplasma* are occasionally found in reptiles. More often than not, the affected reptile is an intermediate host although reports exist for snakes and lizards serving as the definitive hosts. *Isospora* and *Eimeria* are the "typical" coccidia recognized in fecal specimens from infected reptiles and amphibians. Anorexia, listlessness, regurgitation and intestinal hemorrhage and intussusception are the primary signs of intestinal or gall bladder infection with coccidia. *Isospora* have two sporozoites and have not been reported from turtles. *Eimeria* have four sporozoites and is considered the primary coccidian of reptiles. Oöcysts are ingested from contaminated feces or soil. Treatment is with sulfamethoxine and sulfamethazine at 75 mg/kg daily for 7 days.

Boids frequently present with signs of regurgitation, lethargy and depression when infected with *Cryptosporidium*. A midbody swelling may be palpated in advanced cases. The organism causes a proliferative gastritis for which there is no known cure or treatment (although potentiated sulfa drugs may offer some hope if used long term). This organism rivals *Entamoeba* in pathogenicity. Ingestion of sporulated oöcysts, infected mice, snakes or lizards is responsible for infection in captive species. Diagnosis can be made on the basis of a direct smear (if lucky) or an acid fast stain of feces, slime from a regurgitated meal, aspirate or stomach wash, that reveals multiple, round organisms 2 to 5 microns in diameter that stain bright red. Sometimes the organisms will not retain the red dye but will leave a "ghost image" against the counterstain. Care must be taken in male snakes not to mistake tailless sperm, which also stain red, for the coccidian oöcysts. Oöcysts may be shed intermittently. Repeated fecals or a biopsy may be necessary for a definitive diagnosis. Infected animals should be isolated from the collection and preferably destroyed, as they are considered a zoonotic threat. Jacobson reports a possible success in treating one snake with SMZ-TMP at 60 mg/kg PO SID for 60 days. The snake died 1 month after treatment.

Helminths

Trematodes - Operculated eggs in the fecal sample or oral mucosa are diagnostic for trematodes. Some monogenea are external or urinary bladder parasites of frogs and tadpoles or newts. Digenean trematodes often form metacercariae in the skin, eye or various organs. The renifer group of digenetic flukes are common in the mouth, pharynx, esophagus, trachea and lung of indigo snakes. This fluke requires an amphibian as an intermediate host so elimination of this food source will prevent reinfestation. Owners often present animals for anorexia or colds, as presence of large numbers cause copious salivation. Praziquantel (7 mg/kg IM) or fenbendazole (100 mg/kg PO) will control this and other metazoans.

Cestodes - The tapeworms that affect reptiles are hermaphroditic and non-host specific. Transmission is by ingestion of an intermediate host. Diagnosis of an intestine dwelling cestode is by detection of proglottids or eggs in the stool. Plerocercoids of *Diphyllobothrium* are frequently found in tadpoles feeding on crustaceans. Niclosamide (150-300 mg/kg PO), bunamidine HCl (50 mg/kg PO), and praziquantel (7 mg/kg IM) are all reported to be effective in treating for adult tapeworms.

Nematodes - Oxyurid eggs are frequently encountered during fecal exams of lizards and chelonians. In snakes, care must be taken not to mistake rodent pinworm eggs for those parasitic for reptiles. Pinworms are considered non-pathogenic. Oxyurids have a direct life cycle and can exist in significant numbers within the colon, especially of tortoises, putting them at risk of impaction. Anorexia may occur in animals awakening from hibernation. I recommend treatment of tortoises as owners are upset by the worms shed with the feces. Diagnosis is based on passed adults or by the characteristic, often "bowed" eggs.

The presence of thin-walled embryonated eggs or rhabditiform larvae in a fecal sample are indicative of *Strongyloides* or *Rhabdias* infestation. *Rhabdias* inhabits the hosts lungs and may cause respiratory distress. *Strongyloides* can produce diarrhea and respiratory distress as infective larvae migrate through the host's lungs. Both parasites may cause anorexia, weight loss and debilitation. The life cycle is direct and the parasite can be transmitted by ingestion of eggs, larvae, or possibly by direct skin penetration. These parasites can exist as a free-living form, making cage sanitation a necessity.

Two genera of hookworms, *Kalicephalus* and *Oswaldocruzia*, occur in reptiles worldwide and are similar in appearance. Fecal examination will reveal the presence of typical "strongyle type" eggs. Transmission is by ingestion of ova, infective larvae, or possibly by skin penetration. Drinking contaminated water is another means of oral infection. Infestations may cause lethargy, anorexia, general debilitation, anemia, ulceration, intestinal obstruction and peritonitis.

Capillaria is the only known trichurid genera affecting reptiles. These nematodes primarily infect the intestine but have been found in other organs, such as the liver and gonads. They have a direct life cycle. Diagnosis is based on the presence of eggs with opercula at either end.

Ascarid eggs are recognized by their thick shells. Adults may be found embedded in the stomach, esophagus, or small intestine where they may cause no signs of illness to anorexia and regurgitation. Diarrhea and purulent pneumonia may be attributable to heavy infestations. Ingestion of intermediate hosts, such as amphibians and rodents, are the most likely source of infection.

Spirurids are parasites of the mouth and digestive system. Reptiles may act as intermediate or definitive hosts. Ants are a common source of infection for terrestrial animals and copepods for aquatic animals. Diagnosis is by detection of the characteristic eggs. The larva curled within gives the egg the appearance of containing a paper clip. Adults are easily removed from the mouth.

Filariae can be found within lymph vessels, the eye, subcutis or within the coelomic cavity. The microfilaria produced by the adults may circulate in the blood or may be found in the skin, where they may cause dermal tumors. Pathology due to filariae is rare.

Treatment of nematode infestation is readily accomplished. A variety of drugs can be used including thiabendazole (50-100 mg/kg PO), levamisole (10 mg/kg PO), fenbendazole (50-100 mg/kg PO) or ivermectin (0.2 mg/kg IM). DO NOT USE IVERMECTIN IN TURTLES. Milbemycin can also be used in reptiles and has been injected in several species of turtles with no ill effects.

Acanthocephalans - The thorny-headed worms or acanthocephalans are common in aquatic turtles, frogs and toads. They may be found in the stomach or intestine. Clinical signs may include blood or mucus in the stools, anemia, and wight loss. The eggs are typically dark, thick-shelled and tapered at the ends. Fecal material frequently adheres to the shell. Levamisole at 10 mg/kg PO may be successful as a treatment.

Pentastomes - Pentastomids are considered to be a degenerative crustacean that parasitizes the lung and airsac distal to the lung. Larvae and nymphs may be found in the stomach wall. Transmission occurs by the ingestion of an intermediate mammalian host. Symptoms may include lethargy, anorexia, dyspnea, and blood tinged saliva. Affected animals frequently harbor these parasites with no ill effects. Diagnosis is based on observations of the characteristic eggs which contain a primary larva, which is oval, tailed, and has four stumpy legs each bearing one or two retractable pincer claws. Man is an accidental host so care should be taken when handling infested animals or their feces. There is no known treatment but levamisole (5 mg/kg PO) or one of the avermectins (ivermectin or milbemycin) may be effective.

Ectoparasites

Leeches - These are commonly found in certain reptiles, such as aquatic turtles and amphibians. The dorsal lymph sac, body wall or body cavity may be invaded in certain species of frogs, with surgical removal the only treatment. Leeches have also been associated with cutaneous fibroepitheliomas in green sea turtles.

Myiasis - Turtles are particularly prone to infestation by fly larvae. While several species of true bot fly exist, most cases of myiasis are by opportunistic species which exploit pre-existing wounds. Treatment consists of removing the maggots by flushing with dilute hydrogen peroxide/betadine solution. The toad fly, *Bufolucilia*, invades the nasal orifice of toads. Infestation is usually fatal.

Mites and Ticks - Both hard and soft ticks attack reptiles and are usually seen on newly imported snakes. Be certain to carefully check the labial pits in boids. They can cause anemia and are responsible for the transmission of blood parasites or allowing bacteria to invade the wounds they produce. Treatment is by their removal. *Ophionyssus* mites are the most common and the most pathogenic of the mites that attack reptiles. Left unchecked, populations can soar and even moderately sized animals can be exsanguinated. Mites can transmit blood parasites and are also believed to transmit the virus responsible for boid encephalitis. Treatment is with ivermectin or milbemycin, warm water soaks, DDVP fly

strips (Vapona or Shell No Pest Strips, hard to find), or the careful application of pyrethroid insecticide. Silica gel may also be used as it desiccates the mite by scratching the cuticle. It can also desiccate small reptiles so close monitoring is necessary.

BASIC PRINCIPLES OF THERAPEUTICS USED IN REPTILE MEDICINE

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Don't Forget to Evaluate and Discuss Basic Husbandry

Veterinarians practicing on reptiles need to have a thorough understanding of the basic husbandry requirements of their patients. Reptiles are perhaps the most diverse and demanding group of animals in which health concerns are tied so closely with husbandry practices. While there is no substitute for owning and caring for reptiles to accumulate this knowledge, there are numerous sources of information available that the practitioner can take advantage of (see Table 1). A small library of basic information can be obtained for relatively little expense.

One of the most difficult aspects of seeing clients with these exotic pets is how to allow enough time to discuss both husbandry and the medical problems as well. One solution is to schedule longer appointments with new clients to accommodate these needs and to charge accordingly for the time. Another method is to train an interested staff member in client education and to let them discuss the husbandry methods in general, and then the veterinarian can fine tune the deficiencies that have been identified and also discuss the medical problems. The latter method has worked best for the author, as discussing basic husbandry of the green iguana four to five times daily is as exciting as discussing flea or heartworm programs on a repetitive basis. Each practitioner will make decisions based on their own practice style. The point is , if husbandry practice problems are not identified and discussed, then the medical problems may be impossible to resolve.

Sick Reptiles are Immunocompromised

It is generally agreed upon by reptile veterinarians that reptiles a) have primitive immune systems, b) several factors appear to affect the immune system and c) that immune system dysfunction is a major problem in most reptile ailments.[1,2,3,4] Factors affecting the immune system include hydration and nutritional status, seasonal variation, stress, age, and environmental temperature.[4]

Supporting the immune system is one of the most important aspects of treating an ill reptile. Correcting hydration, nutritional deficiencies, and minimizing stress through correct husbandry practices are essential. The nuts and bolts of accomplishing these goals are beyond the scope of this paper, but reference sources[2,3,4,15] can be consulted. One major immune system support measure we will deal with is environmental temperature.

Current veterinary knowledge places a great deal of stress on the importance of providing a thermal gradient to ill reptiles.[1,4] The author places a great deal of stress on providing a usable thermal gradient, which simply means that the provision of such a gradient must be

simple, effective, and easy to maintain. For example, the green iguana, *I. iguana,* has a preferred optimal temperature of 82-84 degrees F.[5] In a true example, a client had created a thermal gradient by using a 20-30 gallon long aquarium placed in a room with an ambient room temperature of 80 F, and one end of the cage near a hot air vent. The end of the cage near the vent often warmed to 85 - 86 F, but was erratic and dependent on the room temperature. While this situation is certainly better than some, the gradient provided remains within narrow confines and is not predictable. This iguana was then placed in the same cage with an aluminum reflector with a 60 watt incandescent bulb placed over the screen lid at one end of the cage which was controlled by a timer that was varied with the season. When this reflector and then an under tank heating pad (designed exclusively for reptile use) was placed at the other end of cage, the owners reported a better appetite, more activity, and better colors provided by the iguana. The second setup obviously produced a focal basking light with intense heat that was available at specific times during the day. At night or at other times when a more subtle heat was desired then the area over the under tank heater was used. The center part of the cage and branches above the area with the under tank cage were cooler and utilized frequently. When one considers these situations in detail, some thermal provisions are more usable or "reptile friendly" than others and should be encouraged. We also need to encourage our clients to use a simple thermometer and measure the enclosure at several spots to avoid both deficiencies and extremes in temperature. Heat stress due to excessively high temperatures can be as much of a problem as chronically low temperatures.

There are many studies to support the claim that heat stimulates the reptile immune system. It has been demonstrated that both turtles[6] and lizards[7] exposed to bacteria will create a "behavioral fever," when a usable thermal gradient is available. This would strongly suggest the survival importance of temperature increases, in response to pathogens.

As early as 1963, Evans demonstrated that antibody formation to typhoid antigen in desert iguanas (*Dipsosaurus dorsalis*) was improved by increasing ambient temperature and suppressed by declining temperatures.[8] In one of the more recent (1985) and impressive studies, Mader evaluated the pharmacokinetics of amikacin in gopher snakes, *Pituophis m. catenifer,* at different temperatures (24 vs. 37 C).[9] By increasing the ambient temperature the dose required to reach therapeutic serum levels could be reduced by 50%, therefore decreasing the risk of using a potentially nephrotoxic drug. The study also noted that the amikacin was distributed to the tissues better and that the MIC values for amikacin for several common reptilian pathogens was decreased. From these works (and others not elaborated on), it is apparent that an increase in core body temperature is vital to stimulate the immune system. The provision of a usable thermal gradient based on each reptiles preferred optimal temperature preferences will not only serve to stimulate the immune system but will potentially augment the treatment through more favorable pharmacokinetic activity, drug distribution, increased bacterial sensitivity, and elimination of therapeutic agents employed.

Developing a Therapeutic Plan

Treating an ill reptile doesn't simply mean pulling a medication off a shelf. As we have discussed so far, the basic husbandry has to be evaluated and corrected. An appropriate thermal gradient has to be provided. To further develop the therapeutic plan a thorough basic examination has to be performed and appropriate diagnostic tests performed and evaluated.

The basic exam should reveal the presence of dehydration, nutritional deficiencies, and other concurrent health concerns. Basic diagnostic tests would include fecals, a CBC, chem panels, and cytology samples. The results of the basic exam and diagnostic tests will have a direct impact on the selection of therapeutic agents. Let us take as an example, a severely malnourished and dehydrated reptile with a concurrent bacterial infection. Renal function was considered to be normal based on uric acid levels. The dehydration should be addressed first, as this will affect both the distribution and elimination of many drugs. With dehydration being addressed and uric acid levels within normal ranges then an antibiotic with potential nephrotoxic activity (aminoglycosides) can be employed. Nutritional concerns are considered and initiated, but with less priority than correcting dehydration and initiating antibiotic therapy.

Let's now take the example of a severely malnourished and dehydrated reptile with a bacterial infection that has also been found to have a high uric acid level (over 15 mg/dl). It was also found to have intestinal parasites. Again, dehydration is our first concern. With uric acid levels elevated, a potentially nephrotoxic antibiotic should be substituted for one with less renal impact (cephalosporins, extended spectrum penicillins, flouroquinolones,etc.). While malnourished, nutritional supplementation is not as quickly initiated as in the previous case. First, a reptile seldom starves overnight. Secondly, feeding and temperature increases (our thermal gradient) will potentially increase the uric acid levels making the hyperuricemia worse, and increasing the risk of visceral gout.[10,11] In this case, it would be more prudent to measure the uric acid levels after fluids and antibiotics have been initiated and nutrition supplements started as soon as uric acid levels have dropped. Anthelmintics for the parasites are also implemented but also have a lower priority in this scenario.

Rarely will the therapy of a reptile be as cut and dried as the previous examples. It is very important to combine all our knowledge obtained from the examination and diagnostic tests in choosing appropriate therapeutic agents. Therapeutic decisions will have to be balanced with the overall picture in mind. When combined with sound husbandry practices, a comprehensive plan with a greater potential for success is put into practice.

Selection of Therapeutic Agents

The ideal therapeutic agent should be inexpensive, easy to administer, safe, and effective. Few therapeutic agents fit this description, but it should be considered when making choices. Safety and efficacy are the most important properties for this author.

Therapeutic agents are administered orally, injectably, or topically. The veterinary literature historically has discouraged oral administration of medications, except for the treatment of gastrointestinal disorders. Perhaps this is based on the study performed in 1976 in which chloromycetin palmitate was orally administered to two gopher snakes, *P.M. caterifer*, and a slow absorption and sub-therapeutic plasma levels were obtained.[12] Perhaps this is also due to the fact that the potential for getting bitten is greater as well. It is the author's contention that this is a vastly underutilized route and should be used whenever it is appropriate. Preliminary research results with both ciprofloxacin (Cipro, Miles Labs) and enrofloxacin (Baytril, Miles Labs) given orally to reticulated pythons indicated that excellent absorption and distribution is seen, just as is noted in humans orally administered these drugs.[13,14] When compared with enrofloxacin being injected or taken orally in the reticulated python, absorption is virtually the same. The advantage of oral administration in this situation is that injections of enrofloxacin at levels needed to produce therapeutic levels against pathogens like *Pseudomonas aeruginosa* can cause severe tissue damage.[13]

If an oral medication is used, it should not be mixed with food whenever possible, but administered directly using a feeding needle (Jorgensen Labs, Loveland, CO) or a red rubber urethral tube (Sovereign, Sherwood Medical, St. Louis, MO). The GI transit time of reptiles varies widely, and this transit time is dependent on temperature, fiber content, etc. Administering a drug orally will produce a fairly predictable uptake, but this will be altered dramatically when administered with food. If a therapeutic agent must be administered in food, then use the absolute minimum required to do so.

Injections need to be made in the front half of the body due to the fact that reptiles have a renal portal system.[15] In snakes IM injections are best made one third of the snakes total length caudal to the head in the lateral muscle groups midway between the spine and the true lateral surface of the body. Lizards, chelonians, and crocodilians should have IM injections given in the caudal muscle groups of the front legs or the epaxial musculature. SC injections in snakes are made in the same area as the IM injections. SC injections in lizards and crocodilians are made over the shoulder blades, being careful to avoid the cervical region due to potential neurological complications (Horner's syndrome, etc.). Chelonians can have SC injections made in the loose skin surrounding the shoulders, again avoiding the cervical region. Intracoelomic injections in snakes are made in the lower third of the body to avoid hitting the extensive lungs present in many species. In lizards and crocodilians ICe injections are made on the right side of the lower abdomen slightly cranial to the rear legs. Care is taken to make sure the urinary bladder is not hit. In chelonians, ICe injections are made at the loose skin attachment to the shell cranial to the rear legs.

Physical size, unique anatomical features, and other considerations need to be taken into account in deciding between oral or injectable medications. For instance, smaller members of the true chameleons have very little muscle mass and react in a negative manner both physically (pain, discoloration at site,etc.) and psychologically to injections. Certain poisonous reptiles may dictate using injections for safety reasons. A large crocodilian may be easier to administer medication to in minimal amounts of food for safety reasons. Each individual reptile needs to have these considerations taken into account as well.

Topical products are useful for wound cleansing, protecting wounds, and treating damaged and infected areas. While hydrogen peroxide can be used, the author prefers using povidone iodine (Betadine, Purdue-Fredericks) or chlorhexadine (Nolvasan, Ft. Dodge) solutions.

A Canadian group of researchers surgically created two sets of five surgical wounds in the skin of common garter snakes (*Thamnophis sirtalis*) and then the healing was evaluated using no treatment, an antibiotic ointment, a polyurethane barrier, and a topical spray.[16] Surprisingly, the polyurethane barrier (Op-Site Spray, Smith & Nephew Inc., Quebec, Canada) was vastly superior in reepithelialization, advancement of the epithelium, dermal maturation, and a less intense inflammatory response. The film is transparent, waterproof, and impermeable to bacteria. The author feels that similar products such as Nexaband Spray (Vet. ProductsLab, Phoenix, Az.) may prove to be the product of choice for the immediate covering of burns, traumatic lesions, and surgical wounds.

Creams such as Silvadene Cream (Marion, Cinn., Ohio) are thought to be of more use than ointments,[15] as healing has actually been shown to be retarded by petroleum based products.[16,17] However, products such as Polysporin and Neosporin (Burroughs Wellcome, Triangle Park, NC) are commonly used due to their content of Polymixin B which is a potent antibiotic for use against gram negative organisms.

Ophthalmic antibiotic drops have been advocated for use in treating infectious stomatitis, but the drugs used should be taken into account in the total dosage calculated for use in the reptile.[1]

Selecting a Dose

To date, approximately twelve pharmacological studies have been done on over twenty-one species of reptiles.[4] In the author's opinion, doses should be based whenever possible on pharmacological data. While few studies have been done, there is enough information on drugs in specific reptiles to allow the practitioner to arrive at safe and effective dosages. When no pk information is available for a specific drug or reptile then two alternatives exist; metabolic scaling and empirical data.

The author has found that consensus empirical doses (those published and used over a course of time) to be of more use than metabolic scaling. It is well beyond the scope of this paper to discuss the many reasons why metabolic scaling is of less value in reptiles than in the other four metabolic groups.[4] Metabolic scaling is an interesting concept but the main criticism is that it does not account for the diversity within the reptile group. There is no way the metabolic activity of a mountain tortoise can be compared to a desert iguana. The author doesn't encourage the use of doses published on a one account basis, but those that have been widely reported and utilized by practitioners can be carefully considered.

General Comments on Antibiotics

As previously discussed, most if not all reptile ailments are accompanied by some degree of immunosuppression. The following guidelines should be observed whenever possible:

1) Bactericidal antibiotics are preferable to bacteriostatic drugs.
2) Antibiotic selection should be based on culture/sensitivity.
3) Lab data must be evaluated whenever dehydration or renal problems are suspected. Drug selection should be based on every aspect of the reptile patients status.
4) Concurrent anaerobic infections are thought to occur in up to 40% of aerobic infections.[18] In such cases, drugs (metronidazole, ceftazidime) with good activity against these bacteria should be used. A mixed infection should be suspected whenever antibiotic failure occurs.
5) Drug combinations may be used where appropriate. The following combinations have produced good clinical results, but no pharmacological studies of such combinations have been conducted to date:
a) Aminoglycosides with cephalosporins (example = amikacin + ceftazidime) for resistant or mixed infections.
b) Aminoglycosides with semi-synthetic penicillins (ex. = amikacin + piperacillin)
c) Aminoglycosides with metronidazole (ex. = amikacin + Flagyl) for mixed infections
d) Aminoglycosides with flouroquinolones (ex. = amikacin + Cipro) for *Pseudomonas* sp.
e) Flouroquinolones with metronidazole (ex. = Baytril + Flagyl) for mixed infections
f) Flouroquinolones with cephalosporins (ex. = Baytril + ceftazidime) for resistant infections including *Pseudomonas sp.*, and mixed infections
g) Cephalosporins with semi-synthetic penicillins (ex. = ceftazidime + piperacillin) for resistant infections including *Pseudomonas sp.* or mixed infections.

A reference source for antibiotic dosages is Chpt. 17, Antibiotic Therapy, by D.R. Mader in Frye's Biomedical and Surgical Aspects of Captive Reptile Husbandry, 2nd edition, vol.1, Krieger Publishing Co., Malabar, Fl. 1991.

General Comments on Anthelmintics

Deaths have been reported in the literature attributable to ivermectin (Ivomec, Merck) Pyrethrin sprays (many generic manufacturers), permethrins (Nix, Johnson &Johnson) Vapona No-Pest Strips (many generic manufacturers), and trichlorfon spray (Chemtronics).[19] The variability in published dosages is profound and so caution is advised, and the use of consensus empirical doses is strongly advised until data based on pharmacokinetics is obtained.[20]

Ivermectin is absolutely contraindicated in chelonians and crocodilians.[20] It should be used with caution in skinks and the true chameleons.[19] Due to a narrow range of safety and dilution difficulties, the author suggests not using Ivermectin in animals weighing less than 0.5 kg. It is the author's opinion that ivermectin is not as effective or safe a drug as fenbendazole (Panacur, Hoechst Roussel).[21] This was fact was proven by the author in a study comparing the efficacy of Ivomec vs. Panacur in eliminating nematode parasites in ball pythons.[21] Ivomec has proven to be very efficacious in the treatment of mites when used as a spray.[19]

Praziquantel (Droncit, Mobay) at doses over 20 mg/kg have caused anaphylactic type reactions in ball pythons and reticulated pythons which recovered when given IM prednisolone acetate.[19]

The deaths due to Pyrethrin sprays, No-Pest Strips, and Permethrins appear to be tied to poor ventilation.[19] These products are more than likely safe if used correctly, but should be used with caution.

A reference source for anthelmintic dosages is Understanding Reptile Parasites (Advanced Vivarium Systems, P.O. Box 408, Lakeside, CA.92040), written by the author. This manual is very basic but contains dosage and administration information that should be helpful.

Cost of Therapeutic Agents

While it would nice not to have to worry about the cost and shelf -life of therapeutic agents, it is unfortunately a necessity. As most reptiles seen on a frequent basis are small, their per kg cost for medications is usually quite low.[4] However, cost can be deceiving as initially stocking certain drugs is quite expensive and some have a very short shelf-life. For instance, ciprofloxacin costs only $0.31 per kg and only has to be administered once every 48-72 hrs. However, each 250 mg tablet costs $2.60 each and some pharmacies will not prescribe only a couple tablets. Carbenicillin costs but $0.44 per kg, but comes in a one gram vial which expires within 72 hrs.

Of those antibiotics on which pk studies have been conducted, those considered to be inexpensive to stock and dispense are gentamicin & amikacin. Chloramphenicol, ciprofloxacin and enrofloxacin (Baytril) are moderately expensive. Carbenicillin, piperacillin, ceftazidime, and cefaperazone are expensive to use due to higher costs and rapid shelf life. In a larger reptile practice the cost of the more expensive antibiotics is off set by being able to use some of the excess mixed and rapidly expiring drugs on other reptiles.

The anthelmintics and antiparasiticidal drugs are an extremely inexpensive group of drugs to use on a per kg basis, and generally have a good shelf life. Ivermectin, Albon, and metronidazole are relatively inexpensive drugs to stock and dispense. Praziquantel (Droncit) and fenbendazole (Panacur) are also very inexpensive to use on an individual basis but

relatively expensive to initially acquire. The veterinarian who acquires certain drugs for his reptile patients only needs to cost account the initial purchase, shelf life, etc. to arrive at a fair fee.

Table 1: Reference Materials for Basic Husbandry

Books:

Manual of Reptile Medicine & Surgery
Edited by Douglas R. Mader
Saunders Publishing
At Press

Understanding Reptile Parasites, A Basic Manual for Herp. & Vet.
Roger J. Klingenberg DVM
*Advanced Vivarium Systems
P.O. Box 408
Lakeside, CA. 92040
619-561-5130

*Advanced Vivarium Systems also carries a full line of concise, informational, no nonsense booklets on most of the commonly kept reptiles which are very inexpensive.

Krieger Publishing Company
P.O. Box 9542
Melbourne, FL 32902-9542

1) Iguanas: A Guide to Their Biology and Captive Care by Fred Frye
2) Biomedical and Surgical Aspects of Captive Reptile Husbandry by Fred Frye
3) John Rossi's book on the Captive Care of Snakes of the United States, vol 1.

Journals / Periodicals

Assoc. of Amphibian & Reptilian Vet.
c/o Michelle Frahm DVM
Gladys Porter Zoo
500 Ringgold St.
Brownsville, Tx. 78520

The Vivarium
c/o American Federation of Herpetoculturists
P.O. Box 300067
Escondido, CA. 92030-0067

Herpetological Natural History
c/o International Herp. Symp.
361 Van Winkle Ave.
Hawthorne, NJ 07506

Captive Breeding
P.O. Box 87100
Canton, MI 48187
313-454-0700

Reptiles; Guide to Keeping Reptiles
P.O. Box 6040
Mission Viejo, CA. 92690
714-855-8822

Reptile and Amphibian Magazine
RD 3, Box 3709-A
Pottsville, PA 17901

Symposia Proceedings

Proceedings of the International Herpetological Symposium (IHS)
c/o Dr. Mike Uricheck
Western Conn. State Univ.
Chemistry Dept.
Danbury, Conn. 06810

Proceedings of the North American Veterinary Conferences

Proceedings of the AAZV Conferences

Proceedings of the SSAR Conferences

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DIAGNOSTIC TECHNIQUES IN REPTILE MEDICINE

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The biggest obstacle that reptilian practitioners have is in the acquisition of usable clinical data to aid in their diagnostics. Extremely intelligent practitioners seem to develop a "brain lock" when faced with a reptile patient. When asked to derive a differential for PU/PD in a mammal patient they would have no problem coming up with a list twelve items long. When asked how to confirm such differentials, again they would have no problem deciding which laboratory or diagnostic tests would best help determine the actual diagnosis. However, when asked the same questions regarding the reptile patient, they wouldn't know where to start.

The fundamental knowledge of "diagnostic approach" is inherent in our basic veterinary training. The main challenges in reptilian diagnostic medicine involve two issues. First, a basic understanding of the common conditions affecting reptilian patients is imperative. If you don't know what to look for, you will not know how to test for it. Second, although the concept of utilizing a particular test to rule in or rule out certain diseases is second nature as I have alluded to, the actual application of these tests to the reptilian patient may need to be learned and practiced.

Fortunately, the vast majority of diagnostic tests used in mammalian medicine are also available to the reptilian practitioner. The differences come in learning how to properly interpret the results. The "advances" in diagnostics are nothing new, just reapplication of existing techniques.

Radiology (including CT scans, MRI and Nuclear Imaging), ultrasound, ECG's, and more are all possible. The only limit is the clinician's own ability. This manuscript will introduce the more common diagnostic techniques utilized in reptilian medicine.

Venipuncture

Venipuncture is an easy technique in all reptiles. Exotic animal clinical laboratories are banking normals for the common reptiles, making blood value analysis an extremely valuable diagnostic aid.

I do not recommend toe nail clips for acquiring blood. This is an archaic practice and causes the patient unnecessary pain. There are a number of sites which can be used for blood sampling, directly from the veins, in reptiles. Site preference depends on the species being collected and the size of the animal.

Cardiocentesis, or heart puncture, can be used in all reptiles, although, it is usually only needed in the snake. This is a very safe procedure and can be performed on any size

animal. In very small specimens it may be the only method which can be used. The procedure will be described here as it is done in the snake.

The heart can be easily located by turning the snake on its back. Depending on the sex and species of the snake, the heart can be found about one-fourth to one-third the length of the body behind the head. If the snake is held quietly the heart can be seen moving the scutes, or belly scales, as it beats. If the animal is to be serially bled, it is advantageous to mark the location of the heart with an indelible pen.

Needle gauge will depend on the size of the animal. A 25 gauge, 3/4-1 inch needle works well for the small snakes (less than 1 kg), and a 22 gauge, 1 1/2 inch needle may be needed for larger snakes such as boas and pythons.

Place the tip of the needle under the caudal edge of the scute one or two scales behind where the heart is seen beating. Direct the needle stick craniodorsally at a 45 degree angle. The needle should be felt penetrating the ventricle. When the needle is removed after the sample is obtained apply some digital pressure over the site of cardiocentesis for a few seconds to assist with hemostasis.

A second common site for blood collection in the snake, and an excellent venipuncture site in the lizard, is via the ventral caudal tail vein. It is located along the mid-ventral aspect of the coccygeal vertebra. With the animal on its back, locate the cloaca, and then go caudally about one-third the distance between the cloaca and the tail tip. This should will avoid the hemipenes on male animals.

Position the needle on the ventral midline, bevel facing the tail tip, and direct it toward the ventral vertebrae. When under the skin apply slight back pressure on the syringe and continue in until the needle contacts bone. If a sample has not been obtained, slowly withdraw and redirect slightly in a cranial or caudal direction. In some species the vein is protected by a chevron bone. The first attempt may have contacted one of these chevron bones. So, the needle needs to be repositioned so that it slips in between the two adjacent arches.

Snakes have two veins which run medial to the upper and lower teeth. These buccal veins are easily accessible in the large snakes. A mouth speculum is used to prop the mouth in an open position. A small gauge needle is then used to enter one of the veins and obtain the sample. A big disadvantage of this technique is that the veins tend to bleed profusely. If pressure is not applied after sampling a large hematoma may form. A second disadvantage is that you have to work in the mouth of the animal, and snakes have many very sharp teeth.

There are two places on tortoises and terrapins which are commonly used for blood collection. With the animal on its back and the head and neck extended it is easy to palpate the jugular veins. These are large vessels and are good for collecting blood or placing indwelling catheters.

There is also a venous plexus in the caudal femoral area of the back leg. Extend the leg by grasping the foot and pulling laterally. Insert the needle caudal to the stifle and direct it proximally. Apply gentle suction as soon as it enters the skin. The plexus is not very deep.

Lizards can also be bled from a plexus in their axilla. With the leg extended out and pulled forward, the needle is directed toward the thorax and inserted just proximal to the insertion of the triceps.

There are reports of blood collection in lizards from the ventral abdominal vein. This is a large vein which runs just under the skin, parallel to the midline. A major disadvantage of this technique is that if the vein is lacerated with the needle tip, it is possible for the patient to exsanguinate. There is no way possible to apply pressure to the venipuncture site, much less know that the patient is in trouble until it is too late.

With all of the above techniques, except for the ventral abdominal vein and cardiocentesis, the clinician should be aware of possible lymph contamination while collecting blood samples. All reptiles have extensive networks of lymphatic vessels which surround and parallel the veins. When collecting blood samples it is possible to penetrate these lymph vessels and contaminate your sample. If you are just collecting plasma for biochemistries this will not have a great influence. However, lymph contamination in the sample will have effects on the hemogram.

Urinalysis

All turtles and some lizards have urinary bladders. Snakes lack a bladder. They store their urine in the ureters and colon. Urinalysis is an important part of any standard data base, and should be included in any reptilian work-up.

Table top urine can be collected from all species. Although this is the least favorable method, when analyzed carefully the results may still be significant. For instance, if a snake urinates on a table just cleaned with some type of potent disinfectant, the urine can be easily collected with a syringe for analysis. Obviously, this will be a contaminated sample. However, if you culture out a pure *Klebsiella* from this sample, it is most likely real, and not just an environmental contaminant.

In the lizards and the turtle urine can also be collected using a catheter. This is not easy, and also results in a contaminated sample (although still preferable to the table top). The reptilian cloaca is divided up into three chambers, the corpradeum (opening of the colon), the urodeum (openings of the urogenital papilla in snakes, and openings of the urogenital papilla and urethra in chelonians and lizards that have a bladder), and the proctodeum (final chamber where all the material mixes and is held prior to elimination). The best way to practice collecting urine from a reptile using a catheter is to try it on a cadaver first. Even then, it is still difficult.

Perhaps the best method for collecting urine is via cystocentesis, just as in mammals. The bladder of the chelonian is large and bi-lobed. The right liver lobe is the largest, and as a result, the bladder tends to be pushed more toward the left side of the animal. Urine can be sampled from the area just cranial to the thigh which is under the carapace. The skin should be aseptically prepped. With the turtle positioned vertically, in a nose up orientation, the collection needle should penetrate this pre-femoral region and directed medially, aiming just cranial to the pubic region. A 22 g, 1-1/2 inch needle reaches the bladder in most patients.

In those lizards with bladders, the approach is similar. I prefer to collect samples from a point just cranial to the left thigh rather than making a midline antepubic approach. The ventral abdominal vein may interfere with urine collection, and should be avoided whenever possible.

Radiology

Radiology is becoming more popular as a diagnostic tool in reptilian medicine. Unfortunately, where in mammalian medicine we have years of experience and normals to rely on for comparison, in the field of reptilian radiology there are still a lot of "seat of the pants" interpretations. There is the added difficulty of obtaining high resolution, diagnostic quality films through the thick, often ossified dermis.

Fortunately, many of these problems can be overcome with practice and patience. Patience is probably the most important ingredient in successful reptile medicine, and in reptilian radiology, it is absolutely essential.

As with small mammals, many reptiles can be easily radiographed without sedation. Turtles and tortoises will often withdraw into their shells in novel surroundings, thus making positioning easy. Some however, will walk endlessly regardless of their placement. These animals can be placed in a radiolucent container such as a lucite restraint box, or a simple alternative is a rigid cardboard box with the bottom cut out.

If an animal is restless it can often be calmed by placing an inverted bucket, small trash can or cardboard box over the pet for a few minutes. By leaving the animal in a dark, quiet place, it will often calm down long enough to take the radiograph. The plate should be marked, the beam centered and the control unit set. When everything is ready the rotor is started and the cover is removed. The radiograph is exposed before the animal has a chance to move.

The dorsoventral (DV) view in turtles and tortoises is useful in evaluating osseous integrity, overall conformation, gastrointestinal disorders and urinary tract (specifically bladder) abnormalities. It is of very little use in evaluating the respiratory system.

To properly evaluate the lung fields and air sacs horizontal beam radiographs must be taken. Some references advocate using a vertical beam and just spatially positioning the animals

is done with mammals. However, in the experience of this author better visualization of the pulmonary spaces can be obtained with the use of horizontal beams. Cardboard boxes, lucite pedestals and foot stools all work well to place the animal in a position for a horizontal beam.

Measurements for establishing radiographic techniques in turtles and tortoises can be a challenge. Technique charts vary with the machine used, but in general, settings which are adequate for skull settings for dogs provide proper penetration of the osseous shell in chelonians. The shell should be measured at its widest part (just cranial to the rear legs), it highest point (at the crown of the carapace) and its maximum length (from the caudal most portion of the carapace to the gular notch).

The craniocaudal and lateral view horizontal beams permit good visualization or the air spaces. Evaluation of the "fullness" of the gastrointestinal system is possible, but detail of the GI structures are lost in these views. Animals in good flesh will have filled intestinal loops. In anorectic or cachectic patients the air space seems uncharacteristically large.

Extremity views (including the head and neck) of chelonians are usually overexposed when using techniques for evaluating the shell or internal structures. Additionally, many of these animals are so confined within their shells, the scutes from either the carapace or the plastron obscure the limbs from view. It is necessary to extend these body parts for proper measurements and exposures. Light gauze tied to the extremity can be used to extend the body part for a brief time to allow exposure. Gentle traction is more effective than brute force.

However, in some chelonians, it is not even possible to gain access to these limbs. In these patients it may be necessary to use sedation or tranquilization to get the appropriate views. Tortoises and terrapins which are reluctant to come out of their shell can be relaxed with 0.5 - 1.0 mg/kg of succinylcholine, given either subcutaneously (SQ) or intramuscularly (IM). This will relax the animal enough not only to get the needed radiographs, but also to withdraw its head and limbs from the shell, obtain a blood sample or any number of other quick procedures. Caution should be taken since succinylcholine may cause respiratory depression at the higher doses.

An alternative, which can also be used for snakes and lizards, is either Ketamine HCl at 40 - 60 mg/kg, or Telazol at 4 - 20 mg/kg. Both agents can be given either subcutaneously or intramuscularly.

Snake radiology is technically much easier than in turtles and tortoises. The DV view is should be the minimum of any study, but a proper assessment warrants an additional lateral perspective. The DV can be obtained much the same as with a tortoise. Placing the patient in a radiolucent box is an excellent method for restraint.

An additional restraint technique involves placing the snake inside of a lucite tube. The inside diameter of the tube should approximate the circumference of the patient. This will

prevent the snake from turning back on itself within the tube, or "kinking" during the radiograph. When the snake is safely restrained within the tube it can then be positioned for DV, lateral and oblique radiographs. This technique is invaluable when working with venomous or dangerous animals.

Radiographs of small snakes in a straight line (as when they are placed in a restraint tube) can often be performed on a single plate. DV views of an entire animal can be accomplished by allowing the patient to curl up in the bottom of a box or bucket, providing that the animal doesn't overlap on itself.

Lager patients need to be radiographed sequentially. This is especially important with animals many feet in length, since many sequential body sections are nearly identical. Each film should be marked with a sequencing number on each side of the plate (eg. 1 - 2, 2 - 3, 3 - 4 etc.) A small piece of labeling tape, placed directly on the patients scales, works well for this. A small area of overlap should be included on each film. If possible, the lateral views should be included in the same orientation and position on the same plate next to the DV views.

Radiology in lizards presents the greatest challenge to the reptilian practitioner. Not only can these animals be very skittish, many of them have such diverse body shapes normal positioning is nearly impossible.

Many of the tricks for restraint mentioned for tortoises and snakes can be employed for lizards. Patience and gentle handling will allow positioning for most DV views. Some animals must be covered with lucite boxes to prevent them from darting off the radiology table. Sedation and tranquilization may be warranted if proper positioning cannot be accomplished by standard techniques.

The body conformation of many of the large body lizards allow for basic DV and lateral views. However, some of the lizards, such as the monitors, are dorsoventrally flattened, thus making consistent lateral radiography difficult. Even with this technical difficulty, a lateral view should always be a part of a radiographic survey.

A working knowledge of reptilian anatomy is essential for proper evaluation of radiographs. Fortunately, reptilian anatomy is fairly basic compared to mammalian anatomy. Written descriptions of pathology are no substitute for actually visualizing radiographs of normals and clinical conditions. Currently there are no texts dedicated to reptilian radiology, however, there are two recent publications which have excellent chapters on the subject. "Radiology and Imaging," written by Rubel et. al in Frye's Biomedical and Surgical Aspects of Captive Reptile Husbandry (Krieger, 1991), and The Atlas of Diagnostic Radiology of Exotic Pets by Rubel et. al (W B Saunders, 1991) are both readily available to the non-domestic practitioner.

Once a basic level of competency and confidence has been attained when performing radiology in these animals has been attained, the practitioner can then utilize the various

special techniques commonly employed in mammalian radiology to enhance visualization of the various organ systems. Gastrointestinal barium contrast studies, venography, bronchography etc, can all be performed just as is done in dogs and cats.

Radiography can greatly augment the reptilian practitioners diagnostic capabilities. As with anything in non-domestic pet medicine, it is just a function of taking our knowledge of domestic animal internal medicine and adapting it to the peculiarities in the species of our special interests.

Endoscopy

Endoscopy, both rigid and flexible, is an extremely valuable tool in reptile medicine. This is warranted in any patient exhibiting signs relating to the gastrointestinal tract. Visualization, lavaging, and sampling for microbiological cultures and cytology are all possible.

Rigid endoscopy of the coelomic cavity is also useful. This is a preferable technique for collecting biopsy samples, especially of the liver and the kidneys. Some reptile patients have extensive fat pads which make good visualization of the coelomic cavity difficult. Since reptiles lack a diaphragm it is important to not over-insulflate the body cavity, as it makes respiration difficult for the anesthetized patient.

Special imaging

Ultrasound, CT scans, MRI and nuclear imaging are all possible. These techniques are all relatively new to reptile medicine, and as yet normals are still being established. But, this should not discourage the ambitious clinician from attempting any of these techniques. When questions arise, it is recommended to perform the identical test on a "normal" animal of the same species for comparison.

COMMON NON-INFECTIOUS DISEASES OF REPTILES

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Non-infectious diseases of reptiles remain as some of the most commonly seen problems in private practice. The most commonly seen diseases will be discussed here.

Metabolic bone disease is a complicated disease process involving many factors, the end result of which leads to pathologic fractures, fibrous osteodystrophy, and/or rickets. Environmental temperatures, diet, renal disease, hepatic disease, photoperiod, parathyroid abnormalities and ultraviolet radiation can all play a role in the development of metabolic bone disease. Treatment involves proper diagnosis, and correction of underlying management practices. Specific treatment may involve the use of calcitonin, injectable or oral supplementation with calcium at 500mg/kg, and optimal environmental daytime temperatures, depending upon species preference.

Vitamin deficiencies and oversupplementation are commonly encountered problems of reptiles. Turtles deficient in vitamin A commonly exhibit palpebral edema, blepharitis, epiphora, and nasal exudates. The epithelium undergoes a squamous metaplasia, which can lead to secondary ocular and respiratory infections. An accompanying history of diets low in vitamin A (i.e., ant/fly eggs) often compliments the clinical signs. Most turtles respond to injectable vitamin A preparations (Aquasol A, Armour Pharmaceuticals) at 500-2,000 IU/kg subcutaneously once weekly for 4-5 treatments. Careful dosing is necessary, as oversupplementation has been seen at injection sites as epithelial necrosis. Vitamin D3 deficiency alone causes rickets, and is essential in transporting calcium across the intestinal wall. Current discussions advise the use of full spectrum ultraviolet lighting with a wavelength of 290-320nm over oral supplementation. Oral supplementation has been shown to be ineffective in achieving adequate blood levels. Full spectrum adequate lighting is believed to be achieved by combining the use of a blacklight (BL) with a fluorescent white light (Vitalite). Hypervitaminosis D can lead to ossification of soft tissues, confirmed by radiographs. No treatment currently exists.

Gout is the end result of an inability of a reptile to properly excrete uric acid. Uric acid is the metabolic end product of protein catabolism, specifically purine metabolism. Visceral gout is the deposition of uric acid in the parenchymous organs, and articular gout is the deposition of uric acid within the joints, and appears less commonly than visceral gout. Dehydration, renal disease, and excessive dietary protein are the common causes. Treatment involves rehydrating the reptile, evaluating the diet, correcting underlying renal diseases, and when warranted, the use of allopurinol and colchicine.

Husbandry related problems are probably the most commonly encountered non-infectious problems of reptiles. Proper education of the client prevents the majority of what can be frustrating and expensive problems to treat. Rostral abrasions can often

be prevented by providing hide boxes. Rodent bites are most easily prevented by feeding dead food items. Thermal burns can be avoided by utilizing proper caging and heating sources. Shell fractures are routinely seen and treated with standard epoxy-resin kits or dental acrylics.

CURRENT TECHNIQUES IN REPTILE ANESTHESIA AND SURGERY

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Anesthesia

The lungs of most reptiles are simple endothelium lined sacs attached to bronchi. The total lung volume is greater than that of mammalian lungs, but the surface area for gas exchange is much smaller. The left lung is absent or vestigial in most snakes. The lungs of reptiles are very fragile and care must be taken when positive pressure ventilation is performed to avoid rupture of the lung. The trachea of chelonians and crocodilians is composed of complete rings while the tracheal rings of squamates are incomplete. Chelonians have a very short trachea so care must be taken to avoid intubation of a single lung.

The position of the glottis varies among reptiles being rostrally located in snakes, more caudally located and partially obscured by the tongue in chelonians, and further obscured by the well developed epiglottis in crocodilians. The glottis remains closed during states of rest and a glottis dilator muscle opens the glottis to allow breathing. Intubation, however, is generally not difficult to accomplish.

Reptiles do not have a functional muscular diaphragm and, thus, have a pleuroperitoneum or coelomic cavity. Reptiles generate negative pressure in the lungs by 2 methods. Most reptiles use intercostal muscles aided by muscles of the trunk and abdomen to generate negative pressure. The wall of the lung also contains smooth muscle which contracts and relaxes to move air. These mechanisms allow reptiles to breathe even if there is a defect in the coelomic cavity as occurs with abdominal surgery. Chelonians are not capable of intercostal movement but change intrapulmonary pressure by movement of the viscera, limbs, and pelvic girdle. Because the lungs lie dorsal to the viscera, positioning chelonians in dorsal recumbency will compress the lungs and reduce their tidal volume.

In addition to alveolar ventilation, reptiles employ other surfaces for gas exchange, such as cloacal and pharyngeal. Cutaneous gas exchange is also a part of reptilian respiration. Many reptiles are capable of converting to anaerobic metabolism when they "breath-hold".

If the patient's condition permits, pre-anesthetic fasting is recommended. Aspiration is not common in reptiles, but, because the tidal volume is affected by visceral volume, fasting may allow for improved ventilation. It is somewhat difficult to monitor depth of anesthesia in reptiles because it can be difficult to visualize respiratory and cardiac movements. The 3 chambered heart of most reptiles does not produce readily auscultable sounds, making stethoscopy difficult. An ECG monitor is very valuable in monitoring the anesthetized reptile patient. The QRS patterns of reptiles are generally inverted and slurred. As reptiles become anesthetized, relaxation progresses from cranial to caudal and during recovery, motor function returns in the opposite direction. The righting reflex is lost early during anesthetic induction but is a useful indicator of recovery. Assisted ventilation may speed

recovery but is not usually necessary. Failure to elevate the ribs when a finger is run down the back or failure to move the tail when the vent or foot is squeezed indicate loss of spinal reflexes and a surgical plane of anesthesia. In chelonians, the head withdrawal reflex is also useful. Corneal reflex should be present at a surgical plane and when abolished the patient is excessively deep. Tongue withdrawal in snakes is present at a surgical plane and lost if the patient is too deep.

Recovery should occur in a quiet environment with the temperature and humidity at the upper end of the optimum range. Excessive warmth may be deleterious by excessively increasing patient activity which increases tissue oxygen demand.

Tranquilizers

Acepromazine at 0.1-0.5 mg/kg IM approximately 1 hr prior to induction of anesthesia has been reported to lower the required dose of induction agent. This agent may also be used as a tranquilizer.

Local anesthetics

Reptiles are very sensitive to skin stimulation but local anesthetics are effective.

Injectable anesthetic agents

These anesthetics require little equipment and may be more familiar to some veterinarians, but once given the effects cannot be reversed and the depth is difficult to control. Many are best given by the IV route which may not be feasible with some reptile patients. The effects of injectable agents in reptiles are often unpredictable. The same dose given to 2 different animals may yield no effect in one while producing profound anesthesia in the other. Reptiles also seem to require unusually high doses of some agents making their use very costly. **Narcotics** are of little value in reptile anesthesia. It is unknown why reptiles are refractory to opiates.

Barbiturates have a long and unpredictable induction time and a very long recovery period. Size, condition, nutritional status, temperature, handling, and physiology of the individual animal may influence the effects of barbiturates making their use in reptiles questionable.

Dissociative anesthetics such as phencyclidine, ketamine, and tiletamine have been used in reptiles. **Ketamine** has been successfully used in all orders of reptiles, though the response is dose dependent and the effects vary with the species and individual. It is most useful for induction of anesthesia for intubation, especially in "breath holding" species. A dose of 22-44 mg/kg IM or SC has been recommended for sedation and 55-88 mg/kg for surgical anesthesia. Generally, at doses >110 mg/kg respiratory arrest and decreased heart rate make ventilatory support necessary. Induction usually occurs in 10-30 min. and recovery from 24-96 hrs. At a surgical plane, some animals will exhibit serpentine movement which

can be controlled by taping the patient to the table. Ketamine may be the most satisfactory injectable anesthetic, but when used in debilitated patients, recovery may be dangerously prolonged (up to 6 days).

Tiletamine + Zolazepam (Telazol) - Tiletamine's action is similar to that of ketamine but it is 2-3 times more potent making the volume of administration smaller. Tiletamine alone causes seizures but in combination with zolazepam with which it is synergistic, they produce anesthesia, analgesia, and muscle relaxation with anticonvulsant and antianxiety effects. Animals are very sensitive to stimulation while under the effects of Telazol resulting in excessive movement. There is great species variation in response to this agent. Because of the rapid onset of effect, Telazol may be most useful as an induction agent or for sedation at 4-5 mg/kg IM.

Neuromuscular Blocking Agents have primarily been used for restraint of large crocodilians and chelonians. **Succinylcholine** is a depolarizing agent which has been used most commonly for this purpose. The dosage appears to be variable among species and sizes of crocodilians. A dose of 3-5 mg/kg used in American alligators weighing 2-5 kg resulted in immobilization in <4 min with recovery complete in 7-9 hr. The IM administration of succinylcholine is important because the drug may not be absorbed rapidly enough to be effective when administered SC. Other neuromuscular blockers have been used with variable success.

Inhalant anesthetic agents

These agents offer several advantages over injectable agents. Most inhalant agents can be used without a precision vaporizer in reptile patients. Its use allows long term anesthesia to be maintained accurately.

A nonrebreathing system should be used on patients <5 kg with an oxygen flow rate of twice the minute volume (300-500 ml/kg/min). The normal respiratory rate for most reptiles is 2-4/min. A circle system may be used for larger reptiles with 2-4 L/min oxygen for induction and 1-2 L/min for maintenance.

Methoxyflurane (MOF) has a slow induction and recovery when compared with other fluorinated hydrocarbon volatile anesthetics. The open drop technique can be used placing 10 ml MOF in a 42,800 cm^3 box. A single exposure provides 10-30 min of surgical anesthesia. If more time is needed the patient should be intubated and maintained using a precision vaporizer.

Halothane may be used for induction using a precision vaporizer or the open drop method. With the vaporizer, induction may be achieved using 2-5.5% halothane in oxygen and maintenance using 1.5-2.5%. For the open drop technique, 5 ml halothane are placed in a 2,840 cm^3 box. Induction with this technique occurs in 5-33 min and has a duration of 5-20 min following a single exposure. Reptiles generally go through an excitement phase during induction with halothane.

Isoflurane is eliminated exclusively by the lungs and, therefore, causes minimal metabolic compromise making it the agent of choice for use in debilitated patients. A concentration of 4-5% isoflurane in 3-4 L/min oxygen has been used to induce anesthesia in 6-20 min. Maintenance at 1.5-4% isoflurane resulted in recovery in 30-60 min. Because it has a vapor pressure and maximum concentration similar to halothane, the open drop method of induction should be as effective for isoflurane as for halothane.

Surgery

Anatomy

The anatomy of reptiles varies among orders, families, and species. A knowledge of the basic features of reptilian anatomy is therefore vital to surgeons.

Except for most snakes, reptiles have a cecum. The stomach of crocodilians has 2 compartments. The first is very muscular and frequently contains stones. The second is similar to the glandular stomach of mammals. All reptiles have a gall bladder. The liver of many reptiles contains melanin and can have black spots or streaks. Reptiles generally have little subcutaneous fat and store fat in discrete masses (called fat bodies) in the caudal abdomen.

The metanephric kidneys of reptiles are lobulated. One or more renal arteries can be present to receive blood from the renal portal system. The nitrogenous wastes of reptiles are in the form of ammonia, urea, uric acid, or a combination of these. Crocodilians, snakes, and some lizards do not have a urinary bladder. In chelonians and those lizards with a bladder, it is connected to the cloaca by a short urethra. Urine passes into the cloaca and then into the urinary bladder, if present, or into the distal colon where water resorption occurs.

The cloaca typically consists of 3 chambers. The coprodeum is the most cranial and receives fecal material and urinary wastes. The urodeum is the middle section and receives genital secretions and urinary wastes from the urogenital ducts. The caudal proctodeum acts as a reservoir for fecal and urinary wastes before they are excreted. This is also the location of the openings of the musk glands.

The skin of reptiles is dry and virtually devoid of glands. Many lizards have femoral glands, which open on the medial aspects of the thighs. Crocodilians have a pair of scent glands in the medial aspects of the lower jaw and another pair within the cloaca. In proposing sites for incisions, these glandular areas should be avoided. The skin of most reptiles is made up of scales and scutes. Soft shelled turtles and some lizards do not have scales but have a leathery, smooth skin. Crocodilians and some lizards have calcific plates, called osteoderms, located within the dermis designed for protection. Incisions can usually be made between osteoderms. The shells of chelonians are composed of bony dermal plates covered with

keratinized epidermal shields. The carapace contains 10 fused thoracic, lumbar, and sacral vertebrae as well as the ribs. The plastron and the carapace are joined at the so-called bridge.

Histologically, the epidermis is composed of 3 layers. The outer **stratum corneum** is heavily keratinized, acellular, and has a serrated surface. The middle **intermediate zone** is composed of daughter cells of the **stratum germinativum** in various stages of differentiation. These 3 layers are present during the skin's resting phase. As ecdysis begins, the cells of the stratum germinativum undergo synchronous mitosis to form a new intermediate zone and stratum corneum under the old generation. The action of enzymes breaks down the cells of the base of the old intermediate zone, and the subsequent influx of lymph causes separation between the old intermediate zone and the new stratum corneum. Blood vessels and sinuses in the head become engorged and cause it to swell. The old skin splits and ecdysis is completed by the animal rubbing off the old skin. In squamates, this process occurs simultaneously over the entire body. In chelonians and crocodilians, proliferation and keratinization are continuous and shedding occurs only at the flexible regions of the body. This produces growth rings between these scales as they grow and the previous, smaller layers are not lost. The frequency of ecdysis is proportional to the growth and metabolic rates of the animal. Age, environmental temperature, availability of food, and space can influence the frequency. In squamates, the cells of the epidermis are mitotically active only during ecdysis.

Patient preparation

Ideally, laboratory data should be obtained before induction of anesthesia. Because of the small size of many patients and the inaccessibility of most peripheral veins, blood samples are often difficult to obtain. Environmental conditions, time of day, and laboratory variations can influence blood cell counts and biochemistry data making interpretation difficult. If blood samples can be obtained repeatedly, trends provide valuable information. The hydration and nutritional status of patients is assessed as it would be for mammalian patients. Balanced electrolyte solutions can be given IV or IP.

Reptiles are susceptible to a variety of microbial infections. It is imperative that aseptic technique be used. Many cutaneous infections result in septicemia and lead to visceral granuloma formation. Perioperative antibiotic therapy is more appropriate if intraoperative contamination is anticipated. It has been suggested that amikacin be used in snakes at a loading dose of 5 mg/kg followed by 2.5 mg/kg every 72 hr. Gentamicin at 2.5 mg/kg every 72 hr maintains adequate therapeutic plasma concentrations in gopher snakes and red eared slider turtles. In view of the long plasma half life of these antibiotics, one dose prior to surgery should provide perioperative coverage.

Patient positioning is a challenge especially for legless and small reptiles. For snakes, a sterile stockinette can be rolled over the surgically prepared patient. The snake can then be placed on a sterile drape providing an aseptic field. The dome shape of the carapace of chelonians makes it difficult to position a patient in dorsal recumbency. A towel can be

rolled into a ring such that the carapace will fit into the ring and prevent the patient from rolling. Clear plastic adhesive drapes are very useful in reptiles. The entire patient remains visible under the sterile drape allowing for proper anesthetic monitoring. Sterile spray adhesives can also be used to allow paper or cloth drapes to stick to the patient avoiding the use of towel clamps.

Instrumentation

With a few exceptions, the instruments needed for surgery on reptiles are found in a general surgical pack. Most abscesses in reptiles contain caseous, inspissated pus. Dental curettes and cerumen loops help in removing this material. Eyelid retractors work well as abdominal retractors for small patients. With most chelonians, some type of saw or drill is needed to approach the coelomic cavity and a restorative material should be available for repair of shell defects and celiotomies.

The edges of incised reptilian skin have a tendency to invert. An everting suture pattern, such as a horizontal or vertical mattress, achieves accurate skin edge apposition. The relatively tough reptile skin and scales help prevent sutures from tearing through. The break down of absorbable materials appears to be prolonged in reptiles and if used in the skin, removal is recommended after the incision has healed. Chromic catgut was still present in a rhinoceros viper 12 weeks after the material was used in the pleuroperitoneum and SQ tissue. It appears to be best to use materials which are absorbed by hydrolysis rather than proteolysis in reptiles. In squamates, suture removal should be performed after the ecdysis subsequent to surgery. The shed skin usually sticks in the sutured area for several ecdyses postoperatively, but can be gently peeled away.

Postoperative care

Anesthetic recovery in reptiles can be prolonged and difficult to monitor. Increasing the environmental temperature to the upper end of the optimal range (30-36° C) will increase the rate of metabolism of anesthetic agents. Once the patient is awake and responsive, it should be placed in a warm, dark, quiet place to complete its recovery. Clean paper should be provided in the recovery area to prevent contamination. Hibernation should be delayed for at least 6 mo as it delays healing. Swimming should be prevented for 7-14 days after surgery. Fluid therapy may be administered IV or IP as needed to maintain hydration. Many reptiles become anorectic after surgery. Force feeding or tube feeding might be necessary.

Skin wounds of reptiles undergo phases of healing similar to those observed in mammals. Wounds strengthen slowly and skin sutures are generally not removed until at least 4-6 wk. Many factors influence wound healing in reptiles. Maintenance of the environmental temperature at the high end of the optimum range promotes healing. In snakes, cranial to caudal wounds heal faster than dorsal to ventral wounds. Open wounds heal well by second intention with a low incidence of infection.

Celiotomy

Indications for celiotomy in reptiles include egg binding, egg peritonitis, gastrointestinal obstruction, ovariohysterectomy, colopexy for colon prolapse, cystotomy for calculi, and exploration for biopsy. The technique varies depending on the family to which the patient belongs.

In snakes, abdominal incisions can be made at the lateral margin of the scutes or between the first 2 rows of lateral scales. Incisions should be made between rather than through scales if possible. The tips of the ribs should be avoided at the junction of the scutes and scales. The lateral approach is generally preferred over a ventral midline approach as it is easier to keep clean. The suture line is not in direct contact with the substrate and is not stressed by rectilinear motion. Three layers are encountered: skin, muscle, and pleuroperitoneum. When separate layers are not identifiable, a single-layer closure is adequate.

Paralumbar and midline incisions have been recommended for approaching the coelomic cavity of lizards and crocodilians. The ventral abdominal vein is a very large vein located inside the body wall on the ventral midline. It should be avoided during celiotomy by using a paramedian approach.

In chelonians with a small plastron, the majority of abdominal structures can be approached through an incision between the plastron and the femur in the flank region. In other chelonians it is necessary to perform an osteotomy of the plastron. The pelvic bones should be avoided and can be identified using radiography. Usually the femoral and abdominal shields are osteotomized for the approach. A high-speed burr or an orthopedic saw is used to cut the plastron. Irrigation is used to dissipate heat and to remove dust. The bone is elevated from the underlying abdominal musculature using a periosteal elevator. The incision into the abdominal wall can be performed using a flap technique or a ventral midline incision. There are venous sinuses on each side of the midline approximately midway between the midline and the bridge. These sinuses should be avoided but can be ligated if necessary. The bone is replaced using restorative material as is described below.

Shell fracture

Small defects or cracks in the shell of chelonians can be maintained in reduction with wires, external bandages, or acrylic materials. Acrylic materials, such as those used for hoof reconstruction and dental repairs, can be used to hold fragments in apposition. The fracture should be maintained in reduction for 3-7 days without exposure to water so that a seal can form. The fixation should not be removed until there is radiographic evidence of union. Large defects should be repaired using prostheses. Various restorative materials have been used, including hoof or dental acrylics, boat or autobody fiberglass, and epoxy resin. Patches of fiberglass cloth can be autoclaved. The fiberglass provides a matrix to enable the resin to bridge the defect. The patch should be large enough to extend beyond the margin of the

defect. The shell should be cleaned with acetone, ether, or similar degreaser. During application, care must be taken to keep epoxy from the edge of the defect as its presence will delay healing. The fiberglass patch is stretched over the defect and held in place, allowing the resin to penetrate the cloth and bond to the shell surface. When this layer has cured, a light coat of epoxy is applied to the fiberglass cloth over the defect. After this layer has cured, several more thin layers of epoxy should be applied to strengthen and seal the defect.

If a large fragment is to be replaced, as in the case of closing a celiotomy, the piece should be bonded to the center of the cloth patch with epoxy before the patch is applied to the defect. Healing of bone in reptiles takes at least 6-18 mo. In growing chelonians, the patch should be removed from the growth rings after healing is complete to allow the shell to continue to grow. Epoxy dust can be toxic and carcinogenic to humans. Copious irrigation should be used to prevent aerosolization, and a face mask should be worn.

Dystocia

Clinical signs of dystocia include anorexia, regurgitation, straining, cloacal discharge that is often malodorous, paresis, respiratory distress, and edema of the cranial extremities. Noninvasive procedures should be attempted before surgical intervention. Intramuscular oxytocin at 1-10 IU/kg and IM or SQ 1% calcium borogluconate at 10 ml/kg have been successful to relieve dystocia when manipulation was not. In species that produce soft and leathery eggs, percutaneous ovocentesis can collapse the eggs and allow them to pass more easily. Salpingotomy is indicated if noninvasive techniques fail or if there is radiographic evidence that natural passage is not possible. In snakes, it might be necessary to make more than one incision to access all eggs or fetuses. The incision in the salpinx and uterus should be repaired with an inverting suture pattern of an absorbable material. Salpingohysterectomy should be considered if dystocia recurs, if the patient is not being maintained for breeding purposes, or if bacterial salpingitis is present. The ovaries of many reptiles are not pedunculated making them difficult to remove. Removal of the ovaries may not be necessary. During salpingohysterectomy the oviduct should be pulled free from the ovary and the uterus should be ligated as close to the cloaca as possible.

The presence of egg yolk within the coelomic cavity produces severe inflammation. Fibrin deposition and serosal thickening are typical. Surgical removal of the yolk material and lavage are indicated, however, the prognosis in such cases is grave.

Cloacal organ prolapse

The cloaca has openings from the colon, uterus, urinary bladder, and reproductive tract. Ureteral prolapse has not been reported in reptiles.

Squamates have paired copulatory organs called hemipenes which lie inverted within the tail. Chelonians have a single penis which is everted during copulation. Although prolapse of the penis or hemipenes has been reported as a sequel to constipation and neurological

dysfunction, it is most frequently the result of infection, forced separation during copulation, or swelling secondary to probing for sex determination. The organ should be cleaned, gently lubricated and replaced. A purse string suture is placed in the cloaca tight enough to prevent prolapse but to allow voiding. The suture should be left in place for 3-4 weeks. If the prolapse cannot be reduced, the cloacal opening can be enlarged by incision. Surgery is indicated in cases in which the organ is severely swollen and damaged. Amputation is performed after mattress sutures are placed at the base of the organ to prevent hemorrhage. Snakes and lizards with one hemipenis are considered fertile.

Prolapse of the uterus is rare but does occur. Replacement should be attempted. If reduction is not possible, celiotomy and salpingohysterectomy should be considered.

Colon prolapse can result from straining because of constipation or bacterial or parasitic enteritis. Conservative management should be attempted before surgical therapy. Often colon prolapse is reducible and successfully managed by treating the primary cause while maintaining a purse-string suture in the cloaca. Frequently the venous return from the prolapsed colon is severely compromised and it becomes engorged and friable. Celiotomy and colopexy are recommended in such cases. An area of healthy colon should be selected and sutured to the body wall. If the colon is severely compromised, it can be resected and anastomosis can be performed.

Gastrointestinal procedures

Principles of gastrointestinal surgery in reptiles are similar to those in mammals. The intestines of most reptiles are thin walled, and the use of fine sutures and an atraumatic needle is recommended. Such sutures as polydioxanone are strong and maintain their tensile strength for several months in mammals which can be advantageous in slow healing reptiles. If the affected section cannot be adequately exteriorized, it should be well packed off before enterotomy. Copious coelomic lavage with saline should be performed before closure.

Cystotomy

Cystic calculi can occur in those reptiles with a bladder but desert tortoises seem to have the highest incidence. Clinical signs associated with cystic calculi are nonspecific and include anorexia, lethargy, and depression. The urinary bladder is generally very mobile within the coelomic cavity and is easily isolated during surgery. A 2 layer closure using an inverting pattern of absorbable suture is preferred.

USE OF COMPUTED TOMOGRAPHY AS A DIAGNOSTIC AID IN THE DIAGNOSIS OF AN ABDOMINAL MASS IN A BOX TURTLE

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An adult box turtle (*Terrapene sp*) has a one week history of cloacal prolapse. during physical examination, a left upper quadrant mass is palpated. The mass is firm and nonpainful. Whole body radiographs show an increase radio-opacity in the upper left coelomic area. Hematologic and serum biochemistry results are within normal limits. Fecal samples are negative for parasite. A whole body computed tomography scan revealed numerous follicles and a large round mass along the left side of the coelom. It appears calcified. A subsequent barium radiographic study shows the gastrointestinal tract not to be associated with the mass. It is decided to explore the coelomic cavity of the turtle. A trapezoidal section of shell is cut from the plastron and the coelom is entered. A large, firm, white mass is present along the mid to upper left aspect of the coelom. It is attached to the perineum, the liver and the lungs. It is only partially debrided, as it is firmly adhered to the lungs. Numerous white / yellow plaques are present on the serosal surfaces of all organs. The liver and yellow plaques are taken. The coelom is the flushed with warm sterile saline and closed. The plastron is reattached with k-wires. Four days after surgery the turtle dies at home. Histopathology reveals the mass to be infiltrated with lipocytes and no normal tissue is observed. *Klebsiella oxytoca* and *E. coli* are cultured from the liver and *Proteus penneri* is cultured from the celomic mass. This case illustrates the use of computed tomography as an aid in the diagnosis of a coelomic mass in a box turtle. Subsequent exploratory examination reveals the mass to be an abscess. There are also multiple abscesses throughout the abdomen and a greatly infiltrated liver is present. The cause of the cloacal prolapse is not definitely identified but may have been due to the disease processes in the coelom.

CHEMOTHERAPEUTIC TREATMENT OF AS SARCOMA IN A CORN SNAKE

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A fifteen year old corn snake (*Elaphe guttata*) has a two month history of a large, ulcerated wound on the distal one-third of its dorsum. the wound had exposed muscles. is oval in shape, and is approximately 5 cm in length. The snake has good muscle function distal to the lesion and the cloaca has normal function. Hematologic and plasma biochemistry values are within normal limits. Radiographs reveal lysis of the vertebrae associated with the lesion. At surgery, the lesion is debulked and debrided of necrotic material and flushed with an antibiotic solution and biopsy and cultures are submitted. Culture results reveal *Proteus mirabilis* and *E. coli.* Biopsy reveal a sarcoma. Antibiotic therapy consisting of parental administration of enrofloxacillin and dilute nolvasan wound flushes were instituted based on culture results. It was also decided to treat the sarcoma with adriamycin at 1 mg/kg intravenously. The medication was given once a week for two weeks, then once every two weeks, then once every three weeks, for a total of six doses of adriamycin. Treatment with this drug necessitated vascular access and this was accomplished with a vascular access port placed in the azygous vein. The end of the catheter entered the right atrium. During the treatment period, the ulcerated mass remained quiescent until 3 months into therapy, when the wound enlarged and the snake died at home. Necropsy revealed the sarcoma to still be present. This case illustrates the use of chemotherapy to treat neoplasia in a reptile. It also describes the use of a vascular access port in a snake to facilitate intravenous injections of a drug.

TWO CASES OF ANEMIA IN REPTILES TREATED WITH BLOOD TRANSFUSIONS: (1) HEMOLYTIC ANEMIA IN A DIAMOND PYTHON CAUSED BY AN ERYTHROCYTIC VIRUS; (2) NUTRITIONAL ANEMIA IN A BEARDED DRAGON

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Case 1

A female diamond python (*Morella spilota spilota*) newly received from another institution was found on arrival August 5, 1993, to have numerous problems: cachexia (weight 1.01 kg), markedly decreased skin turgor and opaque, wrinkled spectacles, indicating significant dehydration; poor muscle tone, no attempt to coil and minimal response to stmuli; faintly green oral mucous membranes and dark green urates; several blisters, foci of necrotic dermatitis, numerous subcutaneous abscesses (4-5 mm in diameter) and a moderate mite burden; and several areas up to 30 cm in length where all ribs on one or both sides had recent unstable fractures. The snake's exact history was unclear, as the other institution, upon being informed of her condition, advised that they had mistakenly sent a recently arrived injured wild animal instead of the intended captive-bred individual.

Blood was collected to investigate the apparent "icterus." Results, when compared with reference values for the species (see Tables 1 and 2) indicated low PCV, elevated TPP, profound biliverdinemia, lymphopenia (0.16 x10^9/L)(total leucocytes 7.8 x 10^9/L, azurophils 4.1 x 10^9/L, heteropils 3.6 x 10^9/L, heteropils 3.6 x 10^9/L and GGT, AST, CK and uric acid within normal range. Examination of films stained with Diff Quik (Lab Co., Australia) revealed normal leucocyte morphology, but very abnormal erythrocytes. Only 5% of RBCs were normal, most of these being immature (both polychromatophilic cells and basophilic erythroblasts). Seventy-five percent of RBCs had circular, acidophilic intracytoplasmic inclusions (0.5 to 3 um in diameter). Most had only a single inclusion; the others had two. Approximately 50% of the cells with inclusions were polychromatopilic, and most of these were small and spheroidal, many with eccentric nuclei; the other 50% were anisocytotic mature cells, mostly mishapen (mildly distorted, spheroidal or spindle-shaped) with pycnotic, eccentric nuclei. Two percent of the total RBCs were mitotic, and many of the small spheroidal cells with inclusions were present as closely adjacent identical pairs, apparently recently divided. The remaining RBCs (18%) were mature cells with similar abnormalities to those described above, but no inclusions. Films were submitted for laboratory identification of the inclusions. Pending these results, a preliminary diagnosis was made of hemolytic anemia caused by an unidentified hemoparasite. It was presumed that the parasitemia had become so extreme as a result of the stress of the extensive injuries and other clinical problems.

The snake was given fluid therapy (initially 50 ml, then 25 ml Ringer's solution SC s.i.d. 8d), piperacilln (100 mg/kg IM q48h 14 d), ocular lubricating ointment s.i.d. 8 d, daily povidone iodine cleansing of skin lesions and a single dose of ivermectin (0.2 mg/kg SC). Over the first 8 days of treatment skin turgor, spectacle clarity and skin lesions all improved, and TPP and PC both gradually decreased, presumably as a result of the fluid therapy and continued hemolysis. By August 14 the snake was well hydrated but very anemic, and muscle tone and responsiveness were still poor. A transfusion of 7 ml blood (mixed 9:1 with Na citrate and given to the recipient within 15 minutes of collection) from a healty conspecific was given (over 2-3 minutes) via a 22 gauge needle into the ventral coccygeal vein, and treatment commenced with iron dextran, quinine sulphate, sulfadoxine and pyrimethamine (for 21 days using doses metabolically scaled from human antimalarial regimens), in an attempt to eliminate the as yet unidentified "hemoparasite."

On August 15 the snake was noticeably stronger and more alert, and bloodwork 3 days later revealed an elevated PCV and increased proportion of normal mature RBCs. Given this apparent response to transfusion, the procedure was repeated 5 days later, as the PCV had decreased again. As only small diamond pythons were available, a large (8 kg) carpet python (*Morelia spilota variegata*) was used as the donor so that a greater volume of blood (15 ml) could be collected and transfused (over 3-4 minutes). Hematology 5 days later again revealed a positive response; and as continued hemolysis was expected, a third transfusion (30 ml from the same carpet python) was given in an attempt to continue to support the snake during the antiprotozoal therapy. As the snake was now stronger, it was anesthetized with isoflurane to permit slow delivery (over 10 minutes) of the blood via a 25 gauge needle into the palatine vein. Three days later the hematocrit was further improved and the snake was bright, coiling, and oral mucous membrane color had returned to normal. After the completion of this treatment, laboratory results were returned indicating that the inclusions were characteristic of *Pirhemocyton* sp. organisms found in a range of reptile species since their first description in 1914.[9] For many years they were considered to be protozoans, but recent ultrastructural studies in some lizard species have demonstrated the inclusions to be "assembly pools" of viral particles consistent with those of the Iridoviridae.[8,9] Therefore, blood collected on September 16 was fixed in 2% gluteraldehyde for transmission electron microscopy. Icosahedral viruses (141-161 nm in diameter) were observed in the cytoplasm of most abnormal RBCs, either as single entities or as aggregations within the areas seen as inclusions by light microscopy. These clusters contained both complete and developing virions at different stages of maturity. The ultrastructure and morphogenesis of this virus is consistent with that of iridoviruses. Despite the viremia, the snake's condition continued to improve, with skin lesions completely resolved by late October. Voluntary feeding commenced in early October and continued regularly thereafter. In early February stomatitis and skin blisters developed in the absence of an identifiable environmental problem and resolved after a month of parenteral antibiotics and topical treatment. Since then, there have been no new problems.

By August 1994, body weight was 1.3 kg and most rib fractures had healed. Throughout this period the snake was bled regularly to monitor the progress of the viremia. The degree of viremia, magnitude of the RBC regenerative response and size of inclusions (up to 6 um in

diameter) varied widely over this time with apparent spontaneous resolution of infection ocurring on 2 occasions. However, although no inclusions were seen on those occasions, the virus may still have been present in the cytoplasm of some cells as single entities not visible by light microscopy. There was no clear correlation between the presence of oral and skin lesions and the RBC picture. The leucocyte profile remained abnormal throughout the case, with a revesed L:H ratio (normally more than 1[3]) and an absolute lymphocyte count (0.16-4.7 x 10^9/L) maintained below the mean of the "normal" range.[3] There was a leucocyte response followng the latter two transfusions with the total count increasing from 6.0-12.0 x 10^9/L from August 23-29 and from 12.0-18.5 x 10^9/L from August 29-September 1. On both occasions this represented an increase of all leucocyte types.

Case 2

A subadult coastal bearded dragon (*Pogona barbatus*) confiscated from an unlicensed owner presented on October 23, 1993, in a cachectic (weight 80 grams) depressed and dehydrated condition. Blood was collected, and results when compared with reference values for the species (see Tables 3 and 4) indicated low PCV, low TPP and elevated uric acid (15.4 mg/dL). After 6 days of fluid therapy (initally 4 ml, then 2 ml isotonic dextrose-saline SC s.i.d. 5d) and 48 hourly tube feeding, PCV, PP and uric acid (4.9 mg/dL) had all decreased, and the lizard was a little brighter and well hydrated. Tube feeding continued at the same frequency, but by day 22 the animal was still depressed and PCV and TPP were further decreased. No immature RBCs were seen on days 1, 6 or 22, and a diagnosis was made of a nonregenerative anemia and hypoproteinemia probably caused by chronic malutrition. A transfusion of 1.5 ml blood from a conspecific was given via a 25 gauge needle over 2-3 minutes into the ventral coccygeal vein. The following day the animal was noticeably brighter and more active, and a second transfusion of 2.5 ml from a different conspecific was given 2 days later. Following this, demeanor further improved and voluntary feeding commenced 6 days later although tube feeding was continued for 2 months to ensure adequate intake. The hematological response to the transfusions is detailed in Table 3. The gradual lowering of the PCV by 16 and 24 days afer the first transfusion accompanied by increased numbers of abnormal mature cells (distorted +/- pale cytoplasm +/- pycnotic nuclei) presumably represents the disintegration of transfused RBCs.

By December 9, despite the improved demeanor and appetite, body weight had decreased (77.5 g), and there was still no evidence of a regenerative response to the anemia. Therefore, nandrolene decanoate (0.5 mg) was given to stimulate erythropocisis. There was a dramatic esponse following this reatment (see Table 3). Three months later the anemia had resolved and body weight was 112 g.

Discussion

Erythrocytic viruses have been described in several lizard,[8,9] snake,[7] and frog,[1,2] species. They have been previously recorded in Australia in geckos,[8] and *Pirhemocyton* was described in *M. s. variegata*,[4] but this is the first report of the virus in *M. S. spilota*. This infection differed from those previously reported in that the virus was not enveloped, and the

acidopilic inclusions were not accompanied by intracytoplasmic albuminoid vacuoles or crystalline structures.[1,2,7,9] Although extreme anemia was reported as characteristic of this infection in some lizards,[1] in most species there did not appear to be associated morbidity.[1,2,7,9] Spontaneous resolution of viremia was reported in a chameleon,[9] and it has been suggested that the mechanism of elimination of this virus may be phagocytosis of infected cells and virions by splenic macrophages and pulmonary endothelial cells.[1] The persistent viremia and anemia in this case may reflect the virulence of this particular virus, or it may be due to a host immunodeficiency secndary to the initial cachexia, extensive injuries or a cryptic disease process. The cause of the prolonged relative lymphopenia is not known, but it may reflect chronic immunocompromise.

The use of transfusions in these cases appeared to be beneficial, resulting in positive clinical and hematological responses. Despite the absence of crossmatching, the use of blood from a different subspecies in the python and the rapidity of blood delivery (compared with the recommended rate for mammals of less than 10 ml/kg/hr[5]), no clinically recognizable reactions occurred. However, the leucocyte response following the latter two transfusions in the python may reflect a potentially destructive immune reaction and suggests that crossmatching should be considered for any future multiple or heterologous transfusions in reptiles as is recommended for other taxa.[5,6]

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TABLE 1. Hematological changes in diamond python case, Aug 93 - Aug 94.

Date (m/d/y)	PCV (%)	TPP (g/dL)	PLASMA COLOR	Normal Cells (%)		Cells with inclusions (%)	
				Mature	Immature	Normal morphology	Abnormal morphology
8.5.93	19	12.2	Dark green	1	4	-	75
8.9.93	12	10.0	" "	2	23	-	71
8.14.93	7	7.0	Mod. green	2	52	-	43
8.14.93	**Transfused with 7.0 ml Diamond Python blood (PCV 24)**						
8.18.93	11	7.4	Pale green	20	54	-	24
8.23.93	8	6.0	" "	23	50	-	19
8.24.93	**Transfused with 15.0 ml Carpet Python blood (PCV 32)**						
8.29.93	12	6.0	Pale green	40	29	-	29
8.29.93	**Transfused with 30.0 ml Carpet Python blood (PCV 32)**						
9.1.93	16	7.8	Pale green	37	10	29	22
9.16.93	13	8.2	Mod. green	13	11	68	6
9.26.93	13	8.0	" "	9	15	63	11
10.6.93	13	8.2	Pale green	13	29	-	56
10.20.93	18	7.8	V. pale green	65	2	-	23
11.11.93	20	8.8	Colourless	84	10	-	-
12.10.93	21	9.0	Colourless	19	41	-	12
12.24.93	19	8.4	V. pale green	15	16	-	67
1.15.94	12	10.0	Pale green	9	24	-	64
2.4.94	12	9.0	Colourless	67	29	-	-
2.14.94	16	8.4	Colourless	85	13	-	1
2.27.94	15	7.4	Pale green	6	33	9	51
3.11.94	17	7.5	" "	-	50	-	48
4.10.94	20	8.0	V. pale green	-	72	-	27
5.8.94	14	7.8	" "	76	-	10	11
6.13.94	18	9.3	" "	1	5	1	91
7.13.94	22	8.9	" "	27	6	52	13
8.22.94	15	9.0	Pale green	4	44	4	35

Footnote: 1. PCV = Packed Cell Volume 2. TPP = Total Plasma Protein g/dL
3. RBC morphology: Figures given are % of 200 erythrocytes counted. Remaining erythrocytes were mitotic figures (1-2% of total in each case) and mature cells with abnormal morphology.

TABLE 2 : Reference hematological values for *M. S. spilota* (n=29)[3]

Value		Mean	Reference range*
PCV	(%)	25	19 - 30
TPP	(g/dL)	7.6	5.6 - 9.6
WBC	(x 10^9)	10.8	3.0 - 18.3
Lymphocytes	(x 10^9)	4.3	0.7 - 11.1
Azurophils	(x 10^9)	3.4	0.7 - 6.0
Heterophils	(x 10^9)	2.2	0.5 - 5.2
Basophils	(x 10^9)	0.4	0.01 - 1.2

TABLE 3 : Hematological changes in Bearded Dragon case, Oct 93 - June 94

DATE (M/D/Y)	PCV (%)	TPP (g/dL)	RBC MORPHOLOGY		
			Normal mature cells (%)	Immature cells (%)	Abnormal mature cells (%)
10.23.93	14	3.4	99	-	1
10.23-28.93	**Daily subcutaneous fluid therapy**				
10.29.93	10	2.6	99	-	1
11.14.93	8	2.4	99	-	1
11.15.93	**Transfused with 1.5 ml blood from Coastal Bearded Dragon #1 (PCV 25)**				
11.18.93	**Transfused with 2.5 ml blood from Coastal Bearded Dragon #2 (PCV 28)**				
11.24.93	23	3.0	98	1	1
12.1.93	21	3.1	96	1	3
12.9.93	17	2.8	91	2	7
12.9.93	**Single IM treatment with anabolic steroid (0.5 mg nandrolone decanoate)**				
12.18.93	17	3.9	85	9	6
12.31.93	19	4.5	82	14	4
1.15.94	20	5.1	82	16	2
3.31.94	22	4.8	95	4	1
6.10.94	23	7.2	98	1	1

FOOTNOTE:
1. Percentages given are % of 200 RBC's count

TABLE 4 : Reference hematological values for *P. barbatus* (n=12) (McCracken, unpubl).

Value		Mean	Range of Values
PCV	(%)	27	20 - 38
TPP	(g/dL)	5.9	4.4 - 7.6

SUCCESSFUL TREATMENT OF A UROLITH ASSOCIATED WITH A FUNGAL CYSTITIS IN *Iguana iguana*

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Introduction

A four year old female green iguana (*Iguana iguana*) was rescued by the local herpetology club. Husbandry for the iguana up until this time had been inadequate. The diet had primarily consisted of dog food. No source of drinking water had been made available to the iguana.

The current owner had successfully raised and bred many species of reptiles. He used this experience to provide an excellent cage environment for this iguana. Upon rescue, the owner noted that the iguana was weak, unable to use its hind legs, and thin even though the abdomen was distended. He assumed that the hind limb weakness was due to "calcium deficiency" and began administering an oral reptile vitamin and mineral supplement. He interpreted the abdominal distension as secondary to constipation so he tube fed the iguana with warm water and a fiber laxative and began soaking her in warm water baths. After a few days, the iguana had only passed a few urates and remained anoretic so the owner force fed a small quantity of cooked carrots and yams. After 2 weeks when this treatment regimen did not improve the iguana's condition, the owner presented the animal to Ohio State University Veterinary Teaching Hospital.

Physical examination

On physical examination, the iguana weighed 1.2 kg. Her snout/vent length was approximately 14 inches. She was extremely depressed. Although her abdomen was severely distended, her pelvic bones were quite prominent which was interpreted as cachexia. Although the musculature along the femurs was reduced, the distal portion of the back legs appeared swollen. Upon further inspection, pitting edema was found to occur bilaterally distal to the mid-tibia/fibula region. Both tarsal joints palpated as swollen, but not particularly painful. Toe pinches elicited a pain response and movement in all four limbs. With further stimulation, the iguana would try to escape, but was unable to lift her body off the table. Abdominal palpation was unrewarding as the abdomen was firm. The iguana's respiratory rate was approximately 60 shallow breaths/minute . The heart ausculted within normal limits, but the lungs sounded harsh. The mucous membranes of the mouth were pink and tacky. The rostral oral cavity showed signs of chronic trauma and mild stomatitis. A small amount of purulent discharge was noticed ventral to the left nostril. The bone density of the skull and limbs appeared adequate based on digital palpation.

Problem list

The most important problems identified in this iguana included: distended abdomen, pitting edema of the rear limbs, swollen tarsal joints, tachypnea and harsh lung sounds, and generalized weakness. The anorexia and dehydration were considered to be important clinical signs with serious metabolic consequences, but they were considered to be secondary to an underlying disease process. The mild stomatitis was considered to be secondary to cage trauma incurred with the previous owner.

Differentials

Distended abdomen:	Cloacolith, obstipation, intestinal obstruction (intussception, foreign body), urolith, eggs, follicles, neoplasia, granuloma, organomegally (gout, metastatic calcification), ascites
Pitting edema:	Decreased vascular return secondary to abdominal distension, heart disease, hypoproteinemia, vasculitis (viral, bacterial, parasitic, uric acid related)
Swollen tarsi:	Septic joints, gout, trauma, fibrous osteodystrophy
Tachypnea:	Compression of lung fields by abdominal mass, secondary to decreased vascular return to the heart, pain, metabolic acidosis, pneumonia, pulmonary edema
Weakness:	Secondary to chronic disease (anorexia and dehydration), metabolic (hypocalcemia, hyperkalemia, hypoglycemia, gout, liver disease), poor cardiac output

Diagnostic testing

In order to limit the number of differentials whole body radiographs, hematology and a serum profile were performed. The radiographic results were available first. They showed a large soft tissue density mass filling the caudoventral abdomen. Dorsal to this mass, there was a tubular mass filled with granular material. The liver was enlarged and coelomic fluid was suspected. The lung fields were compressed and had a slight alveolar pattern. The skeleton appeared to have adequate radiographic density although multiple rib fractures were noted on both sides of the chest. Soft tissue swelling could be visualized over the tarsal area on the dorso/ventral view. Irregular periosteal proliferation was observed on the distal tibias. The right was more affected than the left. Bony lysis of the tarsus or uric acid deposition was suspected for both tarsal joints.

In order to better interpret the large soft tissue mass, the presence of pleural fluid, and the architecture of the liver, a coelomic ultrasound was performed (Ultramark 4, 7.5 MHz transducer, Advanced Technology Laborotories, Inc., Bothwell, WA 98041). The ultrasound showed hepatomegaly with normal architecture, the presence of a small amount of coelomic fluid, multiple ovarian follicles (5-10 mm diameter), and a urolith.

Serum profile results were as follows:	Total calcium	17.0 mg/dl
	Phosphorus	8.5 mg/dl
	Albumin	2.0 gm/dl
	Cholesterol	418 mg/dl
	Alkaline phosphatase	33 iu/L
	Aspartate transaminase	65 iu/L
	Uric acid	2.4 mg/dl
Hematology results were as follows:	Plasma protein	9.5 gm/dl
	PCV	25 %
	Hemoglobin	8.4 gm/dl
	Total WBC	40,400
	Heterophils	14,900
	Lymphocytes	21,800
	Monocytes	2,400
	Azurophils	1,600
	Thrombocytes	Adequate

Occasional clear vacuoles were seen in the cytoplasm of the erythrocytes

The radiographs and ultrasound were interpreted as urolithiasis with concurrent ovarian follicles. The possibility of a concurrent colonic impaction could not be ruled out. The lung changes were attributed to pneumonia or possibly pulmonary edema. The hepatomegaly was attributed to congestion although a diffuse hepatitis could not be ruled out. The changes in the tarsal region were attributed to septic arthritis or tissue reaction to uric acid deposition. The rib fractures were considered to be incidental.

The serum profile results were unremarkable. Elevated calcium, phosphorus, and cholesterol levels are common in green iguanas during egg development and resorption. The high albumin was associated with dehydration or egg production. The high albumin also ruled out severe liver disease and hypoproteinemia as a cause for the pitting edema. Although the uric acid was within normal limits, it did not completely rule out the possibility of gout especially with the diet and hydration history. Normal uric acid levels have been seen in reptiles severely affected with gout.

The hematology results were quite remarkable. The PCV (25%) showed anemia (normal PCV 45-52%). The elevated plasma protein of 9.5 gm/dl (normal 2.8 - 5.0 gm/dl) was attributed to dehydration and/or egg production. The total white count was three times the top of our normal values. The percentages of cell types was within normal limits with a mild monocytosis. This blood picture was too extreme to be inflammatory (i.e. egg yolk peritonitis, tissue trauma from uric acid deposition or the urolith, secondary to congestion) and was considered to be due to a chronic infection.

Preliminary diagnosis

A preliminary diagnosis of dehydration, urolithiasis, septic arthritis, and pneumonia was made. Other potential problems were colonic impaction, retained ovarian follicles, and gout.

Treatment

Abdominal exploratory to remove the urolith and evaluate the gastrointestinal tract, ovarian follicles, liver and lungs was recommended. Because of its weakened state, the iguana was masked down at 2.5 % isoflurane (usually requires 3.5 - 4.5% in healthy iguanas). The iguana was intubated and positive pressure ventilations were administered four times per minute. The iguana was maintained between 1.5 and 2.0% isoflurane for the first 45 minutes and then was decreased to 1.0% for the remainder of the procedure. The iguana was tilted approximately 15 degrees to the left when it was placed in dorsal recumbency to minimize compression of the caudal vena cava.

Because of the iguana's questionable metabolic and cardiovascular condition, an intraosseous catheter was placed into the distal left femur immediately after induction using a 1.5 inch 22 gauge spinal needle. Lactated Ringers was administered at 10 ml/kg/hr during the procedure and recovery. Preoperative antibiotics were not administered so that cultures could be taken intraoperatively.

Abdominal exploratory revealed an enlarged urinary bladder approximately 7 cm in diameter and many normal ovarian follicles (0.5 - 1.0 cm diameter) located on both ovaries. All other coelomic viscera appeared within normal limits. The bladder was partially exteriorized and packed off with moistened lap sponges. A 3 cm incision ventral cystotomy revealed a 5cm diameter sand-like concretion covered with 1 cm of thick mucous within the lumen of the bladder. The urolith was removed and submitted for analysis (Urinary Stone Analysis Laboratory, Room 3106 Medical Sciences Building, School of Veterinary Medicine, University of California, Davis, Davis, California, 95616-8737, (916) 752-3228). Bacterial and fungal cultures were taken from the bladder mucosa and then the lumen of the bladder flushed with warmed saline. The tissues of the bladder wall appeared within normal limits. The bladder wall was closed with a continuous Lembert suture pattern (5/0 polydioxanone). The abdomen was flushed with warmed saline and the body wall closed in a simple continuous pattern (4/0 polydioxanone). The skin was closed with a continuous horizontal mattress pattern (4/0 nylon).

For recovery, the fluid rate was decreased to 2 ml/kg/hr. Enrofloxacin was administered i.m. in the front leg (5 mg/kg once daily) which was to be continued pending culture results. The iguana was placed on a heating pad and ventilations were assisted until the it was breathing normally (approximately 10 minutes post-operatively). The endotracheal tube was then removed. After one hour the iguana had returned to normal activity and so was returned to her normal caging. The iguana recovered uneventfully and began to eat small amounts of food the next day. The edema of the rear legs, severe weakness, and harsh lung sounds had resolved by the following day.

Culture results of the lining of the bladder reported large numbers of *Trichosporon beigelii* and a few *E. coli*. The large numbers of yeast organisms suggested that they were more than contaminants. It was elected to treat the iguana with 50 mg/kg of ketoconazole p.o. once daily for 60 days. The *E. coli* was sensitive to all antibiotics tested and was considered to be a contaminant, so the enrofloxacin was discontinued after a course of 10 days. The urolith analysis reported that the stone was composed of 100% uric acid.

One month postoperatively, a physical examination was within normal limits. An ultrasound guided cystocentesis was submitted for culture. This culture reported no growth. Ovarian follicles were seen during this ultrasound (1.0 cm diameter). The iguana returned to normal health and continues to show no signs of reoccurrence of the urolith 18 months post-operatively. In an attempt to decrease the likelihood of stone reoccurrence by reducing the urinary systems need to process uric acid, long term health care recommendations were made which included free access to clean water, a diet no higher than 5% protein foodstuffs, and avoidance of nephrotoxic drugs in the future (especially aminoglycosides).

Wrap up

The primary problem in this iguana was thought to be the urolith which acted to impede vascular return to the heart which caused the pitting edema, pulmonary changes, and profound weakness. The significance of the presence of *Trichosporon* in the bladder is unknown, but the large numbers recovered on culture suggest that it may have been a pathogen. It is also up for debate whether removal of the urolith without ketoconazole treatment would have been successful in treating this case. The high white blood cell counts is suggestive of a systemic infection, but since both the lungs and the tarsal joints could also have been possible sights of infection, it does not prove that the *Trichosporon* was the cause of the leukocytosis. A follow up hemogram was not allowed by the owner. The boney changes of the tarsal joints did not appear to cause the iguana any problems post-operatively. Due to financial considerations permission for further radiographs was not granted. Without follow up it is impossible to evaluate whether these changes were chronic or secondary to an acute infection that was treated by the enrofloxacin or ketoconazole.

This case is valuable because it introduces the possibility that uroliths can be caused by fungi in reptiles and because it illustrates many diagnostic and therapeutic modalities that can be successfully applied to reptiles.

LIVER ENZYMES AND PATHOLOGY IN RUNT CROCODILES (*C. porosus*)

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Introduction

Failure to thrive, leading to death in hatchling crocodiles is the most significant problem facing crocodile farmers in the Northern Territory. On some farms up to 30% of the animals in the shed can be affected. The vast majority of these animals go on to die in their first year of life. Some do survive until they have to be moved from the hatchling accommodation to make way for the following year's crop, generally these then succumb in the harsher environment of the growing pens.

It is possible to identify animals that will go on to become runts after two months of age. These animals do not show the signs of emaciation typical of anoretic crocodiles. They remain small and never develop the rounded belly of a fast-growing crocodile.

Case history

A large crocodile farm in the Northern Territory moved 50 one year old crocodiles from pens with temperature fluctuations of ± 1°C to pens with fluctuations of ± 5°C. At moving the average weight of the animals was 105 gms.

Blood samples were taken from 10 of the runts. At a similar time, blood was taken from 36 animals of the same age, that had experienced normal growth in their first year.

During or soon after the bleeding procedure two of the runts died. These animals were submitted for autopsy. The postmortems revealed little out of the ordinary. The animals were in poor condition with no fat body. The livers were pale and histology revealed a degree of fatty infiltration. This is a common finding in runts.

Table 1

	Units	Runts mean	Normal mean and range
ALANINE A-TRANSFERASE	U/L	39	30 (10 - 53)
ASPARTATE A-TRANSFERASE	U/L	157	55 (24 - 86)
ALKALINE PHOSPHATASE	U/L	75	56 (23 - 85)

These animals were later used as part of a screening program looking for chemical treatments that could be used to initiate growth. None of the treatments tried were successful. The group suffered 90% mortality, which is normal for this class of animal.

Discussion

It has often been suggested by the stockmen on the farms that these animals are hungry and want to eat, but for some reason refuse the food offered. The hatchlings can sometimes be observed sitting on a pile of mince meat snapping at the flies that are attracted to it. It has also been observed that if tadpoles are released into the pens containing these hatchlings they chase and eat them.

In another farm the feed has been slightly changed to improve its palatability. In this season the overall mortality has reduced from 15 % to less than 3%. The ones that are dying are the good well grown crocodiles and generally they suffer sudden death from bacteremia.

I suspect that the runted animals are suffering from a metabolic disorder induced by starvation. I have read of similar syndromes affecting constricting snakes. I am hoping to develop some strategies in order to offer some treatment to the next generation of these animals in January.

MR OF BOWEL USING MINERAL OIL AS A CONTRAST AGENT: A VIABLE OPTION IN REPTILES WITH LONG TRANSIT TIMES

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Imaging of bowel, especially in reptiles with long transit times, is difficult. Long transit times of bowel effectively exclude barium as an effective contrast agent. Drying of the barium as well as the inevitable development of barium-free regions on bowel due to the difficulty in maintaining regular barium ingestion over a prolonged interval results in flocculation and segmentation of the barium column. Unlike barium, mineral oil is not affected by contact with intestinal mucoproteins. We propose that in certain circumstances, MR of bowel using mineral oil as the contrast agent is actually easier to perform than other forms of bowel imaging. We present our experience in the MR imaging of bowel in a female leopard tortoise (*Geochelone pardalis pardalis*). Due to the intrinsic sensitivity of MR, only small quantities of mineral oil are needed in any segment of bowel for that segment to enhance on both T1-weighted and T2-weighted MR images. Unfortunately, neither contrast enhanced MR nor barium radiology is capable of mucosal detail imaging in long transit time reptile unless the reptiles are kept on long term hyperalimentation during the course of the examination. Mineral oil administered via pharyngostomy tube placed *in situ* and cannot cause bowel obstruction.

ENDOSCOPIC EXAMINATION OF THE UROGENITAL TRACT IN REPTILES

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Endoscopic examination of the body cavity (coelioscopy) have been performed in 373 reptiles out of 34 species. Mainly monomorphic lizards have been examined.

Indications of coelioscopy have been:

1. Confirmation of sex in adult reptiles.

   In many monomorphic species males and female display similarly. In addition both sexes possess hemipenis cavities. To confirm the sex in the species coelioscopy is the method of choice. Parts of this group are *Varanus spp., Tiliqua spp., Egernia spp., Heloderma spp.,* and *Coruzia spp.*

2. Confirmation of sex in juvenile reptiles

   To confirm the sex in juvenile dimorphic reptiles is often impossible using external morphology. Coelioscopy allows confirmation in mono- and dimorphic juvenile reptiles.[1]

3. Clinical examination of inner organs including endoscopic guided biopsies.[3,4]

4. Endoscopic surgery

5. To confirm variation of inner structure during long term examination, for example follicle growth in the ovaries.[2]

6. To confirm suspected diagnosis made for example by ultrasound scanning.

The methods of coelioscopy examination have been described in detail.[5] In *Trachydosaurus rugosus* local anesthesia is sufficient for endoscopy, in all other species we used isoflurane anesthesia applicated by face masks. In all cases the insufflation of gas (filtrated air or CO_2) is necessary. After skin incision on the left side the muscles and serosa were bluntly perforated. The endoscopes were introduced using forceps or sheats (blunt trocar). In larger animals we used a 4 mm diameter arthroscope and in small animals (under 200 g BW) a 3 mm arthroscope, including a working channel for biopsy or irrigation (Stortz 27030B). Results were documented using a photo (35 mm) or video (Stortz, flash generator 600 video/endovision 539).

Organs to be examine during coelioscopy were kidneys (right rarely), adrenal glands (only left), ureter, bladder, ovaries (right rarely), and oviduct (only left). Color, size, and surface

of the organs could be documented easily. In a few very fat animals with large fat bodies, only parts of the urogenital system could be examined.

Documented alteration of the kidneys were gout (subserosal uric acid deposition), bacterial abscesses, neoplasms, and swelling caused by hexamitiasis. Within the bladder we found bladder calculi mainly consisting of uric acid. Testicle varied in color (yellow - *Trachyosaurus, Testudo;* white - *Coruzia;* or grey - *Chelodina, Podocnemys*) and size depending on sexual activity. Juvenile ovaries showed rough, humpy surface. Inactive adult ovaries consisted mainly of amber color primary follicles. Secondary and tertiary follicles are of different size and yellow color. Follicles in resorption are yellow and possess broad, faded vessels. Borders between follicle and ovarial tissue are irregular. The oviducts are of white color and intricated course.

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A REVIEW OF ALLOMETRIC SCALING WITH CONSIDERATIONS FOR ITS APPLICATION TO REPTILE THERAPEUTICS

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The principals of allometric scaling and the consideration of metabolic size in therapeutics have been introduced in a number of forums[1,3,4,6,7,8] in recent years. The purpose of this discussion is simply to review these concepts and discuss the potential application of these theories to reptile therapeutics.

The use of allometric scaling for the calculation of drug doses is a practice with which most practitioners are familiar through the use of chemotherapeutic agents in cancer therapy. Treatment protocols involving these highly toxic chemicals often use a mg/square meter (m^2) basis for calculating doses rather than the mg/kg basis traditionally used for most other drugs. The meter squared variable is derived using Meeh's formula ($K(W_{kg}^{0.66})/10^2$) which relates the body surface area of an animal to its metabolic rate. This formula was originally derived by physically attempting to measure the external surface area of a variety of mammals. The rationale for using this relationship is that as an animal decreases in size, the ratio of surface area to body mass increases as does the animal's metabolic rate. Conversely, as size increases, the ratio of surface area to body mass and the metabolic rate decrease. Thus, changes in surface area more accurately reflect changes in metabolic rate (and therefore drug metabolism) than does simple body mass. Using a metabolic basis for determining doses of these drugs has been found to allow maintenance of blood levels of drug within the same therapeutic range in a variety of patients. As expected, the implications of this phenomena are even more profound when dealing with animals of highly disparate body sizes, as are routinely encountered in exotic animal medicine.

A more accurate assessment of metabolic rate as it applies to pharmacokinetics is based on a formula which determines the minimum energy cost (MEC) of a animal. This allows an assessment of an animal's metabolic machinery at the level of capillary beds, glomeruli, alveoli, etc., the level in the body at which the uptake, distribution, biotransformation and clearance of any drug occurs. This therefore provides a method of incorporating these metabolic parameters into therapeutic considerations.

In order to compare animals across different "metabolic taxa," five different energy classes have been designated based on groups of animals which have the same mean core body temperature range. Constants (K) have been identified by Hainsworth for each of these groups and are used in all extrapolations.

Energy Group	Constant (K)
Passerine bird	129
Non-passerine bird	78
Placental mammal	70
Marsupial mammal	49
Reptile	10

Minimum Energy Cost (MEC) is calculated by raising the lean body mass of the animal to the three-quarter power and multiplying this quotient by the appropriate energy group constant $K(W_{kg}^{0.75})$. When dealing with reptiles and other poikilotherms, it is important to remember that standardization of body temperature is necessary for legitimate extrapolations. Therefore, poikilotherms must have their environmental temperature adjusted so as to achieve an optimal core body temperature of approximately 37°C.

With these principles in mind, it is a simple matter then to apply them to specific therapeutic regimes by employing a few arithmetic calculations, all of which can be rapidly performed on a pocket calculator. In order to scale a dose of a particular drug for a patient animal, it is first necessary to extrapolate from a known effective dose in a model animal. Optimally, this model dose would be based on pharmacokinetic studies in the same or similar species to your patient; however, with drugs that are metabolized by similar physiologic phenomena at the cellular level in all species, it is possible to extrapolate across taxonomic lines.

First, it is necessary to convert your model animal's dose and treatment frequency from a mg/kg basis to a metabolic basis. Note that once this step is completed for a given drug, the metabolic dose and freqency can be recorded in a formulary and used in future calculations for other patients.

The following steps will allow you to extrapolate a metabolic, or MEC dose:

-- Calculate Minimum Energy Cost (MEC) of the model animal. $MEC=K(W_{kg}^{0.75})$

-- Calculate a Treatment Dose (the mg/kg dose) for your model animal.

-- Divide this Treatment Dose by the MEC of the model animal to obtain the MEC Dose.

The next step is to extrapolate a frequency of administration. This again is alculated from your model animal dose:

-- Calculate the Specific Minium Energy Cost (SMEC) of the model animal. $MEC=MEC/W_{kg}$ or $K(W_{kg}^{-.25})$.

-- Calculate a Treatment Frequency by dividing 24 hours by the hourly treatment interval of the model animal; e.g., for a model TID treatment, calculate the treatment Frequency by dividing 24 hours by 8 hours to obtain 3.

-- Divide the Treatment Frequency calculated above by the model animal's SMEC to obtain the SMEC Frequency of this drug.

(Remember that the calculated MEC Dose and SMEC frequency can now be used to calculate doses for future patients in which this drug will be used).

Now calculate the dose and treatment frequency for your patient animal:

-- Calculate the MEC for your patient

-- Multiply the patient MEC by the MEC Dose for this drug as calculated above. This equals the mg of drug per treatment.

-- Calculate the SMEC for your patient.

-- Multiply the patient SMEC by the SMEC Frequency for this drug as calculated above. This equals the number of treatments per 24 hours.

It is sometimes desirable to alter the frequency of administration from the calculated rate; e.g., in an animal in which the calculated frequency is six times/day, the stress of handling this frequently for treatment may outweigh the benefits of the therapy. To calculate a new frequency:

-- Multiply the mg/treatment by the treatments/24 hours to obtain the mg/24 hours.

-- Divide this by the frequency you wish to use to obtain a new mg/treatment.

As a caution, remember that by changing the frequency of administration and thereby changing the amount of drug given at each dose, it is possible to create peaks and troughs in serum concentration which either exceed safe levels or fall below the effective therapeutic range of the drug.

As with any dosing regime, including those derived using the traditional mg/kg dose, a knowledge of the pharmacology of the drug, and in particular the pharmacokinetic differences between species, must be taken into account, especially when extrapolating across taxonomic lines. This appears especally important in treatment regimes involving extrapolations from homeoterems to poikilotherms. As previously mentioned, a body temperature within the optimal metabolic range must be maintained in order for results to be predictable. For some reptiles, especially those that spend a portion of their natural life cycle in a state of torpor, merely warming the animal may not be enough to reach a

metabolically active state. An assessment of heart rate as a metabolic indicator may be more useful than cloacal temperature in these cases.[5] An apparent paradox to the allowmetric principles was noted by Mautino and Page[2] When they reported that higher doses of some drugs were needed to achieve effective serum levels in large tortoises than were seen to be effective in smaller animals. It is possible that differences such as absorption rates, which may be extremely slow in the larger animals, could result in partial metabolism of the drug before effective blood levels are attained, thereby increasing the clinically effective dose.[5] Another speculation, based on clinical observation, is the possible existence of hyperosmotic states which may be achieved as natural phenomena in certain species, such as as desert or marine animals, which normally experience very different environmental conditions from other species.[5] Differences in serum osmolarity would naturally influence the pharmacokinetics of a drug introduced to this system. Clearly more exploration of these issues along with further pharmacokinetic studies will greatly enhance the usefulness of the principles of allometric scaling in regard to therapeutics in reptiles.

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CLINICAL APPROACH TO THE CHAMELEON PATIENT

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Introduction

Success in captive breeding and maintenance of several chameleon species, as well as an increase in the availability of chameleons from Madagascar, has made these lizards more prevalent as pets. They differ in many ways from other lizard species, such as iguanas, that are commonly seen in practice. Chameleons have projectile tongues, prehensile tails, rotating eyes, zygodactyl feet, among other unique features. Veterinarians who treat reptiles must familiarize themselves with the unique characteristics of these animals.

Chameleons tend to be easily stressed. Stress exacerbates both infectious and non-infectious diseases. Minimizing stress, through proper husbandry and management, is the first step in resolving many medical problems.

Signalment

* Important to identify species of chameleon. Husbandry requirements differ among species.

* Two main groups exist: Highland and Lowland

Group: Highland (Montane)
Species: *C.jacksonii, C. fisheri, C. montium, C.hohnelii*, etc. *Temperature*: Prefer be cooler than others, especially at night.
Range: As low as 65F (18-19C) at night; Up to 85F (28-29C) at basking sites during the day.

Group: Lowland (Tropical)
Species: *C. dilepis, C. gracilis, C. calyptratus* (desert), *C. pardalis*
Temperature: Prefer high temperatures
Range: As low as 75F (23-24C); Highs at basking sites approximately 95F to 100F (34-38C)

* Additionally, there are some terrestrial chameleons that may occasionally be seen, including *Brookesia sp., Rhampholeon sp.*, etc.

Age

In general, the life span of chameleons is not known, but it appears to be short. This is likely due to their high reproductive efforts. Female chameleons, in particular, tend to have a shorter life span than males.

History:

* Information the Veterinarian Needs
  -- Captive-born or wild-caught?
  -- If wild-caught, was it recently imported?
  -- Other general history questions
  -- History questions associated with chief complaint

More and more species are being bred in captivity. Most common species include, *C. pardalis, C. calyptratus, C. jacksonii.*

Husbandry:

* Caging

Recommendation:
  -- Free-roaming area or large cage with a lot of ventilation and variable-sized branches and/or plants for climbing
  -- Animals should be kept separately, and unable to see other chameleons
  -- If screen is used on cages, eliminate sharp edges or use plastic-coated screening or PVC mesh
  -- Aquariums are suitable for some small species and juveniles; variety of plants and branches should be provided
-- For bottoms of cages, no substrate should be used; Scrupulous hygiene is critical

* Light/Heat Sources

  -- For heat, incandescent bulbs should be strategically placed for basking; ventral heat sources (i.e. hot rocks, heating pads) generally are not useful
  -- Provide animals a gradient of heat, with cage furniture/branches that allow access to cooler or shaded areas
  -- UV radiation is very important. Natural sunlight is best when possible; A combination of broad-spectrum bulbs (i.e. Vitalite, Repta-Sun, etc.) and a black-light bulb combination is best for indoors; Other UV bulbs are experimental (i.e. mercury vapor lamps, sun-lamp bulbs), but may be helpful; Use caution.

* Water

  -- Chameleons usually will not drink standing water. A drip or moving water system is necessary. Some examples: plastic cups with pin holes in bottom, ice cubes, IV bags, gallon jars with stop-cocks, misting with spray bottle
  -- It is important to observe animals drinking regularly
  -- Keep sources of water clean; periodic disinfection is necessary

* Diet

-- Due to chameleons' seemingly high metabolic functions, chameleons require a high intake of food; anorexia is an early sign of a problem
-- Due to their dietary intake, chameleons are prone to problems involving metabolism of calcium and its association of phosphorous and vitamin D3. Their specific requirements are not known, but total calcium and phosphorous in a ratio of 1.2:1 is adequate. Since most insects are high in phosphorous and low in calcium (inverse ratio), they must be supplemented. Several methods of feeding can be used to minimize these problems:

* Feeding a variety of insects, such as crickets, meal worms, wax-moth larvae, sweepings, etc.

* Feeding insects a complete diet, prior to feeding them to the chameleons, such as Ziegler Cricket Diet, dry dog kibble, Layena by Purina, etc.; Some of the most promising results have come from feeding insects a leafy-green vegetable diet with grated carrots and alfalfa or bean sprouts.

* Offering larger chameleons pinkies or fuzzy mice, which have a better calcium to phosphorous ratio than insects.

* Supplementing insects with a calcium/vitamin D3 powder, such as Rep Cal; Additionally, a multi-vitamin, such as Super Preen, Osteoform, or Reptivite, can be used. Any vitamin/mineral supplements which contain fat-soluble vitamins must be used with caution as overdosing can result in organ toxicity. Some of the most promising results have come from routine use of phosphorous-free, calcium-only supplements, with occasional use of vitamin D3 and multivitamin supplements.

* Providing natural sunlight (best) or use of broad spectrum and/or black lights to promote endogenous synthesis of vitamin D3.

Examination

* Visual Exam
-- Note animal's alertness, posture, color

* Physical Exam
-- As with any other animal, proceed with a systemic approach; generally, start anteriorly, and continue posteriorly.

-- Eyes: Enophthalmia, which can be caused by dehydration/emaciation. Generally means a poor prognosis.

-- Oral Cavity: Examine tongue, glottis, glands at commissure of mouth. Abscesses and stomatitis are common. Look for symmetry in the mouth.

-- Skin: Examine for symmetry, swellings, discoloration, especially of the feet, nails.

-- Musculoskeletal: Examine body condition, symmetry, bones, and strength of grip.

Diagnostics:

Fecal exams, culture and sensitivity, bloodwork (tail vein), radiographs, and biopsy are all beneficial in reaching a diagnosis. For routine diagnostics and surgical procedures, isoflurane -- via face mask -- works well.

Diseases:

* Non-infectious diseases/problems

-- Metabolic Bone Disease and Other Nutritional-Related Disorders

Clinical signs of metabolic bone disease in chameleons include, stunted growth, deformed or fractured bones, spinal deviations, which may lead to paralysis, and death. MBD is more commonly seen in young, growing animals or adults maintained indoors. MBD can be prevented with proper diet and lighting (as discussed earlier). Treatment for animals diagnosed with MBD are similar to protocols described for other lizard species.

Toxicity of organs associated with fat-soluble vitamins is common. Chameleons kept indoors have been noted to have problems, in particular, with vitamin D3 and vitamin A levels in their diets. A relationship exists between these two vitamins and their amount of supplementation to chameleons. The exact requirements of vitamin A and D are not known. However, ongoing research may help us better understand their relationship and how we should properly supplement. At this time, some points are worth noting.

* The exact requirements for vitamin A and vitamin D are not the same for all species of chameleon.

* Excessive vitamin A supplementation may result in:

-- interference of vitamin D3 metabolism, leading to metabolic bone disease
-- organ toxicity (kidney, liver), sometimes causing gular edema

* Inadequate amounts of vitamin A may cause eye problems, neurological dysfunction, and dysecdysis

* Excessive vitamin D3 supplementation can result in organ toxicity (gular edema), metastatic calcification, and pseudo-gout.

* Inadequate amounts of vitamin D3 can lead to metabolic bone disease.

Therefore, until a proper balance of supplementation is discovered, I recommend that clients use caution when adding vitamins and minerals to a chameleon's diet.

-- Dystocia

Due to their high reproductive nature, dystocia is a common problem seen in chameleons. It can be caused by several different factors, including stress, poor nutritional status, and mal-formed eggs. Surgical manipulation and the empirical use of oxytocin may be helpful, but retention of eggs or young commonly results in death. On-going research into the use of a new drug (vasotocin) to help initiate egg laying may also help in the future. However, prevention is the best way to manage this problem. To decrease likelihood of dystocia, females need to be in good body condition prior to breeding and egg-laying. As most chameleons will cycle and ovulate eggs regardless of whether or not they have been bred, females should be kept well fed and hydrated at all times.

Perhaps the most common cause of dystocia is an improper environment/substrate for laying eggs. Several breeders have had success using five gallon buckets of fine-grain sand as an egg-laying medium. Others have had success using potting soil in a tub/bucket within the female's already familiar surroundings. Some chameleons prefer to lay eggs around the roots in the soil of potted trees in their environment.

-- Trauma

Adult, male chameleons are usually extremely aggressive and should be housed separately from other chameleons and any other types of reptiles. Because scratches and bites can lead to life-threatening bacterial abscesses, such confrontations can be disastrous.

I have seen a number of traumatized male and female chameleons damaged by an aggressive male. Females should be placed in the male's cage for breeding, watched closely, and then separated upon cessation of breeding activity.

Infectious Diseases

New animals should be quarantined for a minimum of sixty days, and preferably ninety days. During quarantine several fecal samples should be checked for parasites. Also, note if animals are feeding and defecating normally, and watch for signs of illness. The longer the new acquisitions are isolated, the greater the chance of identifying a problem and keeping diseases from spreading through an existing collection.

-- Bacteria

Due to the delicate nature of chameleons and their tendency to be easily stressed, bacterial infections are common. Most infections come from opportunistic bacteria that are naturally in their environment. The most common bacterial infections are pneumonia, sinus/eye infections, infectious stomatitis, and abscesses.

Abscesses can be caused by many things. Usually, a wound is created from a scratch from a branch, wire, or interaction with another chameleon. Typically, the wound is small and heals quickly, without a visible scar, but later becomes noticeably enlarged. Aggressive surgical intervention for removal or drainage is important in managing abscesses.

The most common bacteria cultured from these infections are gram negative bacteria, such as *Pseudomonas, Aeromonas, Klebsiella, Proteus,* and a host of others. It is extremely important to run culture and sensitivities on these infections. Chameleons are such delicate animals, the window for treatment is short. For best results, culture the lining of abscesses and the trachea for pneumonia and respiratory diseases.

Some of the more common antibiotics that these bacteria are sensitive to, include enrofloxacin, amikacin, piperacillin, carbenicillin, and tobramycin.

-- Parasites

Nematodes, cestodes, coccidia, flagellates, and amoeba are all common intestinal parasites of chameleons. I strongly recommend several fecal exams on each new acquisition to the collection, and all wild-caught chameleons should be prophylactically treated with a nematocidal drug and cestocidal drug. To ensure that parasites are eliminated, several "negative" fecal samples are necessary.

Additionally, aberrant migrating nematodes/cestodes may be found in multiple areas of the body and under the skin. These subcutaneous parasites must be surgically removed.

In addition to the bacterial and parasitic problems discussed above, there may also be concurrent unidentified viral infections that may complicate both infectious and non-infectious diseases.

Common Drugs and Dosages

NOTE: No pharmacokinetic research has been done on any drugs in lizards/chameleons, so these are empirically derived dosages. Due to the seemingly higher metabolic function of many chameleons compared with other reptiles, I have had better success by increasing the frequency of dosing for some of the antibiotics.

Antibiotics:

AMIKACIN (Amiglyde-V): 2.5mg/kg IM, SQ every 48-72 hours.
-- ensure that the animal is well hydrated during treatment

ENROFLOXACIN (Baytril): 5-10mg/kg IM, SQ, PO every 12 hours
-- SQ, when possible, seems the most effective, although may cause color changes or damage to skin

CEFTAZIDIME (Fortaz): 40mg/kg IM every 24-48 hours

PIPERACILLIN: 100-200mg/kg IM every 24-48 hours

TOBRAMYCIN: 2.5mg/kg IM every 72 hours

CARBENICILLIN (Geopen): 100mg/kg IM every 24 hours

* All injections should be given in the upper one-third of the body.

* Some of the above drugs may be used together if C&S indicates it is necessary or to gain a broader spectrum (Ex.: Amikacin + Enrofloxacin, Amikacin + Carbenicillin)

Antiparasitic Agents

PANACUR (Fenbendazole): 100mg/ml suspension
-- For nematodes (round worms)
-- 25mg/kg, give three doses by mouth every two weeks
-- Check stools two weeks or more after third dose to ensure all parasites are eliminated

DRONCIT (Praziquantel): 56.8mg/ml injectable
-- 8mg/kg given SQ in upper one-third of body
-- Repeat in two weeks
-- May only be necessary with wild-caught animals

FLAGYL (Metronidazole): 250mg tablets or 50mg/ml suspension
-- For flagellates
-- 40-60 mg/kg given PO
-- Repeat in two weeks

* May not want to give all three drugs at the same time to debilitated animals.

THE WYOMING TOAD (*Bufo hemiophrys baxteri*): A REVIEW OF CAUSES OF MORTALITY IN THE CAPTIVE POPULATION

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Introduction

Amphibians have been declining worldwide at an alarming rate for unknown reasons.[2,3,6] These animals are sensitive environmental indicator species due to their sensitivity to changes in their habitat. Pesticide spraying, changes in agricultural practices, increased predation, disease, and climatic changes have been suggested as possible causes of decline in amphibian species.[1] However, research to determine specific causes has been limited because essentially no baseline health data exists. In addition, results can be difficult to evaluate because there has been little validation of routine diagnostic tests and procedures which can be utilized in the assessment of the well being of these animals.

The Wyoming toad

The Wyoming toad (*Bufo hemiophrys baxteri*) is a classic example of what is occurring worldwide. It is one of the most endangered amphibians known. This toad was abundant, but only known to exist in Albany County, Wyoming. Here, it inhabited the floodplains, ponds, and small seepage lakes in the shortgrass communities of the Laramie Basin. In the middle 1970's, the population crashed.[7] The entire population, both wide and in captive, is now estimated to consist of less than two hundred individuals. In the wild, they are only known to exist at two lakes.

In January 1984, the Wyoming toad was federally listed as an endangered species. The goal of the Wyoming toad recovery plan is to downlist the species from endangered to threatened, by the year 2000. Recovery criteria are to maintain the existing population at a level of 200 adults and to establish viable populations of 100 adults, each in five other locations.[5] In order to achieve the recovery goals a captive breeding program was established in 1988. Toads are being held at the Wyoming Game & Fish Department's Sybille Wildlife Research & Conservation Education Unit, the Cheyenne Mountain Zoo (Colorado Springs, Colorado) and the Henry Doorly Zoo (Omaha, Nebraska). This paper reviews causes of mortality within the captive Wyoming toad population.

Methods and Materials

Upon death, all toads were shipped on ice express mail to the University of Wyoming/ Wyoming State University Veterinary Laboratory (1174 Snowy Range Road, Laramie, Wyoming 82070, USA). Standard necropsy techniques were utilized and emphasis was placed on collecting samples for bacterial and fungal cultures. Results were then subdivided into age, sex, and diagnosis.

Results

For this report, only data from captive Wyoming toads were included where determination of sex, age, and a diagnosis were possible. Records from autolytic or unsexable toads were eliminated. Thus, the following information is from 26 toads consisting of 11 adult females, 3 adult males, 4 juvenile females, and 8 juvenile males.

Adult females (42%) made up the majority of cases examined, followed by juvenile males (31%), juvenile females (15%), and then adult males (12%). By far, the most frequent finding was a mycotic dermatitis with hepatopathy. This dermatitis and hepatopathy occurred in 73%, gastric foreign bodies causing obstruction occurred in 12%, dehydration in 8%, hepatitis in 4%, and pneumonia in 4%.

Discussion

Currently, mycotic dermatitis is the predominant recognized cause of mortality in the Wyoming toad. Normal bacterial flora has not been established for the toad, therefore it is difficult to assess which bacteria may be contributing to the mortality. The bacteria *Aeromonas* and the fungus *Basidiobolus* have been recovered from clinically diseased Wyoming toads. These agents have rarely been recovered from clinically healthy toads, however samples have been limited to cloacal swabs. Both *Aeromonas* and *Basidiobolus* are present in the environment of most amphibians. Thus, it is assumed that there exists a natural immunity or resistance to these potential pathogens. The potential role of these agents in the mortality of toads needs to be investigated both individually and collectively.

In fish populations, immune function can be compromised by changes in environmental factors such as temperature, pollutants, nutrition, and pathogens. Studies have demonstrated that these factors can depress nonspecific, humoral, and cell mediated defense mechanisms.[4] Suppression of the immune system can increase the susceptibility of these animals to potential opportunistic pathogens. Change in environmental factors may stress or decrease immune function in Wyoming toads and make them more susceptible to infection by Aeromonas, Basidiobolus, or both *Aeromonas* and *Basidiobolus*.

Free-ranging Wyoming toads have also been documented to have increase mortality in late summer and early fall.[7] Changes in bacterial populations may be occurring in the aquatic shoreline habitat. This change may be occurring due to seasonal environmental factors such

as temperature, light, and salinity. During the spring and summer the change may be human influenced, such as through pesticide application. A change in the aquatic bacterial populations may be a contributing factor in the toads becoming diseased.

Our current research is focused on the following five phases: 1) Compare bacterial isolates from clinically healthy and diseased Wyoming toads. 2) Compare 2 bacterial identifications systems (chemical reaction-Pasco Plates verses fatty acid-MIDI) for use on bacteria in amphibians. 3) Conduct bacterial antibiotic sensitivities on predominant bacterial strains from clinically diseased Wyoming toads using antibiotic disks. 4) Experimentally expose surrogate species to *Aeromonas, Basidiobolus* and both *Aeromonas* and *Basidiobolus*. 5) Experimentally expose surrogate species to pesticides and then expose the toads to *Aeromonas, Basidiobolus* and both *Aeromonas* and *Basidiobolus*. Our hopes are that information gained through this research may assist in saving a population of an endangered environmental indicator species, the Wyoming toad from extinction, assist in the development of diagnostic tests for amphibian diseases, and provide information for the assessment of amphibian well being.

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NORMAL AND PATHOLOGICAL ULTRASONOGRAPHIC ANATOMY OF AMPHIBIANS

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Introduction

Ultrasonography has become an increasingly important technique in veterinary medicine. It provides a noninvasive method for direct imaging of internal tissue structures. Ultrasonography is most commonly used for diagnostic examination of soft-tissue structures and for the examination of reproductive anatomy.[6,12] Literature reports of the use of ultrasonography in herpetological species are few[4,7,9,10,11] and there are no reports of its use in amphibians.

There are limited clinical diagnostic procedures available for clinical use in amphibians. Imaging techniques have commonly been limited to radiography. While radiography creates an excellent image of bony structures, it usually provides poor detail of soft tissue organs in amphibians.

Amphibians are well suited for ultrasonography. Their characteristic lack of hair, feathers, or scales, provides an ideal surface for unimpaired imaging. Amphibians, to various degrees, live in an aquatic environment. This allows the use of unique underwater scanning techniques which would be unrealistic for terrestrial animals. Clinically ill amphibians commonly develop coelomic fluid accumulation (ascites, anasarca).[1,2] The presence of this fluid will often enhance the image of internal organ structures.

Materials and methods

Imaging was performed using a B-mode real-time ultrasound scanner (Aloka 500V, Corometrics Medical Systems, Wallingford, Connecticut 06492, USA) with a 7.5 Mhz sector transducer. Initially, a variety of techniques were used for restraining and positioning the animals during ultrasound evaluation. Restraint techniques included sedation, direct manual restraint and placing the individual in a water filled plastic container. Transducer placement included three methods: direct probe-to-skin, probe-to-water and probe-to-plastic. Coupling gel was utilized for all techniques except for the probe-to-water method. The most effective strategy was to place the animal in a normal sternal position within a water filled plastic container. The probe could then either be placed directly in the water or underneath the container in direct contact with the plastic. Two types of containers were utilized: flexible plastic containers (zip-lock bags) and rigid plastic containers (clear plastic food storage containers). Flexible containers provided a slightly improved image but were more cumbersome to use. The rigid containers occasionally produced image artifacts. Either method allowed excellent patient imaging without the need for sedation or direct manual restraint.

Results

In all species examined, the heart provided the most reliable anatomical landmark. The heart can readily be identified (even in very small species) by its contractile motion using real-time ultrasonography. The heart is located on the ventral midline at the level of the forelimbs. Amphibian species have a thin walled, two chambered atria and a single chambered, thick walled ventricle.[3,13] Pericardial effusion has been noted in disease conditions and can be seen as an anechoic region immediately surrounding the myocardium. After the heart has been located and evaluated, the liver can be identified on either side. Amphibians lack a diaphragm and thus all visceral organs lie within the coelomic cavity. In anurans, the liver is divided into two large lobes.[3,13] In salamanders, the liver is a simple elongate structure.[3] Normal hepatic parenchyma appears ultrasonographically similar to that seen in mammalian species.

The gall bladder can be recognized as an extrahepatic spherical anechoic mass adjacent to the heart and liver. The gall bladder can be quite large (equal in size to the heart) in certain individuals. The pancreas and spleen have not been routinely identified in amphibians using ultrasonography.

Both salamanders and anurans have unique fat bodies within their coelomic cavity. These structures can be proportionately quite large especially in well fed individuals just before hibernation. Anuran fat bodies have large fingerlike projections which emerge from a common stalk at the base of each gonad. These were commonly seen in anuran species and are usually moderately hyperechoeic in comparison to the liver. The salamander's fat bodies exist as a longitudinal tissue band between the kidney and gonad. The fat bodies in salamanders are not routinely visualized on ultrasound. The urinary bladder can be easily recognized if full at the time of evaluation. Many species of anurans have large urinary bladders which are voided when the animal is handled. The bladder is seen as a smooth surfaced spherical anechoic structure in the caudal abdomen. Some salamander species have a cylinder-like or bilobate urinary bladder.

Reproductive anatomy changes can be dramatic and are dependent upon the species, age, sex and reproductive status of the animal.[3] It is not uncommon for gravid amphibians to show clinical signs of illness.[1,2] Ova are often produced in large numbers and accumulate in the coelomic cavity. This is usually noted visually as a marked abdominal distension. Ultrasonography has proven useful in easily differentiating gravid anurans from those with ascites. Gravid females have the majority of their coelomic cavity filled with ova. Testes in anurans are oval structures attached to the dorsum, at the base of the fat bodies. Testes can often be visualized adjacent to a full urinary bladder. Ovaries are located in the same approximate position but their size will vary dramatically depending on the animals sexual stage.

The structures of the gastrointestinal system can be imaged via ultrasound to various degrees. The quality of the image is dependent upon the size of the animal, contents within the gastrointestinal tract and the presence of coelomic fluid.

Summary

Ultrasonography is an important, noninvasive diagnostic motility which can provide high quality images of visceral anatomy in amphibian species. Ultrasonography should be used to diagnose and to document normal and pathological conditions in amphibian species.

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PHARMACOKINETICS OF INTRAMUSCULAR ADMINISTRATION OF THREE ANTIBIOTICS IN BULLFROGS (*Rana catesbeiana*)

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Introduction

The Lincoln Park Zoo is committed to the long term maintenance, exhibition and propagation of a number of anuran species. Integral to our ability to safely transport, house and maintain long term survivorship of captive frogs is the ability to provide efficacious health care. A review of the anuran disease literature reveals that most serious anuran disease results from bacterial sepsis (commonly called "red leg") secondary to trauma, inanition, poor water quality, stress, parasitic migration or improper environmental conditions.[2,5,10,12,13] Several studies have identified gram negative aerobic bacterial spp (ie *Aeromonas, Pseudomonas, Citrobacter, Proteus* and *Flavobacterium*) as major anuran pathogens.[1,10,12,27]

Safe efficacious treatment for common anuran bacterial infections requires knowledge of specificity, pharmacokinetics and toxicity of antibacterial agents in frogs. Three readily available antibiotic agents (tetracycline, enrofloxacin, amikacin) which have specificity for common anuran bacterial pathogens were selected for investigation. Tetracycline was the first, and remains the most commonly recommended antibiotic for treatment of bacterial disease in frogs.[4,5,7,8,9,17,25] Recently however, Lincoln Park Zoo has isolated tetracycline resistant organisms from clinically ill amphibians.[28] Widespread bacterial resistance to tetracycline has also been reported in mammals[24] and reptiles.[21,23]

Enrofloxacin is a floroquinolone antibiotic with bactericidal activity against a broad spectrum of gram negative pathogens.[18] Bacterial resistance to enrofloxacin has only rarely been reported[23] and amphibian pathogens isolated at LPZ have been uniformly susceptible.

Aminoglycoside antibiotics demonstrate good bactericidal effect against many gram negative organisms[11,14,23] and have been used clinically in both reptiles and amphibians. Prior reports of the pharmacology of the aminoglycoside gentamicin in amphibians exist.[20,26] Amikacin has a broader spectrum, less resistance and demonstrates less renal, neuromuscular and ototoxicity than gentamicin in mammals[3,14,15,24] and potentially other vertebrates. Pathogens isolated from sick amphibians in the LPZ collection have consistently been sensitive to amikacin.

The pharmacokinetics of the these antibiotics in amphibians has not been well defined. A limited number of pharmacokinetics studies have been completed in reptiles with

enrofloxacin,[22] and amikacin.[16] Extrapolation of reptilian drug dosages to amphibians is questionable at best due to differences in water balance, renal and dermal physiology.[6,19]

This study was designed to assess the pharmacokinetics of a single intramuscular injection of tetracycline, amikacin, and enrofloxacin in bullfrogs (*Rana catesbeiana*). Comparisons of resulting serum levels with the minimum concentrations of each antibiotic required to stop growth of common anuran bacterial pathogens were used to formulate guidelines for objective dosing regimes.

Materials and Methods

The investigation was divided into three parts, one for each of the three antibiotics under investigation. Two dosage trials were conducted for each antibiotic. Six frogs were included per dosage trial. One milliliter of blood was collected at 0, 0.25, 0.75, 1, 2, 4, 8, 24, 48, and 72 hours post injection. Each frog was bled three or four times, resulting in two blood samples for each dosage at each of the ten sampling periods. Plasma was harvested and frozen at -30 degrees celsius. Stored plasma was assayed for antibiotic levels by either fluorescence polarization immunoassay system (amikacin), or high performance liquid chromatography (enrofloxacin, oxytetracycline).

Single injection studies were conducted a dosages of 50 and 100 mg/kg for oxytetracycline, at dosages of 5 and 10 mg/kg for enrofloxacin and 2 and 5 mg/kg for amikacin.

A single injection study for assessment of hematological/biochemical changes was also conducted at the higher dosage of each of the three antibiotics. For each of the three hematological studies, four or five frogs received a single intramuscular injection of antibiotic. The animals were then serially examined and bled at 0, 24, 48, 72 and 96 hours post injection. Blood was analyzed by chemistry profiling and complete blood counts.

Results and Conclusions

The plasma amikacin assay values are presented in Table 1 and Graphs 1 and 2. Similarly the enrofloxacin and oxytetracycline plasma assay values are presented in Tables 2 and 3, and Graphs 3, 4, 5 and 6 respectively.

Amikacin at a dosage of both 2 and 5 mg/kg administered IM in *Rana catesbeiana* resulted in levels greater than the 4 ug/ml, considered effective against a variety of pathogens, within 0.25 hours. Levels were maintained above 4 ug/ml for approximately 7.5 hours at 2 mg/kg, and for approximately 38 hours at 5 mg/kg. No evidence of significant hematologic or biochemical change was found after a single 5 mg/kg IM dose.

Enrofloxacin and its active metabolite ciprofloxacin are frequently effective in inhibiting growth of pathogenic bacteria at serum levels of approximately 0.1 ug/ml. Dosages of 5 and 10 mg/kg once daily maintained the plasma concentration above this level throughout the

dosing interval. A single 10 mg/kg intramuscular dose did not induce any significant hematological or biochemical abnormalities.

The oxytetracycline preparation used had an extended serum half life of approximately 30.5 hours for the 50 mg/kg dose and 48.1 hours for the 100 mg/kg dose. The minimum inhibitory concentration of tetracycline for susceptible bacteria is often between 1-8 ug/ml. At the dosages administered in this study plasma concentrations in this range were maintained throughout the 48 hour dosing interval.

ACKNOWLEDGEMENTS

Support was offered through Institute of Museum Services Grant #IC-30222-93 and the Lincoln Park Zoological Gardens Department of Conservation and Science. All animal trials were reviewed by the Animal Management Committee of Lincoln Park Zoological Gardens. Animal enclosures and husbandry conditions met or exceeded the Universities Federation of Animal Welfare (UFAW) and National Academy of Sciences Subcommittee on Amphibians (NAS) recommendations and were in accordance with all applicable United States Department of Agriculture (USDA) regulations and standards.

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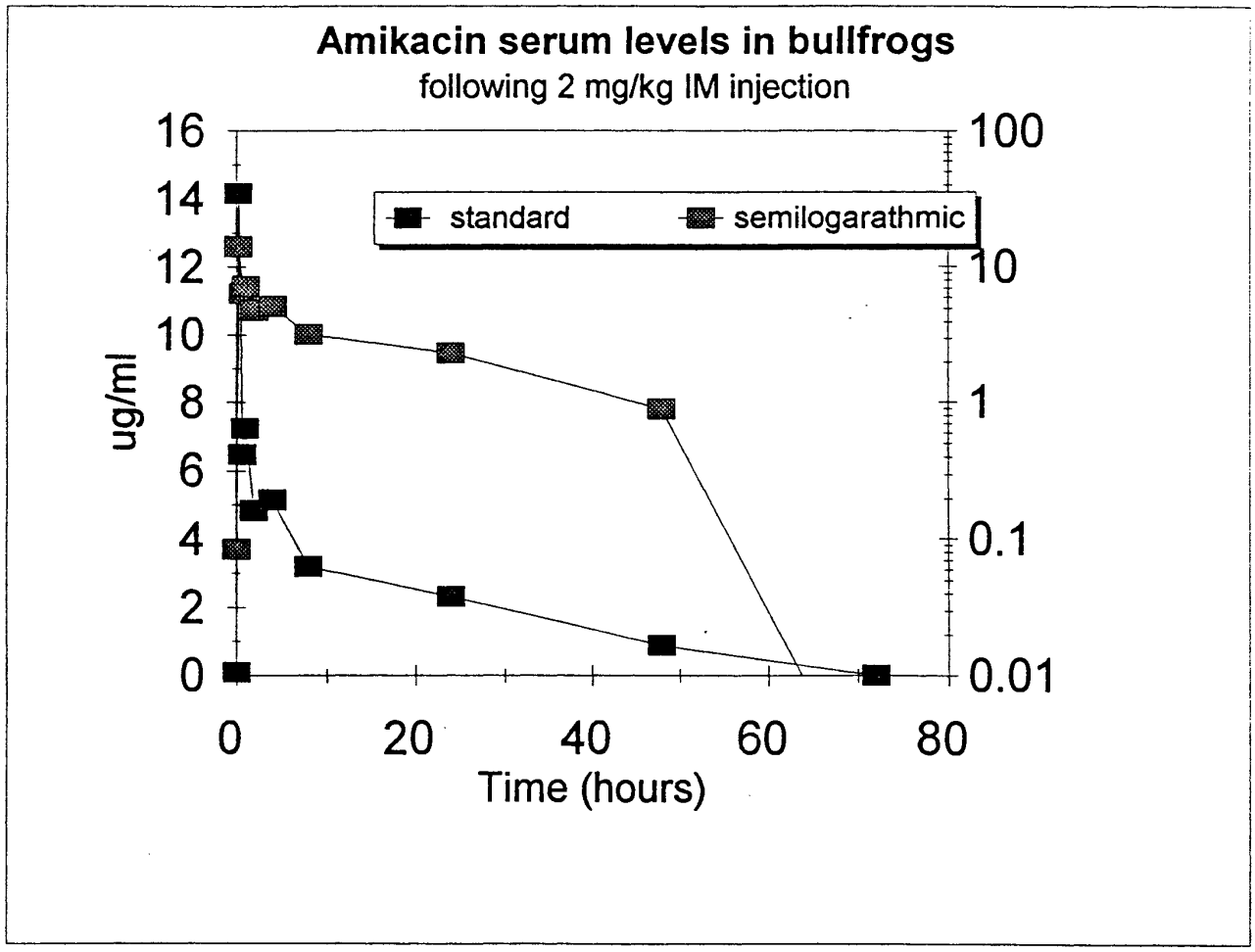
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Table 1
Amikacin blood levels in ug/ml from bullfrogs

		time in hours from injection								
		0	0.25	0.75	1	2	4	8	24	48	72
2mg/kg											
	weight (grams)										
	337			6.83			5.40			0.88	
	421			6.10			4.93			0.89	
	637		17.15			8.64			2.32		
	188		11.17			1.06			**		
	277	0.17			7.24			3.20			*
	411	*			**			**			*
average	378.5	0.09	14.16	6.47	7.24	4.85	5.17	3.20	2.32	0.89	0.00
5mg/kg											
	weight (grams)										
	514			14.37			12.91			2.18	
	351			22.83			15.08			1.71	
	227		0.03			12.70			5.38		
	203		12.97			17.84			4.91		
	361	*			21.03			11.01			0.78
	581	*			26.40			12.70			1.01
average	372.8	0.00	6.50	18.60	23.72	15.27	14.00	11.86	5.15	1.95	0.90

* level below sensitivity of assay
** sample unsuitable for analysis


Amikacin serum levels in bullfrogs
following 2 mg/kg IM injection
standard
semilogarathmic
ug/ml
16
14
12
10
8
6
4
2
0
100
10
1
0.1
0.01
0
20
40
60
80
Time (hours)

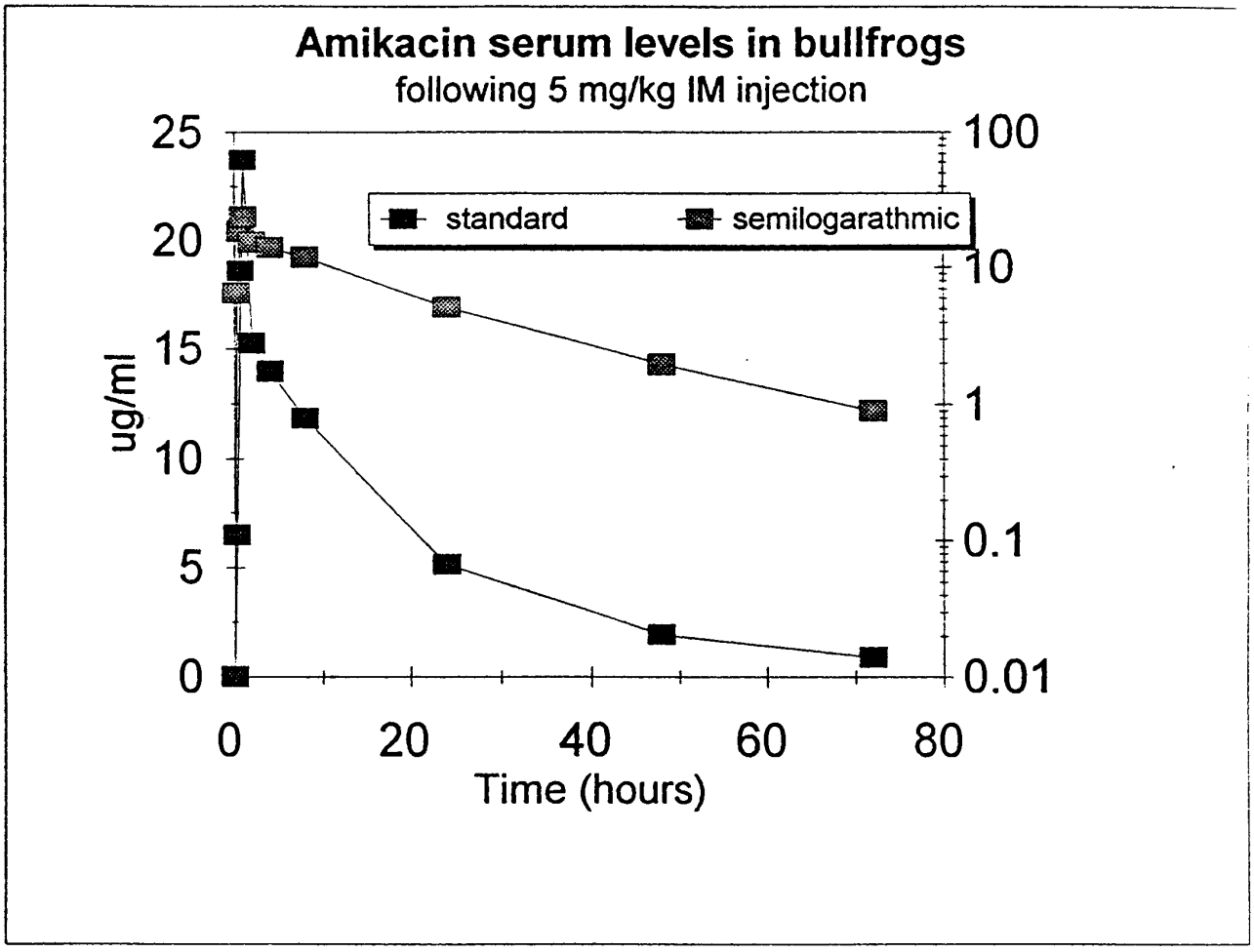
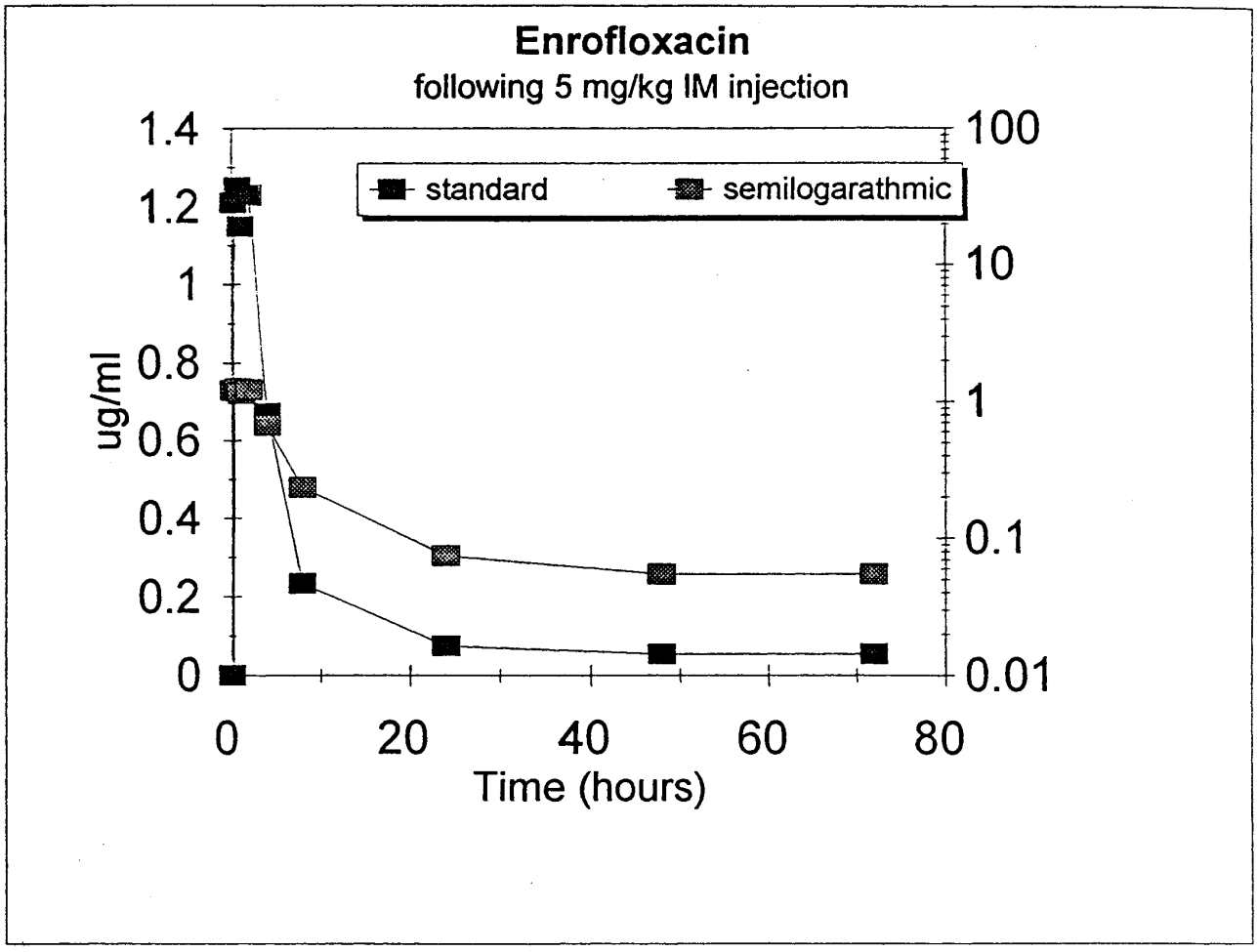

Amikacin serum levels in bullfrogs
following 5 mg/kg IM injection
standard
semilogarathmic
ug/ml
25
20
15
10
5
0
100
10
1
0.1
0.01
0
20
40
60
80
Time (hours)

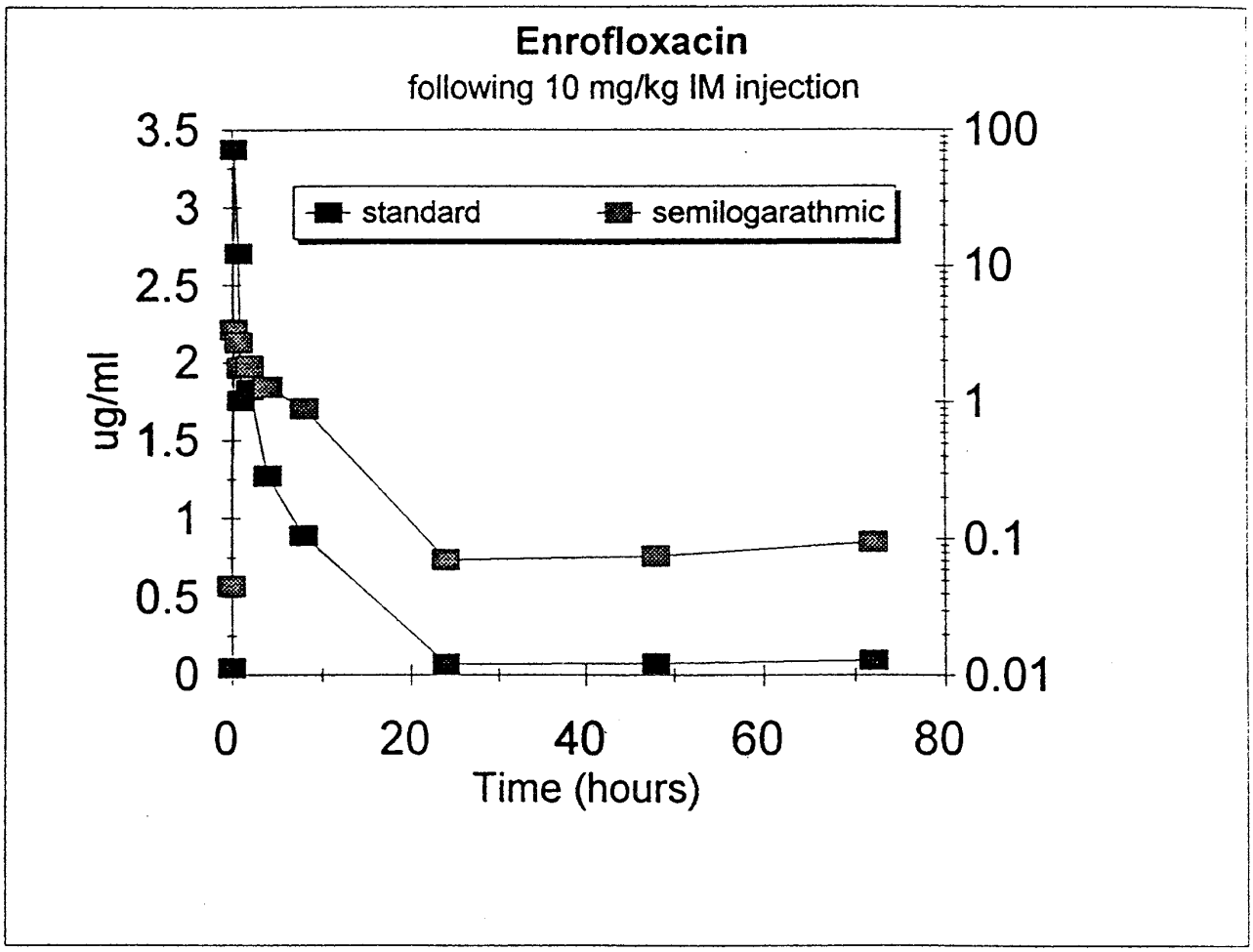
Table 2

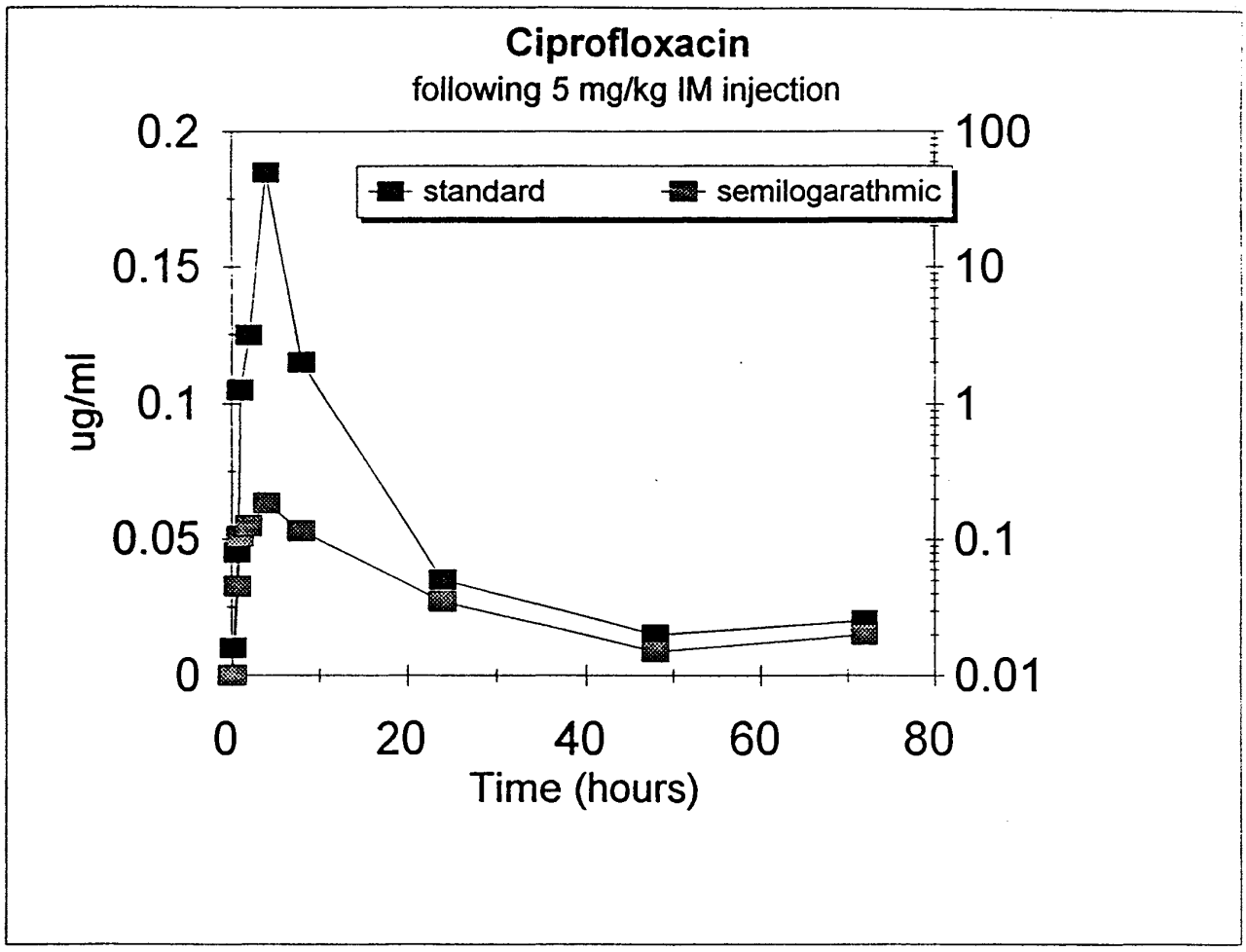
Enrofloxacin and Ciprofloxacin blood levels in ug/ml from bullfrogs

	Time in hours from injection																			
	0		0.25		0.75		1		2		4		8		24		48		72	
Subject	cipro	enro	cipro	enro	cipro	enro	cipro	enro	cipro	enro	cipro	enro	cirpo	enro	cipro	enro	cipro	enro	cipro	enro
weight (grams)																				
450.00					0.08	1.64					0.32	0.94					0.02	0.06		
649.00					0.01	0.86					0.05	0.40					0.01	0.05		
443.00			0.02	2.01					0.02	0.78					0.03	0.07				
414.00			0.00	0.41					0.23	1.68					0.04	0.08				
239.00	0.00	0.00					0.09	1.22					0.11	0.25					0.02	0.07
220.00	0.00	0.00					0.12	1.08					0.12	0.22					0.02	0.04
avera 402.5	0		0.01		0.045		0.105		0.125		0.185		0.115		0.035		0.015		0.02	
		0		1.21		1.25		1.15		1.23		0.67		0.235		0.075		0.055		0.055
Subject																				
weight (grams)																				
618.00					0.07	2.72					0.27	1.39					0.02	0.10		
516.00					0.14	2.69					0.25	1.16					0.01	0.05		
440.00			0.00	2.15					0.08	1.10					0.05	0.07				
372.00			0.03	4.60					0.63	2.55					0.03	0.07				
355.00	0.03	0.09					0.02	0.36					0.34	0.72					0.03	0.08
218.00	0.00	0.00					0.02	3.15					0.33	1.06					0.03	0.11
avera 419.833	0.015		0.015		0.105		0.02		0.355		0.26		0.335		0.04		0.015		0.03	
		0.045		3.375		2.705		1.753		1.825		1.275		0.89		0.07		0.075		0.095

* level below sensitivity of assay


Enrofloxacin
following 5 mg/kg IM injection
standard
semilogarathmic
ug/ml
1.4
1.2
1
0.8
0.6
0.4
0.2
0
100
10
1
0.1
0.01
0
20
40
60
80
Time (hours)


Enrofloxacin
following 10 mg/kg IM injection
standard
semilogarathmic
ug/ml
3.5
3
2.5
2
1.5
1
0.5
0
100
10
1
0.1
0.01
0
20
40
60
80
Time (hours)


Ciprofloxacin
following 5 mg/kg IM injection
standard
semilogarathmic
ug/ml
0.2
0.15
0.1
0.05
0
100
10
1
0.1
0.01
0
20
40
60
80
Time (hours)

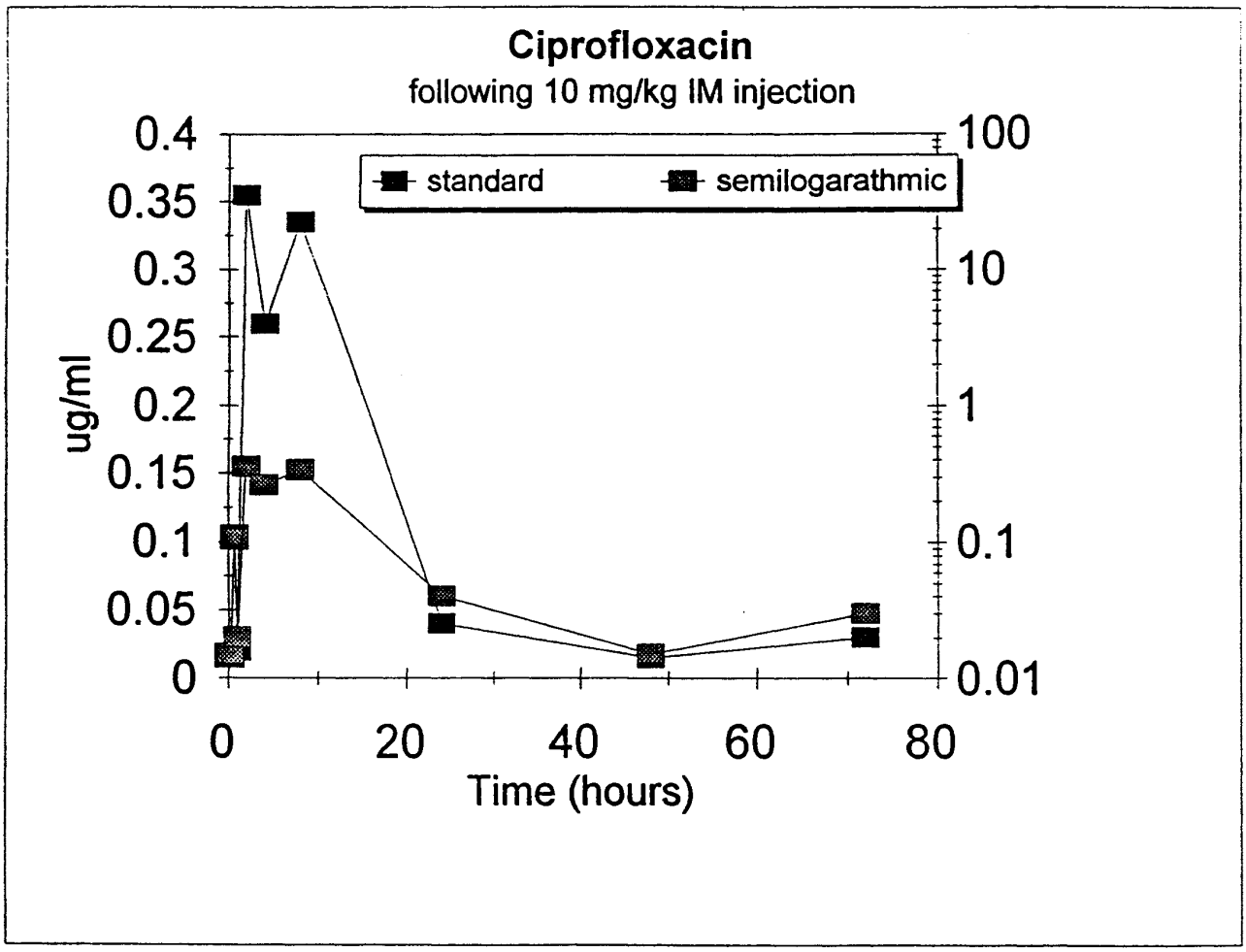
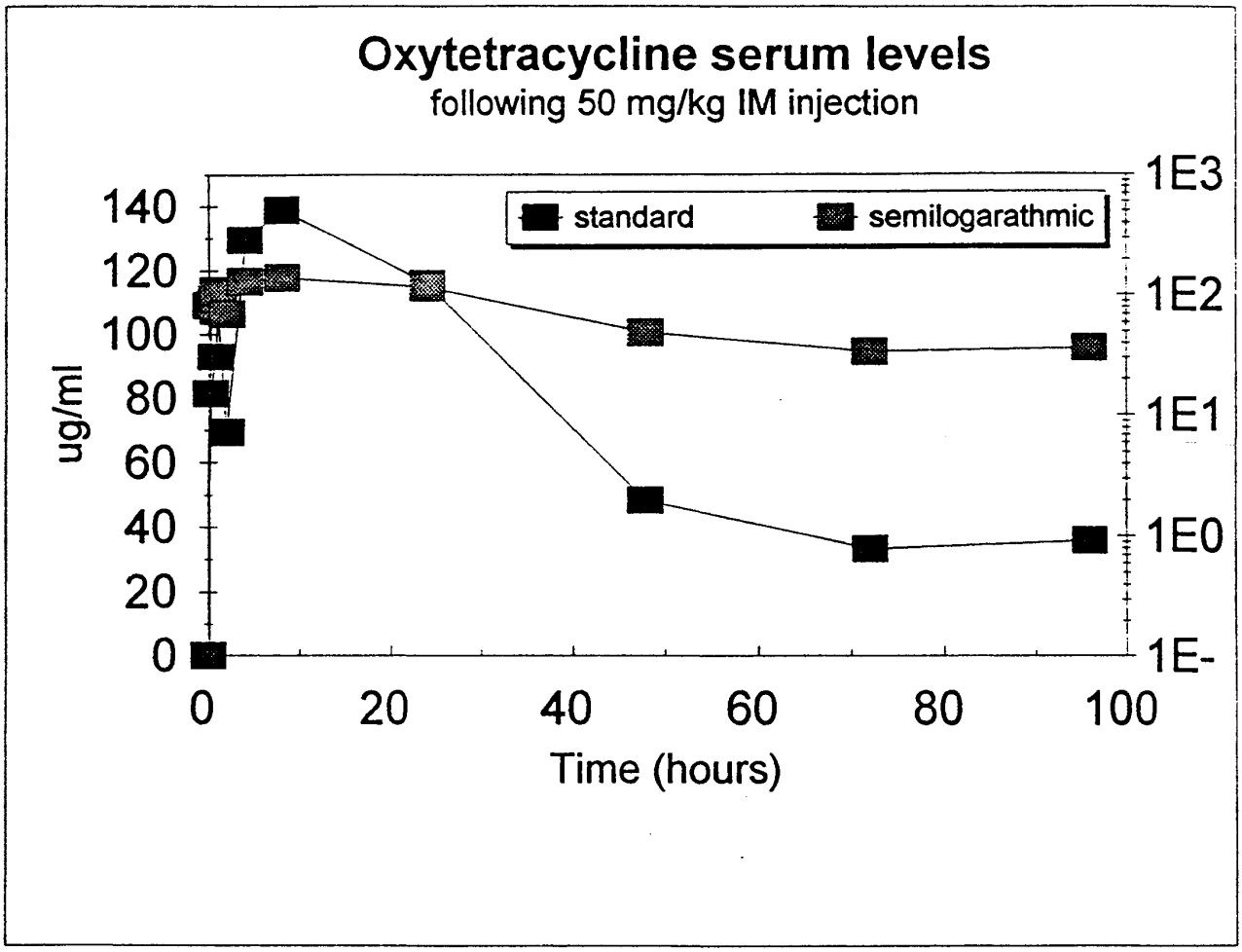
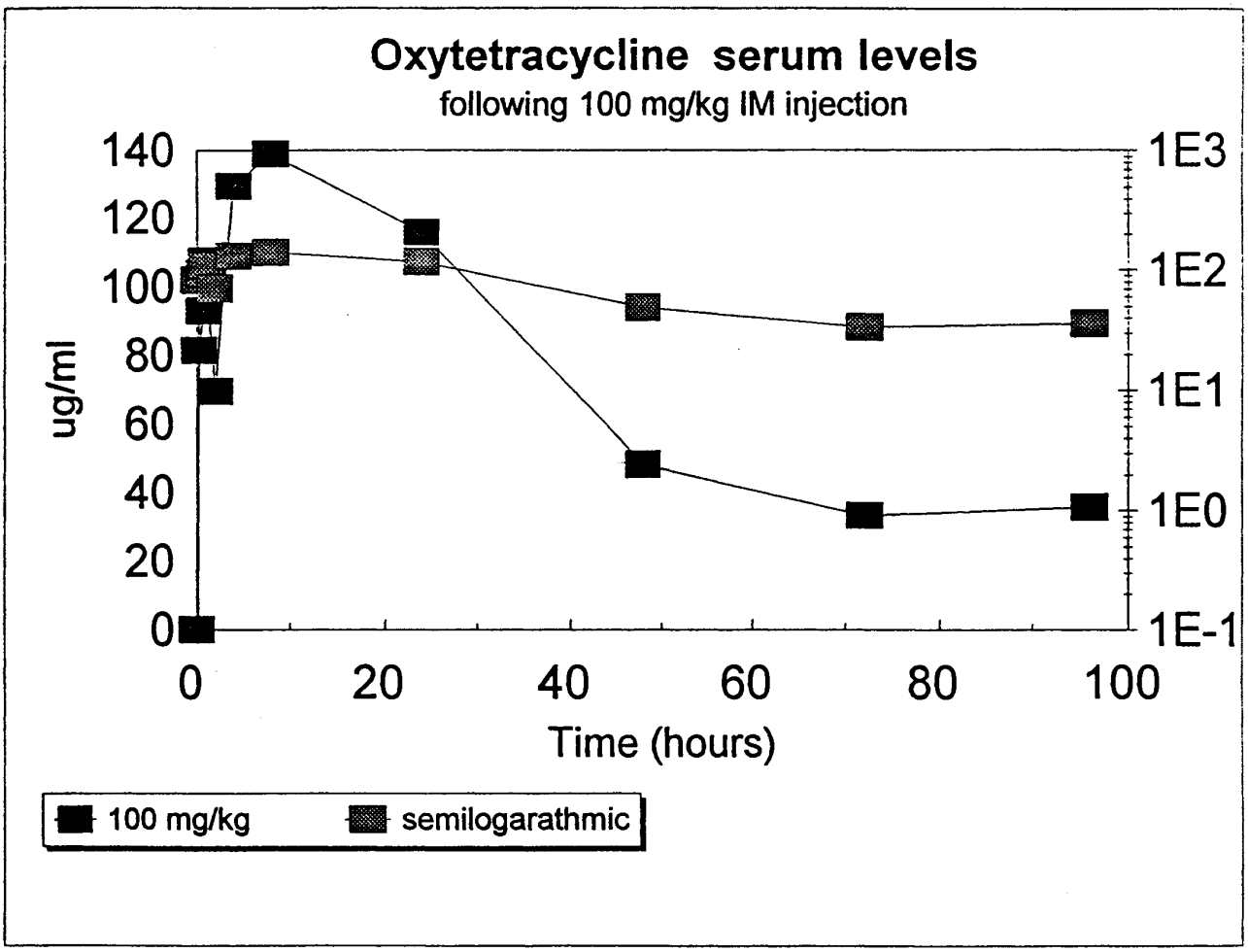

Ciprofloxacin
following 10 mg/kg IM injection
standard
semilogarathmic
ug/ml
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
100
10
1
0.1
0.01
0
20
40
60
80
Time (hours)

Table 3
Oxytetracycline blood levels in ug/ml from bullfrogs

		Time in hours from injection									
50 mg/kg		0	0.25	0.75	1	2	4	8	24	48	72	96
	weight (grams)											
	663.0	0.00			47.00			120.00			12.00	
	471.0	0.00			101.00			73.00			14.00	
	438.0		19.00			56.00			58.00			16.00
	477.0		57.00			54.00			49.00			13.00
	253.0			17.00			31.00			10.00		
	248.0			87.00			78.00			16.00		
average	425	0.00	38.00	52.00	74.00	55.00	54.50	96.50	53.50	13.00	13.00	14.50
100 mg/kg												
	weight (grams)											
	558.0	0.00			49.00			118.00			67.00	
	474.0	0.00			166.00			160.00			**	
	485.0		83.00						148.00			64.00
	365.0		80.00			139.00			84.00			8.00
	420.0			121.00			129.00			22.00		
	254.0			65.00			130.00			75.00		
average	426	0.00	81.50	93.00	107.50	69.50	43.17	139.00	116.00	48.50	67.00	36.00

**found dead, nec#623752,


Oxytetracycline serum levels
following 50 mg/kg IM injection
standard
semilogarathmic
ug/ml
140
120
100
80
60
40
20
0
1E3
1E2
1E1
1E0
1E-
0
20
40
60
80
100
Time (hours)


Oxytetracycline serum levels
following 100 mg/kg IM injection
ug/ml
140
120
100
80
60
40
20
0
1E3
1E2
1E1
1E0
1E-1
0
20
40
60
80
100
Time (hours)
100 mg/kg
semilogarathmic

PROBLEMS WITH METABOLIC SCALING OF ANTIMICROBIAL DOSAGES IN REPTILES

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At present, the number of pharmacokinetic studies, determining accurate dosages of antimicrobials in reptiles is limited. Therefore metabolic scaling is often applied in order to estimate doses of antimicrobials in the treatment of the reptilian patient. In order to determine dosages, an animal's size rather than its mass is being used. The uptake, distribution and excretion of a drug are much closer related to the metabolic rate than to the size of the animal. In order to maintain a certain serum level of an antimicrobial drug, smaller reptiles require higher dose rates (mg/kg) given more frequently than large reptiles.[2] While it is possible to extrapolate treatment regimen from one animal to another by estimating minimum energy costs (MEC) and specific minimum energy costs (SMEC), more factors have to be taken into consideration when treating a reptilian patient.[3] Reptiles are ectothermic animals and consequently, dosage, uptake, distribution and excretion of a drug is dependent on the body temperature. In addition smaller reptiles will have faster uptake distribution and excretion of drugs than larger animals. In order to achieve the most effective treatment, a reptile should have a body temperature within the range of its preferred body temperature.[1] It has been shown that in snakes, an increase of ambient temperature improved the distribution and clearance of the antimicrobial amikacin.[2] In addition, seasonal and species differences should be considered when choosing an antimicrobial agent.[4] Care should also be taken in applying dosages from one species of reptile to another. With the limited number of pharmacokinetic studies conducted, the use of metabolic scaling for appropriate dosages and determination of frequencies is presently the most helpful tool for the antimicrobial treatment of reptiles. Further clinical trials involving multiple species of reptiles are needed, to accurately determine effective antimicrobial treatment regimen.

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THE REPTILIAN RENAL PORTAL SYSTEM AND ITS EFFECT ON DRUG KINETICS

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Controversy has raged for many years over the potential effect of the reptilian renal portal system on antibiotic kinetics. Numerous authors have stated that drugs should not be injected in the hind region of the reptilian body as they will be conveyed to the kidneys, increasing the possibility of nephrotoxicity for aminoglycosides, and reducing the likelihood of achieving the desired therapeutic effect, in the case of other drugs. The purpose of this study was to test the validity of this hypothesis.

Twenty red-eared sliders (*Trachemys scripta elegans*) were used for the study, and gentamicin and carbenicillin were selected as the study drugs. Ten of the sliders received a dose of 10 mg/kg gentamicin intramuscularly, five in a forelimb and five in a hindlimb. Serial blood samples for gentamicin analysis were then taken over the next seven days. When all the drug had been cleared from the animals the experiment was repeated. The sliders which had previously received the drug in a forelimb were treated in a hindlimb and those which had previously been treated in a hindlimb were now injected in a forelimb.

The carbenicillin study followed an identical protocol with each individual receiving 200 mg/kg carbenicillin intramuscularly.

When the results were examined, no significant differences in the peak blood levels and the time of their occurrence, half life, area under the curve, volume of distribution or clearance could be found for the sliders treated with gentamicin in the forelimb or the hindlimb. There was also no evidence of nephrotoxicity. However, a significant difference was apparent for the sliders treated with carbenicillin. Those that received the drug in a hindlimb had significantly lower blood levels for the first twelve hours post injection than those which received it in a forelimb. However, as blood levels for both injection sites were still well above the MIC for organisms generally treated with carbenicillin, this difference is not likely to be clinically significant.

A study was also performed to describe the anatomy of the renal portal system in the red-eared slider and examine blood flow through these vessels by fluoroscopy. In this study, radio-opaque dye injected into the femoral vein flowed directly into the abdominal vein to the liver, and bypassed the kidney. A small amount of dye entered the kidney via minor femoral vein tributaries. Dye injected into the dorsal coccygeal vein took one of two routes. It flowed through the circumflex iliac vein entering either the abdominal vein, bypassing the kidney, or the iliac vein, entering the kidney.

When the latter event occurred dye also entered the abdominal vein, but only as far as its junction with the femoral vein, and then stopped abruptly. This provided circumstantial evidence for the presence of a valve, similar to that described in birds, capable of shunting blood through or around the kidneys. A structure within the lumen of the abdominal vein, compatible with a valve, was observed histologically at this site. When the valve is closed blood would be forced into the iliac vein and enter the kidney and when it is open blood would enter the abdominal vein and bypass the kidney.

PHARMACOKINETICS OF ENROFLOXACIN IN JUVENILE BURMESE PYTHONS (*Python molurus bivittatus*)

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Bacterial infections are a commonly encountered problem in the veterinary care of snakes. Minimal information is available regarding the dose and frequency of administration of antimicrobials necessary to obtain therapeutic concentrations. This scarcity of pharmacokinetic data poses an important problem to veterinarians attempting to treat bacterial diseases in snakes. The objective of this study was to determine the plasma concentrations and disposition kinetics of enrofloxacin in juvenile Burmese pythons required to make appropriate treatment recommendations.

Eleven juvenile Burmese pythons were randomly divided into two groups. Twelve to twenty-four hours prior to each individual study, a catheter was surgically placed into the anterior carotid artery of the snake to facilitate blood sample collection. Six pythons received a single intramuscular injection of enrofloxacin at a dose of 5 mg/kg body weight. Blood samples were collected at 30 minutes, 1, 3, 6, 12, 24, 48, 72, and 96 hours post-injection. A mean maximum plasma concentration of enrofloxacin of 1.56 ug/ml was measured at 6 hours post injection. The apparent terminal half-life was calculated to be 6.37 hr. The second group of five snakes received intramuscular injections of enrofloxacin at 5 mg/kg body weight every 24 hours for 5 days. Blood was collected immediately prior to and at 6 hours after each injection. Throughout the 5 day period there was a stepwise increase in mean trough and peak plasma concentrations of enrofloxacin.

Pharmacokinetic data in this study were assessed against minimum inhibitory concentrations of enrofloxacin for *Pseudomonas sp.* isolates in snakes obtained from historical data of the Veterinary Medical Teaching Hospital, University of Florida. This study indicates that an effective dosage regimen for the use of enrofloxacin in treatment of *Pseudomonas sp.* infection in juvenile Burmese pythons is 5 mg/kg body weight intramuscularly every 48 hours.

ACKNOWLEDGEMENTS

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USE OF LONG-ACTING IVERMECTIN SUSPENSION IN SNAKES

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Introduction

The snake mite, *Ophionyssus natricis*, has been an increasing problem in the San Diego Zoo reptile collection for the past four years. Part of our treatment protocol involves 3-4 weekly ivermectin injections. In the larger pythons and boas this requires several keepers for manual restraint. The repeated handling of infested snakes permits the spread of mites into the environment. Keepers can also act as vectors, spreading mites to other parts of the building.

A study by Boyce et al[1] describing the use of ivermectin implants in bighorn sheep for the treatment of psoroptes lead to an inquiry regarding applicability in snakes. This implant has been investigated in domestic cattle for control of livestock pests.[2] Rather than an implant, a suspension containing microencapsulated ivermectin is currently being studied. In cattle given the suspension, serum levels of ivermectin were maintained for 90 days and 3 distinct peaks were seen at 1, 6, and 10 weeks post injection.[3] Peak concentrations in cattle averaged 50 PPB.

A one-time treatment that would maintain adequate serum ivermectin levels for an extended period of time would eliminate multiple handling of infested snakes. The goals of this study were to evaluate the safety of the long-acting ivermectin suspension in snakes and to measure serum ivermectin levels of ivermectin. The authors are not aware of any publications reporting serum ivermectin levels in snakes.

Materials & Methods

Four large boids were selected for use to facilitate collection of enough serum for the ivermectin assay. Three reticulated pythons, *Python reticulatus*, and one African rock python, *Python sebae,* were used (Table 1). Snakes had been in the collection for a minimum of one year prior to the study. With the exception of python #1 all snakes were healthy prior to the start of the study. Reticulated python #1 was dehydrated based on the outward appearance of its skin (dull and dry). Problems existed with the humidity control in the exhibit. The snake was treated with subcutaneous fluids and allowed to soak in water for several hours. This snake was still included in the study due to the small number of individuals available for use.

The ivermectin suspension is composed of Medisorb Lactide/Glycolide (PLGA) copolymer microspheres. Half the spheres are 50:50 D,L-PLGA and half are 65:35 D,L-PLGA polymer. The spheres range in diameter from 25-180 microns and contain 25% ivermectin by weight. The two types of spheres degrade at different rates. The spheres are mixed thoroughly with sterile methylcellulose (KY jelly) and injected subcutaneously.

Three of the four snakes were given a subcutaneous injection of sterile KY jelly as a control. Two weeks later the ivermectin suspension was administered. Each snake was dosed at 2000 ug/kg of the 50:50 D,L-PGLA polymer and 2000 ug/kg of the 65:35 D,L-PGLA polymer. Reticulated python #3 did not receive the control injection because mites were discovered the week prior to the start of the study. This snake received the ivermectin suspension when the others received the control injection. The location of the injection was recorded for each snake to monitor any local tissue reaction.

Using the caudal tail vein blood was obtained prior to the first injection of methylcellulose in snakes #1,#2, and #4 and prior to the ivermectin suspension in snake #3. Snakes were then bled every 2 weeks throughout the duration of the study (4 months). CBC and chemistry panels were performed on plasma, and serum was banked for the ivermectin assay done at the end of the study. Plasma chemistries were performed on a Kodak Ektachem DT60II analyzer (Eastman Kodak Company, Rochester, NY 14650 USA). Serum ivermectin levels were determined by a liquid chromatography assay as previously described.[4] In bovine serum this assay will detect ivermectin levels as low a 2 ppb in a 5 ml serum sample.

Results

No adverse reactions were noted in any of the snakes. No abnormalities developed at the injection sites. All snakes continued to eat during the study. Values for CBC and plasma chemistries were within normal limits.

Ivermectin levels are shown in Table 2. High serum ivermectin levels were obtained in the African rock python. Reticulated python #3 had measurable serum levels for 8-10 weeks and then levels dropped off. Reticulated pythons #1 and #2 did not develop significant serum levels.

No further mites were noted on reticulated python #3 throughout the study. Two weeks after the ivermectin suspension injection, the African rock python was found with a heavy mite infestation. This animal was removed from the reptile building due to potential for continued spread of mites into the environment and was housed at the hospital for the remainder of the study. No additional treatment was given. Each time the snake was restrained for blood sampling the mite burden was subjectively evaluated. The number of mites seen progressively declined and by 12 weeks post-ivermectin no live mites were present.

Discussion

The goal of this study was to evaluate the safety of the suspension and to determine serum ivermectin levels. No detrimental effects from the ivermectin suspension were observed. Though it was not part of the original study design, two snakes inadvertently became infested with mites. These two pythons developed measurable serum ivermectin levels which are considered to be clinically effective since the mites were eliminated.

Possible explanations for the variation in serum levels include: leakage of suspension from injection site; variation in metabolism between the snakes due to differences in micro-environmental temperature or activity level; species differences between reticulated and african pythons; variation in vascularity of injection sites. The hydration status of reticulated python #1 at the start of the study may have also affected release of ivermectin from the suspension.

A mite treatment program should target systemic and topical treatments as well as strict hygiene measures. The African rock python was deliberately not treated with anything else in order to fully evaluate the ivermectin suspension. Topical treatment using dri-die and pyrethrins would also have been instituted in a non-experimental situation.

These results are encouraging and warrant further investigation. Evaluation of the pharmokinetics of ivermectin following a single 200 ug/kg dose is needed in addition to studies utilizing more animals and quantitative evaluations of efficacy.

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Table 1. Pythons used for this study

Common name	Snake #	Weight
Reticulated python	1	26.7 kg
Reticulated python	2	17.6 kg
Reticulated python	3	26.2 kg
African rock python	4	21.8 kg

Table 2. Serum Ivermectin Levels, PPB

Weeks post ivermectin	RETIC. #1	RETIC. #2	RETIC. #3	AFRICAN ROCK #4
-2[a]	0	0	ND	0
0[b]	0	0	0	0
2	0	7*	24	44
4	5*	3*	21	35
6	0	3*	13	46
8	0	0	NA	64
10	0	0	9	51
12	0	0	0	38
14	0	0	0	45

a-prior to control injection
b-prior to ivermectin suspension
ND-no control done due to mite infestation.
NA-unable to analyze because serum gelled
*-Values less than 7 PPB difficult to quantify because of insufficient volume of serum.

TRICHOMONAS ASSOCIATED WITH OCULAR AND SUBCUTANEOUS LESIONS IN GECKOS

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Introduction

Trichomonas sp. are protozoan parasites that have a broad host range. Many species of *Trichomonas* have been identified, however many more have not yet been thoroughly characterized. While all *Trichomonas sp.* have a parasitic lifestyle, the great majority are considered non-pathogenic commensals. These commensal organisms generally inhabit the lumen of the lower gastrointestinal tract, and their association with disease has been speculative at best.[10] Several trichomonads are well known pathogens affecting the reproductive organs of humans (*Trichomonas vaginalis*), and bovines (*Tritrichomonas foetus*), and the upper gastrointestinal tract of avians (*Trichomonas gallinae*).

Trichomonads are generally considered surface-dwelling non-invasive organisms; however, their penetration into deeper tissues is not without precedent. *T. gallinae* is known to invade the liver, lungs, air sacs, heart, pancreas, spleen, kidney, trachea, and bone marrow.[11] Narcisi has shown the more virulent strain (*T. gallinae*, Eiberg strain) is able to spread to these organs hematogenously after ulcerating the gastrointestinal tract.[11] *T. vaginalis* has been demonstrated sub-epithelially in the human prostrate and cervix indicating transepithelial migration.[4] *T. vaginalis* has also been inoculated subcutaneously in mice and induced subcutaneous abscesses,[4,9] but no reports of naturally acquired subcutaneous trichomoniasis exist. Culbertson [4] suggests that the apparent ability of several species of *Trichomonas* to invade tissues and/or migrate to aberrant tissues suggests a need for revising the concept of trichomonads as strictly surface-dwelling parasites.[2]

Trichomonads have been associated with reptiles for years, although their pathogenicity has not been proven in these hosts. The observation of, and treatment for gastrointestinal trichomoniasis in reptiles is documented, however the correlation of disease and parasitism is lacking.[5,6,8,13] When associated with clinical disease in reptiles, trichomonads have been accompanied by other known pathogens such as *Entamobea invadens* and *Pseudomonas sp.* or *Aeromonas sp.*[5] Levine (1985) suggests that concurrent gastrointestinal disease can increase the number of *Trichomonas* by local changes in the intestinal mileau favoring trichomonad production and shedding, but he attributes little pathogenicity to these organisms in this process.[10]

Hilgenfild[7] reported necropsy findings of trichomonads in pathologically altered lungs of a western diamondback rattlesnake (*Crotalus atrox*) and Walton[14] found liver lesions associated with *Tritrichomonas augusta* in a leopard frog (*Rana pipiens*). Both of these studies failed
to meet Koch's postulates after laboratory investigation. Thus, while these cases don't prove trichomonads cause disease in poikilotherms, they are strongly suggestive of the possibility of trichomonad induced lesions in poikilotherms.

The following cases report of *Trichomonas* occurring in subspectacular and subcutaneous lesions in three species of geckos. All three animals were presented within a period of three months, and all came from the same collection of privately owned lizards. To the best of the authors' knowledge, these cases represent the first report of naturally acquired ophthalmic and subcutaneous trichomoniasis in animals, and one of few reports documenting pathological changes associated with *Trichomonas* in poikilotherms. Diagnostic and treatment considerations are discussed, as are potential explanations of apparent pathogenesis of trichomonads in non-gastrointestinal organs of geckos.

Case reports

Case 1

A 33 gm lined gecko (*Gecko vetalus*) was presented for evaluation of unilateral ocular swelling. The swelling had an acute onset and the animal continued to eat and act normally. On examination the gecko appeared to be in good flesh with no abnormalities except for the eye. The right eye was distended to almost twice normal size by an accumulation of flocculent fluid between the spectacle and cornea. The spectacle was aspirated with a 27 ga. needle on a tuberculin syringe and the space was drained of 0.3 cc fluid. After aspiration, the eye was normal in function and appearance. The fluid was examined microscopically directly and after Dip-Quick stain (Jorgensen Laboratories, Loveland, Colorado 80538 USA). The stained sample was non-diagnostic, revealing only a few inflammatory cells, amorphous debris, and no bacteria. The direct wet-mount contained macrophages and thousands of live, mobile, flagellated trophozoites. Some of the macrophages had phagocytized single flagellated trophozoites. Besides draining the fluid, no other treatment was done at that time.

Dried slides and the remaining fluid sample were forwarded to the Department of Veterinary Pathobiology at Texas A&M University for parasite identification. No recognizable organisms were seen, but they may have been too fragile to survive mailing in form suitable for identification. The gecko was rechecked one week later, at which time its eye was again greatly swollen. The space was drained of 0.37 cc of the fluid and a sample was taken directly to the lab where it was examined within a few hours of collection and live *Trichomonas sp.* were observed and suitable stained slides were prepared.

Treatment was instituted at this time by fenestrating the spectacle with a 25 ga. needle to allow ventral drainage through the small window and by administering 1.25 mg

metronidazole (Flagyl Suspension, Rhone-Poulenc Pharma de Mexico, Jose MA, Rico 611 03100 Mexico) p.o. repeated in 2 wks. To cover the possibility of a secondary bacterial infection, the gecko was also placed on 0.25 mg enrofloxacin (Baytril Injectable Solution, Mills Inc., Shawnee Mission, Kansas 66201 USA) i.m. once daily for seven days.

After the first dose of metronidazole, the lesion improved dramatically and after the second dose the eye became normal in appearance and function. At 1 yr follow-up, there has been no recurrence of ocular or other lesions, and the gecko remains healthy.

Case 2

A 72.4 gm tokay gecko (*Gekko gecko*) was presented for evaluation acute onset bilateral ocular swelling. This animal was presented 1 mo after the line gecko in case 1 and was from the same collection. No other problems or lesions were identified in the history or upon physical examination.

The ocular lesions of this lizard appeared the same as that of the line gecko in that flocculent fluid was retained within the subspectacular space, the only difference being that the tokay gecko had bilateral ocular swelling. Flocculent fluid was drained from the right and left subspectacular spaces via a 27 ga. needle of 0.5 ml and 0.7 ml respectively. Microscopically, the fluid contained macrophages and live trichomonads when viewed directly, but in smaller numbers than in the first animal. No bacteria or other pathogens were observed in wet mounted or stained specimens.

Treatment, following the process used in the line gecko, consisted of fenestrating the ventral aspect of the spectacle to create drainage and administering metronidazole at 2.5 mg p.o. repeated in 2 wks. The lesions resolved with this treatment, and there has been no recurrence or other lesions at one year follow- up.

Considering transmission likely, the owner was instructed to sanitize housing, food and water bowls, and to wash his hands between handling different animals. For 2 mos, no other cases were presented.

Case 3

A 39.4 gm leopard gecko (*Eublepharis macularius*) was presented for evaluation of subcutaneous swellings. History and physical examination failed to reveal other problems or lesions. Initially, there were 5 subcutaneous masses located on the dorsal hip and axillary regions. The masses were approximately 3mm x 2mm and were pale tan in color. Stained cytologic specimens of the aspirated material revealed necrotic cellular debris and numerous clear cuboidal crystals. No inflammatory cells or organisms were seen. A presumptive diagnosis of epidermal inclusion cysts was made and treatment consisted of lancing and expressing the cysts and daily soaks of chlorhexidine diacetate (Nolvasan Solution, Aveco, Fort Dodge, Indiana 50501 USA) diluted to 0.005 mg/ml.

Two months later, the cysts had increased in size and number. Aspiration cytology at this time was identical to that previously performed. The diagnosis was reconsidered and calcinosis cutis was suspected based on physical, cytological, and historical findings of zealous vitamin and mineral supplementation. Restriction of dietary supplements was instituted to no avail. The gecko returned after 1 mo of dietary restriction and the tumors were twice the original size. Aspiration cytology at this time was identical to earlier specimens, but empiracle antibacterial therapy was instituted at this time and the gecko was started on 0.72 mg trimethoprim-sulfa (Sulfatrim Pediatric Suspension, Barre-National Inc. Baltimore, Maryland 21207 USA) p.o. once daily for 7 days. After no response to this treatment, the gecko was started on enrofloxacin at 0.2 mg p.o. once daily for 10 days.

After 3 mo of dietary vitamin and mineral restriction and two courses of antibacterial therapy, the lesions were still spreading and enlarging. At this time, microscopic examination of a direct wet mount revealed thousands of trichomonads from both old and newly developed cysts, indicating the etiology of these masses had been undiagnosed until direct microscopic exam of the cystic fluid. The gecko was given 8.5 mg metronidazole orally with a repeat dose in 14 d. Three weeks after institution of metronidazole the cysts were almost completely resorbed and 6 wk follow-up showed very small cysts with scant white exudate. These were examined microscopically and no trichomonads or other organisms were found. At 6 mo follow-up all cutaneous lesions were healed and there has been no recurrence in this gecko.

Discussion

Findings in these cases that are suggestive of trichomonad induced disease in this group of geckos are:

1. All lesions contained numerous viable *Trichomonas*, some were found intracellularly in macrophages.
2. No other etiologic agents were observed by light microscopy, and the subcutaneous lesions failed to respond to antibacterial or dietary therapy.
3. Response was rapid and complete with no recurrence after institution of metronidazole therapy.
4. All three cases came from the same collection, and were presented within 3 months of each other. This acute outbreak suggests transmissibility or environmental factors facilitating an aberrant infection.

Limitations of these findings include the possibility that an underlying and undetermined disease process facilitated the infections as histopathology and bacterial cultures were not done for economic reasons. Koch's postulates were not attempted, so *Trichomonas* may not have caused the described lesions, but, rather may have been a secondary contaminant. Several attempts to culture, isolate, and speciate these *Trichomonas* were unsuccessful, thus, they may represent different species of trichomonads, and their source can only be speculated upon.

Clinical implications of these findings are that ocular and subcutaneous lesions of unknown etiology in geckos, and possibly other poikilotherms, should be examined for the presence of *Trichomonas*. Although viable trichomonads have been recovered during fecal examinations of frozen reptiles,[8] the organisms from these cases were quite fragile. Attempts to ship dried slides and fresh samples in the exudate of origin destroyed the organisms as did attempts to culture them at mammalian and room temperatures. Stained specimens of trichomonad containing exudate were not as helpful diagnostically as were direct wet mount microscopic examination, so we recommend this simple procedure for all unexplained ocular, dermal, and possibly other lesions in reptiles. It is important to note that many geckos have a clear, cutaneous structure, the tertiary spectacle, which covers and protects the cornea.[1,12] The potential space between the spectacle and cornea, the subspectacular space, communicates with the lacrimal drainage system and is a common site for exudate accumulation when the lacrimal system becomes infected.[2] During ocular examination of reptiles, this anatomic space must be noted and differentiated from the anterior chamber.[3]

Successful treatment of these animals was accomplished with metronidazole. Frye suggests a dosage of metronidazole in reptiles of 40-250 mg/kg p.o., repeated in 2 wks.[6] The two geckos with ocular lesions were successfully treated at slightly less than 40 mg/kg, and the subcutaneous abscesses were treated at 200 mg/kg. Surgical fenestration of the spectacle was performed to allow drainage and reduce pressure on the globe.

The occurrence of *Trichomonas* in these lesions presents more questions than solutions as to the potential pathogenesis of these microorganisms in reptiles. We believe that these protozoa were the etiology of these lesions, but we can only speculate as to how they became established. The possibility of a strain of a normal gut *Trichomonas* that had become invasive seems likely. Another possibility is that these geckos represented an aberrant host for a known virulent *Trichomonas* species. Potential routes of infection that are known or suggested for certain trichomonads include hematogenous, transepithelial migration, or spread via lymphatics.[4,11] Another consideration is that trichomoniasis may be more common in non-gastrointestinal lesions in reptiles, but has been overlooked due to the relatively specific diagnostic methods required ("i.e." direct wet mount microscopic examination of fresh specimens). Further studies of the pathological behavior of trichomonads in reptiles are required to elucidate these questions.

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PERIODONTAL DISEASE IN LIZARDS - A REVIEW OF NUMEROUS CASES

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Introduction

Diseases of the oral cavity are not uncommon in lizards, occurring mainly due to trauma or bacterial infections, in most cases caused by organisms which are members of the animals' normal oral flora. The most commonly reported oral infections are ulcerative stomatitis,[3,13] labial or facial abscessation and mandibular or maxillary osteomyelitis.[2,8,12] These latter two conditions have mainly been reported in agamids and chameleons and in most cases the cause has not been determined although oral mucosal trauma induced by prey items or substrate is suspected to have been a precipitating factor in some cases.[8,12] The most common oral disorder seen in lizards at Melbourne Zoo in the past 5 years has been a condition which is very similar in its presentation to periodontal disease in mammals, including facio-oral abscessation and osteomyelitis. This problem has only been seen in agamids (39 cases: Common Bearded Dragon, *Pogona barbatus*, Inland Bearded Dragon, *P. vitticeps*, Dwarf Bearded Dragon *P. minor*, Eastern Water Dragon, *Physignathus lesueurii*, Frilled Lizard, *Chlamydosaurus kingii*, and Sail-tailed Water Dragon, *Hydrosaurus pustulatus* and chameleons (3 cases in Jackson's Chameleon, *Chamaeleo jacksoni*).

The term periodontal disease refers to diseases of the periodontium which in mammals includes the gingiva, periodontal ligament, alveolar bone and cemental surface of the tooth. It includes gingivitis, periodontitis and periodontal abscessation.[9] Gingivitis is reversible inflammation of the marginal gingiva, caused by bacteria in plaque, an invisible material which accumulates on teeth and gums if they are not kept clean by mastication of a diet of suitable texture and consistency. Periodontitis is the extension of gingivitis to involve the periodontal ligament, causing irreversible loss of connective tissue attachments and bone.[4] The bacteria in plaque are initially predominantly non-motile, Gram-positive aerobic cocci but as the plaque matures there is a change to a predominantly anaerobic flora including Gram-negative motile rods and spirochetes.[4,10] Clinical signs of periodontal disease in mammals include swelling, erythema and sometimes hyperplasia of the gingiva, accumulation of dental calculus (calcified plaque), and periodontal pockets and/or gingival recession [9].

The dental and gingival anatomy of agamids and chameleons differs markedly from that of mammals (see Figs. 1 and 2). Agamids, chameleons and tuataras are unique amongst the lizards in that they have acrodont dentition, ie. simple laterally compressed triangular teeth ankylosed to the crest of the mandibles and maxillae which are not continually replaced throughout life as are the teeth of other reptiles. Other lizards have pleurodont teeth which are shed and replaced. Many agamids also have a few pleurodont teeth rostrally [6], as illustrated in Fig. 1. A major difference between these two types of dentition is the level

of the bone - gingiva junction. In pleurodonts this is at the base of the teeth (similar to mammals), whereas in acrodonts it is at a faint longitudinal ridge of bone several millimetres away from this point (see Fig. 1). The exposed area of bone appears to be surfaced by a thin dull enamel-like layer which is presumably impervious to microbial invasion. Therefore the lizard equivalents of the periodontal ligament and alveolar bone are this point of gingival attachment and the mandibles and maxillae respectively.

This paper presents the clinical findings of the cases, and describes the treatments and management strategies adopted.

Figure 1. Agamid skull

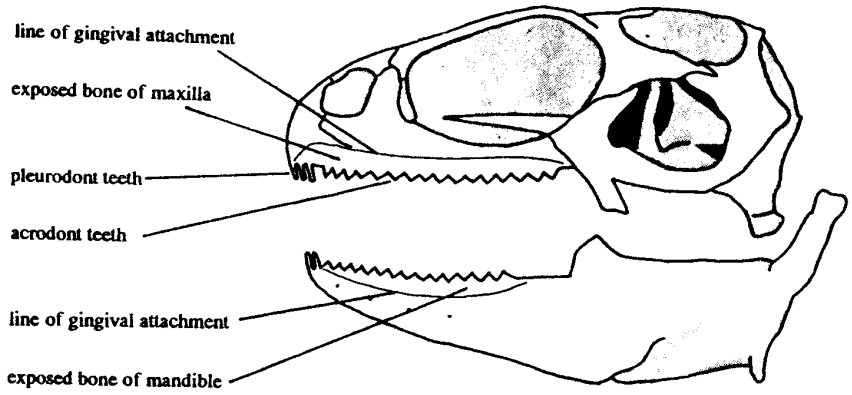
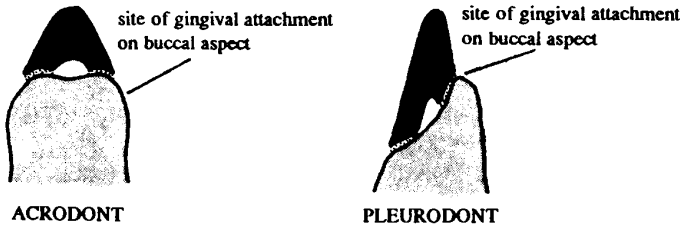


Figure 2. Types of dental attachment in lizards



Clinical Findings

This disease has presented with a range of severity of signs. The mildest cases presented with slight gingival erythema at the bone-gingiva junction and very light calculus deposition on the teeth and exposed bone of the jaws. In more severe cases, there was heavier calculus deposition, more intense gingival erythema and swelling, and varying degrees of recession of gingiva from its point of normal attachment, thus exposing the underlying porous bone of the jaws. This recession was greater on the mandibles than on the maxillae in most cases. In even more advanced cases, all of the above signs were more severe, including hyperplasia and sometimes suppurative inflammation of the receded gingivae with pocket formation, and very extensive gingival recedence exposing as much as the complete lateral aspect of the mandibles and maxillae and even the ventral border of the mandibles in some cases. A few of these cases also presented with localized subcutaneous abscesses over the mandible or maxilla. In the most severe cases (n=11), in addition to all of the above signs, there were localized areas of osteomyelitis of the mandible and/or maxilla. In most of these cases (n=9) the presenting sign was a localised, often very subtle, subcutaneous swelling over the mandible or maxilla which on investigation was found to be an abscess with adjacent focus of osteomyelitis. Lesions were multifocal in six cases, and in three cases resulted in fracture of the affected bone. One of these was of the anterior shaft of the mandible; the other two were disruption of the normally fused mandibular symphysis. A summary of the cases seen is presented in Table 1.

Epidemiology

The first nine of these cases were seen in 1989 in *P. vitticeps* and *P. barbatus* housed in the classrooms at Melbourne Zoo. One of these animals presented with a subcutaneous abscess over the maxilla, and on examination was found to have severe periodontal disease. As it was suspected that this was diet-related, all other lizards in the classrooms (including several large skink species) were examined and all eight apparently normal agamids were found to have mild to severe periodontal disease. As a result, all lizards in the zoo's reptile house (agamids, iguanids, skinks and varanids) were also examined and found to have normal mouths. At the time it was found that there was considerable difference in the consistency and composition of diets fed to lizards in the two different areas. Both groups were fed a combination of live insects and a mixture of chopped vegetables, fruit and puppy chow, but while in the reptile house this mix was composed of distinct, firm 7-10mm^3 pieces of predominantly vegetables, in the classrooms it was soft, sloppy and mainly fruit. The periodontal disease was attributed to this inappropriate diet which was immediately changed. Since that time, several new dragons have been moved to the classrooms and six monthly oral examinations for the past three years have revealed no lesions. The other 14 cases seen in *P. vitticeps* and *P. barbatus* were in animals confiscated from unlicensed owners by the local wildlife control authority and brought to the zoo for holding. Five of these presented with facial swellings, but the other cases were detected on oral examinations routinely performed on arrival. It was assumed that these animals had also been fed inappropriate diets.

In 1990, the Zoo acquired several *P. minor* which were housed in the reptile house and fed similarly to the other *Pogona* spp. One year later, two presented with facial abscessation and severe periodontal disease and on examination, all others (n=3) were also found to be affected. At the time, all other agamids were still normal. In an effort to prevent the problem, the diet of *P. minor* was changed to exclusively live insects, with the assumption that this may more closely approximate their natural diet, and no new cases have since been seen.

In 1991, the Zoo acquired four *C. jacksoni* which were fed exclusively insects. Two died within several months of arrival and since that date, the remaining two and their one surviving offspring developed severe periodontal disease which resulted in death in two cases. The cause of these cases is not understood.

In 1994, all Zoo agamids were routinely examined for periodontal disease. Mild disease was seen in most *C. kingii* (exclusive insect feeders), *P. lesueurii* and *H. pustulatus* (fed as for *P. vitticeps* and *P. barbatus*). In each case, this disease had apparently developed in the 2-4 years since their arrival, but as their diets seemed appropriate, the cause is not understood.

As many of the most recent cases, and a few of the *P. barbatus* and *P. vitticeps* seen earlier, had only calculus deposition with mild to moderate gingival recedence but no inflammation or hyperplasia, the possibility was considered that such changes were being misinterpreted as signs of disease, while they may actually be within the range of normality for these species. Therefore the opportunity was taken to examine spirit-preserved, wild-caught adult specimens of eight *P. vitticeps*, five *P. barbatus*, five *C. kingii* and eight *P. lesueurii* at the National Museum of Victoria in order to assess the normal appearance of the mouths of these species. In every case there was no calculus deposition or gingival recedence or swelling. The results of this brief study suggest that the mouths of the captive lizards described above were in fact abnormal as suspected.

Culture results

Swabs for both aerobic (28°C and 35°C) and anaerobic (35°C) culture were taken at the bone-gingiva junction from 17 cases with signs ranging from mild to severe (two *P. vitticeps*, three *P. barbatus*, one *P. minor*, four *P. lesueurii*, five *C. kingii* and two *H. pustulatus*). Similar swabs were also taken from five lizards with normal, healthy mouths (two *P. vitticeps* and three *P. minor*). From the normal mouths there was only aerobic growth, including *E. coli*, other coliforms and *Corynebacterium* sp. Fifteen of the abnormal mouths had both aerobic and anaerobic bacteria present, and in seven of these there were also spirochaetes. There was only aerobic growth from the remaining two cases. Aerobes cultured included *E. coli*, other coliforms, *Proteus* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Aeromonas* sp., *Staphylococcus* sp., and *Streptococcus* sp. Anerobic growth was moderate to profuse in many cases (n=10) and consisted predominantly of Gram-negative bacilli including *Bacteroides* sp. and *Fusobacterium* sp. *Clostridium* sp. and anaerobic streptococci were also seen, each in one case only.

Material for aerobic culture only (28°C and 35°C) was taken from osteomyelitis lesions and facial abscesses. From six osteomyelitis cases in *P. barbatus* and *P. vitticeps, Proteus* sp. (n=1), *E. coli* (n=1), *Corynebacterium* sp. (n=1), *Yersinia enterocolitica* (n=1), *Proteus mirabilis* in heavy mixed growth (n=1) and *Pseudomonas aeruginosa* in heavy mixed growth (n=1) were cultured. Two abscess cases from the same species yielded *Proteus* sp. (n=1) and *Klebsiella ornitholytica* (n=1). In *P. minor, P. aeruginosa* was cultured from one osteomyelitis case, and *Micrococcus* sp. and *Clostridium* sp. cultured from an abscess. *P. aeruginosa* was grown from two *C. jacksoni* osteomyelitis cases. Each of these organisms was sensitive to a variety of antibiotics, including at least one of the following: amikacin, piperacillin and ceftazidime.

Treatment and subsequent management

All cases were anaesthetised to permit thorough removal of calculus using a combination of ultrasonic and instrument scaling. In cases with gingival hyperplasia, the pockets were curetted and flushed liberally (using a fine lacrimal needle to permit access), with sterile saline and 0.2% chlorhexidine mouth wash (Chlorohex, Colgate-Orapharm, Australia), then irrigated with aqueous metronidazole solution (5 mg/ml) and aqueous ceftazidime (90 mg/ml) or a combination of aqueous amikacin (50 mg/ml) and piperacillin (100 mg/ml). If abscesses were present, they were incised, curetted of all contents, flushed as above and left open. Most cases of osteomyelitis were readily detected by the presence of soft bone directly underlying a soft tissue abscess, or by the observation of lytic lesions on areas of jaw exposed by receded gingiva. However, some deeper foci were only detected by radiographs which were routinely taken of all cases presenting with facial swelling(s). All bone lesions were debrided using a fine bone curette or human dental caries curette (very fine), saucerized to prevent food entrapment and then thoroughly flushed as above and left open. Pending culture and sensitivity results, all cases were commenced on a parenteral course of either ceftazidime (20 mg/kg IM q 72 hrs) or a combination of amikacin (initially 5 mg/kg IM, thence 2.5 mg/kg q 72 hrs) and piperacillin (100 mg/kg IM q 48 hrs), all of which have good distribution to bone [1]. Antibiotics were later changed if indicated by culture results. The length of antibiotics courses ranged from 2-4 weeks in mild to moderate cases to 6-10 weeks in cases with osteomyelitis. In addition to parenteral therapy, local treatment was given in every case. In individuals which did not become overly stressed by frequent handling (most *P. vitticeps* and *P. barbatus*), the mouth, including any open soft tissue or bone lesions, was irrigated daily with Chlorohex solution. In more readily stressed species, this was only performed at the time of antibiotic injections. Chlorhexidine was used as it is the disinfectant of choice for the treatment of periodontal disease in mammals because it is active against a wide range of organisms, and binds electrostatically to bacterial and oral surfaces thus inhibiting bacterial adhesion [4]. Following application, it produces an immediate bacteriocidal effect, followed by prolonged bacteriostatic action, reducing dental plaque formation and associated gingivitis [1]. Cases with osteomyelitis were reanaesthetised every 2-3 weeks for thorough cleansing of lesions including further curettage if necessary. If the lesions continued to enlarge, repeat cultures were taken and antibiotics changed if indicated. Radiographs were taken at the completion of therapy to confirm resolution of lesions.

Most cases resolved with this treatment, except two *P. vitticeps* with extensive bone lesions which were euthanised due to poor prognosis, one *P. barbatus* and one *P. minor* which died with secondary septicaemia and one *P. vitticeps* and two *C. jacksoni* which died of undetermined causes during the course of treatment. Ten mild to moderate cases are still under treatment at the time of writing (see Table 1) but all are responding well. Gingival recedence has been permanent in all cases and animals with this problem are prone to repeat calculus deposition and gingivitis, even following dietary modification. To prevent further progression of the condition, these animals are anaesthetised for a thorough oral clean six monthly. Lizards with oral bone deficits are checked frequently for food impaction and reinitiation of infection, but in most cases these lesions, including some fractures, have eventually filled in with bone and/or fibrous tissue.

Discussion

Periodontal disease in lizards has not been previously reported in the literature, however this series of cases and reports from veterinarians of similar cases in agamids and chameleons in other zoos and private collection suggest that it may be a significant problem in these species and indicate the need for particular attention to oral examinations even where there are no related presenting clinical signs.

The bacteriological findings in these cases reflect a similar pattern to that seen in mammals with periodontal disease, with the alteration of oral flora from being predominantly aerobic in the healthy mouth to one which includes anaerobes and spirochaetes with the onset of disease.[10] However, while the predominant aerobes in mammalian mouths are Gram-positive cocci,[10] those seen in these cases were mainly Gram-negative bacilli, as has been reported for the oral flora of other reptiles.[5]

Although the cause of this disease seemed clear in many cases, it was not understood in *C. jacksoni, C. kingii, P. lesueurii* and *H. pustulatus* because the two former species eat exclusively live insects and the latter two were fed a diet which had previously appeared adequate in the prevention of the condition. A possible explanation for the *C. jacksoni* and *C. kingii* cases is that until recently, these species were fed mainly crickets and mealworms. It is possible that these relatively soft-bodied insects resulted in minimal mastication and hence plaque formation. The natural diet of these species consists of a wide range of insects including hard-bodied beetles and cockroaches, and the occasional small lizard,[7,11] which require long periods of mastication. In the past 12 months, grasshoppers and occasional beetles and moths have been added to these animals' diets and it has been observed that both lizard species now spend more time chewing. Six new *C. jacksoni* have been on this diet since arrival at the zoo eight months ago and to date all have healthy mouths. In the case of the other two species, it has been assumed that the disease occurred as a result of long-term exposure to the current diet which must predispose to some plaque formation. As they are naturally omnivorous, eating a variety of fruits, flowers and leaves as well as invertebrates,[7] they will still be fed some fruit and vegetable mix but in the future their diet will contain a greater proportion of insects, including the hard-bodied species mentioned above.

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Table 1. Cases of periodontal disease in lizards seen at Melbourne Zoo 1989-94

SPECIES	MILD-MODERATE PERIODONTAL DISEASE[1]		SEVERE PERIODONTAL DISEASE WITHOUT OSTEOMYELITIS[2]		SEVERE PERIODONTAL DISEASE WITH OSTEOMYELITIS[3]	
	No. of cases	No. resolved	No. of cases	No. resolved	No. of cases	No. resolved
P. vitticeps and *P. barbatus* (zoo classrooms)	5	5	4	4	-	-
P. vitticeps and *P. barbatus* (not zoo stock)	5	5	2	0	7	5
P. minor	2	2	2	2	1	0
*P. lesueurii**	4	1	-	-	-	-
*C. kingii**	5	0	-	-	-	-
*H. pustulatus**	2	0	-	-	-	-
C. jacksoni	-	-	-	-	3	1
TOTAL	**23**	**13**	**8**	**6**	**11**	**6**

LEGEND

1. Cases with mild to moderate gingivitis +/- mild to moderate gingival recession +/- mild to moderate calculus deposition.

2. Cases with moderate to heavy calculus deposition, extensive gingival recession and inflammation +/- hyperplasia. Some presented with localised subcutaneous abscess over mandible or maxilla.

3. As for 2, with associated osteomyelitis lesion(s) in maxilla and/or mandible. Most presented with localized subcutaneous abscess adjacent to bone lesion.

* Four *P. lesueurii* and all *C. kingii* and *H. pustulatus* cases are still current at the time of writing.

DETECTION OF ANTIBODIES AGAINST PARAMYXOVIRUS (OPMV) ON MEXICAN CAPTIVE SNAKES

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Fifty healthy snakes from the genera *Crotalus* (25), *Vipera* (10) and *Bothrops* (15), belonging to public and private collections were used in this experiment. Some of the snakes were born in captivity and others were collected from the wild and donated to the different institutions.

Blood samples were collected to perform Hemagglutination Inhibition tests to determinate antibodies (Ab's) against Ophidian Paramyxovirus (OPMV).

The objective of this investigation was to determinate the presence of Ab's against this viral illness into Mexican collections.

The results showed the presence of Ab's in this apparently healthy snakes. Further investigation will need to be performed to increase the sample size, include other genera and collections, and to collect samples from individuals with nervous and/or respiratory signs. Animals dying of suspected OPMV should be thoroughly investigated including viral culture and histopathology.

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GALLAMINE REVERSAL IN CUBAN CROCODILES (*Crocodylus rhombifer*) USING NEOSTIGMINE ALONE VERSUS NEOSTIGMINE WITH HYALURONIDASE

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Introduction

Gallamine triethiodide is a water soluble competitive neuromuscular blocking agent. It is excreted unchanged via the renal system in mammals. Its effect on skeletal muscle ranges from relaxation to complete paralysis. It does not provide analgesia, anesthesia, loss of proprioception nor consciousness. For this reason it is only suitable for procedures eliciting minimal pain if used alone however may be combined with local or general anesthesia for more invasive procedures. The dose and duration vary with species. Reversal can be achieved via neostigmine methylsulfate administration. In this study recovery time was monitored to evaluate the rate of absorption and response to the reversal agent neostigmine both with and without hyaluronidase. Hyaluronidase (purified bovine testicular hyaluronidase) hydrolyzes interstitial hyaluronic acid promoting spread and absorption of concomitantly injected substances. It is used to enhance the absorption of subcutaneously administered fluids and urographic contrast media to accelerate absorption in humans.

Materials and Methods

Four Cuban crocodiles (*Crocodilus rhombifer*), ranging in weight from 45 - 74 kg, were immobilized for physical examination, radiography, gastric coin removal per os, sexing, and transport to new enclosures. A total of 12 immobilizations were performed using gallamine; seven of these were recovered with neostigmine and hyaluronidase, five times with neostigmine alone. A dose of 75 mg hyaluronidase was mixed in the same syringe with the neostigmine in every dose independent of the total neostigmine dose or the weight of each animal.

Results (see fig. 1)

Recovery was based on the animal's ability to lift their body and ambulate effectively. The animals reversed with neostigmine in combination with hyaluronidase recovered significantly sooner than those reversed with neostigmine alone. No repeat doses of reversal agents were required for any of those animals augmented with hyaluronidase and all recovered within eight hours. Two of those given neostigmine alone, however, required three doses of neostigmine, five took greater than 24 hours to recover and one of those was still unable to effectively ambulate even at three days post procedure.

Discussion

Use of gallamine for immobilization of crocodilians, including Nile crocodiles (*Crocodylus niloticus*), caiman (*Caiman crocodilus*) and American alligators (*Alligator mississippiensis*) has been reported by numerous authors both with and without neostigmine reversal.[7,5,4,1,2] No reports of neostigmine in combination with hyaluronidase were found.

Excellent immobilization has been achieved over a dose range of 0.64 - 4.0 mg/kg I.M. in Nile crocodiles with minimal side effects. Side effects after gallamine administration included tachycardia, tachypnea, hypersalivation and gaping. It is unclear whether these are direct effects of gallamine or due to the procedure stimulation. Recumbency occurred from 8 - 30 minutes post injection, comparable to our results in Cuban crocodiles. Reversal with neostigmine was used in some but not all of these procedures. A dose range of 0.03 - 0.25 mg/kg neostigmine was used.[7,5,4,2] Recovery from the high dose was as short as 5 minutes post injection but the route was unspecified. This is comparable to the results we obtained with the I.V. dose of 0.063 mg/kg neostigmine with hyaluronidase in Cuban crocodiles (4 min). The shortest recovery without reversal in Nile crocodiles was 45 minutes, significantly shorter than specimens in our study.[7] Adverse side effects of neostigmine reversal are chiefly due to its muscarinic activity and may be manifested even at the low end of the dose range above.[5] These include emesis and lacrimation but may be avoided via atropinization if necessary.[4] By fasting the Cuban crocodile 24 - 48 hours prior to immobilization no regurgitation/ emesis was observed in our study. The exception is one individual which "rushed" the keeper leading it out into the immobilization area and snatched the bait rat from the tongs only to purge it post neostigmine administration. Fatalities were reported in Nile crocodile but were associated with drowning during an attempted aquatic immobilization or asphyxiation by a conspecific which laid on top of an unrecovered individual.[5]

Dosage of 50 mg/kg I.P. gallamine in *C. crocodylus* combined with pentobarbital resulted in apnea and required positive pressure ventilation, however these specimens were intentionally not recovered.[3]

Two American alligators receiving only 0.75 - 1.0 mg/kg of gallamine were given no reversal agent and did not survive. For this reason some authors do not consider gallamine safe in this species.[2]

Conclusions

Augmentation of the reversal agent neostigmine with 75 mg hyaluronidase may increase efficacy and speed recovery of Cuban crocodiles immobilized with gallamine.

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Figure 1

RANGES AND AVERAGE VALUES FOR REVERSAL WITH AND WITHOUT HYALURONIDASE

	w/ H range	w/o H range	Ave H grp	Ave w/o H grp
Wt.(Kg)	45-74	45-68	54	53
total G dose mg/kg	0.60-0.77	0.91-1.28	0.70	1.07
recumbency (minutes)	21-103	30-130	42	58
time to reversal admin.(min)	95-331	173-405	257	304
total N dose(mg/kg)	0.04-0.07	0.07-0.17	0.06	0.11
time til ambulatory (min)	99-434 (1.7-7.2hr)	1440-2050 (24-34hr)	321 (5.4hr)	1867 (31.1hr)

KEY

total G dose - total dose gallamine, in all except one immobilization the total dose was given all at once

recumbency - time from gallamine dose to sternal recumbency and inability to stand or ambulate

time to reversal admin. - time from initial dose of gallamine to the administration of the reversal agent(s)

total N dose - total dose of neostigmine given to effect, reversal defined as ability to stand and ambulate, initial dose may have been repeated after several hours or days if insufficient response occurred

time til ambulatory - response to reversal agent(s) gauged by ability to stand and ambulate since initial gallamine administration

w/ H range and Ave H grp - range of values and average value for each parameter in immobilizations with hyaluronidase supplemented reversals in minutes with hour equivalents below

w/o H range and Ave w/o H grp - range of values and average value for each parameter in the immobilizations without hyaluronidase in minutes with hour equivalent below

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Products Discussed

Gallamine triethiodide: Flexadil (R), Davis+Geck, American Cyanamid Co., Pearl River, NY, 10965

Neostigmine methylsulfate: Bristol-Meyers Squibb Co., Prinston, NJ, 08540

Hyaluronidase: Wydase (R), Wyeth Labs Inc., Philadelphia, PA, 19101

SNAKE HEMATOLOGY- PRACTICAL CONSIDERATIONS

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Snake hematology poses many challenges to even experienced clinicians and technicians. Technical problems include identifying the effects of anticoagulants on the quality of peripheral blood smears, making peripheral blood smears without disrupting heterophils, and recognizing the effects of different stains on leukocytes. For hematology of the yellow rat snake, *Elaphe obsoleta quadrivittata*, edetic acid(EDTA) appears to be a suitable anticoagulant and peripheral smears are best made in our laboratory using the push slide method. Either alcohol or aqueous based Wright's-Giemsa stains, used at a cytology stain setting, produce readable smears for differential leukocyte counts but there are marked differences in the appearance of snake granulocytes with these two stains.

Definitive identification of leukocytes is the most controversial component of making both a differential leukocyte count and a total white blood cell count. Differentiation between lymphocytes and thrombocytes and between azurophils and monocytes is difficult in the rat snake. These distinctions are made more difficult by the abundance of thrombocytes, pleomorphism of lymphocytes, and low numbers of heterophils and monocytes. In healthy yellow rat snakes, heterophils and monocytes each make up <3% of the leukocytes in peripheral blood smears. There is also evidence of a shift in azurophil morphology with bacterial infection.

Two techniques are commonly used to perform total white blood cell counts in reptiles: direct counts using Natt-Herricks diluent and derived counts using the eosinophil unipette technique. The former relies on the ability to differentiate between lymphocytes and thrombocytes and is unreliable if the distinction between these two cell types is equivocal. The eosinophil unipette technique relies upon an accurate differential granulocyte (heterophil/eosinophil) count to calculate the total white blood cell count. In reptiles with low heterophil counts, small changes in heterophil numbers result in arithmetic fluctuations which render the eosinophil unipette technique of questionable reliability.

AVIAN EMERGENCY TREATMENT AND CRITICAL CARE

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The initial approach for the assessment of the avian patient presented as an emergency differs little from the steps taken in domestic animal emergency medicine. Prior to emergency treatment, it is recommended to take a thorough history. Particular attention should be paid to environmental conditions the bird has been exposed to, as well as the diet, temperature, behavior, and condition of other birds kept at the same facility. A complete physical examination with critical evaluation of the patient's vital signs and diagnostic tests are essential for the successful outcome. Optimal environmental conditions should be provided before treatment is initiated. This includes placement of the bird in a temperature controlled oxygen cage, with the temperature ranging between 80-90 °F. Hopefully the history in combination with the clinical findings will lead to a list of differential diagnoses.

Initially, the bird should be evaluated visually. This will often help to identify the major problem. Every effort should then be made to minimize stress and handling of the patient. Therefore, physical examination should be undertaken in an efficient, routine, and prepared manner. This includes proper set-up of the equipment needed prior to restraint and handling. If needed, oxygen can be administered via face mask during the examination period. Samples (feces, blood, microbiological swabs) should be collected during the examination. If indicated, anesthesia may be necessary to facilitate sample collection, physical examination, and, if necessary, radiography. If the patient is unstable, radiographs should be postponed. Every effort should be made to keep the examination period as short as possible.

For the initial assessment of the patient's status, some diagnostic tests are required most of which can be performed without having specialized equipment available. Minimally, the packed cell volume (PCV), total protein, and blood glucose levels should be determined. Only small amounts of blood are needed for these tests and the results will give valuable and fast information about the bird's status. If indicated radiographs should be taken to identify organ abnormalities, foreign bodies, etc. In addition samples should be obtained for microbiologic determinations.

Emergency treatment

With the forementioned steps completed, sufficient information should be available for the initial treatment. In cases were the condition of the bird does not allow completion of the above, treatment must be initiated without some of the all information. This is especially indicated in trauma cases with severe blood loss and patients in acute respiratory distress where a delay in treatment may put the animals life at risk.

The majority of birds presented as an emergency, especially wild birds, will be dehydrated, either because of an inadequate intake of fluids or loss of fluids due to vomiting or diarrhea. Determination of the PCV and total protein will help to estimate the degree of dehydration. Anemia or hypoproteinemia will influence the accuracy of these parameters. The most effective route of fluid administration is i.v. or by intraosseous cannulation. Subcutaneous or oral fluid administration is indicated in patients with mild dehydration or for maintenance fluid therapy. For emergency treatment lactated Ringer's solution either alone or in combination with 5% dextrose are administered slowly i.v. at 10 - 20 mg/kg. Daily fluid requirements should be divided in multiple doses until the patient is rehydrated. Maintenance fluid requirements for most birds is estimated to be 50 ml/kg/day.

The management of a bird in shock is similar to the treatment of a mammalian patient, were support and stabilization of the cardiovascular system is most important. Administration of fluids to expand the circulatory blood volume and corticosteroids for protection of cellular membranes is a priority. Intravenous or intraosseous administration will more rapidly show beneficial effects than the intramuscular or subcutaneous routes. If there is no venous access, corticosteroids (dexamethasone, 2-4 mg/kg) should be given intramuscularly and fluids subcutaneously. The use of corticosteroids should be limited to the initial treatment of the patient. Long-term use has been associated with immunosuppressive effects in birds.

Traumatic incidents are the most common cause for blood loss in the avian patient. An anemic patient will be diagnosed based on clinical signs and a decreased packed cell volume (<25%). The first priority in the treatment of a bird presented with active blood loss is localization and cessation of the bleeding. Direct pressure, hemostatic agents, and/or ligation of vessels may be indicated. In addition to fluids for volume replacement, vitamin-B preparations and iron dextran are recommended. In patients with acute respiratory distress, endotracheal intubation or placement of an airsac tube will assist ventilation.

Critical care

The avian patient requires supportive care following initial emergency stabilization. Due to the higher metabolic rate of birds in comparison to mammals, reserves are rapidly depleted in a debilitated bird. Optimal environmental conditions such as an appropriate cage, oxygen supplementation, and optimum temperatures are recommended. The avian patient recovering from an injury or a disease requires nutritional support, fluid therapy and other appropriate therapy such as antimicrobials.

In hypoglycemic patients with a blood glucose level of less than 100 mg/dl, intravenous glucose solutions should be given initially. If the patient remains anorectic following stabilization, enteral or parenteral feeding becomes necessary. Enteral feeding is best accomplished in using a feeding tube. Commercially available formulas, fluids and oral medications may be administered concurrently. Indications for total parenteral nutrition include regurgitation, gastrointestinal stasis, malabsorption and maldigestion. Through an intraosseous or intravenous catheter, all essential nutrients can be administered.

Although antimicrobial treatment is not indicated in all emergencies, they are often used prophylactically in the avian patient. Many non-infectious diseases will compromise the immune system and secondary bacterial and/or fungal infections are present. The initial antibiotic of choice should be a broad spectrum antimicrobial effective against known disease causing organisms in birds. Further identification of the organisms and sensitivities are necessary either to confirm or adjust the antimicrobial treatment regimen.

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AVIAN ANALGESIA

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Introduction

Very little information is available regarding avian pain perception and analgesia. The limited studies that have been performed suggest that pain perception in birds is mediated by neural pathways and neurotransmitters that are similar to mammals.[8] Pain perception (nociception) is carried by dorsal spinal tracts to several areas of the midbrain and forebrain. Activation of these pain centers induces recognition of pain, stimulates nocifensive reactions and activates descending inhibitory modulation pathways. Once initiation of nocifensive reactions have occurred, ongoing pain perception has a negative effect on homeostasis and healing.

Analgesia can be provided to animals by decreasing stimulation of the ascending spinal pathways or by activating the endogenous descending pain modulation pathways. Medical activation of pain modulation pathways is most frequently accomplished by the use of drugs which facilitate central serotonin transmission, such as opiates and alpha adrenergic agonists. Pain modulation systems are also naturally activated by nociceptive stimuli which is mimicked by counterirritation techniques such as acupuncture and acupressure.

The following is a compilation of avian analgesic doses reported in the literature and from personal communications with zoo and avian veterinarians. Most are extrapolations of mammalian dosages which appear to be clinically effective in limited case numbers. The dosages listed are not given as specific recommendations for use, but to disseminate information and to stimulate further studies in the area of avian analgesia.

Opiates

Pain perception and analgesia studies performed in pigeons and chickens suggest that neural opiate receptors are present in birds, though the specific subclasses of avian opiate receptors remain in question.[10] Learning studies indicate that pigeons can recognize morphine and other opiates, and that naloxone and other antagonists block this recognition.[10] Morphine has been given to galliformes at 2.5-30 mg/kg IM; sensitivity and response appear to vary with species.[15] Clinically, butorphanol at 0.2-2.0 mg/kg IM provides analgesia for 3-8 hours in various species of birds.[3,11,15] Buprenorphine at 0.01-0.05 mg/kg IM provides a similar level of analgesia with a clinically apparent longer duration of 8-12 hours.[3,11] Butorphanol given to healthy budgerigars (*Melopsittacus undulatus*) at 3 mg/kg decreased perch grip in 50% of the birds for 2-4 hours with no effect on heart rate or respiratory rate.[2]

Alpha$_2$-adrenergic agonists

Alpha$_2$-adrenergic agonists induce sedation, analgesia and anxiolysis in mammals, although these effects vary considerably in different species.[12] Limited data suggest that these drugs will have similar effects in birds. Xylazine 1-4 mg/kg IM provides sedation for ketamine anesthesia, and has been used up to 10 mg/kg for sedation in small psittacines.[14,16] Detomidine 0.3 mg/kg IM produced marked sedation in chickens, but data on duration, cardiopulmonary effects or complications were not given.[13] Metomidine 0.1 mg/kg IM produced drowsiness without immobilization in ostrich (*Struthio camelus*) chicks. Again, no information concerning duration or cardiopulmonary effects was given other than a statement that an unquantified drop in heart rate was seen in all birds.[17] Unfortunately, none of these studies assessed analgesia, but this class of drugs should be able to provide analgesia and warrants further investigation. Possible complications of concern include short duration of analgesia, hypotension, bradycardia, hypothermia and GI tract inhibition. Reversal agents for alpha$_2$-adrenergic agonists appear to be effective in birds. Yohimbine 0.1 mg/kg IV and tolazoline 15 mg/kg IV has been used for reversal of ketamine/xylazine anesthesia in raptors.[1,4] A suggested dose of atipamezole in birds was not found.

Antiinflammatory drugs

While not adequate for sharp or acute pain, antiinflammatory drugs are indicated in the relief of pain caused by inflammation. Dexamethasone 1-2 mg/kg IM, betamethasone 0.1 mg/kg IM and methylprednisolone acetate 0.5-1.0 mg/kg IM have been used in a variety of species.[6,15] Because of possible immunosuppression and other complications of steroids, nonsteroidal antiinflammatory drugs are often preferred. Suggested clinical doses include flunixin meglumine 1.0-10.0 mg/kg IM SID, or meclofenamic acid 2.2 mg/kg PO SID (Orosz, pers. comm.).[3,11,15] Acetylsalicylic acid 5.0 mg/kg PO TID has also been used but does not appear to be as effective.[3,15] Gastrointestinal side effects are not frequently recognized following use of these agents in birds. Administration of high dose flunixin meglumine 10 mg/kg IM to budgerigars resulted in initial regurgitation and tenesmus. The birds resumed eating immediately after regurgitation and continued straining was not apparent.[2] An African crowned crane (*Balearica pavonina*) passed droppings containing fresh blood after a month long course of daily injections with flunixin meglumine. Droppings cleared upon cessation of treatment and no further adverse GI side effects were noted (Stover, pers. comm.).

Other drugs

While sedatives and tranquilizing agents do not provide analgesia, the induced behavioral changes and reduction in limbic activation can decrease pain perception and allow for improved efficacy of concurrent analgesics. Diazepam 0.5-2.0 mg/kg IV or IM, and midazolam 1 mg/kg IM have been used in birds.[16] Additionally, both drugs provide skeletal muscle relaxation which affords pain reduction in appropriate cases. Muscle relaxation can also be obtained with methocarbamol 50 mg/kg IV BID.[5] The hypnotic metomidate has been used as a premedicant in birds at 5-15 mg/kg.[7]

Topical administration of bupivicaine and dimethyl sulfoxide (50:50) to the cut portion of chicken beaks immediately after trimming eliminated the reduction in food intake typically observed.[9] Similar improvements were seen with application of a mixture containing phenylbutazone, isopropylaminophenazone and dimethyl sulfoxide.[9]

Conclusion

Clinical experience supported by limited research data strongly suggest that pain perception in avian species differs little from mammals. Analgesics used in mammals appear to be effective in birds, though specific dosages and effects remain to be described. Additional studies in avian analgesics are needed.

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THE EFFECT OF FLUNIXIN MEGLUMINE (BANAMINE^R) ON THE RENAL FUNCTION IN NORTHERN BOBWHITE (*Colinus virginianus*) : AN AVIAN MODEL

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Introduction

Flunixin meglumine (FM) (Banamine^R:Schering-Plough) is a potent non-steroidal anti-inflammatory drug (NSAID) used therapeutically in human and veterinary medicine to produce analgesia. FM and other NSAID's act to inhibit the biosynthesis of prostaglandins from the precursor arachidonic acid by blocking the enzyme cyclo-oxygenase. Prostaglandins are mediators of the inflammatory process but also have cytoprotective effects in the gastrointestinal tract and kidney by regulating gastric acid secretion and renal blood flow, respectively.[1,2,3,8] Renal prostaglandins, PGE_2 and PGI_2: (a) act as vasodilators to maintain glomerular filtration rate and renal blood flow by antagonizing the vasocontrictor effects of angiotensin II, norepinephrine and alpha-adrenergic stimuli; (b) mediate renin release from the macula densa; (c) redirect renal blood flow from the outer cortex to the inner cortex and medulla in mammals; and (d) inhibit proximal tubular reabsorption of filtrate during renal ischemic and hypovolemic states.[8,12]

Nephrotoxicity induced by FM and other NSAID's has been reported in man and several animal species.[2,4-9,11,12] The major mechanism for this nephrotoxicity is associated with the decreased prostaglandin synthesis and subsequent reduction in the protective effects on renal function. During periods of vasoconstriction of the afferent renal arterioles, the anti-prostaglandin effects of FM permit unopposed vasoconstrictive reactions in the kidney leading to ischemia and necrosis.

Nephropathy and nephrotoxicity associated with FM administration in humans and other mammals has occurred at therapeutic dosages. [2,3,4,8] The use of FM as an analgesic in avian species has relied on extrapolation of therapeutic dosages used in mammalian species. However, the structural anatomy of the avian kidney and renal hemodynamics vary significantly from that of mammals. These differences may predispose to adverse effects on the avian kidney following FM administration.[10] Several cases of presumptive FM nephrotoxicosis in cranes and flamingos have been reported subsequent to therapeutic administration of the drug for clinical musculoskeletal problems (F.J. Dein and J. Langenberg, pers comm).

The present report describes the gross, microscopic and clinico- pathologic changes associated with FM administration in the northern bobwhite (*Colinus virginianus*) which were selected as an avian model.

Methods

Forty-two northern bobwhite were housed indoors in a light, temperature, and humidity controlled room. They were maintained in seven groups of six birds in a multi-level brooder cage unit, and provided food and water ad-libitum. One group served as the control group, receiving a sham dose of sterile water. The remaining six groups received varying doses of FM as follows:

Group 1: Control (sham dose); Group 2:0.1 mg/kg/day; Group 3: 0.32 mg/kg/day; Group 4: 1.0 mg/kg/day; Group 5: 3.2 mg/kg/day; Group 6: 10.0 mg/kg/day; Group 7: 32.0 mg/kg/day.

Control and treated birds were administered either sham or assigned doses of FM once daily for seven days by intramuscular injection in the deep pectoral muscles. Prior to the first dose, water was withheld for 24 hours to simulate histories of dehydration reported in clinical cases. Birds were monitored daily and humanely euthanized at day 7. One ml. of whole blood was collected by venipucture of the jugular vein prior to the first FM dose (day 0), on day 2, and on day 7 at euthanasia. Hematology and clinical chemistry analyses were performed on blood samples collected. The total volume of whole blood collected antemortem did not exceed 1.0 % of the total body weight of the bird. Tissues collected at necropsy were fixed in 10% buffered formalin, and routinely embedded in paraffin. Tissue sections were cut at 6 micron thickness and stained with hematoxylin and eosin (H&E).

Results

Clinical pathology

There were no significant differences in the hematology parameters measured between dosage groups, or within groups over time. Total WBC, differential counts, packed cell volume (PCV) and total solids (TS) remained within normal range throughout the test period.

There were no significant differences in the clinical chemistry parameters measured between dosage groups, or within groups over time. Albumin, total protein, uric acid, blood urea nitrogen (BUN), creatinine, calcium, phosphorus, Na, K, and Cl remained within normal limits throughout the test period. A slight decrease in albumin and total protein occurred on day 7 in group 7 but this was considered inherent laboratory variability.

Gross pathology

Severe, focally extensive muscle necrosis in the pectoral muscles was seen in all birds in Group 7 which received the highest FM dose. No gross lesions were present in any of the visceral organs in any of the birds examined at necropsy. Endocrine and central and peripheral neurologic systems were grossly normal.

Histopathology

Brain, heart, lung, liver, spleen, kidney, proventriculus, ventriculus, intestines, and skeletal muscle from all birds were examined microscopically. There was a significant difference between the kidneys in the control and treated groups. Basophilic, granular to amorphous mineralized deposits were present in the renal glomeruli of all treated groups. These deposits often obscured the mesangial matrix and occasionally occupied the entire glomerulus eclipsing Bowman's space. The distribution and severity of the lesions increased with increasing dose. No renal changes were present in kidney tissues examined from the control group. Severe, acute, focally extensive necrotizing myositis was seen at the FM injection sites in the pectoral muscles examined from birds in group 7. No significant microscopic changes were seen in other tissues examined from the control and treated groups.

Discussion

Clinicopathologic measurements, including hematology and serum chemistry parameters, did not differ significantly between dosage groups, or within groups over time. Accepted indicators of renal function such as uric acid, BUN, creatinine, phosphorus, and electrolytes were evaluated to test their value in assessing acute nephrotoxic insult from FM. Although microscopic pathologic changes were present in the kidneys of birds in all test groups, no demonstrable changes were seen in their blood parameters. It may be the severity of the renal changes was insufficient to produce measurable alterations in serum chemistries, or the currently used test methods are not sufficiently sensitive to detect modest changes in renal function.

Significant pathologic changes occurred in the renal glomeruli of northern bobwhite following intramuscular administration of FM at doses ranging from 0.1 mg/kg to 32.0 mg/kg daily for 7 days. Therapeutic administration of FM in birds has been extrapolated from the recognized mammalian dose of 1.0 mg/kg/day. It appears that there may be a direct correlation between FM administration and an acute nephropathy syndrome in some avian species. Presumptive cases of FM nephrotoxicosis have been reported in whooping cranes (*Grus americanus*), Siberian cranes (*Grus leucogeranus*), and Rred-crowned cranes (*Grus japonensis*) subsequent to therapeutic use of the drug for musculoskeletal disorders. At necropsy gross lesions described in those birds included visceral and renal gout. Microscopic changes in the kidneys of affected birds included acute necrotizing glomerulitis and evidence of gout tophi in the renal tubules. The pathologic changes in the renal glomeruli appear to be a common feature in the clinical crane cases and the experimental bobwhite study, but of a lesser severity in the Bobwhite. It may be that the crane species are more susceptible than the bobwhite to renal ischemia and subsequent renal necrosis due to FM anti-prostaglandin effects. Further studies are planned to investigate the effects of FM in cranes and to examine the pharmacodynamics of FM in blood and tissue samples following drug administration.

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RADIOGRAPHIC IMAGING TO EVALUATE CHICK POSITION IN CALIFORNIA CONDOR (*Gymnogyps californianus*) EGGS

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Radiographic imaging of seven incubating California condor eggs from 1990-1993 was used to evaluate embryo position when a developmental abnormality near hatch was suspected. Three of the seven eggs resulted in live hatches. In these eggs, embryo position appeared normal on radiographic images. Radiographs of the remaining four eggs in which the embryos did not survive revealed two with malpositioned embryos. The remaining two embryos were in apparently normal radiographic position; however, one came from an egg with a previously cracked shell that consequently was incubated in a vertical rather than horizontal position, the other came from an egg with the air cell at the small rather than large end of the egg.

The incubation period for California condor eggs incubated in captivity is 55-58 days. The embryo, or more specifically, the chick's head enters the air cell at 52-55 days after which it takes 24-60 hours to pip. The pip to hatch interval is approximately 72 hours. At the San Diego Wild Animal Park a chick is assisted from its shell 72 hours after pip to reduce stress on the chick if it is judged not making sufficient progress toward hatch.

The California condor egg is asymmetrical in shape. The air cell appears normally at 1-2 days of incubation at the large end of the egg. As a comparison ostrich eggs are generally symmetrical and therefore the air cell may appear at either end of the egg.[1] Current general incubation parameters at the San Diego Wild Animal Park include a dry bulb temperature of 97.5ºF-98ºF, and a relative humidity range up to 52% using a Petersime Model #1 forced air incubator.[2] (Some of these parameters change after the egg pips). An egg weight loss goal of 14% is desirable. At near hatch the normal position of the California condor embryo is not unlike other avian embryos. That is, the head is tucked upside down under the right wing.

In 1984, a symmetrically shaped egg was presented on day 58 of incubation which had not pipped. Radiographs indicated the chick had entered the air cell, but the position of the embryo prior to air cell entry could not be determined. A decision was made to remove the chick by artificially pipping the egg after it was determined by candling that shell membrane blood vessels had shut down. The chick breathed for approximately 30 min prior to expiration. During removal from the egg it was determined that the chick was malpositioned. Neonatal death was attributed to pulmonary edema associated with an

inability to make the transition from chorioallantoic respiration to pulmonary respiration. In addition, multiple congenital deformities were found in musculoskeletal and central nervous systems, which may have also played a role.

From 1984-1990, no eggs were presented where the embryo position could not be determined with confidence by conventional candling techniques. However, between 1990-1993, 7 instances arose where embryo position was in question and radiographic imaging provided further information to keeper personnel responsible for egg incubation. In these cases, eggs were radiographed with their long axes horizontal. Eggs, 7 cm in thickness, were exposed to a vertical x-ray beam (56 kvp and 100 mA) for 1/30 second at a distance of 101.6 cm. At least two films were made, the second image with the egg rotated 90 degrees from the first. Films made "end on" of the egg or in vertical axes did not assist in clarifying embryo position. Cronex® 10T (E.I. Dupont De Nemours & Co. Inc, Wilmington, DE 19898) and Kodak (Min-R® M MRM-1) Diagnostic Film (Eastman Kodak Company, Rochester, New York 14650) were used in this study.

Of 7 eggs radiographed between 1990-1993, 3 hatched successfully. In these cases an impression of normal embryo position was documented by radiographic imaging. That is, the chick's head is positioned upside down adjacent to the air cell. Of the remaining 4 eggs, the third and fourth embryos appeared to be in normal position radiographically, but at necropsy both were found to be malpositioned, with the head over the right wing (rather than under) and the right foot over the head. The cause of the malposition in the third embryo remains speculative, but in the fourth embryo it was attributed to incubation in a vertical position, which was necessitated by earlier egg shell damage.

From radiographic studies of near hatch California condor eggs, (51-55 days of incubation), we have learned that it is possible to critically evaluate normal verses abnormal embryo position when there is doubt following conventional candling techniques. Radiographic imaging of near hatch eggs may have application in other avian species. Necropsies of dead embryos provided further information to correlate with radiographic findings.

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METABOLIC SCALING OF KETAMINE IN OSTRICHES AND EMUS

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Ketamine (Ketaset, Aveco Co., Inc., Fort Dodge, Iowa) is recognized as a useful dissociative anesthetic in a wide variety of animal species.[1,3-7] The effect and excretion of ketamine are dependent on metabolic functions of the body. Therefore, it is a drug which can be allometrically scaled for individual animals based on their taxonomic grouping and body mass.[8,10] Sedgwick introduced the idea of determining ketamine dosages for small birds using metabolic equations.[9] Dosages for larger avian species, such as ratites, can also be mathematically extrapolated.[2]

Four emus (*Dromaius novaehollandiae*) and seven ostriches (*Struthio camelus*) were anesthetically induced with intramuscular doses of ketamine and xylazine (Rompun, Mobay Corporation, Shawnee, Kansas). The ketamine doses were mathematically calculated using minimum energy cost (MEC) calculations. The xylazine dose for each animal was approximately 20% of the ketamine dose.[8] Xylazine was given 20 min prior to ketamine administration in all of the emus and in five of the seven ostriches. All of the birds in this study were eventually maintained on isoflurane (AErrane, Anaquest, Madison, Wisconsin) anesthesia and underwent various surgical procedures.

Ketamine doses were derived from domestic feline doses. The following series of calculations shows the mathematical derivation of a ketamine dose for the 103.6 kg ostrich listed in the accompanying table:

1) The average domestic feline weight is estimated at 4.5kg.
An anesthetic dose for ketamine in the domestic cat is chosen--30mg/kg (this is somewhat arbitrary since the range is 25-33 mg/kg)

2) The anesthetic dose of the felid is then 135mg (4.5kg x 30 mg/kg).

3) Minimal energy cost (MEC) of the cat is then calculated using the mammalian coefficient of 70:

$$MEC = 70(\text{body weight}^{.75})$$

$$MEC = 70(4.5^{.75})$$

$$MEC = 216.3 \text{ kcal}$$

4) The MEC dose for ketamine is then derived by dividing the anesthetic dose of by the MEC of the domestic felid:

$$135mg/216.3kcal = \underline{.687mg/kcal}$$

This is a standard for ketamine administration which can be used on any animal when its MEC kilocalories are calculated.

5) The MEC of the ostrich is calculated using the non-passerine coefficient of 78.

$$MEC = 78(103.6^{.75})$$

$$MEC = 78(32.5)$$

$$MEC = 2{,}533kcal$$

6) Multiplying the ostrich MEC x the MEC dose of ketamine gives the anesthetic dose of ketamine for this bird:

$$2{,}533kcal \times .687mg/kcal = \underline{1{,}740mg}$$

The xylazine dose chosen for this bird (350mg) is approximately 1/5 the dose of ketamine.

Nine of the birds in this study received xylazine intramuscularly 20 min prior to the i.m. dose of ketamine. This produced moderate to deep sedation and made each animal significantly tractable. Induction times of 1.5-3.0 minutes were recorded after the ketamine was administered i.m. The birds rapidly slumped into sternal recumbency with minimal struggling. Ten of the ratites could be intubated immediately after ketamine induction. One ostrich required isoflurane via mask to reach an intubation depth.

Effective ketamine induction doses ranged from 15.7 to 25.6 mg/kg. Larger ratites received lower dosages of ketamine than smaller birds. This is because smaller birds have a higher minimum energy cost per kilogram of body mass. It is noteworthy that the anesthetic effect of these divergent dosages was the same in each animal. This indicates that metabolic scaling can be used to produce effective doses of ketamine in ratites. Ostriches 4 and 6 in Table 1 received proportionately lower doses of ketamine. The induction of these two birds was unsatisfactory and supplemental doses of drugs were required.

During isoflurane anesthesia, these ratites frequently required respiratory assistance. This is not uncommon in ostriches and emus. However, the large dose of xylazine used for induction may have contributed to respiratory depression during anesthesia and recovery (ostrich 2). It is also likely that xylazine may have prolonged the recovery of emu 1. It is our clinical impression that ratites induced by this method and maintained

with isoflurane tend to have a high incidence of bradycardia and/or hypotension which require intra-operative treatment. Therefore, close monitoring of cardiopulmonary function throughout anesthesia is advised. Lower doses of ketamine[2] and xylazine can be given i.v. for induction in ratites. This method normally produces a shorter recovery period after isoflurane anesthesia.

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Table 1. Ketamine and xylazine dosages for ostriches and emus.

	Weight (kg)	Ketamine (mg) i.m.	Dosage (mg/kg)	Xylazine (mg) i.m.	Dosage (mg/kg)	Induction/recovery comments
			Emus			
1	36.8	850	23.1	160	4.3	Prolonged recovery
2	35.0	750	21.4	150.0	4.5	Smooth recovery
3	34.5	763	22.1	152.6	4.4	
4	16.8	430	25.6	80.0	4.8	
			Ostriches			
1	127.2	2,000	15.7	400	3.1	
2	112.5	1,847	16.4	400 [a]	3.5	Mild respiratory depression
3	103.6	1,740	16.8	350	3.4	Isoflurane required for intubation
4	101.8	1,129 [b]	11.1 [b]	225 [b]	2.2 [b]	Incomplete induction smooth recovery
5	95.4	1,800	18.9	360	3.8	Incomplete induction
6	84.5	1,493 [c]	17.7 [c]	298 [a,c]	3.5 [c]	
7	45.5	960	21.1	190	4.2	

[a] These doses of xylazine were given concurrently with the ketamine.
[b] 400 mg of ketamine given i.v. 10 min later.
[c] 222 mg of ketamine and 8.9 mg of diazepam given i.v. 10 min later.

ENDOSCOPIC EXAMINATION OF THE DISTAL UTERUS OF OSTRICHES AND EMUS

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The development of a ratite industry in the United State in recent years has placed new demands for expertise on veterinary practitioners. One particular area of ratite medicine that is challenging to veterinarians is that of reproduction. Veterinarians have traditionally received minimal formal education in avian reproduction. The growing popularity of ostriches (*Struthio camelus*) and emus (*Dromaius novaehollandiae*) requires that veterinarians learn about the normal reproduction of ratites, as well as common reproductive disorders that they suffer.

Disease of the distal uterus of ostriches and emus occurs frequently. Bacterial infections are the most common form of uterine disease, and occur retrograde from the cloaca. They may be predisposed by intromission or by injury to the oviduct during egg laying. Gram negative organisms, such as *Salmonella spp.* and hemolytic strains of *Escherichia coli*, are most often the infective agents. During the breeding season, uterine infections are likely to cause poor shell deposition and early elimination of the egg. It is noteworthy that these infections may not terminate ovulation or fertilization of the ovum.

Functional problems of the uterus are more difficult to diagnose because the hormonal cycle of ratites remains ill-defined. Nutritional problems that lead to calcium imbalances in ratites may cause problems with shell deposition in ratites. This may include low dietary calcium, high dietary phosphorus, and deficiency of vitamin D_3. High fat diets may also decrease the absorption of calcium and indirectly alter the deposition of calcium carbonate on the shell membrane. Reproductive hormone imbalances likely cause poor shell production in the uterus. Egg impaction was seen in an ostrich hen which displayed other signs suggestive of hormonal imbalance. These signs were (1) a history of dark feather coloration, and (2) poor ovarian development during the breeding season as identified by ultrasound examination.

Flexible endoscopes provide a diagnostic alternative to radiography and ultrasonography for the examination of the oviduct, uterus, isthmus , and magnum of ostriches and emus. A 9.8 mm diameter, 100 cm flexible videoendoscope (Videoendoscope, Olympus Corporation, Lake Success, NY) is an appropriate instrument for use in adult, female ratites. The scope should have insufflation capabilities in order to distend the lumen of the reproductive tract. It should also be equipped with biopsy cable forceps for tissue retrieval.

Birds are anesthetized with ketamine and valium and then, following tracheal intubation, are maintained on isoflurane vaporized in oxygen and delivered through a semiclosed system procedure. They may be kept in either sternal recumbency or right lateral recumbency throughout the procedure. Because the reproductive tract of female ratites lies on the left side of the abdomen, right lateral recumbency may allow easier access into the oviduct. Abdominal viscera may cause less pressure on the lumen of the reproductive tract in this

position. After external cleansing, the cloacal sphincter is opened with gloved hands. The cloaca is irrigated with saline to lavage residual feces and urates from its vestibule. Using both hands as a speculum, the walls of the cloaca are retracted until the vaginal and rectal openings are evident.

The vaginal opening is distinctive because epithelial tissue normally evaginates from it, giving it a rosette appearance. This opening to the reproductive tract lies to the left of the rectal opening. By comparison, the rectal opening shows no epithelial evagination. It quickly becomes cavernous and more easily accepts an endoscope. The paired ureteral openings are small and dorsal to the vaginal and rectal openings. They are not likely to be mistaken for the vaginal opening. The ureters may constantly secrete urate-laden urine throughout the scoping procedure. This causes no problem once the endoscope has been properly inserted.

The scope may be inserted directly into the vagina, but in some cases a gloved finger must be inserted first to act as a guide. Occasionally, the operator may be unable to insert the endoscope into a patent reproductive tract. This may be due to positioning of the of the tract or inability to insufflate the vagina.

Mucosal inflammation of an infected uterus is often less dramatic than expected, when viewed through the scope. Mild infection can irritate the uterus of ratites, causing eggs to be expelled before the shell calcification is complete. Lesions seen are often punctate mucosal ulcerations with localized erythema. These inflamed sites may make up a relatively small portion of the mucosal surface of the uterus. It is important to culture these areas for bacteria and fungi. Samples can be taken for microbiological cultures by inserting equine intrauterine swabs (Teglin Swab, Har-Vet, Spring Valley, Wisconsin) along the insertion tube of the endoscope until the culturette can be seen. At this point, the swab can be visually guided to the desired site for sampling. Abnormal tissue can be biopsied by insertion of a flexible biopsy instrument through the biopsy channel of the endoscope.

VIRAL DISEASES IN FREE-RANGING DUSKY-HEADED PARAKEETS (*Aratinga weddellii*) AND TUI PARAKEETS (*Brotogeris sanctithomae*) IN PERU

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Thirty-five wild dusky-headed parakeets (*Aratinga weddellii*) and 13 Tui parakeets (*Brotogeris sanctithomae*) were caught and released in Parque Nacional del Manu, southeastern Peru (S 11'50", 71'26" W) in July and August, 1993. Blood samples were collected, and serum was assayed for antibody titers to the following viruses: herpesvirus (by complement fixation, CF), polyomavirus (by CF and virus neutralization, VN), and paramyxovirus-1 (by hemagglutination inhibition, HI). Results of the survey are reported in Table 1 . A total of 8 of 21 (38%) of the *Aratinga* tested for polyomavirus by one or both of the tests were positive, and 11% were positive for herpesvirus. Complement fixation titers for polyomavirus ranged from 1:8 to 1:64. Herpesvirus titers were $\geq$1:8. Although both polyomavirus and herpesvirus infections are described in captive parrots, neither virus is believed to have been previously reported in wild parrots. Whether either virus has caused significant disease in the free-ranging *Aratinga* or *Brotogeris* populations in Manu is not known; at capture, all birds were in good physical condition, with no obvious signs of systemic disease. Based on the findings of this study, quarantine screening of wild-caught parrots for psittacine herpesvirus and avian polyomavirus is recommended, especially considering the potential for latent infection with both of these viruses.

Table 1. A serologic survey for select viral antibodies in wild *Aratinga weddellii* and *Brotogeris sanctithomae* in southeastern Peru .

	Aratinga	*Brotogeris*
Herpesvirus [a]	4/38 (11)*	0/13 (0)
Polyomavirus		
CF[b]	5/10 (50)	0/3 (0)
VN[c]	3/16 (19)	0/11 (0)
Paramyxovirus-1 [d]	0/9 (0)	0/8 (0)

*Reported as positives/total tested (percent positive).

[a] Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas.
[b] Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas.
[c] School of Veterinary Medicine, Texas A&M University, College Station, Texas.
[d] California Veterinary Diagnostic Laboratory, Davis, California.

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OSTEODYSTROPHY IN AN ORPHAN ASIAN ELEPHANT (*Elaphas maximus indicus*)

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A nine month old orphan male Asian elephant calf maintained on an artificial diet was euthanized after developing osteodystrophy. Following a review of the calf's clinical record, diet history and necropsy findings, the cause of the osteodystrophy remains unclear.

On June 13, 1990 a 120 kg male Asian elephant was born after an uneventful pregnancy to a captive bred, wild caught cow. Following maternal rejection and when efforts failed at reintroduction of the calf, the neonate was given 1500 cc of the dam's plasma intravenously and fed the dam's colostrum by gastric tube. Initial feedings consisted of maternal colostrum, bovine colostrum and rice cereal with oral electrolyte solution (Biolyte®, The Upjohn Company, Kalamazoo, MI 49001, or Lytren®, Mead Johnson and Company, Evansville, IN 47721). Within a week to ten days the calf's formula consisted of powder low iron infant formula (Enfamil®, Mead Johnson Nutritionals, Evansvile, IN 47721) with rice cereal (Gerber Products Company, Fremont, MI 49413) and a vitamin supplement (Vi-Sorbin®, Smith Kline Beecham, West Chester, PA 19380).

The calf maintained good vitality and achieved slow but steady weight gains despite ongoing diarrhea, which began the first week of life. At six weeks of age and weighing 138 kg, a diagnosis of formula intolerance based upon acetic pH of the stool and an absence of enteric pathogens, resulted in changing to a milk free powdered diet (Prosobee®, Mead Johnson, Evansville, IN 47721) with rice cereal added. While weight gains were achieved, intermittent diarrhea persisted.

At eight months of age the calf demonstrated lameness and discomfort which became more progressive. Radiographs revealed pathological fractures of both distal humerus with callous formation. Euthanasia was elected as the calf's condition deteriorated.

At necropsy all bones could be cut easily with a sharp knife. Further gross finding included greenstick fractures of both right and left radius and ulna along with multiple folding

fractures and lump calluses. A lumbar degenerative intervertebral disc protruded into the spinal canal. Histological sections of bone demonstrated fibrous thickening of periosteum, replacement of cortex and marrow space by fibrous connective tissue, and thin marrow trabeculae of woven bone with occasional large osteoclasts.

A diagnosis of osteodystrophy was made based upon necropsy findings.[4] What precipitated this condition remains unclear. The many causes of rickets in children are due to deficiencies of vitamin D, calcium, and/or phosphorus.[1] The artificial diet of the elephant calf in this case was thought to be complete; however, the bioavailability of the skeletal nutrients is unknown. Furthermore, the diarrhea could have caused or contributed to the malabsorption of these nutrients.[2] Disorders caused by malabsorption are complex and the diarrhea may have resulted in a mucosal absorption defect.[3]

To further elucidate the pathogenesis of the bone disease in this case a retrospective study of banked serum samples will be undertaken to provide information on calcium, phosphorus, calcitonin, vitamin D, alkaline phosphatase and parathyroid hormone levels.

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SUBTOTAL RADIAL OSTECTOMY IN A CALIFORNIA SEA LION

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In October of 1993, a juvenile male California sea lion (*Zalophus californianus*) weighing 22 kg was admitted to the Marine Mammal Center with an injury to the left forelimb. Initial physical examination revealed a slight pneumonia and an open wound of the left antebrachium with bone exposed and a purulent exudate. Initial treatment included flunixin meglumine for analgesia; ceftiofur and gentamicin systemic antibiotic therapy; irrigation of the open wound with dilute (1%) povidone iodine solution and topical application of silver sulfadiazine cream; and mucomyst and coupage for the pneumonia.

Radiographs revealed a luxation of the left elbow with a Salter I fracture of distal humerus. Periosteal proliferation was consistent with the clinical diagnosis of osteomyelitis. After 2 weeks of therapy, the animal appeared to be stable and the wound was covered with a healthy bed of granulation tissue. Surgical exploration was scheduled.

Pre-operative radiographs revealed progression of the osteomyelitis to involve the distal half of the humerus and the majority of the radius. The presence of gas within muscle planes was considered to be indicative of the presence of gas forming bacteria. Small fragments of the proximal metaphyseal ulna appeared to be isolated but in a normal location. There was concern that these might form sequestra. The elbow was approached laterally and several pockets of purulent material were encountered. The radius proximal to its distal epiphysis was devitalized and devoid of any soft tissue attachments. Following collection of diagnostic samples, it was easily removed and discarded. The distal humeral epiphysis was similarly devitalized and, therefore, removed. Several attempts were made to retrieve the fragments of ulna identified on radiographs; however, they could not be identified. The wound was irrigated with hydrogen peroxide because of the presence of interstitial gas and the suspicion that this was the result of anaerobic infection. The area was also irrigated with several liters of sterile saline prior to closure. Two Penrose drains and one continuous suction drain were placed and the wound was closed routinely.

Post-operatively, the animal was given butorphanol for analgesia. There was minimal drainage from the 3 drains. Dilute povidone iodine was irrigated through the drains and silver sulfadiazine was applied topically to the wounds. Hot moist compresses and passive range of motion therapy were well tolerated by the patient. *Listeria ivanovii* was isolated from the bone. Other organisms isolated included *Proteus mirabilis*, *Serratia odorifera*, *Streptococcus fecalis*, *Edwardsiella hoshinae* and beta hemolytic *Streptococcus* group C. These isolates were consistent with a bite wound. During the convalescent period, antibiotic therapies were instituted based on culture results.

The drains were removed 5 days post-operatively. Some dehiscence of the surgical incision occurred but by 4 weeks the wounds appeared to be granulating well. Radiographs made at this point revealed proliferation and lysis along the distal humerus and proximal ulna. It was difficult to determine if this was the result of osteomyelitis or normal bone callus production.

Five weeks post-operatively, the wounds had healed and antibiotic therapy was discontinued. At this point, the sea lion was using the affected limb well. By 9 weeks post-operatively, a draining tract had developed. Radiographs demonstrated that the periosteal reaction had decreased but the fragments of ulna did not appear to be incorporated into the callus. There was an apparent synostosis between the distal humerus and the proximal ulna. The sea lion was using the limb very well in spite of the draining fistula. Antibiotic therapy was re-instituted and the fistula was irrigated with dilute povidone iodine daily.

Following 4 weeks of antibiotic therapy, the wound appeared to be closing and the therapy was discontinued. Unfortunately, the sea lion was beginning to demonstrate stereotypic behavior making early release necessary. One week later, the fistula had reformed and was draining again.

Because it was likely that a sequestrum was responsible for the recurrent drainage, sequestrectomy was scheduled. Approximately 4 months following the initial surgery, a second surgery reveal 2 large pieces of devitalized bone. These were imbedded within the callus formed between the distal humerus and the proximal ulna but were distinct from the pieces of ulna under suspicion. Following their removal, recovery was rapid. The sea lion was placed on antibiotic therapy for 1 week and within 2 weeks the animal was using the leg well. The wound was no longer draining. Sixteen days after the second surgery, the animal was released.

This sea lion developed a synostosis of the humerus and ulna but had good limb function. Because of the conformation of the forelimb of sea lions with the brachium and antebrachium within the torso, the majority of motion occurs at the carpus. Motion in this joint is responsible for movement of the flipper which is used for locomotion as well as maneuvering during swimming. In this animal, we were able to preserve the distal epiphysis of the radius, and thus, all of the ligamentous support of the carpal joint. Though there was no motion at the elbow, this animal was able to ambulate and swim with minimal lameness. This procedure should be considered a viable treatment option for pinnipeds with severe injury to the radius.

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THE USE OF BONE SCAN DIAGNOSTICS IN AN AFRICAN LEOPARD (*Panthera pardus*)

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A nineteen year old, female, spayed african leopard (*Panthera pardus*) developed bilateral alopecia due to self mutilation of the flanks and tail. Three months of alternate conservative therapy with diazepam, naltrexone, and steroids resulted in only temporary improvement. Self induced alopecia and eventually erythematous skin lesions resulted as therapy was discontinued.

Chest radiographs during diagnostic evaluation revealed a transverse fracture of T7 spinal process. Because of the animals signalment, radiographic appearance of the fracture, and clinical history, pathologic fracture secondary to neoplasia was considered in the differential. Four years prior, the leopard had several masses removed from the right scapular region which were diagnosed on histopathology as chondrosarcoma. In order to rule out neoplasia, scintigraphy ie. bone scanning was used to evaluate for signs of metastasis. Scintigraphy was performed by injecting 40 millicuries of Technetium 99m hydroxymethelene diphosphonate (Tc-99mHDP) into a 20g catheter through a saline drip in the cephalic vein. Time to imaging was 3 hours post-injection and each area was imaged for 8 to 10 minutes. Results showed mild isotope uptake in the T7 region and radiographs confirmed a healing fracture. T12-13 region revealed considerable isotope accumulation indicating an active lesion corresponding to a collapsed disc space and spondylosis deformans on radiographs. None of the other regions scanned showed abnormal tracer uptake, thereby ruling out neoplastic metastasis and pathologic fracture.

In order to utilize Tc-99mHDP at the Zoo, a local hospital facilitated an extension on their NRC license and provided the mobile scintigraphy unit and professional expertise to monitor radiation control. The leopard was isolated for 60 hours (10 half lives) post injection and the area scanned for radiation prior to return to the regular holding area.

After four weeks of a reducing dose steroid therapy, the self- induced alopecia resolved. It was concluded that pain from the T7 or T12-13 region may have been responsible for the self-mutilation of the skin and hair in the flank and tail region.

Nuclear imaging provides a great deal of sensitivity and allows for the detection of active lesions over a large region. Major disadvantages of nuclear imaging include a very low resolution and specificity. However if combined with radiography, nuclear medicine imaging can provide valuable diagnostic information.

AN UNUSUAL PRESENTATION OF OSTEOMYELITIS IN A SNOW LEOPARD (*Panthera uncia*) CUB

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A 5.5 month old, male snow leopard was presented for rear leg lameness upon its arrival at the Zoo. The cat had been pulled from its dam at birth due to maternal neglect and hand-reared at another institution. Physical examination revealed a mild bilateral rear limb lameness and a small, overall size (10.75 kg, 3 kg less than his mother raised littermates). The following day the leopard was anesthetized with 100 mg ketamine IM, supplemented with isoflurane via endotracheal tube. Radiographs showed proliferative and lytic lesions in all long bones and the skull. The white blood cell count (WBC) was elevated at 19,200; globulins were elevated at 6.2 gm/dl; FeLV antigen was negative; FIV antibody was negative. Trephine bone biopsies were taken from lesions in the right ulna and tibia. Histopathology results of the biopsies indicated multifocal, severe, chronic suppurative osteomyelitis surrounded by extensive reactive woven bone. Cultures of the bone biopsies yielded *Klebsiella oxytoca* resistant to ampicillin and no fungal growth. Lesions continued to smolder and WBCs remained elevated as different antibiotics were tried (See table below). Lesions were re-biopsied (again growing *Klebsiella oxytoca*) and followed radiographically. Following prolonged therapy with enrofloxacin 136 mg PO BID X 60 days, WBC remained normal and lesions are slowly resolving.

Event	Date	WBC 10^3	Comment
Initial Rads & Biopsy	25 Nov 91	19.2	Culture: *Klebsiella oxytoca*
Cefadroxil	04 Dec 91		400 mg PO SID X 28 days
Chloramphenicol	08 Jan 92	29.8	250 mg PO BID X 30 days
Rads & Biopsy	21 Jan 92	31.9	Culture: *Klebsiella oxytoca*
	08 Feb 92	27.5	
Tribrissen	20 Mar 92	29.6	480 mg PO BID X 10 days
Rads & Biopsy	07 Apr 92		Culture: *Klebsiella oxytoca*
Enrofloxacin	09 Apr 92		136 mg PO BID X 60 days
	20 Apr 92	15.6	
Rads	05 May 92	12.6	Lesions resolving
	26 May 92	12.1	
Rads	25 Jun 92	15.8	
	14 Jan 93	10.2	
Rads	27 Jul 93	8.1	Lesions resolving

PROVENTRICULAR ASCARIDIASIS IN LAPPET-FACED (*Torgus tracheliotus*) AND AFRICAN WHITE-BACKED (*Gyps africanus*) VULTURES

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The Dallas Zoo has a current inventory of 2.3 Lappet-faced (*Torgus tracheliotus*) and 1.0 African White-backed (*Gyps africanus*) Vultures. Historically, 1.1.1 African White-backed Vultures have also been in the collection. During April 1993, the Lappet-faced Vultures demonstrated acute onset vomiting or regurgitation of short duration with large, white helminths present in the eliminated material. These nematodes were identified as members of the genus *Porrocaecum*. Although no additional clinical signs have been seen in these birds, they have continued to demonstrate evidence of patent infection through shedding of eggs.

In June 1993, a 0.1 African White-backed Vulture presented with signs of lethargy and weakness. Once improved with supportive care, the bird was housed with a 0.1 Lappet-faced Vulture for companionship. In October 1993, the African White-backed Vulture suddenly succumbed and gross necropsy findings of severe, visceral gout and incidental proventricular helminths were reported. These nematodes were identified as members of the genus *Contracaecum*. The remaining African White-backed Vulture, also housed with the same Lappet-faced Vulture, has not demonstrated clinical signs nor shed any ascarid eggs.

Ascarids of the *Contracaecum* and *Porrocaecum* genera are large (100-170mm in length), white helminths which are historically difficult to distinguish from one another. Differentiation relies primarily on subtle anatomical structures and therefore they are often discussed interchangeably in terms of their life cycles and clinical effects. In contrast to most ascarids, these genera demonstrate an indirect life cycle with fish or rodents serving as intermediate hosts. Additionally, they invest into the proventricular lining of birds instead of establishing in the intestine which is typical of most ascarids. Because of their location, they are associated with clinical signs of vomiting, regurgitation, and poor or lost condition. Though no additional signs have been observed in the Dallas Zoo collection, an effort has been undertaken to resolve the infection as practically as possible.

These ascarids can be difficult to remove with anthelminthics as the current infection has revealed. Several regimens have been given to the Lappet-faced Vultures with continued shedding of ascarid eggs. These birds are handled monthly for flight plumage monitoring and clipping. A one day, monthly deworming was sought to minimize additional handling and stress for the birds while assuring complete ingestion of the anthelminthic. Currently,

pyrantel pamoate (Pyran-50, 50mg/ml, Wintec, Inc., Pacific, MO 63069 USA) is under evaluation and an initial dose of 4.5mg/kg was used. After two months of minimal decline in apparent shedding of eggs, the dose was increased to 6.75mg/kg. Fecals are evaluated and ascarid egg levels estimated (direct and flotation) the week prior to each dose. The one African White-backed Vulture has not been treated but has fecal samples submitted with the other birds.

With an indirect life cycle, removal of the intermediate host would be helpful to prevent re-ingestion of the ascarid. These birds however do not consume a diet which would likely carry the nematodes. Historically, at least one of the Lappet-faced Vultures (0.1), entered the collection associated with a previous episode of clinical signs of ascarid infection, treatment, and a diet of herring which would provide an appropriate intermediate host. It is possible that the bird has maintained a predominantly non-apparent clinical infection and not been re-infected.

Both genera of ascarids have been identified in the *Gyps* genus but the literature did not report an infection in the genus *Torgus* with either ascarid.

CHAGAS' DISEASE IN AN AFRICAN HEDGEHOG

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A mature (1 year, 4 months), 277 gram, male African hedgehog (*Atelerix albiventris*) was presented in September for evaluation of anorexia. Housing consisted of a glass fronted, wire topped wooden box with bark mulch litter and several furniture items of wood and shed antlers. The exhibit was permanently open to the outdoors.

Two months previously, the animal showed signs of lethargy and partial anorexia for a period of three weeks. Ambient temperature during this period had been quite high so heat stress was considered a factor. The animal was moved to air-conditioned quarters and given a wider choice of foods. Its appetite and activity level increased and it was returned to the exhibit.

On this present physical examination, the hedgehog was depressed, lethargic, and slightly ataxic, but responsive. No respiratory difficulty or other abnormalities were noted. Initial treatment included Lactated Ringer's solution (LRS), procaine penicillin, oxytetracycline, vitamin B complex, and ivermectin. After three days of treatment no improvement was noted and therapy was changed to cephalexin, continued LRS and force-feeding. Blood was drawn from the femoral vein for a complete blood count and serum chemistry analysis.

Unfortunately the sample for the CBC was destroyed in transit to the laboratory, however the blood smear used for the WBC differential revealed an apparent leukocytosis. The blood smear showed 75% segmented neutrophils, 18% lymphocytes, 5% mononuclear cells, and 2% band cells (mature neutrophilia and monocytosis[1]). The packed cell volume was 45%, and total protein 4.7. A subsequent review of the blood smear revealed *Trypanosoma* organisms on the slide.

The chemistry screen revealed an elevated CPK of 18,532 U/l. The remainder of the serum chemistry appeared within normal limits. A urinalysis was also performed which revealed many red cells and sperm in the urine, urine protein of 500 mg/dl, a bilirubin of 1+ on urine dipstick, and a specific gravity > 1.060. Fecal parasite examination was negative, as was fecal occult blood.

The hedgehog's condition continued to deteriorate despite force-feeding, subcutaneous fluids, antibiotic therapy, and dexamethasone. The hedgehog died 7 days after initial presentation.

Postmortem examination revealed multiple purplish-red 1 mm lesions in the mucosa of the stomach with apparent defects in the mucosa. The reproductive organs were notably large. Abnormal results noted on histopathology included paraportal fatty cyst formation in the liver (hepatic lipidosis); tubular dilation, hyaline droplet formation, and localized areas of fibrosis in the kidney (toxic tubular nephrosis); extramedullary hematopoiesis in the spleen; swelling of alveolar septal cells in the lungs (pneumonitis); proliferation of interstitial cells, infiltration of lymphocytes and histiocytes in the myocardium (granulomatous myocarditis), and packets of microorganisms morphologically consistent with *Trypanosoma cruzi* in the myocardium.

Discussion

Chagas' disease is caused by the blood-born protozoan, *Trypanosoma cruzi*, and has been documented in humans, dogs, cats, raccoons, opossums, armadillos, and several rodent species. Chagas' disease is most frequently seen in South America, Central America, and Mexico, although it has also been reported in many states in the USA including Alabama, Arizona, California, Florida, Georgia, Louisiana, Maryland, New Mexico, Oklahoma, and Texas.

High incidence of infections in South America are considered to be due to the insect vectors custom of defecating after feeding giving trypomastigotes in the vector's stool access to a fresh skin wound. North American vectors do not defecate immediately after feeding so oral transmission is considered more probable. Infection in opossums can occur this way and is possible in dogs as well. The vector's bite can be quite painful and cause the victim to snap at the bite site. Insect vectors and host animals occur in most areas of the USA, so the possibility for infection is always present. In particular, insectivorous mammal may be at risk from oral infection wherever infected vectors are present. Provisions for exclusion of insect access in endemic areas should be considered. Danger to humans in North American is minimal, however, animal husbandry and health personnel should be aware of the infection possibilities; vector stool and blood from host animals can potentially be infectious.

Initial signs of acute Chagas' disease in dogs are local inflammation at the sight of inoculation of infected feces, and inflammation of regional lymph nodes at 14-17 days post-infection. Severe myocarditis may develop 2-4 weeks post-infection and cause acute death due to complete heart block or cause progressive left and right ventricular dysfunction. In dogs that survive the acute episode, cardiac functions appear to normalize within one week and the disease progresses to the chronic form. Multifocal neurologic signs have also been reported in dogs as acute symptoms. Chronic stage Chagas' disease signs and eventual death are caused by left and right side congestive myocardial failure. In this particular case, we surmise that the illness in July may have been an acute episode of trypanosomiasis which then progressed to the chronic disease which demonstrated symptoms 8 weeks later. Antemortem diagnosis of Chagas' disease can be made by visualizing trypanosomes on a blood smear. However, as parasitemia occurs intermittently the organisms are not likely to

be present in the blood once they have invaded the tissues. In addition, *Trypanosoma cruzi* organisms seen in the blood may be confused with other trypanosome species.

There is currently no totally effective treatment for Chagas' disease as most available antiprotozoal drugs do not have long-term success. Nifurtimox has been shown to be effective on dogs during the acute phase, however, it is currently only available from CDC for human infections. In addition, benzimidazole and allopurinol have been demonstrated to have some effectiveness. In general, therapy is often instituted too late to change the disease progression and dogs surviving the acute phase succumb to chronic disease.

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MYASTHENIA GRAVIS IN A SIBERIAN TIGER

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A 17-year old female Siberian tiger (*Panthera tigris altaica*) at the Milwaukee County Zoo was diagnosed with myasthenia gravis. The onset of the disease was insidious, characterized by intermittent episodes of drooling, abnormal food prehension, dysphagia, gagging and regurgitation. Episodes began in November 1989 and increased in frequency and severity over the next two years prior to diagnosis.

Diagnostic examinations initially focused on the oral cavity and teeth, but no cause for the clinical signs was found. Hematologic and serum chemistry parameters were normal. In March of 1991 endoscopic examination of the pharynx and esophagus was performed and findings were unremarkable; however, it was noted that the entrance to the trachea through the laryngeal cartilages was fully open. Reflex closure of the aryepiglottic folds and arytenoid cartilages could not be elicited. Exploratory surgery of the larynx, trachea and cervical esophagus performed in December of 1991 did not reveal any abnormalities.

Serum was submitted to the Comparative Neuromuscular Laboratory, at the University of California, San Diego, to determine titers for antibodies against acetylcholinesterase receptor (AChR). For comparison, sera from three healthy tigers were sent as well. AChR titers in the healthy individuals measured <0.1 nmol/l, (feline normal <0.3 nmol/l), but >6.17 nmol/l in the affected tiger. Thus, a diagnosis of acquired myasthenia gravis was made. Treatment with pyridostigmine and prednisolone was initiated in January of 1992.

Because of the potential toxic effects associated with anticholinesterase compounds,[4] a low dose of pyridostigmine (0.12 mg/kg orally, once a day) was given initially, and slowly increased over 5 weeks to a maintenance dose of 0.24 mg/kg once a day. Prednisolone was given concurrently at 0.4 mg/kg, but after three weeks and only moderate clinical improvement, daily immunosuppressive doses of prednisolone (2.8 mg/kg) were begun. Clinical improvement was dramatic, and the prednisolone dose was decreased over 2 weeks to 2.2 mg/kg every other day where it was maintained. In addition, the tiger was placed on a monthly rotation of oral antibiotics to help prevent systemic bacterial infections occurring secondary to immunosuppression.

Clinically the animal did well for approximately 4 months, at which time her appetite and activity level began to decrease. Generalized weakness was apparent and tremors of the front legs were seen occasionally. Physical deterioration continued despite increasing the prednisolone to a daily dose of approximately 2.8 mg/kg.

Six months after initial diagnosis, she was immobilized for examination. Radiographs suggested pleural effusion, therefore thoracocentesis was performed. Twelve ml of purulent fluid was obtained and submitted for bacterial and fungal cultures, results of which were negative. At this time hematology revealed a leukocytosis with 10% band cells. A CT scan

performed 2 days later revealed little pleural effusion and a small mass, thought to be a thymoma, located cranial to the heart at the level of the tracheal bifurcation.

Despite antibiotic therapy and continued treatment for myasthenia gravis, her physical condition deteriorated, and she was euthanized 7 months after initial diagnosis.

Necropsy findings included: one pulmonary abscess, esophageal dilatation, and thymic masses with mineralization. Histologic findings were cryptococcal pulmonary abscessation and thymoma.

Acquired myasthenia gravis is an autoimmune neuromuscular disease in which antibodies attack the acetylcholine receptor (AChR) of the postsynaptic membrane of the neuromuscular junction. The resultant decrease in the number of functional receptors hampers transmission of the nerve impulse across the neuromuscular junction.[1] The clinical manifestation is weakness which increases with activity. It has been reported in humans, dogs and cats. In the dog and cat, it has generally been described as affecting both the cranial and spinal nerves, with generalized weakness resulting.[2] In humans, cranial nerve signs are the most common manifestation, with ptosis, difficulty in chewing, dysphagia and dysarthria occurring frequently. Limb weakness may also occur. The course of the disease can be episodic, with weeks or months between episodes. The events that initiate the formation of antibodies is unknown. Thymoma occur in an estimated 15-40% of human cases, and thymic hyperplasia is present in most of the patients without thymoma.[3]

Demonstration of circulating antibodies to AChR is the most important laboratory test, although the level of antibody titer does not correlate with the severity of disease.[3] Diagnosis is confirmed by improvement following administration of anticholinesterase drugs.

Treatment is on two fronts: one is the use of anticholinesterase drugs to treat the symptoms, and the other is the use of immunosuppressive drugs to induce remission of the disease itself.[4] In humans, thymectomy is recommended for patients in whom the disease is refractory to drug treatment alone.[1,3] Myasthenia gravis can be difficult to manage, the course of the disease is variable, and complications secondary to treatment may occur.

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EFFECTS OF A GNRH AGONIST ON SERUM SEX HORMONE LEVEL OF MALE AND FEMALE WESTERN BLACK-FACED, GRAY KANGAROOS, MALE AFRICAN WILD DOGS AND SPECTACLED BEARS

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Introduction

A major problem facing the zoological community has been the reproductive management of the animals in their collections. Due to limited holding space, it is imperative to regulate the time and frequency of breeding to maximize the use of available space.

Many of the endangered species of animals at the zoological institutions are now being genetically managed under the auspices of the American Association of Zoos and Aquariums (AZA) Species Survival Plan (SSP) committees and a myriad of difficulties has developed in producing the exact number and ratio of genetically desirable individuals needed to ensure their long term survival. To help in the genetic management there are currently several tools available to zoo managers. Regarding the reproductive management of these animals, managers can select to move animals, chemically or physically contracept them, or attempt to employ artificial reproductive techniques such as artificial insemination or embryo transfer. Contraception is just one of the tools, but possibly the most important.

A few examples of the types of contraception follow. They include melengestrol acetate implants, oral contraceptives, vas plugs, tubal ligation, ovariohysterectomy, medoxyprogesterone, and castration. There are two major divisions in the types of contraception available: those which are reversible and those which are not.

One of the most widely used forms of reversible contraception is the melengestrol acetate (MGA) implants. This product has been used in a variety of animals, especially primates and large felids. There is evidence which suggests these implants may cause a variety of animal health problems, such as uterine hyperplasia and subsequent neoplasia, failure due to removal by cagemates (primates), failure due to poor surgical implantation technique, infection at surgical site, and failure due to species specificity. Although widely used, it is not necessarily safe or extremely effective in all species.

Two other currently used methods include Depo-Provera injections (medroxyprogesterone) and the surgical implantation of vas plugs. The Depo-Medrol injections are extremely variable in their efficacy. Not only is there a species efficiency difference, as with unclear duration of effect and dosage, but there also is an individual difference between animals that otherwise physiologically appear equal (weight, age, nutritional status, and health status).

The vas plugs are still being tested and the success rate is unknown. The species which can utilize the vas plug are also unknown. There are anecdotal reports which state there appears to be failure due to variability in the size of the vas deferens, not only between

species, but also among individuals within a species simply due to the variation in size and age. Many of these problems are currently being addressed, but there are some other difficulties with the contraceptive. Installation may be a tedious surgical procedure, depending on the size of the patient and the skill of the surgeon. The ability to reverse this procedure is also unknown as the surgery undoubtedly traumatizes the vas deferens.

A zona pellucida vaccine is also being developed. Unfortunately the adjuvant currently being used may render the animal as a positive reactor for the standard intradermal tuberculin skin test.

After considering some of the drawbacks of current methods of contraception, the continuing need for a safe, effective, reversible, and easily administered contraceptive led to the testing of leuprolide acetate in depot suspension (Lupron^{R}).

Several GnRH agonists (Lupron Depot^{R}, Zoladex^{R}, and D-Trip6) have been reported to decrease blood levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, and progesterone levels to that of a gonadectomized animal. This has been shown in humans, nonhuman primates, rats, dogs, guinea pigs, and mice.

Lupron^{R} (TAP Pharmaceutical, Chicago, Illinois) is currently being used in human males in the treatment of prostatic neoplasia in lieu of orchiectomy and in human females for treatment of endometriosis. This drug has been approved by the Food and Drug Administration for both of the stated diseases due to its high success rate of treatment and minimal side effects.

Lupron Depot^{R} could become a primary contraceptive for the following reasons: 1) shown relatively few side effects, 2) controllable duration of effect, 3) ease of administration, 4) no anesthesia or surgery involved, therefore animal manipulation and stress are minimized, 5) works on a "universal" hormonal pathway which theoretically will allow its use in multiple orders of animals and therefore a tremendous variety of species, and 6) the molecule can be engineered (once base lines are established) to variable durations of effect per injection for specific needs.

If this proves to be as efficacious as research reports on some of the GnRH agonists, the zoo and wildlife field will have an improved, safer means of effectively controlling the reproduction of threatened and endangered species.

Objectives

The objectives of the study presented are part of an overall contraception study using the GnRH agonist Lupron^{R} as a reversible contraceptive in a variety of species in both males and females. This will be determined by the surveillance of blood hormones, urinary hormones, and for most males, testicular size and semen production. This report includes

only that of blood levels of testosterone in male western black-faced gray kangaroos, African wild dogs and one spectacled bear and progesterone levels in female western black-faced gray kangaroos.

Materials and Methods

Fifteen animals, of each species examined, will be used in the study (five male and ten females). One male and two females per group will serve as controls. Of the species discussed here, there have only been 2.4 kangaroos (all experimental group), 3.0 African wild dogs (2 experimental, 1 control), and 1.0 spectacled bear (experimental) involved in the study and therefore this paper will serve only as a preliminary report.

The approximate dose of Lupron[R] in humans is 0.075 mg/kg, intramuscularly, every 28 days. This study was conducted over a period of ten months with the majority of the animals (60%) being finished with the study in six months, as described below. The study animals were injected subcutaneously at a dose of 0.075mg/kg/28 days of Lupron[R] while the controls were given an equal volume of Depot only. The depot is a bio-degradable copolymer of lactic and glycolic acid.

The leuprolide acetate was delivered via hand syringe when the animals were restrained for blood collection. When the animals did not have a blood sample taken, the Lupron[R] was given using a Telinject[R] blowdart.

Blood samples were collected from each of the animals prior to Lupron[R] or placebo injection and subsequently at the following intervals: week 4, 8, and 24. Two males and four females had the Lupron[R] discontinued at week 24, but had additional blood samples collected at week 32 and 40. The last two blood samples allowed us to determine post-treatment hormone levels and to evaluate time to return to pre-treatment hormone levels.

The males were evaluated by measuring testicular size and by the collection of blood with the subsequent evaluation of testosterone levels. The female animals' blood was collected and progesterone levels were determined.

Results

The female kangaroos showed no significant change in the progesterone levels throughout the study.

The male kangaroos showed a decrease of testosterone from 0.41 ng/ml to <0.156 ng/ml from the date of injection to the next month. This was a reduction of a minimum of 62%. The levels returned to pre-study levels within 8 weeks of cessation of treatments. The testicular size did not appreciably change during the period, however it was slightly reduced.

The male African wild dogs showed a decrease of testosterone levels from original values of 0.3 to 0.2 ng/ml down to a low of <0.06 ng/ml or a reduction of 80% or more of the initial level. The testicular measurements were approximately 50% of their normal size. The control animal maintained high levels of testosterone throughout the study. The levels returned to pre-study levels within 8 weeks of cessation of treatments.

The one spectacled bear in the study showed a reduction in testosterone levels from 1.75 ng/ml down to <0.156 ng/ml. This is a 91% reduction in testosterone levels. The levels returned to pre-study levels within 8 weeks of cessation of treatments.

Discussion

All animals in this study were maintained in a normal social structure and were either allowed access to reproductively active animals within their group or by adjoining holding areas. There were no alterations in the living conditions or social arrangement prior to the initiation of the study. In the case of the kangaroos, the animals were housed in a reproductively active mob which they were members of since birth.

Unfortunately the study design lends itself to miss changes in the progesterone levels of the kangaroos. Due to the sampling frequency, it was possible to miss normal fluctuations of progesterone due to natural cyclisity. Especially with only four animals on a once a month blood collection protocol. With the increased number of animals needed to complete this section of the study, and a possible increase in sampling frequency, we will clarify this point.

The kangaroos were maintained in a mob of approximately 25 individuals where the females were with two actively breeding males. Joeys were being born during the study, but none of the study animals became pregnant. The study animals ranged in ages from three and seven years old, with three of the four proven breeders. After cessation of treatments, three of the four animals became pregnant and had live, healthy joeys in a time frame which indicated the females were fertile within four months of the last Lupron^{R} injection. The fourth female died from "lumpy jaw" three months after the initiation of the study and no uterine or ovarian abnormalities were noted.

The African wild dogs were sampled during the breeding season of the wild dogs at the Brookfield Zoo (August-October). Although not housed with a breeding female, they were housed in an adjoining enclosure which they had occupied for several years which was adjacent to a breeding pair. The bear was housed adjacent to a normal cycling female throughout the course of the study.

Although their is no definitive proof the use of the Lupron^{R} was directly responsible for stopping pregnancy in the kangaroos, it was extremely suggestive it was effective due to their housing situation. It is also quite suggestive of efficacy when animals testosterone levels drop to as low as 9-20% of their normal levels. We could not evaluate changes in

semen production, due to logistics of semen collection. Whether the use of this drug lead to aspermia or not, in unknown. Aspermia may not be a requirement for effective contraception of the male, if libido is reduced significantly.

Current studies in these and other species will lead to the answers to these questions.

USE OF METHOHEXITAL SODIUM AS AN ANESTHETIC IN TWO SPECIES OF COLUBRID SNAKES

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Chemical restraint and anesthesia of reptiles can be challenging due to the uniqueness of poikilothermic metabolism and difficulties in intravenous drug administration for many species. Currently, the most popular anesthetic regimes rely on gas inhalants, dissociative agents, and/or benzodiazepine derivatives combined with dissociative agents.[3,4] In reptiles, most of the barbiturates have relatively low margins of safety and cause prolonged recovery times; their use in reptilian anesthesia is not widespread.

Methohexital sodium (Brevital; Eli Lilly and Company, Indianapolis, Indiana 46285) is an ultrashort-acting oxybarbiturate primarily utilized as an anesthetic in humans.[1] It has been approved by the U.S. Food and Drug Administration for use in dogs and cats.[2] Although the only recommended route of administration is intravenous, extravascular injection of methohexital does not produce the soft tissue irritation and necrosis that is commonly seen with many of the other barbiturates.[2] Methohexital has been reported to be a satisfactory anesthetic in some reptiles -- particularly snakes.[4,5] The ability to administer the drug subcutaneously is advantageous for use in small reptiles and when inhalant anesthetics are undesirable or impractical.

During the course of an on-going project to study ophidian paramyxoviruses and their effects on brown tree snakes (*Boiga irregularis*) and prairie kingsnakes (*Lampropeltis calligaster*), anesthesia of the snakes has been required for viral inoculation and blood collection procedures. In each case, a 1% or 1.5% solution of methohexital was used at doses ranging from 4.52 to 16.3 mg/kg BW; injections were made subcutaneously lateral to the vertebral dorsal spinous processes in the cranial 1/2 of the body. For each anesthetic event, "induction time" was measured and, in most instances, the times until "partial recovery" and/or "full recovery" were also recorded. Induction time was defined as the interval between administration of methohexital and loss of response to tactile stimuli. "Partial recovery" occurred when snakes regained their response to tactile stimuli; fully recovered snakes displayed tongue flicking and spontaneous movement.

A total of 32 brown tree snakes and 3 prairie kingsnakes have been anesthetized more than 130 times and 19 times respectively. The tree snakes ranged in weight from 23.9 g to 1660 g. In this species, the average dose of methohexital given was 9.95 mg/kg BW and the mean

induction time was 23 minutes (range: 7 - 45 minutes). Partial recovery was seen, on average, 116 minutes after administration of the drug and the mean time until full recovery was 184 minutes.

The kingsnakes varied from 147 to 235 g in weight and the average dose of methohexital was 9.56 mg/kg BW. The mean induction time was 22 minutes (range: 15 - 35 minutes) and the average times to partial and full recovery were 190 minutes and 291 minutes, respectively.

Our data suggests that there are differences between the two snake species regarding anesthetic recovery times. Compared to brown tree snakes of similar mass, prairie kingsnakes have thicker bodies with more adipose tissue per gram of body weight. Since the ultrashort-acting barbiturates tend to localize in body fat,[2] the prolonged recovery times in the kingsnakes may be associated with the relatively high body fat content in this species.

In our experience, methohexital has proven to be safe and effective for short term anesthesia in these two species of colubrid snakes. Relatively rapid induction times and excellent muscle relaxation occurred at doses of approximately 9 - 10 mg/kg BW. When using methohexital in other species, however, dosage adjustment may be necessary for heavy-bodied snakes with large amounts of coelomic fat.

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ORAL CARFENTANIL CITRATE USE IN WHITE-HANDED GIBBONS (*Hylobates lar*)

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Historically, ketamine hydrochloride has been used routinely as an anesthetic agent in primates, either using a direct or remotely injected system. This report covers the oral use of carfentanil (Wildnil, Wildlife Pharmaceuticals,Inc.) in twelve anesthesias in captive white-handed gibbons.

Six individuals, two males and four females, ages 80 to 192 months were immobilized with carfentanil for annual physical exams, TB tests, and MGA hormonal implants. Weights ranged from 5.5 kg to 8.9 kg. All doses were delivered within food items, typically bananas or pitted dates, and handfed to each gibbon on an island exhibit. After the initial effects of anesthesia, each individual was crated and transported to the park's clinic.

Respiratory rates (range 8-20), heart rates (100-140) and level of consciousness propreoception were monitored during the course of the anesthesia. At the end of each procedure, a reversal (diprenorphine, naloxone or naltrexone) was given subcutaneously and intravenously. Naltrexone was dosed at 100mg per 1mg carfentanil given, divided 25% IV and 75% SQ.

Induction times were a mean of 15 min. (range 5 - 195 min.) with no apparent increase of depth during anesthesia (15 min. - 1 hr). Mean reversal time was 10 min. (range 7 - 15 min.) with partial reversal recognized in two different individuals. One individual received a partial dose of oral carfentanil with a prolonged induction of 195 min. to only a heavy sedation stage.

All handling of these food items were done with the usual precautions regarding carfentanil use. Any uneaten portions of food were safely disposed of.

One renarcotization was seen approximately 18 hr. after the immobilization. Full recovery occurred following diprenorphine administration. There was one anesthesia-related death. A nine year old female with no known prior health problems was anesthetized and reversed with naltrexone. Upon reversal, she became extremely agitated, had violent uncoordinated body movements and collapsed. Histology showed lipofuscinosis of the myocardium and during extreme stress is thought to have led to acute myocardial degeneration.

INVESTIGATION OF SUSPECTED MYCOBACTERIOSIS IN A GROUP OF TROPICAL BIRDS AT THE TOPEKA ZOOLOGICAL PARK

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Introduction

The rising popularity of large, mixed species exhibits housing various semi-free ranging species of mammals, birds, reptiles, and amphibians has resulted in the construction of many such enclosures in zoos around the world. These exhibits often feature a tropical theme, with natural substrates, extensive use of live plants, and a consistently warm and humid atmosphere. Although aesthetically attractive, these conditions make surveillance and control of infectious disease a challenge.

Mycobacterial disease, especially infection with *Mycobacterium avium*, is of particular concern, not only for it's potentially devastating effects on the animal collection, but also for it's zoonotic potential. Although, one study has found *M. avium* isolated from various human patients to be antigenically distinct from animal pathogenic *M. avium* strains.[3]

Case Report

In September 1993, a male red-crested turaco (*Touraco erythrolophus*) housed in the Tropical Rain Forest the Topeka Zoo was examined because of the presence of blood on the feathers of the head. Physical examination revealed a fleshy, pedunculated mass arising from the left external auditory meatus. Histopathology performed on a biopsy of the mass revealed granulomatous inflammation with acid-fast bacteria, suggestive of avian tuberculosis. The bird was re-examined 2 wk later, and was found to be in poor body condition. Multiple cutaneous masses were noted in the ventral pectoral skin as well as the ventrum of the radius and ulna bilaterally. A blood sample was obtained for hematology and serum chemistries, and whole body radiography was performed. A fecal smear was negative for acid-fast bacteria. On the following day, the bird was euthanized and submitted to Kansas State University (KSU) for necropsy.

Postmortem examination revealed a slightly enlarged liver with multiple tan to white indiscrete foci scattered throughout the parenchyma. A generalized thickening of the small intestinal wall was noted, and both radii and ulnae were thickened and irregular, with multiple periosteal swellings. Histopathologic evaluation revealed granulomatous dermatitis with histiocytic myositis of the underlying skeletal muscle. Ziehl-Neelsen stained sections highlighted myriads of intracellular rod shaped acid-fast bacteria. Bacteria laden macrophages were found throughout sections of bone, liver, spleen, heart, liver, and small

intestine. Sections from the proventriculus, kidney, and brain were histologically normal. Tissues were submitted for bacteriology, and a tentative diagnosis of avian tuberculosis was made.

The possibility of infection in other birds in the Tropical Rain Forest exhibit prompted indepth discussions to determine an appropriate course of action, pending the results of mycobacterial culture. Many Zoo personnel and several members of the KSU faculty contributed to the decision making process. All specimens from the Tropical Rain Forest had been maintained in holding facilities for the previous 10 mo, pending renovation of the building. The decision was made to euthanize those birds that had been most closely associated with the diseased turaco. This included a female turaco, a crested wood partridge (*Rollulus roulroul*), a lilac-breasted roller (*Coracius caudata*), and a blue-crowned motmot (*Momotus momata*). The information obtained from thorough evaluation of these birds would then be used in determining the extent of evaluation needed for the remainder of the specimens previously housed in the Rain Forest. Necropsy and histopathology revealed no evidence of infection with mycobacteria in any of these birds. However, samples were collected for mycobacterial culture.

Samples from the liver from each bird were pulverized, decontaminated with 0.25% cetylpyridinium (HPC), and centrifuged. The re-suspended pellet was washed with sterile water, and slants of Lowenstein-Jensen and tubes of Middlebrook 7H9 with glycerol broth media were inoculated. Additionally, samples from the kidney, bone, lung, and tumor from the male turaco were prepared in a similar manner, and feces were prepared using two different concentrations of HPC. Tissues from all birds were also prepared without decontamination, which resulted in overgrowth of the media. All inoculated media were incubated at 37° C with 5-8% CO_2.

On the 25th and 26th days of incubation, acid-fast rods were recovered from the broth inoculated with spleen, bone, and liver from the male turaco. However, inoculation on to Lowenstein-Jensen and Herrold's medium with mycobactin J resulted in no growth. The broth initially appeared to support growth from samples of the roller, motmot, and partridge, but further investigation revealed these cultures to be negative.

Inoculated broth from each of the birds were submitted to the Department of Pathobiological Sciences, School of Veterinary Medicine at the University of Wisconsin for radiometric culture, using the Bactec 460 system (Becton Dickinson Diagnostics, 383 Hillen Road, P.O. Box 20086, Towson, MD 21204, USA). The initial procedure was to inoculate Middlebrook 7H12 medium containing C^{14}-labeled palmitate, to which was added amphotericin B (20 μ/ml) and naladixic acid (30 μ/ml), with 0.1 ml of broth. These cultures became heavily contaminated; samples were decontaminated with 1% HPC, filtered, and re-inoculated into Middlebrook 7H12 medium. All successfully rescued cultures resulted in no growth.

Discussion

There is little doubt, based on gross and histopathologic findings, that mycobacteria caused disease in the male turaco. Possible reasons for failure to culture any mycobacteria include: 1) samples for culture may have contained non-viable organisms; 2) the organism is an uncommon serotype of *M. avium* with unknown growth requirements; or 3) the organism is some other fastidious mycobacterial species.

At the time of this writing, no further clinical cases of disease resembling mycobacteriosis have been discovered in the specimens housed in the Rain Forest exhibit at the Zoo. As a precaution, most birds returning to the exhibit after renovation, as well as all new arrivals, were examined and samples collected for complete blood counts and acid-fast staining of feces. Hematologic findings were within normal limits, and acid-fast organisms were observed in only one bird, a Bali mynah, which had been previously housed in the exhibit. Mycobacterial culture of feces collected 3 days later resulted in no growth, and bi-weekly examinations of fecal samples over the following 60 days revealed no acid-fast organisms.

Mycobacterium spp. are being isolated with increasing frequency in the laboratory. The recent rise in the incidence of tuberculosis in humans is caused by organisms other than *M. tuberculosis*, primarily the *M. avium-intracellulare* complex.[2] Disease in birds is primarily caused by *M. avium* complex, and musophagids (turacos, go-away birds, and plantain-eaters) are particularly susceptible to infection.[1] Mycobacteria other than tubercle bacilli (MOTT) are being recognized with increased frequency in captive wild animals.[4]

Conclusions

Although a presumed *Mycobacterium* spp. caused disease in one bird at Topeka Zoo, exhaustive testing indicated the presence of an organism not typical of the more common serotypes of *M. avium*. New technologies, including radiometric culture, amplification by polymerase chain reaction (PCR), and nucleic acid probes for rapid identification have contributed greatly to the understanding of mycobacteriosis in humans. The application of these techniques in the diagnosis of animal diseases is well underway, and is rapidly becoming an important tool in the control of infectious disease in zoological collections.

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TREATMENT OF AVIAN TUBERCULOSIS IN A WHOOPING CRANE (*Grus americana*)

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Introduction

This report describes the treatment and ongoing evaluation of a whooping crane diagnosed with avian tuberculosis by biopsy and culture of *Mycobacterium avium* from a granulomatous mass protruding from the cloaca. The debilitated bird was initially given supportive care including fluids, force-feeding, and nutritional supplements. Once stabilized it was given tuberculocidal drugs for one year (rifampin at 45 mg/kg, and ethambutol at 30 mg/kg) and two doses of an experimental immunostimulant (*M. vaccae* antigen). The bird's condition returned to normal during the course of treatment and the tubercular process remains in apparent remission at 20 months post-entry.

Treatment of tuberculosis is seldom attempted in avian species because of the uncertainty of gaining a curative outcome instead of repression of disease with later recrudescence. The long-time interval for assessing outcome of treatment and the potential for further disease transmission discourages efforts to treat valuable or endangered specimens which may otherwise justify special consideration. The natural occurrence of avian tuberculosis in this whooping crane provided an opportunity to test the potential for successful treatment.

Case Report

A debilitated adult male whooping crane (leg band 559-42784) judged to be unable to continue its southward fall migration was captured at night at the Monte Vista National Wildlife Refuge, Colorado, and transported to the Rio Grande Zoo on November 5, 1992, by U.S. Fish and Wildlife Service biologists. The bird was recumbent at initial presentation yet required anesthesia (isoflurane by mask, Aerrane, Anaquest) to allow examination without excessive struggle. It weighed 5.78 kg and was thin with a prominent keel. There was a large, easily palpable mid-abdominal mass thought to be associated with the ventriculus and a cloacal prolapse of long standing. A firm 2-cm tissue mass was associated with the ventral wall of prolapsed rectum. Heavy urate stains covered wing primaries, tail feathers, and legs.

Initial diagnostic tests included blood samples for hematology and serum biochemistry, feces for ova and parasite examinations, whole body radiographs, surgical biopsy and culture of the tissue mass in the wall of the prolapsed cloaca and tuberculin skin test using 0.1 ml avian OT and avian PPD intradermal in featherless skin on top of the head. Treatment consisted of intravenous fluids, steroids, antibiotics and stomach-tube feeding. The prolapse was reduced and held in place with a purse-string suture.

Radiographs confirmed a large mid-celomic soft tissue density causing a bulging abdominal profile and displacing viscera. There was notable splenomegaly. Dissection of the cloacal

mass revealed pockets of brownish inspissated exudate among firm fibrous-like stroma. A wedge biopsy of the tissue when evaluated histologically revealed numerous coalescing granulomas with necrotic centers surrounded by zone of macrophages. An acid-fast stain showed rod-shaped acid-fast bacteria in singles and small aggregates in the centers of many of the granulomas. *Mycobacterium avium* complex was isolated from the biopsy tissue (New Mexico Veterinary Diagnostic Laboratory [NMVDL], organism on file).

Tuberculin skin test sites developed marked induration at 48 hours after injection. Skin fold calliper measurement increased from 2 mm to 5 mm. Biopsy of the sites at 72 hours revealed histologic changes compatible with delayed type hypersensitivity reaction (i.e., infiltrates of macrophages and lymphocytes in the superficial and deeper dermis).

The initial serum biochemistry values were not diagnostic. Tests run were serum protein, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glucose (Reflotron, Boehringer Mannheim). The white blood cell (WBC) count was estimated to be elevated (8-10 WBC/oil immersion field). Feces were negative for ova and parasites.

The bird was tube fed twice daily until it began to self-feed on November 12. Antitubercular treatment was then initiated and continued for one year. Rifampin (Rifadin, Marion Merrell Dow, Inc.) and ethambutol (Myambutol, Lederle) were each dosed at 30 mg/kg daily. The measured portion of powder and tablet were loaded into gelatin capsules and placed into the body cavity of a mouse or herring, which the bird ate. Later (on December 10) the dosage of rifampin was increased to 45 mg/kg once daily for the remainder of the year of treatment. Isoniazid (Isoniazid, USP, Rugby Laboratories) was unsuccessfully added as a third antitubercular drug at a dose of 30 mg/kg SID on two occasions early in the course of treatment. On both attempts the bird became anorectic within three days and isoniazid was discontinued.

An experimental immunotherapeutic agent, *Mycobacterium vaccae* vaccine was added to the therapeutic regimen in mid-January, 1993, (Provided by Dr. Ruth Cromie, The Wildfowl and Wetlands Trust, Slimbridge, Gloucestersire GL2 7BT, United Kingdom). Two 0.05 ml doses of the antigen were given intradermally in the thick skin on top of the head at an eight-week interval. A blood lead level assessed in mid-January was within normal limits at 0.07 mcg/gm (graphite furnace atomic absorption, NMVDL). The purse-string suture at the cloaca was removed when pericloacal swelling had regressed sufficiently.

Patient monitoring including physical exams, radiography, hematology, serum biochemistry and feces collection for AFB stain and mycobacterial culture (NMVDL) has been completed at monthly or quarterly intervals throughout the course of treatment and continues to the present. Antitubercular drugs were discontinued on November 15, 1993. Body weight, hematology and serum biochemistry values during the entire period of captivity to date are given in tables 1 and 2.

Radiographically, the midabdominal mass and spleen were greatly reduced in size by March 1993, four months after beginning antitubercular drugs. At this time the cloacal mass was

still palpable but estimated to be less than one centimeter. All systems were normal including radiographs and cloacal palpation (mass no longer palpable) by August 1993, nine months after starting treatment. Feces have been collected (three day collection per sample) for AFB stain and mycobacterial culture on a regular basis (dates include: Nov. 1992; Jan., Mar., Aug., Nov., Dec. 1993; and monthly in 1994). *M. fortuitum* was isolated from one sample. All other samples have been negative although four of these were overgrown by contaminant bacteria.

Monitoring of the patient will continue through at least the end of 1994. From November 1993 to present it has experienced weight loss of 0.34 kg (5% body weight). The significance of this trend will be important to follow since it may indicate an early sign of recrudescence, although other factors may be responsible.

Table 1: Whooping Crane Body Weight, Hematology and Serum Biochemistry Values Monitored During and After Treatment for Avian Tuberculosis.[a]

Date	Body Weight (kg)	WBC (#/µl)	PCV (%)	SP (gm/dl)	AST (U/L)	UA (mg/dl)
11-05-92	5.78	8-10/OIF[b]	-	-	-	-
12-16-92	-	32,787	44	5.8	455	<2
01-15-93	6.13	18,000	46	5.0	393	3.92
03-09-93	5.33	14,513	43	3.6	342	4.44
08-06-93	5.78	6,206	42	4.0	412	6.47
11-09-93	6.24	12,042	44	4.8	-	-
02-25-94	6.13	12,445	45	4.2	-	-
06-10-94	5.90	10,304	48	4.0	310	3.59

[a] All tests performed by Rio Grande Zoo veterinary staff. Abbreviations: WBC = white blood cell count (by Eosinophil Unopette Method); PCV = packet cell volume; SP = serum protein (by refractometer); AST = aspartate aminotransferase (Reflotron, Boehringer mannheim); UA = uric acid (Reflotron).
[b] WBC estimate from blood smear (#/oil immersion field)

Table 2: Whooping Crane Differential White Blood Counts During and After Treatment for Avian Tuberculosis.[a]

Date	WBC #/µl	Het % (#/µl)	Lym % (#/µl)	Mono % (#/µl)	Eos % (#/µl)	Baso % (#/µl)
12-16-92	32,787	63 (20,656)	26 (8,525)	10 (3,278)	1 (333)	0 (0)
01-15-93	18,000	59 (10,620)	34 (6,120)	0 (0)	7 (1,260)	0 (0)
03-09-93	14,513	63 (9,144)	26 (3,773)	9 (1,306)	2 (290)	0 (0)
08-06-93	6,206	57 (3,537)	41 (2,544)	2 (125)	0 (0)	0 (0)
11-09-93	12,042	73 (8,790)	20 (2,408)	4 (482)	3 (362)	0 (0)
02-25-94	12,445	65 (8,090)	31 (3,859)	2 (248)	2 (248)	0 (0)
06-10-94	10,304	54 (5,564)	44 (4,534)	1 (103)	1 (103)	0 (0)

[a] All tests by Rio Grande Zoo veterinary staff. Abbreviations: WBC = white blood cell count (by Eosinophil Unopette Method); Het = heterophil; Lym = lymphocyte; Mono = monocyte; Eos = eosinophil; Baso = basophil.

MYCOBACTERIUM TUBERCULOSIS IN A BLACK RHINOCEROS (*Diceros bicornis*)

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A female, 31 year old black rhinoceros (*Diceros bicornis*) was found to be positive on tuberculin skin test using 0.1 ml PPD bovis in October, 1992. The test was given in the eyelid and caudal fold and both sites were indurated and erythematous at 24, 48 and 72 hr. A blood sample was submitted and the ELISA test was positive. Gastric lavage was performed and culture results were negative.

Previous medical records for this animal indicated treatment with isoniazid for 1 year (1981) following a positive tuberculin test in an exhibit mate. No confirmation of mycobacterial infection was ever made. No dosage or indication of compliance was given. The rhinoceros was skin tested in 1989 using PPD bovis and was negative.

Due to positive tuberculin skin tests in an Asian elephant (*Elephas maximus*) housed in the building with this rhinoceros, 2 more gastric lavages were performed in January 1993. *Mycobacterium tuberculosis* was cultured and confirmed. The rhinoceros was started on 7 g p.o. isoniazid (INH) daily. Rifampin (RIF) was added to the regimen at 11 g daily. Sensitivity results indicated resistance to INH at 0.2 μg/ml, sensitivity at 1.0 μg/ml. In humans, if an INH resistant strain of *M. tuberculosis* is encountered, pyrazinamide (PZA) and ethambutol (EMB) are usually added to the RIF, or streptomycin is used. Initially the cost of PZA and EMB was prohibitive and it was felt that multiple injections of streptomycin were not feasible. Therefor, the INH dose was increased to 14 g in an attempt to raise serum levels to the organism's sensitive range.

In August 1993 (3 months into therapy; 2 months at the higher INH dose), another gastric lavage was performed and was culture positive. INH serum levels were 2.25 μg/ml and RIF was undetectable. In October, PZA (32 g) and EMB (26 g) therapy was initiated in addition to INH (14 g) and RIF (11 g). Sensitivity results showed the organism had developed total INH resistance, so INH was discontinued in November 1993. Gastric lavage results were negative in November and serum levels were as follows: EMB = 5.34 μg/ml; PZA = 5.32 μg/ml; RIF = 0.46 μg/ml.

Since therapeutic levels in rhinoceros are unknown, adequate human serum levels were used as guidelines: EMB 2-6 μg/ml, PZA 20-60 μg/ml, RIF 8-16 μg/ml. Doses of PZA and RIF were increased to 64 g and 33 g respectively and EMB was continued at 26 g. Compliance was not a problem.

In January 1994, another gastric lavage was performed and found to be negative. Serum levels were EMB = 0.0 μg/ml, PZA = 6 μg/ml and RIF 0.0 μg/ml. The rhinoceros died suddenly in March 1994. Necropsy results showed pulmonary tuberculosis, pulmonary

Abscesses with interlesional fungi (Aspergillus-like) and metastatic carcinoma / adenocarcinoma. No liver pathology attributable to the anti-tuberculosis therapy was found. Despite the severity of lesions found on necropsy, this animal was asymptomatic until approximately 1 hr prior to death. At that time she was lethargic, developed dyspnea and died.

VETERINARY ISSUES IN AQUATIC EXHIBIT DESIGN

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Introduction

Aquatic exhibits challenge the design and construction capabilities of even the most advanced zoo. The special considerations involved in containing, displaying and managing water in an exhibit add to the already complex issues of animal husbandry. Because of the relative lack of experience with aquatic exhibits in most zoos, outside consultants and specialized engineers are often heavily relied upon in the development of these displays. Unfortunately, these consultants may have little or no experience in day to day husbandry of the display species or in the issues of health management. It is very important that hydraulic engineering considerations not be allowed to dominate the design process to the exclusion or detriment of husbandry and medical considerations. To this end, it behooves the veterinarian to be aware of the major issues of aquatic exhibit design as they relate to display animal and personnel health and to be intimately involved in the exhibit design from conceptualization through completion.

Exhibit Purpose

Not all aquatic exhibits are created equal. An early consideration in the conceptualization phase must be the type of species, number of species and number of individual animals to be maintained. The scope and scale of the project will be determined by these decisions. At this point in the process, the veterinarian must be able to provide input on infectious and non-infectious disease interactions among species being considered for the exhibit as well as the potential impact of materials selection and hydraulic design on the species selected. Special requirements for restraint, isolation and delivery of health care should be identified during the conceptualization period, prior to solidification of the species list. This approach is more often followed in large expensive exhibit design projects for marine mammals where species specialized exhibit design is more accepted. Unfortunately in smaller projects for fish, small amphibians, reptiles or even smaller aquatic mammals, flexibility and interchangeability can be over emphasized, to the detriment of providing optimal conditions for any species.

Hydraulic Considerations

This complex area includes consideration of the flow tolerances of the species to be exhibited. Can they tolerate rapidly surging water, or do they require quiet still water. Obviously this later condition in often in direct conflict with the desire to meet cleanliness standards by rapidly turning over the exhibit water through filtration. Beware of over emphasizing turnover and ignoring species related water flow requirements. High turn over systems can stress animals to the point of exhaustion. Still water species of fish may be unable to eat enough food to restore their energy losses from struggling against a current

if they are not adapted to that environment. They may experience physical damage to delicate fins and even to epidermal barrier layers. You may observe poor growth, susceptibility to diseases and aberrant behavior in animals subjected to excessive flow. On the other hand, turn over is an important consideration in reducing bacterial loading and algal buildup in aquatic exhibits. Animals adapted to relatively pristine, fast moving water can be very susceptible to water quality problems in relatively stagnant exhibits.

Materials Selections

Glass affords excellent visibility and is relatively chemically inert, harder and less easily scratched during maintenance than acrylic plastics. It is generally less expensive than high quality clear plastics, but is more breakable and difficult to drill for some plumbing applications. Plastics cover a wide range of materials. Clear plastics, used as glass substitutes are usually highly specialized acrylics. Acrylic panes can be broken, but withstand more force than glass.

Opaque plastics are also used as structural components of exhibits. New or unknown materials should be tested for toxicity with a bioassay before they are used in a fish or amphibian exhibit. Materials graded as acceptable for foodstuffs are usually acceptable. Avoid recycled plastics. Fiberglass has the highest tension loading capacity of the plastics, is relatively inexpensive, and probably the most commonly used structural plastic in aquatic exhibit construction. Newly constructed tanks incorporating fiberglass should be treated carefully to rid the system of polymerizing agent and trapped metals in the fiberglass resin. This can be accomplished by alternately running the filled system for a day with freshwater of pH 3.0 or lower, followed by freshwater of pH 11 or higher, and finally, fresh water at pH 3.0, discarding the water after each pH shift. A final leeching with salt water for an additional day should be done if the tank is destined to be a marine display. The entire treatment process is facilitated by the use of warm water (37 to 40 C). A bioassay is still advisable before introducing exhibit animals.

Vinyl often finds its way into inexpensive makeshift holding facilities. It is not durable, easily damaged, and can have large residuals of toxic plasticizer and heavy metals trapped in the polymerization process. These leach out into the water. Dioctyl phthalate is a common contaminant, and although 10 days of soaking and etching is recommended for its removal,[2] it makes a poor choice in any fish system. High density linear polyethylene and polypropylene can be stripped with the same protocol described for fiberglass. Polyvinyl chloride generally is not used in tank construction, but in the construction of the plumbing systems that operate displays. Polyvinyl chloride is basically inert to salt water, however, it comes in several types or schedules. These have different properties. High-impact or unplasticized polyvinyl chloride is most commonly used for plumbing applications, but can contain trace amounts of metals, particularly lead, which can be leached in acid water systems.[3] Acrylonitrile butadiene styrene pipes are less likely to cause subtle toxicity problems and are recommended in construction of small systems for delicate species. Use only high-grade silicone for sealing tanks and systems. Low-grade, silicone caulks contain heavy metals, cyanide, and organic toxins, which can kill fish. Plastics require solvent sealing

and cannot be properly sealed with silicone sealants. It is very important to remove the solvents after construction with these materials.

Concrete is durable, inexpensive and easily formed. It has a strong resistance to compression, but lacks tensile strength and shear resistance, which must be provided by metal reinforcement buried in the concrete. Concrete is very alkaline and contains small amounts of foreign materials, including chromates, which can leach out slowly over a long period after exhibit construction. Concrete structures should be thoroughly washed or leached with dilute muriatic acid, and coated with several coats of sodium silicate or other sealant before being used. A soaking period of several weeks, adjusting pH and discarding water, is recommended.

Wood is often used in exhibit design to create "furniture" and for aesthetic accents. Only well-dried, seasoned heart wood should be used. Keep in mind that many wood preservatives are toxic.[4] Metals and water do not get along. Corroded metals lose structural integrity and strength, and the metal being lost through corrosion can be toxic. Even stainless steel will corrode in water, particularly salt water. Where strength and maximal corrosion resistance are needed, titanium is preferred over stainless steel. Other common metals used in exhibit construction include galvanized fittings and brass or copper. Unfortunately, enough zinc can dissolve from galvanized fittings to be lethal to fish within very short periods, even when calcium protection is in effect in seawater. Bronze can be a fatal source of zinc and copper for fishes.

Access to Animals and Isolation

Access to animals is an important consideration in aquatic exhibits. Failure to design for this need will result in the inability to provide even basic medical care. This issue intertwines with human ergonomic considerations. Basically, it should be possible to place hands on an individual specimen within 8 hours, using manpower appropriate to the species value. In marine and aquatic mammal facilities this requires design and implementation of catching devices and areas which can be operated routinely. This need is equally important for fish, reptile and amphibian exhibits. Provision for isolation facilities should be made at initial exhibit development. The rather common assumption that the animal will never get sick has been disproven time and time again.

Human Ergonomic Considerations

One of the largest ergonomic problems in aquatic exhibit design is provision of adequate overhead clearance. A net should reach the bottom of a tank with enough of the handle exposed to allow easy manipulation. That means the minimum overhead clearance for any tank should exceed the depth of the tank if nets are going to be used without impediment. Net access to fish tanks can be enhanced by having tops that are completely removable, and lighting systems that are also easily moved to allow open access to the tank. Although mammal exhibits are often drained for animal capture and manipulation, this does not eliminate the need for overhead space for hoists and cranes etc.

Make the controls and monitors for the environmental systems of the exhibit easily accessible. This makes it much more likely that they will be examined regularly and adjusted when necessary. If a keeper has to peer under a dark shelf to examine a pump's bearings, chances are it will not happen.

Spare no effort to provide good visibility into the exhibits from the back. Do not attempt to save money on the viewing ports of your exhibits.

Avoid narrow bridges, ladders and precarious perches whenever possible.

Finally, no matter how difficult it is, be sure to design your systems so they can be drained completely without having to resort to baling buckets, mops, or sponges.

Safety

The issue of safety in aquatic exhibit design affects both display animals and staff. The major safety hazard of aquatic exhibits is electrocution. It is very important to design your facility to minimize the chance of an electrical accident. Use ground fault breakers in the receptacles used to provide power to the room. Make sure all grounds are properly wired. Train your employees in the safe use of electricity near water. Make sure the floors and drains of the facility do not add to the hazard by retaining standing water. Use nets with nonconducting handles. Avoid any form of exposed wiring. Falls on wet slippery floors are also a major hazard. Design for good drainage and sure traction on all walking surfaces of the exhibit.

Accidental loss of power or equipment failure that resulted in the draining of the water from the exhibit would be devastating for many display animals. Design the plumbing approach so adequate water remains in the tank to allow the animals to survive if power fails. Use gravity to your best advantage. If a pump fails, no siphons should allow the tank to continue to drain without return flow.

A critical safety feature for the display of aquatic animals is the availability of reserve water. Reserve water should be thermally tempered and preferably designed to allow delivery of water of appropriate pH and hardness. The reserve should minimally allow an immediate 50 percent water change of the entire exhibit in cases of emergency and it should be possible to replenish the reserve within 24 hours.

Water Filtration Systems

There are three major types of filtration: mechanical, biological, and chemical, each with their own purpose and application. These need to be applied with some knowledge of the species being displayed.

Mechanical filtration, sometimes referred to as primary filtration, removes suspended particles from water by passing it through a fine medium. The mesh of the medium used

depends on the size of the particulates to be removed and the amount of resistance that can be placed on the pump. More pumping effort is required to move water through finer mesh. Very finely meshed filters occlude quickly. When occlusion occurs, the head differential across the filter becomes very high and the filter must be backwashed to avoid cracking the filter medium or damaging the pumps. Mechanical filter design involves compromises between the size of particles cleared (degree of polishing) and the need to achieve reasonable turnover times while keeping backwash labor at reasonable limits. A uniform medium with an effective size of 0.3 mm will remove about 95% of particles down to 6 microns in diameter.[6] A coarser medium, 0.45 mm in diameter, will retain 15 micron particles.[6] For complete retention of all bacteria, a mechanical filter must exclude particles down to 0.2 microns in diameter. This is impractical in most systems and infectious disease control through mechanical filtration should not be a goal. Mechanical filters should have easily changeable media, otherwise, the labor costs associated with disinfection will result in reluctance to disinfect in the face of disease outbreaks. This issue will be more important in the long run of an exhibit than very small exclusion sizes.

Chemical filtration covers a wide range of methods which include ion exchange, both specific (resins) and nonspecific (activated carbon), and oxidative systems (ozone). Foam fractionation by protein modification and ultraviolet filtration, can also be considered methods of chemical filtration, since they rely on basic modifications of chemical structure to remove contaminants. Activated carbon filters are the most commonly applied form of chemical filtration. A finite number of binding sites are available, depending upon the surface area of the carbon particles. These are capable of binding cations and anions with binding strengths that vary with the ion being bound. These ions undergo constant exchange with ions in the water at a rate inversely proportional to their binding strength to the carbon sites. Ions with strong binding affinity are effectively removed from the water. This works well until all of the binding sites are saturated and competitive binding between the more toxic compounds reaches a point where not all toxic ions can be bound simultaneously. At this point, some ions must be released by mass action.[5] The filter also fails when a very strongly binding ion is introduced into the system that displaces more weakly bound toxic compounds from the binding sites.[1] In either of these cases, the filter designed to remove toxic compounds becomes a source of the toxin.

In multiple exhibits, individual filtration systems are the best plan, reducing the risk of cross contamination between exhibits. Disinfection of the complex surface areas involved in filtration media is a false economy. The potential for transmitting disease from one patient to the next through incompletely disinfected chemical filtration media is high. For larger facilities, it may be practical to build a contact chamber for delivery of ozone or ultraviolet filtration to various exhibits in series. If you intend to invest in this technology, it is best to arrange for a knowledgeable consultant. It will be critical that the contact chamber provide complete disinfection before returning the water to the exhibits, or disease transmission will be a major problem. Systems adequate to kill bacteria may not be adequate to kill protozoa, fungi or metazoa. It is best to realize that this will be a calculated risk. Even ozone systems are not 100% effective against all disease causing organisms. When you chain filtration systems you must do so knowing the risk of cross contamination.

Biological filtration in aquatic exhibits refers to the biological fixation of nitrogenous wastes into less toxic compounds by bacteria. Other forms of biological filtration, including the concentration of metals and certain toxic organics in algal scrubbers, are also employed. A traditional biological filter must maintain enough heterotrophic bacterial colonies to process the solid nitrogenous wastes into soluble wastes such as ammonia. The ammonia from this process and that directly excreted by fish is then converted by autotrophic bacteria to nitrite (by *Nitrosomonas* spp) and then to nitrite (by *Nitrobacter* spp). The surface area for bacterial growth is usually the limiting factor in biological filters, along with the ability to circulate the waste-laden water into contact with the bacteria responsible for nitrogen fixation. Numerous augmenting systems to increase the efficiency of waste fixation have been developed, primarily to provide additional surface area for bacterial growth and easier water contact. Biofilter dynamics need to provide adequate turnover of the tank water to avoid accumulation of toxic wastes, but at the same time must provide enough contact time with waste-laden water to allow effective waste metabolism by the bacteria. An imbalance results in an ineffective biofilter. The bacteria working on the biofilters are very susceptible to environmental changes, and a large drop in fixation efficiency can occur with minor environmental fluctuations.

Air Systems

Do not overlook the importance of a safe air delivery system when designing aquatic exhibits. Any air system should have a backup wired to an emergency power generator. Locate your air intake where there will be no risk of exhaust fumes from parking facilities, pesticide spraying, use of solvents and cleaning solution, etc. It may be advisable to maintain carbon filtration on your air intake.

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SURGICAL MANAGEMENT OF LIPID KERATOPATHY IN GREEN MORAY EELS (*Gymnothorax funebris*)

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In December of 1993, an adult, 22.5 kg, eight-year captive Green Moray Eel of undetermined sex was presented for bilateral corneal opacities. This represented the third recorded case of lipid keratopathy due to cholesterol ester deposition in Green Moray Eels at the Shedd Aquarium as confirmed by both histological and biochemical evaluation. Due to the presence of bilateral keratopathies, visual impairment was apparent in that all three of the affected animals were observed having difficulty in navigating their enclosures. As a result, a bilateral keratoplasty procedure was performed in all three cases. The following report describes this surgical procedure as well as the anesthetic protocol that was utilized in the most recent case.

Induction of anesthesia was accomplished with tricaine methanesulfonate (MS-222) as an immersion bath at a concentration of 100 ppm. In order to maintain a safe, surgical anesthetic plane, the branchial epithelium was continuously irrigated with an aqueous solution of MS-222 delivered by a customized, water-circulating, flow-through anesthetic machine. The concentration of MS-222 during the procedure was kept between 50-100 ppm. The heart rate was continuously monitored with a doppler flowmeter (Model 810-A, Parks Medical Electronics, Inc., Aloha, OR) using a hand-held pencil probe. The anesthetic depth could then be adjusted accordingly. During the procedure, the eel was placed in lateral recumbency on wet polyurethane foam sheeting and draped with saltwater-soaked towels to prevent cutaneous dessication.

Analogous to the structure found in the eye of squamate reptiles, the moray eel has a transparent brille or spectacle overlying the cornea. Separating the spectacle and the cornea is a subspectacular space that is lined with conjunctival epithelium.[1] Examination of the eyes of the present case under an operating microscope revealed scarring and dystrophic mineralization of the epithelial (outer) surface of the spectacle. This was believed to be a result of the visual deficit and subsequent trauma during attempts at tank exploration. Care was taken to keep the ocular structures periodically moistened via irrigation with 0.9% NaCl during the surgical procedure. The spectacle was incised circumferentially along the caudal perilimbal margin (1mm from the limbus) for a distance of about 180 degrees with a #15 B.P. scalpel blade (Fig. 1). The surgically incised spectacle was then reflected rostrally exposing the corneal surface (Fig. 2). Lipid deposits were observed on both the epithelial surface of the cornea and the posterior epithelial (inner) surface of the spectacle. The deposits were located throughout the loosely arranged stromal fibers. The diseased tissue was slowly dissected until clear cornea was all that remained. This was done utilizing Colebri forceps and Castroviejo corneal scissors (Fig. 3). Any cholesterol deposits noted on

the underside of the reflected spectacle were similarly removed. The spectacle was then replaced and sutured back into normal position using 7-0 Dexon (polyglycolate) in a simple continuous pattern (Fig. 4). The dystrophic areas on the epithelial surface of the spectacle were then debrided in the same manner. The contralateral eye was treated in a similar fashion two months later. The keratoplasties were thusly staged due to the length of the procedure (approximately 60 minutes) so as to minimize stress, trauma and anesthetic risk. Healing required approximately three weeks for each eye. At four months post-surgery, there has been no observable reoccurence of corneal opacification.

The etiology of this condition in Green Morays remains speculative. Similar lesions in anuran amphibians may be related to dietary fat or, since the preponderance of affected animals are females, excessive lipid mobilization associated with vitellogenesis.[2,3] In order to elucidate the pathogenesis of this syndrome in Green Morays, future efforts will be initially directed at diet analysis, comparative blood chemistry analyses of normal vs. affected animals (cholesterol, triglycerides, lipoproteins), feeding trials and gender determination.

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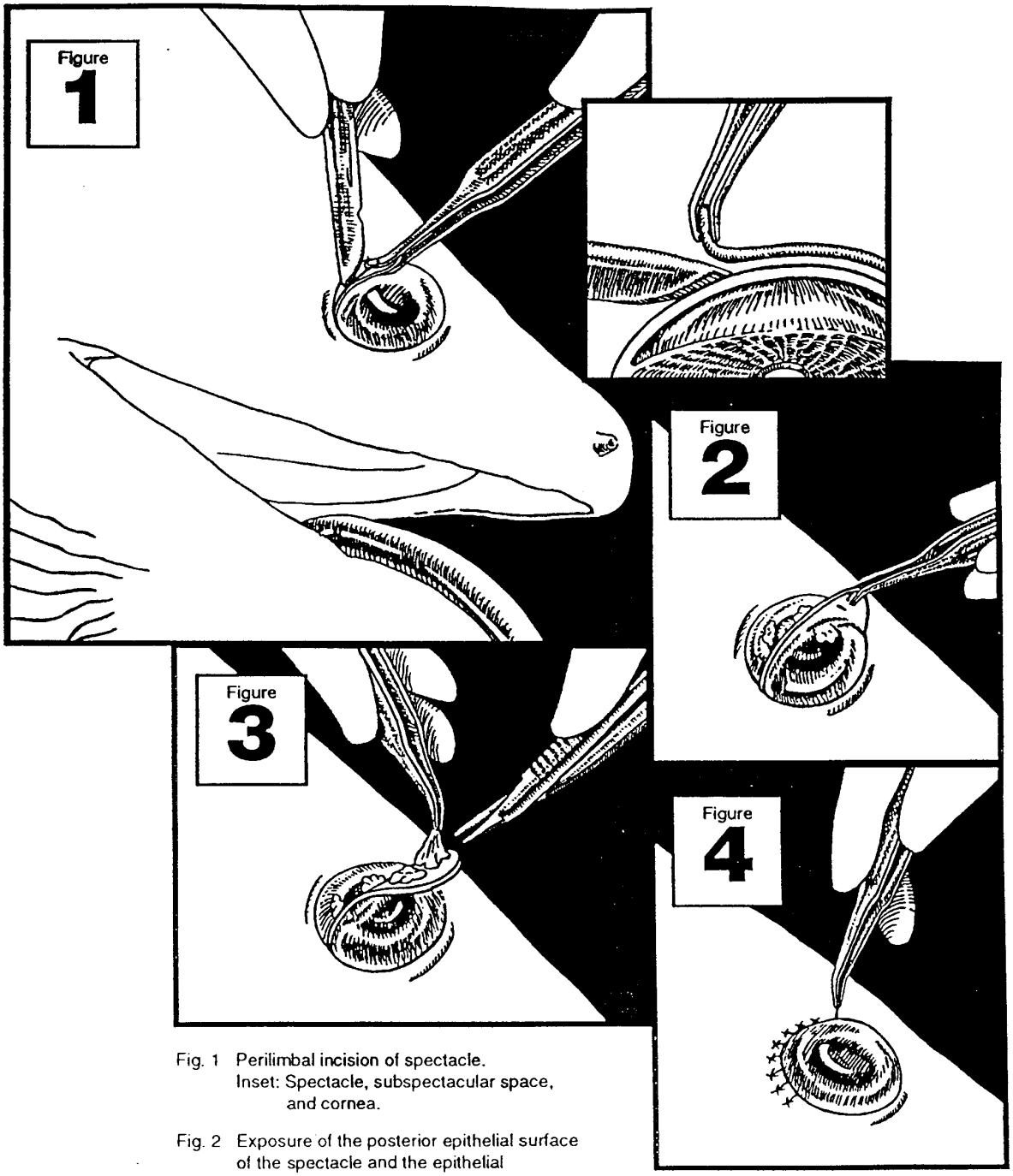


Fig. 1 Perilimbal incision of spectacle.
Inset: Spectacle, subspectacular space, and cornea.

Fig. 2 Exposure of the posterior epithelial surface of the spectacle and the epithelial corneal surface.

Fig. 3 Excision of cholesterol deposits.

Fig. 4 Replacement and closure of the spectacle.

MEDICAL OBSERVATIONS AND IMPLICATIONS ON "HEALTHY" SEA TURTLES PRIOR TO RELEASE INTO THE WILD

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As is well established, sea turtle populations are diminishing for many reasons including fisheries interactions, loss of habitat and breeding grounds, indiscriminate disposal of waste, and recreational use of the waterways. In response to this crisis, several reactions have arisen including Turtle Ejection Devices, beach preservation, research on natural history, "Head Starting", rehabilitation, and public education.

The success of head start programs has been controversial leading to the discontinuation of many such efforts. Despite the intensity with which these programs operated, little information is available concerning the health of both wild and captive juvenile loggerhead sea turtles. This paper will present several interesting findings in a group of juvenile loggerhead sea turtles which were examined just prior to release. Recommendations for the development of a comprehensive medical program are provided which will hopefully identify individual animals less likely to survive in the wild.

Recently, two loggerhead sea turtles, *Carretta carretta,* were thoroughly examined prior to release. They appeared visually normal, however, serum chemistries showed severe calcium/phosphorous imbalances, and high white cell counts using the Natt-Herrick method.[3] Survey radiographs did not indicate any abnormalities. A comparison of physical parameters, blood work, and radiographs was carried out on loggerhead sea turtles housed at another facility. These animals were clutch-mates to one of the turtles held at NAIB.

Immediately evident was a marked size difference between clutch-mates held at the two institutions. The NAIB turtle weighed 1.8 kg with a straight carapace length of 22.0 cm. Average weight of it's clutch mates was significantly less, 0.344 kg, with an average straight carapace length of 12.96 cm. Radiographs of the smaller turtles indicated bony lesions including bowed femurs, cortical thinning, and healed pathological fractures not seen in the NAIB turtles.

Blood was collected from yearling turtle's occipital sinus using a heparinized tuberculin syringe and a 25-gauge needle. This was done by angling the head downward and placing the needle perpendicular to the long axis of the neck to a depth of approximately 1-1.5 cm. The needle was inserted into the area paramidline to the distal portion of the nuchal crest.[2] Blood discrepancies included significant variations in white blood cell counts, lower than expected albumin and calcium levels, and calcium/phosphorous imbalances; presently the only data available for juvenile loggerheads are for turtles weighing 15-125 kg. This is summarized in table 1.[5]

White blood cell counts were performed using two different methods; the eosinophilic unopette method and the Natt-Herrick method.[3] On animals large enough to obtain a

sufficient quantity of blood, significant differences were noted between the two methods. White blood cell counts using the Natt-Herrick technique had a mean value approximately twice that observed using the Eosinophilic Unopette system.[1]

The problems identified upon radiographic and serum chemistry analysis of all of the turtles examined were consistent with chronic nutritional deficiencies. Normal Ca:Phos ratios of young and growing turtles is still controversial. Currently we are studying monthly blood samples of a turtle obtained by NAIB at ninety days of age. Preliminary observations indicate a improvement of an inverse Ca:Phos ratio following a change in diet from "Cut Mix" (krill, fish, shrimp, and squid) to a marine animal gel food listed in table 2.[4] These results appear to be consistent with the those obtained at the other facility.

Based upon our initial findings of grossly "normal" individuals and an increased knowledge of turtle diseases, we would recommend medical screens upon entry, every six months, and at least one month prior to release. This should provide ample time to address any suspected abnormalities. Examination of these animals should include radiographs, CBC, serum chemistries, eye examination, oral exam, palpation, and weight as compared to carapace length. Fecals, direct or by cloacal flush, should be examined for parasites. Cultures for fungi and bacteria should also be included in the exam. Other important observations would be the animal's ability to dive and swim properly.

In conclusion, we suspect that suboptimal medical and husbandry care of juvenile sea turtles results in metabolic and physical changes that are not conducive to their long-term survival in the wild or captivity. Because their abnormalities are not often apparent upon visual examination, the use of routine blood analysis and radiographs are essential to fully determine the health status of the these animals.

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Table 1.
Sea Turtle Plasma Chemistry Normals*

Wild juvenile *Carretta carretta* from Chesapeake Bay, n=75

TEST	MEAN	SD	RANGE
PCV %	29	5	24-34
WBC per cubic ml	3768	2770	1098-6538
T. Protein G/dl	3	1.1	1.9-4.1
Albumin G/dl	1.35	0.19	1.2-1.5
Glucose mg/dl	100	18	82-118
BUN mg/dl	92	13	79-105
Creat mg/dl	0.2	0.1	0.1-0.3
LDH u/L	310	484	0-794
CPK u/L	1680	2043	0-3723
Alk Phos u/L	53	25	28-78
SGOT/AST u/L	285	120	165-405
Na mEq/L	157	4	153-161
Cl mEq/L	112	17	95-129
K mEq/L	3.6	0.5	3.1-4.1
Ca mg/dl	7.7	1.3	6.4-9
P mg/dl	5.9	1.3	4.6-7.4
Mg mg/dl	2.9	0.8	2.1-3.7

* Data provided by Bob George, D.V.M. in conjunction with the Virginia Institute of Marine Science

Table 2.
Sea Turtle Gelatine Diet**

INGREDIENT	WEIGHT (g)	% of Diet
Trout Chow	425	8.00
Fish (various)	565	10.60
Squid (internals removed)	282	5.30
Peeled shrimp	282	5.30
Spinach (fresh or frozen)	142	2.80
Carrots (fresh)	142	2.70
Gelatin (unflavored knox)	450	8.50
Water 2800 ml	2800	53.0
Supplements:		
Sea Tab (4-500 mg tabs)	2	0.04
A.A. 1000 (4-500 mg tabs)	2	0.04
Rep-Cal (200 ml powder)	180	3.40
Spirolina (50 ml powder)	28	0.50

**Modified from the Virginia Institute of Marine Science marine animal gelatin diet.

FIBROMYXOMATOUS GINGIVAL PROLIFERATION IN A SAND TIGER SHARK (*Eugomphodus taurus*)

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Introduction

Entanglement is always a concern when using nets to move or capture sharks. Sharks trapped in nets are at risk of injury or death. Net injuries can result in abrasions and lacerations caused by the netting material; pressure necrosis created by constrictions; and shock associated with excessive struggle to escape the entrapment. Sand tiger sharks have protruding teeth that are constantly exposed and predispose them to net entanglement. Once entangled in a net, sharks often struggle to free themselves and in doing so, may damage the mouth.

History

A 180 kg (396 lb) female Sand tiger shark (*Eugomphodus taurus*) with a total length of 270 cm, fork length of 230cm, and precaudal pit length of 207 cm was presented with a large swelling on the buccal gingiva on the cranial aspect of the palatoquadrate. The shark was maintained in a 660,000 gallon aquarium containing artificial sea water and other sharks and bony fish. The swelling had an insidious onset. It was also noted that the shark was unable to elevate her palatoquadrate.

Diagnostic Plan

The shark was guided into a smaller holding pool and restrained in a nylon stretcher designed for restraining sharks. Blood was collected from the vein just caudal to the dorsal fin for a complete blood cell count and serum chemistry profile. The shark was lowered into a transport box containing 200 gallons of water and 70 ppm tricaine methane sulfonate (Finquel, Argent Chemical Labs, Redmond, WA) as an anesthetic. The shark was induced to the proper anesthetic level in 18 minutes. She was placed upon a padded board for examination of the swelling and radiographic evaluation. The swelling was a firm finger-like mass that measured 6cm by 7cm. One was able to reduce or push the mass to the dorsal aspect of the palatoquadrate. Radiographs of the rostrum in the area of the mass were taken using portable equipment (AMX-4, General Electric Co, Milwaukee, WI). The dorsoventral view measured 13 cm and required 80 KVP and 2.0 MaS. The lateral view measured 17.5 cm and required 90 KVP and 2.0 MaS. An excisional biopsy was taken using a scalpel blade and the tissue was closed using #1 Maxon (Davis + Geck, Inc, Manati, PR) as a simple interrupted suture. A contact smear was made for cytodiagnosis and the tissue was placed in 10% neutral-buffered formalin for histopathology. Recovery from the anesthetic (total anesthesia time was 60 minutes) was unremarkable (full recovery was attained in 30 minutes).

Results

The blood profile was compatible with normal values for this shark and other Sand tiger sharks in the exhibit (based upon blood profiles taken twice a year). The radiographic evaluation indicated normal skeletal images and the mass had a soft tissue density. The cytology revealed numerous erythrocytes and fibroblasts. No evidence of inflammation was observed in the cytologic specimen. The histologic examination of the biopsy revealed a benign proliferative fibrous tissue response (fibromyxomatous proliferation) of unknown etiology. It was then determined that the cause of the fibrous mass may be associated with rupture of the tendinous attachment of the levator muscles (i.e. palatoquadrate levator) to the palatoquadrate because the shark was unable to elevate the palatoquadrate and one could push the mass into a position just above the palatoquadrate suggesting the origin of the tissue.

One month following the diagnostic evaluation of the shark, it was decided to surgically remove the mass. Because of the chronic nature of the lesion and the extensive amount of fibrous tissue, no attempt was made to explore and possibly repair ruptured tendons. The shark was again anesthetized in the same manner as the previous procedure and the mass was surgically removed. The surrounding gingival tissue was closed using #1 Maxon in a simple interrupted suture. Complete recovery occurred in 15 minutes. The sutures were removed three weeks later during a routine physical examination of the sharks in the exhibit. After one year, the mass did not recur and the gingiva was healed. The shark was unable to elevate her palatoquadrate, therefore her upper teeth were constantly held in the down position. This condition did not interfere with the animal's ability to eat.

Discussion

The cause of the fibrous response in this shark most likely was associated with the tearing of the tendinous attachments to the palatoquadrate and the animal's attempt to stabilize the structure. Because Sand tiger sharks have protruding teeth that are constantly exposed, they are predisposed to entrapment of their teeth in nets. This most likely occurred with this shark and during the struggle to free herself, she was strong enough to rupture the large tendons supporting the cartilage (palatoquadrate) that house the upper teeth.

When handling sharks with nets, care should be taken to prevent net entanglement. When a shark becomes entangled in a net, an attempt to free the animal as quickly and calmly as possible should be made to prevent the animal from struggling and causing injury to itself. Also, the shark should be freed from the net without lifting the net and the shark, if possible to minimize injury to the animal.

LASER AS A TREATMENT FOR SQUAMOUS CELL CARCINOMA IN A PACIFIC WHITE-SIDED DOLPHIN

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Lasers have been used extensively in human medicine for the last two decades but only recently have they been utilized in veterinary medicine. A major benefit of lasers in surgery are the wide variety of applications as well as the precision they can achieve in cutting, coagulation and vaporization of tissue. A 16 year old female Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) was diagnosed histopathologically with squamous cell carcinoma of the right upper lip and rostral hard palate. Due to the vascularity and difficulty achieving homeostasis after the biopsy, it was determined that a conventional surgical approach was not the best option. With the assistance of a human health laser surgery team, a neodymium: yttrium-aluminum-garnet (Hd:YAG) laser was tested to determine the best technique for this purpose. The animal was sedated and under a local anesthetic a blunt contact tip was used to ablate the majority of the lesion. The laser provided excellent control of hemostasis and depth of penetration while allowing clear visualization of the surgical field. Although the lesion will require further treatment, the majority of the area treated is completely healed or is covered with a bed of granulation tissue. The positive results achieved are encouraging and could lead to other uses of this technology in aquatic animal medicine.

HANDLING, RESTRAINT, ANESTHESIA AND SURGERY IN THE BLUEFIN TUNA (*Thunnus thynnus*)

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The New England Aquarium is currently engaged in a captive breeding program for bluefin tuna (*Thunnus thynnus*). We have maintained wild caught tuna in captivity for the past two years. These fish were held in a semi-closed 230,000 liter recirculating sea water system and were fed a diet of previously frozen, vitamin supplemented fish. To effectively maintain a species new to captivity it is necessary to develop techniques to be able to safely handle, restrain and anesthetize the animals for routine medical procedures.

To date, the most successful method of capture and subsequent handling of bluefin tuna was accomplished by separating animals from our school with the use of a seine net. The animals were removed from the seine net with the use of a vinyl stretcher and then quickly transferred to an anesthesia induction tank containing a natural sea water solution with 90 ppm tricaine methanesulfonate. When a surgical level of anesthesia (stage III, plane 2) was reached, the animals were transferred to a "wet" surgery table. Oxygenated water from the induction tank was then pumped over the animal's gills at 12-20 liters per minute. Depth of anesthesia was monitored by EKG, noting the heart rate and amplitude of the QRS complex. Nearing the completion of the procedure, anesthetic-free sea water was pumped over the gills until the animals began to respire on their own (stage 2, plane 1-2). The animals were then moved to a recovery tank and monitored closely.

We have performed minor surgical procedures, ultrasonographic examinations and taken serial blood samples on three individuals using the above methods. Although blood can be drawn semi-consistently from the caudal vein, a more reliable location was found just dorsal to the pectoral fin "slot." The artery in this region is generally large enough to place a 22G catheter on tuna greater than 10 kilograms. The ventral aorta is another location we are examining for blood sample collection. Regardless of sampling site used, serial samples were obtained for routine chemistries, blood gas analysis, lactate level determininations and hormone analysis. With further modification of these techniques, the performance of routine medical examinations and procedures will enhance the study and husbandry of these exceptionally delicate animals.

HANDREARING NORTHERN ELEPHANT SEAL PUPS (*Mirounga angustirostris*)

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The Marine Mammal Center (TMMC) in Marin, California, is a rehabilitation center for pinnipeds. The staff has cared for over eight hundred elephant seal pups during the past 18 years. There were 279 pups four months of age or less brought to TMMC for rehabilitation in 1992 and 1993 combined. There was a 60% success rate for rearing these pups and releasing them to the wild.

Pups were put on a special admission protocol which included blood samples, microbiology samples, fecal samples and a daily schedule of fluids and formula via gavage. Pups that weighted less than 38 kg were put on six feedings per day scheduled every four hours. Pups greater than 38 kg were started on five feedings per day. Numerous formulas were developed for the pups but the most successful was a fish, whipping cream and vegetable oil based formula.

The ingredients included a high quality herring (at least 9% fat content), ground through a meat grinder, and placed together in a large blender with the following:

0.45	kg herring paste
200	ml water
300	ml Pedialyte or 5% glucose in lactated ringers
280	mg calcium gluconate
10	ml safflower oil
10	ml lecithin

All ingredients were blended to an even consistency. Contents were poured into a clean container and 400 ml treated whipping cream was added and the container was rocked until the ingredients were mixed. The container was labeled with the date and time prepared and refrigerated. The formula was warmed to room temperature before feeding, and excess formula was discarded after 24 hours.

The whipping cream was treated with 0.75 ml of the enzyme lactase added to one quart of whipping cream. The container was inverted several times during a 24 hour period. This gave the enzyme time to break down the lactose in the shipping cream. Once the whipping cream has been treated, it can be used in the formula.

The pups were supplemented with vitamins each day. These were added to the formula in the blender, or crushed and added to the formula at the time of feeding. Each pup received:

1	multiple vitamin	BID
500	mg vitamin C	BID
200	mg vitamin B1	TID
400	IU vitamin E	SID

The pups were housed in fresh water pools and were supplemented with 2.2 grams of salt three times a day.

Pups may simply be separated from their mothers by natural events and require only a special feeding regimen of formula and fish to gain weight and be released back to the wild. Criteria for release are: the pup must be eating fish competitively with other pups; a blood sample with parameters that fall within established normal limits within one month of release; fecal sample negative for parasites; adequate body weight; and determined clinically normal and free of disease by the veterinary staff.

Numerous pups are admitted with medical problems. The most common medical, disease, and parasite problems in elephant seal pups are dehydration, hypoglycemia, emaciation, gastrointestinal problems causing diarrhea and vomiting, salmonellosis, hepatitis, and parasitic infestations of *Otostrongylus circumlitis*. The leading cause of death in elephant seal pups in the past two years has been due to infestations of *Otostrongylus*.

Specific protocols have been developed for all aspects of rearing elephant seal pups. In spite of the large numbers of pups admitted to TMMC in recent years, the success rate has remained high.

CARFENTANIL, KETAMINE, XYLAZINE COMBINATION (CKX) FOR IMMOBILIZATION OF EXOTIC UNGULATES: CLINICAL EXPERIENCES IN BONGO (*Tragelaphus euryceros*) AND MOUNTAIN TAPIR (*Tapirus pinchaque*)

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Introduction

Anesthetic combinations are used in veterinary medicine to achieve adequate sedation, muscle relaxation, analgesia, and reduce the dose of each drug.[1] In many cases, patients are premedicated before general anesthesia is induced. Drug combinations, usually a sedative/muscle relaxant and general anesthetic, are widely used in exotic animals as well. This is especially valuable in exotic hoofstock where a rapid smooth induction is important to minimize the risks due to flight response and trauma. This report describes the use of a three-drug combination in two species of exotic ungulates at the Los Angeles Zoo. The "cocktail" consists of a narcotic opioid (carfentanil citrate), a dissociative anesthetic (ketamine), and an alpha-2 agonist (xylazine). Although this mixture has been used in other exotic hoofstock, it has not been reported in bongo antelope and mountain tapirs.[2] In addition, the supplemental use of a new ultrashort-acting intravenous anesthetic (propofol) in mountain tapirs is described.

Materials and Methods

The immobilizations described in this paper were performed at the Los Angeles Zoo between March 1991 and April 1994. Animals were confined to holding pens or in stalls for administration of anesthetic drugs using a pneumatic dart system (Telinject USA, Inc.). Body weights were estimated before immobilization, and actual weights were obtained when possible. Drug doses were selected based on species, age/size, and temperament, rather than calculated by body weight. All animals were fasted for 24-48 hours prior to immobilization.

Bongo antelope were immobilized for a variety of procedures including elective examination and diagnostic testing, hooftrims, dystocia, and other reproductive surgery. Twelve immobilizations on 9 animals were performed using CKX. Animals ranged in age from 1 to 11 years of age and included 5 males and 7 females. Six other anesthetic procedures using acepromazine-carfentanil (2 animals) or carfentanil alone were used for comparison (3 males, 3 females; age 1-7 years).

Mountain tapirs were immobilized for footwork, gastrointestinal endoscopy, and reproductive surgery on 6 occasions. One female and three males were immobilized using CKX. This included one juvenile male that was immobilized 3 times between the ages of 9 and 14 months.

Immobilization data recorded during the procedure included time from anesthetic drug administration to clinical signs of sedation/ataxia (induction time), time from drug administration to recumbency (time to recumbency), time from recumbency to standing (immobilization time), and time from administration of reversal drugs to standing (reversal time). Anesthetic monitoring was performed using TPR, thoracic auscultation, evaluation of oral mucous membranes, peripheral pulses, muscle relaxation, salivation, and pulse oximetry. The overall anesthetic procedure was assigned a subjective score between 1 (poor) and 4 (excellent) which included amount of struggling during induction and recovery, stability of vital signs and ability to perform required procedures.

The anesthetic cocktail CKX consisted of carfentanil citrate (Wildnil, Wildlife Pharmaceuticals, Inc.), ketamine hydrochloride (Ketaset, Aveco Co., Inc.), and xylazine (Rompun, Haver). Drugs were mixed in a pneumatic dart immediately prior to administration. Two adult bongo antelope were premedicated with 30 mg acepromazine IM (Promace, Fort Dodge Labs., Inc.) prior to induction with carfentanil, and these procedures were used for comparison to CKX procedures. Animals which required supplemental anesthesia were given isoflurane (Aerrane, Anaquest). Propofol (Diprivan, Stuart Pharmaceuticals) was used in 4 tapir procedures to provide additional muscle relaxation at a dosage of 0.2-0.48 mg/kg b.w. IV.

Anesthetic antagonists were administered at the completion of each immobilization. Yohimbine (Yobine, Lloyd Laboratories) was used to reverse the effects of xylazine at a dosage of 0.1-0.2 mg/kg b.w. IV or IM. Carfentanil was antagonized with naltrexone (Naltrexone, Wildlife Pharmaceuticals, Inc.) or naloxone (Naloxone, Wildlife Pharmaceuticals, Inc.) in earlier bongo procedures, at a 100:1 ratio (mg antagonist:carfentanil) given 1/4 IV and 3/4 SQ.

Results

Average effective doses for bongo antelope immobilized with CKX are shown in Table 1. These data include individuals that may have required larger or smaller doses than those shown in the table due to temperment and physical condition. Immobilization data for bongo procedures using CKX or carfentanil with or without acepromazine is compared in Table 2. Although the time to induction, recumbency and reversal were similar, the overall quality of the anesthetic procedure was judged to be significantly improved using CKX as compared to carfentanil alone. In addition, this was achieved with a one-third decrease in the carfentanil dose. Muscle relaxation and analgesia were also improved in the bongo in which CKX was used, thus allowing more rapid completion of the required procedures (T immobilization average: 33.6 mins vs 61.8 mins). Four of the six animals in which carfentanil was used as the sole induction agent required supplemental IV ketamine as well as isoflurane to achieve an adequate plane of anesthesia.

Since bongo antelope were immobilized for preshipment exams, medical diagnosis, and reproductive procedures, most animals were not significantly compromised at the time of immobilization. Neither mortalities nor significant post-anesthetic complications occurred. Reversal of anesthesia was achieved with administration of naltrexone in all of the CKX procedures and naloxone or naltrexone in the carfentanil alone procedures. Time from administration of narcotic antagonist to standing was not significantly different between the two groups (5.3 mins vs 4.5 mins). No signs of renarcotization were observed in either group.

CKX was used to immobilize 3 adult and 1 juvenile mountain tapirs. Anesthetic data are shown in Table 3. Subjective quality of anesthesia was judged to be good-excellent. Induction and recovery were smooth and without complications. Due to the length of procedures performed (gastrointestinal endoscopy and surgery), anesthesia was maintained with isoflurane and additional muscle relaxation provided by intravenous administration of propofol. Propofol was administered as a slow intravenous bolus to effect (approximately 30 mg/dose) and resulted in 6-8 minutes of excellent muscle relaxation. No apnea was observed at this dose (0.3 mg/kg). Heart rate, respiratory rate, and oxygen saturation were stable throughout the procedures. All animals were reversed with naltrexone at a rate of 100:1 (mg naltrexone: mg carfentanil) and yohimbine (0.13 mg/kg IV). No post-anesthetic complications or signs of renarcotization were observed. No procedures were performed with carfentanil as a sole induction agent in mountain tapirs.

Discussion

The risks of anesthesia in exotic ungulates include such serious complications as hyperthermia, myopathy, regurgitation and aspiration, and trauma especially in field conditions. Consequently, rapid smooth inductions and recoveries are critical to minimizing these risk factors. In addition, during the period of recumbency, adequate muscle relaxation and analgesia are required to facilitate rapid completion of the procedure and maximize patient and handler safety. The goal of this "balanced anesthesia" then, is to achieve these effects while reducing the dosages of the individual drugs thus minimizing adverse responses.[1,3] Balanced anesthesia in exotic ungulates has been achieved by administering a premedicant such as xylazine, followed by induction with carfentanil. Studies using large populations of exotic ungulates have reported that immobilizations using xylazine and carfentanil resulted in better relaxation and control of the patient as compared to carfentanil alone.[4,5] In situations where animals are semi-free-ranging or may not be accessible for multiple drug administrations, xylazine and carfentanil have been administered in the same dart with similar effects to premedication.[5,6] Snyder et al. reported the initial use of CKX in kudu, sable antelope and gemsbok oryx.[2] They also described the advantages of narcotic cocktails in a variety of exotic ungulates using a combination of etorphine, ketamine, xylazine.

Although anesthetic cocktails are frequently used in exotic species, this report focuses on the efficacy and safety of a carfentanil, ketamine, xylazine mixture in two species in which there is a paucity of anesthetic literature, bongo antelope and mountain tapirs.[4-9] In both

species, a single dart could be used to administer the total anesthetic dose for induction of general anesthesia. Rapid smooth inductions and good to excellent muscle relaxation using CKX allowed significantly reduced total immobilization times (as compared to carfentanil alone) for the same types of procedures in bongo antelope. In addition, handler safety was increased as kicking and thrashing of the anesthetized animal was absent using this combination. Although Snyder et al. used equal proportions of ketamine and xylazine in their cocktail, similar results were achieved using lower doses of xylazine while decreasing the risks of bradycardia, apnea/hypoventilation, and regurgitation.[2] Significantly lower doses of carfentanil were also used, therefore, decreasing the possibility of renarcotization.

CKX was successfully used in both juvenile and adult mountain tapirs. The standard dose used in an adult animal was 20 mg xylazine, 50 mg ketamine, and 1 mg carfentanil as a single injection. Due to the length of the procedures (average 106 mins total immobilization time), propofol and isoflurane were used effectively and safely as supplemental agents in this species. Similar effects and advantages were observed in tapirs as has been reported for ruminant species. This represents the first known report of this anesthetic combination in Tapiridae.

In conclusion, CKX provided a safe, effective single dose regimen for immobilization of bongo antelope and mountain tapirs while reducing the incidence of adverse effects often observed with the use of carfentanil alone.

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Table 1. Average effective doses for CKX in bongo antelope

	Carfentanil (mg)	Ketamine (mg)	Xylazine (mg)
Adult male	1.4	80	20
Adult female	1.3	70	24
Juveniles (60-100 kgs)	0.9	50	15

Table 2. Comparison of immobilization data for bongo antelope

Average values:	CKX	Carfentanil +/- ace
T induction (mins)	3.7	3.5
T recumbent (mins)	5.5	4.2
T immobilization (mins)*	33.6	61.8
T reversal (mins)	5.3	4.8
Anesthetic score*	3.2	2
Heart rate (at 10-20 mins)	80.8	96.5
Respiratory rate	17.1	14
Xylaxine (mg/kg)	0.17	N/A
Ketamine (mg/kg)	0.54	N/A
Carfentanil (ug/kg)*	9.8	15.5

* Values significantly different at $p<0.05$, Student's t test.

Table 3.
Anesthetic data for mountain tapirs using CKX.

Average values:	CKX
T induction (mins)	1.8
T recumbent (mins)	3.7
T immobilization (mins)	106.3
T reversal (mins)	4.3
Anesthetic score	3.25
Heart rate (at 10-20 mins)	82.5
Respiratory rate	13
Xylaxine (mg/kg)	0.103
Ketamine (mg/kg)	0.26
Carfentanil (ug/kg)	5.4
Propofol (mg/kg)	0.31
Yohimbine (mg/kg)	0.13

IMMOBILIZATION OF FREE-RANGING MOOSE (*Alces alces*) WITH MEDETOMIDINE-KETAMINE AND REVERSAL WITH ATIPAMEZOLE

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Medetomidine (MED) in combination with ketamine (KET) were evaluated for immobilization of free-ranging moose (*Alces alces*) in four trials in Norway and Finland in 1992 to 1994. The animals were eartagged and adult moose were fitted with radio collars (Televilt International AB, Storå, Sweden). Atipamezole (ATI) was used for reversal of immobilization.

Trial I (August-September 1992 and 1993, Norway): Fifteen adult moose (2 males, 13 females) and two male calves were darted (DAN-INJECT®, J. Lund-Jørgensen, Børkop, Denmark) either from a motor vehicle or by approaching the animals on foot. The doses for adults were 30 mg MED (Medetomidine hydrochloride 10 and 30 mg/ml, Orion Corp. Animal Health Division, Turku, Finland) and 400 mg KET (Ketamine hydrochloride dry powder, Parke, Davis & Co., Pontypool, Gwent, UK), and for calves 15 mg MED and 200 mg KET respectively. Standard procedure after darting was to wait 10-15 min before tracking of the animals with dogs (Norwegian elkhounds) started. Consequently, no "induction" time is available for this group. However, three animals were actually seen going down, one adult female after 1.33 min (at the place of darting) and the two calves (close to their immobilized mothers) after 4.0 and 5.0 min, respectively. The other animals were found 19.5 (7.7) (7-35) min [mean (SD) (range)] after darting. The mean walking distance after darting for these animals was 340 (190) (100-750) m. All animals were completely immobilized and were in sternal or lateral recumbency. Physiological measures recorded 34 (8) (20-50) min after darting in adult animals were: rectal temperature 38.8 (0.5) (38.2-39.8) °C, heart rate 44 (7) (30-60) beats/min, and respiratory rate 31 (20) (10-68) breaths/min. Oxygen saturation, recorded continuously with pulse oximetry (NELLCOR® N-10, Nellcor Inc., Hayward, CA, USA) during immobilization in six animals, ranged from 83 to 95%. One female developed periodic apnea 40 min after darting. ATI (Antisedan® 5 mg/ml, Orion Corp. Animal Health Division, Turku, Finland) at five times the dose of MED was administered 49 (10) (32-64) min after darting for reversal. The ATI dose was divided and given half i.v. and half s.c. in seven adult animals and half i.m. and half s.c. in eight adult animals. The mean head-up times for these two groups were 1.9 (0.8) (1.25-3.33) min and 4.1 (2.1) (1.0-7.5) min respectively, while the mean on-feet times were 3.9 (1.8) (1.7-6.3) min

and 6.9 (3.4) (1.5-10.5) min respectively. The difference in the mean head-up time was significant ($p < 0.05$). All animals recovered completely. Ambient temperatures during this trial ranged from 12 to 20 °C.

Trial II (March 1994, Norway): Moose were baited with grass silage and eight adult cows were darted from a motor vehicle with 30, 35, or 40 mg MED and 500 mg KET. Four animals were observed going down after a mean induction time of 7.1 (3.3) (4.0-11.3) min, while two animals were found after 43 and 22 min respectively. These animals were completely immobilized in sternal or lateral recumbency. Two animals did not go down after the initial dose, and one of them was manually restrained while the other was captured after an additional dose of 6 mg etorphine (Etorphine 9 mg/ml, C-Vet Ltd., Bury St. Edmunds, UK) had been given. These two individuals were excluded from the data analysis. The mean walking distance after darting was 240 (210) (50-600) m. Physiological measures recorded 26 (11) (17-47) min after darting were: rectal temperature 37.8 (0.4) (37.4-38.4) °C, heart rate 32 (5) (24-36) beats/min, and respiratory rate 39 (22) (18-66) breaths/min. ATI at five times the dose of MED was given i.m. 36 (10) (27-52) min after darting. The mean head-up and on-feet times were 6.5 (2.8) (4.5-12.0) min and 7.2 (2.8) (5.2-12.8) min respectively. The depth of snow was 0.5-1.0 m in the study area and the ambient temperature ranged from -3 to -18°C during the trial.

Trial III (March 1994, Norway): Thirteen adult moose (5 males, 8 females) were darted from a helicopter with 30 mg MED and 400 mg KET (n=2) or 40 mg MED and 500 mg KET (n=11). Two animals given the highest dose required additional dosing and were excluded from the data analysis. The mean induction time for animals receiving one injection was 8.3 (3.8) (3.7-15.0) min. These animals were completely immobilized in sternal or lateral recumbency. Physiological measures recorded 25 (7) (15-35) min after darting were: rectal temperature 39.3 (0.7) 38.0-40.2), heart rate 46 (7) (38-64) beats/min, and respiratory rate 70 (11) (56-90) breaths/min. Excessive drug effects with respiratory depression, rapid and shallow breathing, and periodic apnea were seen in three animals given the highest dose. ATI at five times the dose of MED was given half i.m. and half s.c. 23 (9) (20-46) min after darting. The mean head-up and on-feet times were 4.6 (1.7) (3.2-9.0) min (n=10) and 5.4 (2.1) (2.7-9.3) min (n=10) respectively. One cow that stopped breathing after 40 min was treated with 400 mg doxapram hydrochloride (Dopram® 20 mg/ml, Wyeth-Ayerst International Inc., Philadelphia, PA, USA) i.m. in addition to ATI, while inspiration was induced by intermittent pressure on the chest. This individual recovered completely. One bull that was immobilized with the lowest dose of MED-KET became alert after ATI administration but was unable to get up. The bull was left in sternal recumbency 2.5 hr after darting. The next morning the bull was tracked with helicopter for > 1 km to an area with dense vegetation but it was not seen. The body condition of all animals included in this trial was poor. The depth of snow was approximately 1 m in the study area and the ambient temperature ranged from -5 to 0 °C during the trial.

Trial IV (March 1993 and 1994, Finland): Forty-four adult moose (22 males, 22 females) were darted from a helicopter with 40 mg MED and 600 mg KET (1993, n=23) or 50 mg MED and 600 mg KET (1994, n=21). Animals that required additional dosing because of

insufficient effect from the initial dose (suboptimal injection site, incomplete injection, malfunctioning of darts etc) were excluded from the data analysis. The mean induction time for 1993 was 6.3 (2.4) (3.0-12.0) min (n=16) and for 1994 5.6 (1.9) (3.0-10.0) min (n=14). The difference in mean induction time was not significant ($p > 0.05$). Physiological measures (pooled data for both years) recorded 26 (6) (17-40) min after darting were: rectal temperature 39.2 (0.7) (38.0-40.0) °C (n=18), heart rate 50 (9) (32-68) beats/min (n=28), and respiratory rate 50 (28) (16-88) breaths/min (n=30). ATI at five times the MED dose was given half i.m. and half s.c. 35 (6) (24-52) min after darting. The mean on-feet time was 4.4 (1.4) (2.0-8.0) min. One bull that was darted twice with a full dose in 1994, was unable to get up although it appeared alert after ATI administration. The bull was left in recumbency 2 hr after ATI treatment, but was found dead the next morning. Necropsy revealed massive lungworm infestation, but no specific cause of death was found. The depth of snow was 0.5-1.0 m in the study area and the ambient temperature ranged from -15 to 2 °C during the trial.

In conclusion, medetomidine-ketamine and atipamezole appear to be useful alternatives to opioids for reversible immobilization of free-ranging moose. The variation in drug response between the trials may have been caused by differences in capture method, metabolic rate, body condition, nutritional status, or genetic factors.

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CARDIOPULMONARY AND ACID-BASE STATUS IN CAPTIVE ADDAX ANESTHETIZED WITH CARFENTANIL-ACETYLPROMAZINE-KETAMINE

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Results of a recent study, in which carfentanil and xylazine were given to domestic sheep (*Ovis ovis*), indicated that hypercarbia, severe hypoxemia, hypotension and ECG changes indicative of myocardial hypoxia occurred with this drug combination.[1] Doses used were those recommended for wild mouflon sheep (*Ovis ammon musimon*).[2] Although excessive dosage may have been a factor in the severity of the changes observed, the study raises questions about the cardiopulmonary effects of carfentanil in combination with tranquilizers in artiodactylid ungulates. In this study, the cardiopulmonary and acid-base status of captive addax, anesthetized with carfentanil, acetylpromazine, and ketamine were investigated.

Six adult female addax (*Addax nasomaculatus*), corralled in 2 groups of 3 animals at Fossil Rim Wildlife Center, were restrained in a drop floor chute or manually (1 animal), and given carfentanil[a] 25 μg/kg and acetylpromazine 0.11 mg/kg IM (CFA) for non-surgical embryo collection. After immobilization occurred, the addax were moved to the on-site clinic where they were hand held in a sternal, head-up position on a padded operating table. Ketamine, 0.3 to 0.9 mg/kg was given IV as needed to maintain immobilization and improve muscle relaxation. ECG and direct arterial pressure (AP) were monitored continuously, and samples for blood gas and pH analysis were taken at approximately 20 min intervals from the auricular artery. Samples for plasma lactate were drawn from the jugular vein or auricular artery. At the conclusion of the clinical procedures, the addax were moved to individual paddocks, and naltrexone[a] 1.25 mg/kg IV and 1.25 mg/kg SQ. was given. Results were analyzed by ANOVA for repeated measures.

The first addax in each group was calm before restraint and drug injection. The remaining 2 addax in each group became agitated and active after removal of the first animal from their corral. Ataxia was present 2.2 $\pm$ 0.45 ($\bar{x} \pm$ SD) min., and recumbency occurred 3.5 $\pm$ 0.55 min. after CFA injection. Total ketamine dose ranged from 0.6 to 2.5 mg/kg. Duration of immobilization was 77.7 $\pm$ 7.6 min. The addax stood 4.8 $\pm$ 1.1 min. after naltrexone administration. No post-immobilization complications or renarcotization were noted. Rectal temperature upon immobilization was 39° and 39.2°C in the calm addax and 40.0-41.9°C in previously excited addax. Initial mean AP was 118 $\pm$ 5.7 mmHg and did not change significantly with time. Initial heart rate was 149 $\pm$ 83 bpm and decreased to 96.8 $\pm$ 9.1 bpm by 50 min. ($P<0.05$). No arrhythmias or abnormal waveforms were noted on the ECG. Initial P_aO_2, P_aCO_2 and pHa collected within the first 30 minutes of immobilization, and analyzed at 37°C were 69 $\pm$ 5.5 mmHg, 31.1 $\pm$ 6.8 mmHg and 7.36 $\pm$ 0.06 mmHg respectively, and did not change significantly over the period of immobilization. Initial calculated base excess

(BE) was -6.4 $\pm$ 5.0, and final BE was -1.8 $\pm$ 1.1mEq/L. The change was not significant. Initial plasma lactates were 5.8 and 5.0 mM/L in the calm addax, and 17.1 $\pm$ 5.8 mM/L in excited addax. Final plasma lactates were 4.2 mM/L in both calm addax and 8.5 $\pm$ 2.2 mM/L in excited addax.

CFA and ketamine, in the doses given, provided rapid immobilization. Anesthesia and muscle relaxation were sufficient for non-surgical embryo collection. Mild hypoxemia was present, but there was no evidence of hypotension, ECG abnormality or significant respiratory depression. Ketamine, in the doses given, did not interfere with naltrexone remobilization, and renarcotization was not noted. Excitement and activity before drug injection can result in moderate to severe hyperthermia and lactic acidosis. In these healthy addax, ventilatory responses to hyperthermia and acidosis were generally well maintained during CFA-ketamine immobilization. No evidence of capture myopathy or persistent metabolic derangement was seen, but the results suggest that administration of an anti-anxiety or tranquilizing agent should be considered for animals in a small group before removing one or more members from the group.

a. Wildlife Pharmaceuticals, Fort Collins, CO 80524

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TECHNIQUES IN FISH ANESTHESIA

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Fish Anesthesia--General Considerations

When extending your clinical practice to fish, you will encounter some familiar basic requirements. These include restraint for examination, transport, obtaining diagnostic samples, and surgery. Since many of these procedures are more easily performed out of the water, a condition which most fish by their very nature find objectionable, the need for effective restraint is obvious. The use of chemical immobilization is often less stressful and traumatic (for both the fish and the clinician) than physical restraint for many minor procedures. For major procedures the use of an anesthetic agent greatly facilitates the successful completion of the operation and gives the patient the benefit of the doubt in the philosophical debate over whether or not fish feel pain. When necessary, overdose of anesthetic agents is an acceptable means of euthanasia.

Water quality is an important consideration in all aspects of fish medicine, and anesthesia is no exception.[3,4,16] Physical and chemical parameters of the anesthesia water should closely match those of the aquarium or pond water, in order to reduce physiologic stress. These include temperature, dissolved oxygen, salinity, pH, and hardness (nitrogenous waste products should be negligible). Because anesthetic agents tend to cause respiratory depression, hypoxia is a distinct threat. Adequate aeration or oxygenation is mandatory, particularly when multiple fish are to be anesthetized in the same water. Since some anesthetic agents markedly affect pH, addition of buffers may be required to avoid inducing acidosis (see below). Water temperature has a direct effect on metabolic rate, and therefore affects the rates of induction and recovery, with higher temperatures speeding both.

In preparation for anesthesia, stressors on the fish should be minimized.[3,4] Relaxed fish experience a smoother induction than fish which are aroused. Food should be withheld for at least one feeding cycle prior to anesthesia. Although aspiration pneumonia is not a hazard in fish, regurgitation can clog gill rakers and foul the water. Withholding food also reduces production of nitrogenous wastes. For water-borne anesthesia, water and containers for induction, maintenance, and recovery should all be prepared ahead of time. This involves selecting containers of adequate volume, adjusting water quality parameters to approximate optimal conditions for the patient, and pre-mixing the desired concentrations of anesthesia. When dealing with multiple individuals of an unfamiliar species, carry the entire anesthetic procedure through to recovery on one fish before proceeding to the rest, as species variability in susceptibility to anesthetics may necessitate adjustment of induction or maintenance doses. Handle the fish carefully, with wet hands, to avoid abrasions or loss of protective mucus.

Anesthetic effects in fish have been staged in varying schemes by different authors.[3,4,9,11] All schemes are gauged by activity, reactivity to stimuli, equilibrium, muscle tone, respiratory

rate, and heart rate. Broad stages include sedation, narcosis or loss of equilibrium, and anesthesia, and may be further subdivided into light and deep planes. Not all stages are observed with all anesthetic agents or routes of delivery in all fish. An excitement phase, which can be of some consequence in larger fish, may be seen between sedation and narcosis, especially when the anesthetic agent is delivered in the water.

Anesthetic Agents

Many compounds have been employed as fish anesthetics. Some of the more commonly used and/or readily available are included here.

Tricaine methanesulfonate (MS-222, Finquel®) is the most widely used fish anesthetic, and is the only one currently approved for use in food fish in the United States. It is a benzocaine derivative with a sulfonate radical which accounts for its water solubility and increased acidity over the parent compound.[4] Administered as a water-borne solution, tricaine is absorbed across the gill epithelium and is biotransformed in the liver and probably kidney.[1] It is cleared primarily through the gills as the free and acetylated form, with additional metabolites eliminated in the urine and bile. Tricaine is conveniently administered as a pre-mixed stock solution, of 10 g/L (10,000 ppm).[16] The stock solution is unstable in light and should be kept in a dark container. Shelf life can be extended by refrigeration or freezing. Because tricaine solutions are acidic (stock solutions can be as low as pH 3), they should be buffered prior to administration to fish. Saturating the solution with sodium bicarbonate buffers the stock solution between pH 7.0 and 7.5.[16] Other buffers (imidazole, sodium hydrogen phosphate, sodium hydroxide) may also be used.[3,4] Oily residues in buffered stock solutions indicate the presence of a desulfonation product and decreased potency.[16] Hypoxia is noted to be a problem with tricaine anesthesia, whether resulting from respiratory depression or a primary effect of the drug. Hypoxemia can be mitigated by aeration or oxygenation of the solution, and by directing water flow over the gills. Our clinical impression is that tricaine also induces bradycardia (even when adequately oxygenated) and hypotension. Although generally considered a safe anesthetic, tricaine margins of safety are narrower for young fish in warm, soft water, and there is variation across species.[16] Recovery from short procedures is rapid (less than 10 minutes if properly dosed), with prolonged recoveries (up to 6 hrs) from longer procedures.

Quinaldine sulfate is popular in the aquaculture brood stock industry because of the perception of a wider margin of safety than tricaine with imprecise dosing (i.e., the sprinkle method). It is not approved for use in food fish. Quinaldine sulfate is a highly water soluble powder, unlike its parent compound, quinaldine, which must be dissolved in acetone or alcohol prior to mixing in water.[4] Like tricaine, quinaldine sulfate in solution is strongly acidic, and should be buffered. It may also be conveniently administered as a 10 g/L stock solution.[16] Unlike tricaine it is not metabolized by fish, and is excreted entirely unchanged. Because it does not eliminate all reflex responses, and is believed to confer minimal analgesia, quinaldine sulfate is not suitable for major or delicate surgery.[4]

Benzocaine, the parent compound of tricaine, is less expensive than tricaine. Although insoluble in water, a stock solution in ethanol or acetone (100 g in 1 L) may be used. The stock solution should be kept in a dark bottle and held at room temperature.[4]

Etomidate and metomidate are closely related non-barbiturate imidazole hypnotics which have been used as water-borne anesthetic agents in fish.[4,16] Etomidate is used as a parenteral anesthetic induction agent in humans, and metomidate is available under the trade name Marinil® in Canada. Both are readily water soluble and should be stored in tight light-protected containers. Although use of these agents does not result in increased cortisol typically observed with other anesthetics, this is most likely due to a metabolic blockade of cortisol synthesis rather than a lack of stress.[14] Some fish anesthetized with metomidate turn very dark transiently. The proposed mechanism for this phenomenon is that reduced cortisol production terminates the negative feedback loop on ACTH synthesis, which is linked to melanocyte stimulating hormone (MSH) production, so that both ACTH and MSH production are increased. Neither drug has analgesic properties, and muscle fasciculations occur at low doses. They are therefore not appropriate for surgery, although they are useful for sedation and tranquilization. Gouramis are believed to be very sensitive to metomidate.[3]

Ketamine is an injectable short-acting dissociative anesthetic familiar to most clinicians. It can be used alone or in combination with demetomidine, which has the advantage of reversibility with atimpamezole.[21] Respiratory support (directing a stream of well-aerated water over the gills) may be necessary during ketamine anesthesia.

Lidocaine has been used as an immersion anesthetic with variable results,[4] but may have application as a local anesthetic in combination with a non-analgesic agent such as quinaldine or metomidate. Care must be taken not to overdose small patients with local injections

Carbon dioxide has been used from various sources (Alka-Seltzer®, sodium bicarbonate, CO_2 gas) for fish anesthesia, apparently with some success.[7] The use of CO_2 has many disadvantages, however. Levels in water are difficult to control, oxygen levels must be maintained at high levels,[4] and blood gases and acid/base balance are markedly altered compared to other anesthetics.[10] Electroencephalography of carp anesthetized with CO_2 shows a pronounced tendency towards flatness, indicating an overall inhibition of spontaneous activity of the central nervous system.[23] Its primary advantage, in food animal situations, is the lack of tissue residues of regulatory concern. In clinical situations, however, it should be considered an anesthetic or euthanasia agent of last resort.

Isoflurane and halothane are readily available in veterinary clinics, and may be used for fish anesthesia, either by direct addition to water, or by vaporizing and bubbling through the water.[4, 7] However, anesthetic levels are difficult to control, localized areas of higher concentrations may occur resulting in overdose, and volatization constitutes a hazard to personnel. Use of volatile anesthetics is not recommended.

Ethanol is included as another anesthetic/euthanasia agent of last resort. Anesthetic depth is variable and difficult to control.[4] For one reason or other, however, in non-clinical situations it is sometimes available when other agents are not.

Delivery Methods

Anesthesia can be delivered by any of the usual routes of administration: orally (PO), parenterally (IV, IM, IP) or inhalation (topical to gills, bath, water-borne).

Oral administration of anesthetic agents is rarely used in fish medicine, due to difficulty in precise dosing and uncertainty regarding rate and degree of absorption. Oral metomidate has been administered by high pressure syringe as a supplement in fish partially immobilized by remote injection (dosage unspecified),[9] and diazepam impregnated pellets have been fed to American shad, resulting in a slow anesthetic induction.[15]

Parenteral administration is usually IM or IP (with the chance IV administration resulting in more rapid induction times). The parenteral mode is best suited for larger fish, for which injection site trauma is less of a hazard, and for larger volume aquariums, where adding water-borne anesthetics to the entire tank would be impractical, and confinement and capture of the fish in a smaller volume of water would be problematic and stressful. Injections may be made by hand syringe, pole syringe, or dart. A Hawaiian sling may be modified to serve as a dart delivery system,[16] though care must be taken not to shoot with excessive force. Use of a laser-aimed underwater dart gun for remote delivery of immobilizing agents has been described.[9] Intramuscular injections are associated with leakage of some injected material as the needle falls out or is withdrawn and the surrounding muscles contract. A site for hand injection at the caudal base of the dorsal fin has proven successful in avoiding this problem in *Morone* spp., and other fish. The needle enters a soft spot on the dorsal midline between major muscle bundles and is advanced cranioventrally and slightly laterally into the muscle. Any drug extruded from the muscle when the needle is withdrawn is then trapped in the interstitial space rather than coming out through the injection site.

Water-borne anesthesia is the most widely used route of anesthetic administration for fish. For bath treatment the drug can be brought to the desired concentration in water containing the fish, or the fish can be placed in an induction tank containing anesthetic water (slightly more stressful). For short procedures lasting less than 5 minutes, this may be all that is necessary. The fish may be removed from the water for the necessary manipulations, and either returned to the anesthesia water to extend the procedure, or moved to recovery water when the task is completed.

For longer out-of-water procedures water must be delivered in continuous flow to the gills. This can be achieved by a non-recirculating or recirculating system. In a non-recirculating system (the equivalent of a non-rebreathing or Bain system) the anesthesia/water mix is held in a reservoir bag or tank, and is conveyed through appropriately sized tubing into the fish's mouth and over the gills. Used water is then collected only to prevent making a mess, not

to recycle to the fish. This system works well for small fish. A simple design uses empty IV fluid bags as reservoirs and a drip set for delivery, with flow rate regulated by the drip set clamp.

A recirculating system is well suited to large fish, where economics make conservation of anesthesia and water (i.e., inland salt water) a concern. Numerous recirculating systems have been described,[2,3,4] but the basic idea is that anesthesia water from a reservoir is delivered to the gills and is collected in a sump and returned repeatedly to the fish. The simplest recirculating system has the operator returning the sump water to the reservoir by hand, or switching the position of the sump and reservoir tanks. Less labor intensive designs use pumps to return water to the fish.

Bath treatments and recirculating systems used for multiple sequential fish anesthesia procedures eventually become depleted of anesthetic, as anesthetic laden fish are taken out of the bath or off the system. Ammonia concentrations rise as more fish-hours are logged.[2] Both factors eventually necessitate a change of water and anesthesia. Time for change can be assessed by monitoring induction time and anesthetic depth, or direct measurement of water quality. Increasing protein concentration from fish slime causes foaming in aerated water, providing a visual indication of deteriorating water quality.

In both recirculating and non-recirculating systems, flow should be normograde (in the oral cavity and out the opercular opening) to achieve optimal gas and anesthesia exchange. During oral surgery, flow can be reversed if absolutely necessary to allow surgical access, but recognize that retrograde flow short circuits the normal counter-current exchange mechanism of the gills.[14]

A problem common to both types of systems is difficulty adjusting anesthetic concentrations as the depth of anesthesia varies. One simple method for coping with this design deficiency is to prepare 60 ml syringes containing anesthesia-free water or more concentrated anesthesia solution. These solutions can be delivered to the gills directly as needed, based on the patient's condition. It is also possible to design a low volume recirculating system which allows the infusion of water or concentrated anesthetic solution to regulate the anesthetic concentration in a more controlled manner. A simple design to achieve rapid fine control, similar to that possible with an isoflurane precision vaporizer, is not yet available for aquatic anesthesia.

There are no published guidelines for minimum or maximum effective flow rates during fish anesthesia. Monitoring the fish's anesthetic depth is the best guide (see below). We have found that 1300 ml/min works well for 500 hybrid striped bass (*Morone saxatilis* X *M. chrysops*) anesthetized for up to 1 h. A flow rate of 12.1 ml/min was successful for a 47 min procedure in an 8 g gourami (*Colisa labiosa*).[8] Brown[2] described a recirculating system delivering 1 L/min for 10 to 41 min to channel catfish (*Ictalurus punctatus*) weighing 42 - 1100 g.

Monitoring Techniques

A variety of parameters can be monitored visually or palpably during anesthesia induction and maintenance.[4,16] Respiration (opercular movement) is probably the most important reference of anesthetic depth. Others include loss of equilibrium (during induction), jaw tone (which may be present in the absence of opercular movement), pupil size, color of fin margins (hybrid striped bass overdosed with anesthesia develop a broad pale margin in all fins, possibly due to hypoxia or hypotension), response to touch or surgical stimuli, and gill color (as an indication of blood loss during surgery rather than oxygenation).

Although pulse is not readily palpable in fish, heart rate can be monitored by ECG, cardiac ultrasonography, or Doppler probes (used in blood pressure measuring equipment) placed directly over the heart. ECG leads can be placed subcutaneously, and the ECG can be used to detect not only heart rate, but also indications of hypoxia such as increased T wave amplitude and irregular rhythm.[18]

Our attempts to use pulse oximetry in some fish species have been disappointing. Both transmittance and reflectance probes have been tried in multiple locations (all fins, caudal peduncle, over heart, over medial opercular membrane, dorsal aorta, tongue, palate, forehead, gills) to no avail. Whether this failure arises out of differences in fish hemoglobin (although the existing technology works in amphibians), low pulse pressure, bradycardia, or poor access to capillary beds, is unknown.

Blood gases can be measured directly in patients which are sufficiently large. The StatPal® II[a] portable blood gas analyzer has been used in bluefin tuna[5] and in hybrid striped bass. It requires 0.8 ml blood and corrects for patient temperatures between 25.0 - 40.0 C. Below 25.0 C trends in measured blood gasses may still be useful. Measured parameters include pH, pCO_2, and pO_2. The machine also calculates bicarbonate, total CO_2, actual base excess, extracellular fluid base excess, standard bicarbonate, and oxygen saturation. The dorsal aorta is the only readily accessible sampling site where oxygenated arterial blood can be obtained with certainty, but this may be detrimental in some fish with relatively small vessels. We used cardiac puncture to monitor the efficacy of our recirculating anesthesia machine in maintaining blood gases and pH in hybrid striped bass without supplemental aeration. During a 60 minute anesthesia period, pO_2 tended to increase in the first 10 minutes on the machine (after induction in a well aerated bucket), then gradually declined. Throughout the procedure, however, pO_2 compared favorably with unanesthetized fish sampled within 15 seconds of capture. Fish which were pursued a greater length of time prior to capture had a lower starting pO_2. Blood pH tended to increase and pCO_2 tended to decrease.

While the patient is sometimes difficult to monitor, especially small fish, the anesthesia water is easily accessible. Once the initial water quality parameters have been set, possibly the most important parameter to watch for change is dissolved oxygen. Ammonia and pH could also be of concern if multiple fish are anesthetized on the same system. We found that with our anesthesia machine, supplemental aeration reduced the amount of time for

DO to stabilize, and it stabilized at a higher level, than without supplemental aeration. Presence of 500 g hybrid striped bass on the system did not reduce DO measurably, either with or without supplemental aeration. Even though we were apparently able to deliver adequate oxygen to the fish without aeration, as evidenced by survival and blood gas measurements, supplemental aeration is recommended as the prudent course of action, especially if DO is not measured.

Recovery and Resuscitation

The distinction between recovery and resuscitation is occasionally blurred. If opercular motions cease, or other signs indicate that depth of anesthesia is too great, anesthesia-free water should be directed across the gills. This can also be done towards the end of a long surgery, in the same manner as turning off the anesthesia and administering pure oxygen to a mammalian patient at the end of an anesthetic procedure. The recovery tank should be well aerated and free of anesthetic agents. When returning a fish to the recovery tank, the fish can be faced into the water flow from the filter/aerator. Larger fish can be held with the mouth open and pulled forward, ramming water across the gills. Pulling the fish backwards, even briefly when moving alternately forwards and backwards, should be avoided. It results in greatly reduced efficiency of gas and anesthetic exchange (by running flow counter to the counter-current exchange mechanism), and may damage the gill filaments.[3] Monitor jaw tone and opercular movement. Jaw tone and even biting can precede return of opercular movement. Once the fish begins breathing on its own, it is best left alone, as the recovery can proceed with less stress, and the fish is more efficient at directing the proper water flow over the gills than the anesthetist. Continue to monitor respiration, motion, and equilibrium until the fish is fully recovered, and periodically thereafter.

It is sometimes difficult to determine immediately whether or not a fish has expired. Do not give up on resuscitation attempts prematurely, as respiratory arrest (lack of opercular motion) can precede cardiac arrest by an extended period of time. Electrocardiography or ultrasonography can prove useful in detecting heart beats in very bradycardic patients.

Euthanasia

Euthanasia can be accomplished by an overdose of any of the anesthetic agents discussed. Larger fish which can not easily be transferred to a bath treatment may have the anesthesia solution poured directly over the gills.[3] To be certain the euthanasia is complete, once the fish is deeply anesthetized, cranial concussion, spinal transection, or exsanguination can be performed.

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Formulary*

Tricaine methanesulfonate (MS-222)		
induction	100 - 200 mg/L	
anesthesia	50 - 100 mg/L	
sedation	15 - 50 mg/L	
Quinaldine Sulfate		
induction	50 - 100 mg/L	
anesthesia	15 - 60 mg/L	
Benzocaine		
anesthesia	30 - 50 mg/L	
sedation	15 - 30 mg/L	
Etomidate		
transport tranquilization	0.4 - 0.6 mg/L	channel catfish, golden shiners
sedation	0.1 mg/L	striped bass
sedation	2 - 4 mg/L	freshwater tropicals
induction	6 - 10 mg/L	channel catfish, golden shiners
Metomidate		
transport tranquilization	0.06 - 0.20 mg/L	marine tropicals
light sedation	0.5 - 1 mg/L	sunfish
sedation	2.5 - 5 mg/L	freshwater and marine tropicals, sharks
immobilization	7 mg/L	red drum
immobilization	10 - 30 mg/L	Atlantic halibut
immobilization	40 - 60 mg/kg IM	
Lidocaine		
local anesthetic	<1 - 2 mg/kg	(therapeutic dose for cardiac total dose effects)
Ketamine		
anesthesia	66 - 88 mg/kg	
Ketamine/Demetomidine		
anesthesia	1 - 2 mg/kg ketamine/ 50 - 100 μg/kg demetomidine IM	
reversal	atipamezole 200 μg/kg IM	
CO_2 (Alka-Seltzer®, sodium bicarbonate, CO_2 gas)		
CO_2 gas	120 - 150 mg/L	trout
$NaHCO_3$	500 mg/L	trout (pH adjusted to 6.0 with HCl)
Alka-Seltzer®	1 tablet/L	
Isoflurane, Halothane		
anesthesia	0.5 - 2 ml/L added directly to water or vaporize and dissolve to effect	
Ethanol		
anesthesia	1 - 1.5% in water	
euthanasia	>3% in water	

*Disclaimer: See text for important notes on dosing. This list is compiled from multiple sources[3,4,6,7,9,12,13,16,17,20,21,22] and personal experience. These doses should not be considered suitable for all species, nor for all conditions. When working with unfamiliar anesthetic agents or fish species, start with low doses and low numbers of fish.

POST ANESTHETIC MONITORING OF CORE BODY TEMPERATURE USING TELEMETRY

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Introduction

Radiotelemetry has been used to monitor core temperature in a variety of birds, reptiles, amphibia and mammals implanted with commercially available temperature transmitters.[3,4,6,12] Specially designed ingestible radiotelemetry capsules have also been used in several species of marine endotherms (pinnepeds, African penguins) to record changes in core temperature indicative of activity, feeding, behavioral thermoregulation, diurnal variation and fever .[2,5,10,11] Previous studies in anesthetized North American river otters (*Lutra canadensis*) revealed marked differences in rectal temperature among different anesthetic protocols.[8,9] However, a baseline range for body temperature in this species has not been established. A pilot study was conducted using ingestible temperature transmitters for core body temperature determination in river otters during anesthesia and recovery.

Materials and Methods

The Biological Field Monitoring System (BFMS), developed for military research by the Department of Behavioral Biology, Walter Reed Army Institute of Research (Washington, DC, 20307-5100, USA) and subsequently modified by the Research Triangle Institute (Research Triangle Park, Raleigh, NC, 27709, USA), was used to monitor core body temperature in river otters.

Core temperature was recorded using a micro-power disposable telemetry capsule (29 x 11 mm, 3.9 g) which transmitted both a pill ID code and temperature (FM band, 86-96 MHz) with an error of +/- 0.05 C.[7] The capsule consisted of an antenna, battery, temperature sensor, transmitter and encoder assembly contained in an epoxy inner shell with an outer silicone coating. The data acquisition system was a self-contained, microcontroller-based receiver able to detect, transform, and store data. A single data acquisition pack was powered by three, 3.6 volt lithium C cells, or multiple packs were powered by a 12 volt car battery. A portable computer loaded with BFMS base station software provided real-time display and on-line analysis of core temperature.

Temperature telemetry was performed on 25 North American river otters during the 1994 Otter Relocation Project administered by the North Carolina Wildlife Resources Commission. Otters were anesthetized with one of the following 5 protocols: ketamine 10 mg/kg-midazolam 0.25 mg/kg, ketamine 10 mg/kg-diazepam 0.5 mg/kg, ketamine 2.5 mg/kg-medetomidine 25 ug/kg with reversal by atipamezole 100 ug/kg, telazol 4 mg/kg, or

fentanyl 0.05 mg/kg-azaperone 0.4 mg/kg-midazolam 0.25 mg/kg with reversal by naloxone 0.06 mg/kg. After anesthetic induction, the temperature capsule was placed in the caudal pharynx. Anesthetized otters maintained a gag reflex and subsequently swallowed the capsule. Core temperature was recorded at one-minute intervals from the time of insertion until passage of the capsule in the feces. During anesthesia, rectal temperature was also recorded every 5 min using a digital thermometer. Upon recovery (reversal or spontaneous), otters were placed in holding cages constructed of coated wire-mesh (0.5 x 1.0 x 0.5 m high). The data acquisition pack was attached to the outside of the cage frame and the antenna placed diagonally across the top of the cage, protected in a length of PVC piping. Ambient temperature was also recorded throughout the monitoring period.

Results and Discussion

There were no adverse effects of capsule administration in river otters, and all capsules were recovered by the following day (range 6-24 hours). Gastrointestinal transit time for the capsule was considerably longer than reported food transit time in river otters fed a semisolid, ground fish-based diet (mean 202 min).[1] In the present study, otters readily consumed water and whole fish upon recovery from anesthesia. Delayed passage of the transmitter capsules was most likely due to gastric retention. Capsule size and/or shape may be modified to further prolong transit time and thus provide additional telemetry data.

Elevated rectal and core body temperatures (103-105 F) were recorded after induction in most otters anesthetized with ketamine-midazolam, ketamine-diazepam, or telazol. Considerable variation in core body temperature occurred in all otters, regardless of anesthetic protocol, during the first 4 hours post anesthesia. Although minimized, interactions among humans and otters did occur during this time (recovery observation, feeding, cage cleaning). Resultant changes in activity level may have affected body temperature. Within 8 hours post anesthesia, core temperature stabilized between 99.5-101.5 F in most otters. The median core temperature at this time was 101.2 F (25th percentile 100.0, 75th percentile 101.6 F, n = 21). These values provided an estimate of baseline core body temperature in unrestrained North American river otters.

Core temperature fluctuated throughout the monitoring period (range 98-103 F) in several otters following telazol or fentanyl-azaperone-midazolam anesthesia. Clinically, there were no overt signs that could be attributed to changing body temperature. Given a larger sample size, similar findings would suggest anesthetic-related disruption of thermoregulation and/or muscular activity.

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IMMOBILIZATION OF BEARS USING ORALLY ADMINISTERED CARFENTANIL CITRATE

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Recent reports of oral administration of narcotics for preanesthesia sedation of humans prompted the investigation of orally administered carfentanil for chemical restraint of bears. Potent narcotics have been used parenterally to immobilize both captive and free-living bears. Typically these drugs are administered by a remote delivery technique, such as darting. Such delivery has several inherent potential problems or disadvantages, including dart-related injuries, long pre- and post-darting excitement periods, injections into poorly vascular tissues, and dart failures.

Seven American black bears(*Ursus americanus*) and three polar bears (*Thalarctos maritimus*) were immobilized with orally administered carfentanil citrate (Wildnil™, Wildlife Laboratories, Inc., Fort Collins, CO 80524, U.S.A.), mixed with honey. The black bears weighed 105 to 230 kg and received individual carfentanil doses ranging from 0.7 to 3.0 mg (6.66 to 8.69 μg/kg). The polar bears weighed an estimated 170 to 350 kg and received individual carfentanil doses of 1.5 or 2.0 mg (5.71 to 8.82 μg/kg). One polar bear was immobilized twice. The mean induction time (time from 80-90% carfentanil ingestion to sternal recumbency) for the black bears was 7 minutes (range: 4 to 12 minutes). The mean induction time for the 4 polar bear immobilizations was 8 minutes (range: 6 to 10 minutes). Inductions were smooth but all bears showed muscle rigidity and retained some head movement when in sternal and lateral recumbency. All bears were given intravenous diazepam at initial contact. The principle complications observed during immobilization were bradypnea and low O_2 saturation. All bears were insufflated with O_2 following measurement of initial oxygen saturation. Narcosis was reversed using naltrexone HCl (INADA 6277) at a dose of 100 mg naltrexone per 1 mg carfentanil administered, with 75% of the naltrexone given s.c. and the remainder given i.v. No episodes of renarcotization were observed but 4 black bears vomited following reversal.

COMPARATIVE CARDIOPULMONARY EFFECTS OF INTRAMUSCULAR ETORPHINE AND CARFENTANIL IN GOATS

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Seven female goats were used to determine the comparative cardiopulmonary effects of IM etorphine (5, 10, 20 and 40 μg/kg) and carfentanil (5, 10, 20 and 40 μg/kg).

Both drugs produced rapid catatonic immobilization, characterized by limb and neck hyperextension, with occasional vocalization and bruxation (chewing). Etorphine produced transient violent struggling and vocalization immediately after injection. Immobilization appeared dose-dependent, and was more rapid with carfentanil ($\leq$ 5 min) than etorphine (5 to 10 min). Recovery to standing was faster following etorphine injection (1-3 hrs) than carfentanil (> 2 hrs).

At all dosage levels both drugs significantly ($P \leq 0.05$) increased systemic and left ventricular (LV) end-diastolic pressures, LV peak - dP/dt, total peripheral resistance, hemoglobin concentration, and left atrial (LA) and pulmonary O_2 content. They also significantly decreased heart and respiration rates, and P_aCO_2. A significant increase was observed at some dosage levels for LV stroke volume and index, LV peak positive dP/dt, mean pulmonary artery pressure, P_aO_2, $P_{mv}O_2$, P_aCO_2, $P_{mv}CO_2$, LA hemoglobin saturation, LA transport index, and body temperature. A significant decrease was observed at some dosage levels for $P_{mv}CO_2$ and $S_{mv}O_2$. Neither drug had a significant effect on cardiac output and index, aortic peak flow, rate-pressure product, and oxygen consumption index. Except for a short period of bigeminy in 1 goat, the only arrhythmia observed was a Mobitz type I 2° atrioventricular heart block.

The cardiopulmonary effects of etorphine and carfentanil were similar over the evaluated dosage range. The major difference was the more rapid onset of effect of carfentanil, and a difference in the pattern of response of blood pressure, LV peak + and - dP/dt and total peripheral resistance at the higher dosages of etorphine. There appears to be no advantage of carfentanil over etorphine based on their cardiopulmonary effects for the dosage range 5 to 40 μg/kg. However, carfentanil does offer the advantages of greater potency and faster onset of immobilization which are offset by a longer duration of effect.

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CAUSES OF MORTALITY IN CAPTIVE LOWLAND GORILLAS : A SURVEY OF THE SSP POPULATION

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Introduction

Necropsy reports were requested for all Western Lowland Gorillas, *Gorilla gorilla gorilla* listed in the North American Regional Studbook that died after 1980. This period covers the years since the last published review of captive gorilla mortality.[1] Records were received from 74 gorilla mortalities and divided into age classes. The most significant pathologic finding, or cause of death, was noted for each animal by sex.

Results

Premature, abortions, stillbirths, and perinatal , (One day old or less) ; 12 records (16.2 %)	
7 males, 5 females	7.5
Infection related (ascending reproductive tract infections, and placentitis)	2.4
Dystocia	3.0
Intrauterine hypoxia	1.1
Premature (Hyaline membrane disease)	1.0
Neonates, (one day to one month) ; 5 records (6.8%)	
2 males, 3 females	2.3
Maternal neglect	1.2
Infection related (Generalized bacterial)	1.0
Congenital	0.1
Infants, (one month to one year) ; 10 records (13.5%)	
5 males, 5 females	5.5
Trauma	4.2
Gastrointestinal infection (bacterial)	1.1
Viral disease (respiratory)	0.1
Amebiasis (cerebral)	0.1

Juvenile, (one year to seven years in females and one year to nine years in males); 8 records (10.8%)	
4 males, 4 females	4.4
Gastrointestinal disease	
parasitic	2.1
bacterial	1.0
mixed bacterial and parasitic	1.0
Cagemate inflicted trauma	0.1
Systemic infection	0.1
Idiosyncratic drug reaction	0.1
Adults, (seven to thirty years in females and nine to thirty years in males); 22 records (29.7%)	
12 males, 10 females	12.10
Gastrointestinal disease	
parasitic	2.2
bacterial	1.2
other (ruptured appendix)	1.0
Cardiovascular disease	
myocardial fibrosis	2.0
aortic dissection	0.1
cardiac infection related	2.1
other cardiovascular	1.0
Trauma	1.1
Periparturient death	0.1
Neoplasia	1.0
Infection related (suppurative meningitis)	1.0
Parasitic (hydatid disease)	0.1
Idiosyncratic drug reaction	0.1
Older adults, (greater than thirty years); 17 records (23%)	
10 males, 7 females	10.7
Cardiovascular disease	
myocardial fibrosis	2.0
aortic dissection	3.1
cardiac infection related	1.0
other cardiovascular	1.1
Infection related (peritonitis,urinary tract, and CNS)	1.2
Neoplasia	0.1
Parasitic (hydatid disease)	0.1

Anesthetic related death	0.1
Euthanasia (severe osteoarthritis)	1.0
Post operative hemorrhage	1.0

Discussion

An effort was made in reviewing the necropsy reports to determine the most significant pathologic finding leading to death. There were many cases that had multiple organ system involvement, making this determination difficult. These other potentially non-fatal causes of morbidity should be the subject of further study. There are however some findings of note in this survey. The incidence of cardiovascular disease in adults (41% of all deaths in adults and older adults) should prompt further investigation for known cardiovascular disease risk factors. The incidence of traumatic death in infants (60% of infant deaths) may also warrant a review of husbandry in group rearing situations.

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UPDATE ON DIAGNOSTIC AND THERAPEUTIC APPROACHES TO AIRSACCULITIS IN ORANGUTANS

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Abstract

Infection of the laryngeal air sac has been previously reported in a free-ranging mountain gorilla, and in captive orangutans, chimpanzees, owl monkeys, and a baboon. This condition is not limited to free-ranging, zoo or research center settings, and it continues to be associated with significant levels of morbidity and mortality in the captive North American orangutan population. This paper attempts to summarize and update relevant observations, diagnostic and therapeutic options available to the clinician.

Clinical signs associated with airsacculitis can be subtle, and previous reports have emphasized the importance of early detection of disease. In the orangutan, relevant subtle signs can include halitosis, diarrhea, lethargy and anorexia, as well as the more obvious signs of nasal discharge, intermittent cough, rapid and shallow breathing patterns, and swollen air sac. The latter signs may only occur when the disease process has progressed to involve the lower respiratory tract. The exudate found within infected air sacs varies from liquid to a tenacious substance with a consistency similar to peanut butter. Thus, palpation and ballotment of the air sac are not sufficient to diagnose all cases. Aspiration (with or without irrigation), ultrasound, and endoscopy have been used in problematic cases.

Mixed cultures of enteric bacteria (*Proteus vulgaris, Proteus morganii, Pseudomonas aeruginosa, Escherichia coli, Streptococci, Aerobacter cloacae* and others) are frequently isolated, and sensitivity testing is essential for optimal therapy. Fibrous bands of tissue frequently form in chronically-infected air sacs, thus forming compartments; these compartments must be cultured separately as the bacterial populations may differ.

The air sac communicates directly with the trachea through bilateral slits, or ostia. These anatomical structures predispose the animal to aspiration of exudate during normal postural movements, or positioning for medical procedures. Permanent surgical closure of these ostia, in two layers, has been performed in several animals with chronic airsacculitis, with the goal of preventing potential aspiration pneumonia and other fatal sequelae. Permanent marsupialization of the sac has been elected in many chronic cases, while intermittent re-evaluation, drainage, and antibiotic therapy have been successful in controlling disease progression in others.

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MORBIDITY AND MORTALITY OF DOUC LANGURS (*Pygathrix nemaeus*) AT THE SAN DIEGO ZOO

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Introduction

The douc langur (*Pygathrix nemaeus*) is an endangered, arboreal folivore native to southeast Asia. Douc langurs belong to the colobinae subfamily of leaf-eating primates which have ruminant-like adaptations for cellulose digestion of plant material. Douc langurs are one of the most delicate species of langurs to maintain. Only in the last few decades have they been maintained with any degree of success in captivity.

The San Diego Zoo has maintained a colony of douc langurs for the past 25 years. Reviews of the pathology of douc langurs in San Diego have been published on cases prior to 1978.[2,4] Heldstab has discussed management and disease problems of douc langurs at the Basle Zoo.[5] The following review of post-mortem and clinical findings at the San Diego Zoo is presented to provide an overview of medical problems in this group of highly adapted primates.

Post-mortem Summary

Forty-five necropsies were performed on douc langurs at the San Diego Zoo during the past 25 years. Table 1 shows the number of necropsies performed during each decade or partial decade. A large proportion of necropsies were on abortions and stillbirths. Some abortions may not have been necropsied and therefore were not included in this study group. Figure 1 displays the number of deaths occurring by age group. This graph also reflects the large number of aborted fetuses and stillbirths. Furthermore, it reveals that a disproportionate number of young adults died during this period. Forty-two percent of the deaths in this age group were due to lung mite disease.

A summary of pathologic findings are displayed in Tables 2 and 3. Three major findings account for > 60% of the deaths. These include lung mites (*Pneumonyssus simicola*), abortions and stillbirths, and gastroenterocolitis. Lung mite disease was the most common problem in douc langurs prior to 1980. The usual post-mortem findings of animals affected with lung mites included a chronic pneumonia with cavitation as well as fibrosis and bronchiectasis seen histologically.

Several cases of abortion and fetal death were attributed to a chromosomal rearrangement (translocation) in a breeding male.[2,9] Additionally, cases of chorioamnionitis and placental infarct leading to fetal death have been seen in douc langurs in San Diego.[2] One set of stillborn twins were born after which the aging female delivered one live infant followed by four aborted fetuses. This one female accounts for 5 of the 11 abortions and stillbirths that were recorded here.

Gastrointestinal disease was frequently seen in post-mortem specimens. Most often it occurred as non-specific inflammatory bowel disease. Gastric amebiasis has been reported in colobinae previously.[7] Furthermore, Heldstab reported problems with *Entameba histolytica* in douc langurs.[5] At least one case was seen in douc langurs in this series as well.

Clinical Disease Summary

A review of our medical records available from 1974 to 1993 revealed a number of medical problems similar to the pathologic findings described above. Table 4 presents a list of specific conditions that were diagnosed clinically. This table attempts to relate clinical signs to these specific conditions and to diagnostic techniques. Obviously, other clinical illnesses and problems were observed in this group of douc langurs that were not definitively diagnosed and therefore were not included in the table.

By far, the most frequent clinical problem has been recurrent vomiting and diarrhea. Vomiting was often seen without relation to mealtime or other readily identifiable factors. Stress from social maladjustments, overcrowding, and other environmental factors seems to play a role, however. Diet in relation to fiber and gluten content may also be a factor in this problem. At Basle Zoo, it was reported that vomiting and diarrhea stopped when "monkey cakes" and bananas were discontinued.[5] In some of our severe or recurring cases, ulceration in the tubus gastricus portion of the stomach with and without invasion by ameba was documented by endoscopy. Treatment with histamine H2 receptor antagonists, ulcer protectants, and antiprotozoals have been moderately effective in decreasing the severity of the vomiting and related signs. Dietary and environmental adjustments have also been attempted with varying success.

Lung mite disease often went undetected in the live animal and resulted in sudden and unexpected death prior to 1980.[8] Since then, with the availability of an effective treatment, deaths and illness attributable to lung mites have fallen into the background. The disease was often evident radiographically, but the active infection was difficult to differentiate from old, inactive lesions. Attempts at retrieving mites from tracheal washes have been unsuccessful. In one case, an expelled blood clot was found to contain mite-like remnants on microscopic examination. The most severe cases have been in long-term captive animals. However, animals as young as 4 mo have been found to be infected.[8] Single treatments with ivermectin have not eliminated the infection in some animals. Treatment with ivermectin at 200 ug/kg has been shown to be an effective treatment in macaques.[6]

Intestinal obstruction due to plant material has occurred in two cases. In one, the animal vomited repeatedly and had a palpable abdominal mass. Surgical removal of undigested *Acacia* sp. from the stomach and intestines resulted in recovery.[3] The second case occurred in an animal that died suddenly after aborting a late term fetus. Clinically, the mother showed evidence of sepsis, but not intestinal obstruction. Post-mortem examination revealed a long, rope-like phytobezoar which caused a duodenal perforation and peritonitis. The plant material was identified as ginger, *Hedychium flavum*, a plant with long, fibrous leaves. Both of these plant materials have been discontinued as browse choices for langurs.

Discussion

Early in the history of this population, pulmonary acariasis was a devastating disease affecting all age groups of animals. Other infectious diseases were not major disease factors. Notable exceptions include gastric amebiasis, listeriosis, toxoplasmosis, and placental chorioamnionitis. Non-infectious diseases were numerous and affected most of the organ systems, but few patterns of disease emerged from this study group. Neoplasia was conspicuously absent from this group despite the presence of older animals in the population. Chronic vomiting, probably due to multiple etiologies, stood out as a predominant clinical and management problem. Abortions and stillbirths were of various causes, and severely limited the reproductive potential of this population. Renal disease, particularly affecting the glomeruli, surfaced as a significant cause of death as well as a possible underlying problem secondary to long-term ill-health.

Any retrospective review such as this which summarizes the diseases of a subpopulation is limited to the information supplied by historical records and restricted by the conditions which occurred in the sample population. It is, therefore, certainly not complete, but may aid in developing medical management plans for this and other species of langurs.

ACKNOWLEDGEMENT

I thank Dr. Bruce Rideout for his assistance in reviewing the pathology summaries in this report. Also, I thank Thomas L. Rost, PhD at the University of California, Davis for identifying the plant fibers present in the phytobezoars.

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Table 1. Number of necropsies performed each decade at the San Diego Zoo. Animals at risk is defined as the number of animals alive in the collection during each time period.

TIME PERIOD	ANIMALS AT RISK	ABORT/ STILL-BIRTHS	TOTAL NECROPSIED
1968 - 1969	10	0	1
1970 - 1979	35	4	23
1980 - 1989	25	2	8
1990 - 1993	33	5	13
TOTALS		11	45

Figure 1. Histogram showing age of death for 45 animals necropsied at the San Diego Zoo. Abortions and stillbirths (A/S); Infants (I) 0 - 6 mo; Juveniles (J) 6 mo - 3 y; Young adults (YA) 3 - 10 y; Adults (A) 10 - 20 y; Geriatric (G) > 20 y.

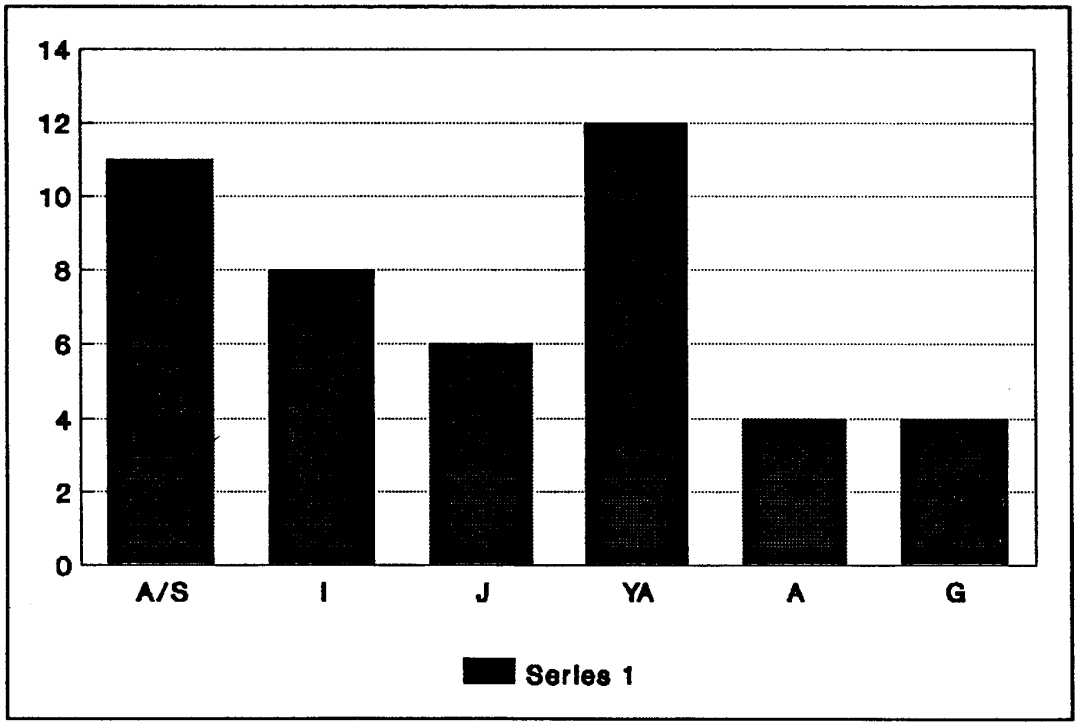


Table 2. Summary of pathology findings on 45 necropsies performed at the San Diego Zoo between 1968 and 1993. Each case may have had multiple pathologic findings. A primary finding is defined as either the cause of death or the most significant finding present in a particular case. Findings are listed in order of frequency of primary findings.

PATHOLOGY FINDING 45 necropsies 1968-1993	FREQUENCY (TOTAL CASES)	FREQUENCY (PRIMARY FINDING)
PULMONARY ACARIASIS (*Pneumonyssus simicola*)	44% (20)	24% (11)
ABORTIONS AND STILLBIRTHS chorioamnionitis, dystocia, genetic	24% (11)	24% (11)
GASTROENTEROCOLITIS hemorrhagic, lymphocytic, plasmocytic, ulcerative, necrotic	20% (9)	13% (6)
INANITION	11% (5)	7% (3)
CARDIOVASCULAR DISEASE dissecting aortic aneurysm, aortic valvular thrombosis and heart failure, and necrotizing myocarditis	7% (3)	7% (3)
GLOMERULAR DISEASE atrophy, membranous, sclerotic	13% (6)	4% (2)
PNEUMONIA (other than acariasis) bronchopneumonia, interstitial	9% (4)	4% (2)
HEAD TRAUMA subdural hematoma	4% (2)	4% (2)
LISTERIOSIS (CNS)	2% (1)	2% (1)
PHYTOBEZOAR,DUODENAL (ginger Hedychium flavum)	2% (1)	2% (1)
TOXOPLASMOSIS	2% (1)	2% (1)
PECTUS EXCAVATUM	2% (1)	2% (1)
HEMOSIDEROSIS liver, spleen, intestines, etc.	29% (13)	0% (0)
INTERSTITIAL NEPHRITIS	7% (3)	0% (0)
GI CANDIDIASIS (tongue, esophagus)	7% (3)	0% (0)

Table 3. Summary of primary necropsy findings comparing etiology to organ system.

ORGAN SYSTEM	INFECTIOUS	NONINFECTIOUS	TOTAL
RESPIRATORY	13	0	13
REPRODUCTIVE	2	9	11
GASTROINTESTINAL	2	5	7
CARDIOVASCULAR	1	2	3
URINARY	0	3	3
NERVOUS	1	2	3
MUSCULOSKELETAL	0	1	1
GENERAL	1	3	4
TOTALS	20	25	45

Table 4. Clinical signs and specific conditions diagnosed clinically in douc langurs at the San Diego Zoo. Diagnostic techniques listed in the third column correspond with numbered diagnoses in second column.

CLINICAL SIGN OR PROBLEM	CLINICAL DIAGNOSES	COMMENTS/DIAGNOSTIC TECHNIQUE
Chronic vomiting and diarrhea with weight loss	1. ulcerative gastritis 2. gastric amebiasis 3. stress and diet	1. endoscopy and biopsy 2. endoscopy, biopsy, and protozoal culture 3. presumptive
Dyspnea and coughing or sudden collapse	chronic pulmonary acariasis (*Pneumonyssus simicola*)	radiographs and microscopic examination of sputum. Repeat tracheal washes failed to diagnose.
Azotemia, hematuria, inanition	1. nephrosis/nephrolithiasis 2. glomerulosclerosis	1. kidney biopsy 2. kidney biopsy
Stranguria, urinary obstruction	cystic and ureteral calculi, calcium carbonate	physical exam, radiographs, cystotomy
Heart murmur	1. aneurysmal aortic dilatation 2. myocardial fibrosis, ventricle	1. cardiac ultrasound 2. cardiac ultrasound
Acute vomiting, linear abdominal mass	phytobezoar, *Acacia* sp.	Physical examination, enterotomy (ref)
Pelvic limb weakness	1. retained placenta and endometritis. 2. post-partum neuromuscular weakness 3. listeriosis	1. cytology, culture, hematology 2. presumptive diagnosis 3. post-mortem diagnosis; lumbar CSF tap and myelogram failed to diagnose.
Tuberculin test responder (6 cases)	non-specific responders (i.e. no evidence of disease)	thoracic radiographs, gastric/tracheal/fecal AFB cultures, comparative tuberculin testing

PARTIAL PNEUMONECTOMY IN A SIAMANG (*Hylobates syndactylus*) AND OTHER APPLICATIONS FOR SURGICAL STAPLING EQUIPMENT

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A 14 year old female siamang (*Hylobates syndactylus*) was evaluated for lethargy, depression, anorexia and coughing. A tentative diagnosis of a respiratory tract infection was made. Because of limitations based on the physical facility, it was elected to treat the animal with oral antibiotics within the exhibit. Unfortunately, the animal refused all types of oral medications offered. The exhibit is 25 ft high and this animal had a 2 year old infant with her making it not feasible to administer antibiotics by injection such as dart or pole syringe. It was, therefore, decided to hospitalize the animal and give her injections with a pole syringe.

The animal was immobilized and radiographs revealed a mass approximately 3.5 cm in diameter in the left caudomiddle lung field. CBC and serum chemistries were within normal limits. The animal was febrile and a tracheal wash was performed and submitted for cytology and culture. Cytology was consistent with infection; however, no organisms were observed. Culture was negative for bacterial growth. She was started on amoxicillin with the hope of converting her to an oral form as soon as possible.

Eight days later, the animal was still depressed, not eating, and not taking oral medications so she was immobilized again for re-evaluation. Fever was still present and the lesion was relatively unchanged radiographically. Percutaneous aspiration of the mass was submitted for cytology and culture. The animal had lost weight and appeared dehydrated. It was given subcutaneous fluids and the antibiotic was changed to enrofloxacin. Radiographs were sent to the University of California at Davis for review as the cytology stimulated concern regarding the potential for neoplasia.

Culture of the aspirate revealed growth of *Pseudomonas aeruginosa* and *E. coli* which were resistent to amoxicillin. The radiologist felt the lesion was most consistent with infection but could not rule out neoplasia. Eight days later, the siamang was evaluated again. Radiographically, the mass was larger but less dense. Fever was present and the body weight was stable.

It was still uncertain whether the lesion was the result of infection or neoplasm; following a month of therapy the lesion had changed little; the animal had to be injected with antibiotics; and this matron was the mother of the other 3 siamangs in the exhibit with her absence resulting in abnormal group dynamics. It was, therefore, elected to surgically remove the lesion. A left fourth intercostal thoracotomy provided access to the lesion. Adhesions were dissected to mobilize the entire left lung from the chest wall. No apparent pulmonary lobulation was identified. The lesion was confined to the caudal half of the left lung. A TP 60 surgical stapling device (Ethicon Endo-Surgery, Cincinnati, OH 45242) was

used to seal the lung parenchyma, blood vessels, and bronchi proximal to the lesion. The affected lung was transected distal to the double row of staggered staples allowing the diseased tissue to be easily removed. The total operative time was 50 minutes.

The animal was treated with enrofloxacin for 6 days, then returned to the exhibit. She remains healthy 10 months later. Histologically the lesion was diagnosed as chronic suppurative interstitial pneumonia. Acid fast stain was negative for organisms. Aerobic and anaerobic cultures were negative for bacteria.

This lesion may have resolved without surgical intervention but would have required several weeks of antibiotic therapy. Surgery provided the advantages of providing a definitive diagnosis, elimination of the diseased tissue, and an early return to the exhibit. The TP 60 surgical stapling device allowed the procedure to be performed quickly with minimal risk for the patient.

The first surgical stapling device was developed in 1909 by Humer Hultl of Budapest. During the 1950's great advances were made in the development of a broad range of stapling instruments primarily in the Soviet Union. Surgical stapling instruments are currently marketed by various medical equipment manufacturers (Ethicon Endo-Surgery; United States Surgical Corporation; 3-M Corporation). They are of lightweight design, pre-sterilized, preloaded and reloadable, disposable, and rapid loading and firing. The staples are biologically inert being made of stainless steel or titanium. When fired, they bend into a B configuration providing hemostasis without collapsing the vital microcirculation through the opening of the loops of the B (figure 1). They generally fire a double row of staggered staples which also allows for some circulation between the staples assuring the viability of the 2-4 mm of tissue remaining distal to the staples.

Surgical staples have been developed for skin apposition, ligating and dividing vessels, performing intestinal end to end anastomosis, sealing tissue for transection distal to the staples, and sealing tissue on two sides and cutting between. In this discussion, the TP Linear Stapler and the TLC Linear Cutter (Ethicon Endo-Surgery) will be emphasized.

The letters used to describe these staplers have significance in regard to their application. The letter T represents the titanium construction and the letter L refers to the linear configuration of the double row of staggered staples. The letter C indicates that these devices not only fire two double rows of staggered staples but it also cuts the tissue between. Additional modifiers include V which indicates the leg length of the staples is shorter so that when fired they close more tightly. These are more applicable for hemostasis and the designation V is for vascular occlusion. The letter H indicates the staples are made of a "H"eavier gage of wire and have a longer leg length. These staples do not close as tightly and are applicable for thicker, tougher tissues (e.g. a thick, calcified bronchus). The letter P indicates Plus and R is used for the reload cartridges. The TCT Linear Cutter is used for thick tissue. The linear staplers (TL and TP) are available in 30, 60, and 90 mm lengths and the linear cutters (TLC and TCT) are available in 55 and 75 mm lengths.

Stapling is faster than traditional suturing techniques which decreases operating and anesthesia time. Tissue trauma is reduced as there is less manipulation required with stapling instruments. Additionally, there is less opportunity for tumor cell or bacteria to be released from the tissue. In many instances, the stapling device can be used where access for suturing may be limited (e.g. thoracic surgery). These devices are marketed for single use in humans; however, their expense makes single use in veterinary medicine difficult to justify. Reload cartridges are available.

These devices have been used in veterinary medicine for partial or complete lung lobectomy or pneumonectomy, partial or complete hepatic lobectomy, partial splenectomy, resection of right atrial appendage for hemangiosarcoma, enterotomy closure, gastrotomy closure, typhlectomy, gastrointestinal resection-anastomosis, cholecystoenterostomy, large uterine or ovarian pedicles, partial prostatectomy and resection of prostatic cysts.

To operate the linear stapler, the tissue to be removed is passed through the jaws of the stapler to the level at which the transection is proposed. The jaws are then closed and the adjusting knob is set to the desired amount of tissue compression. For thick and friable tissues such as liver, less compression is used as excessive compression will cut the tissues including the vessels allowing hemorrhage to occur. The safety is released and the trigger is squeezed once until it locks into the handle. The tissue is then sealed with the staples. The device has a cutting guide which may then be used with a scalpel to transect the tissue distal to the staples. The jaws are released and the instrument removed. The stump should be inspected for leakage of fluid, blood, or air. The linear stapler fires the line of staples perpendicular to the long axis of the device which allow application deep within body cavities and is applicable to such procedures as resection of the right atrial appendage, pulmonary lobectomy or pneumonectomy, hepatic lobectomy, prostatic cyst resection, partial splenectomy, uterine or ovarian pedicles, and similar procedures (fig 2).

The linear cutters fire two double rows of staggered staples and also cut the tissue between them sealing both sides of the transected tissue. The staples are fired along the same axis as the device. As a result, they are applicable to tissues which can easily be exteriorized from body cavities. They are useful for various gastrointestinal procedures including anastomoses. The device consists of two halves which are fitted together over the tissue to be transected. The intermediate position allows for adjustment to be made to the tissue between prior to closing the instrument. Once the device is closed the firing knob is pushed forward firing all 4 rows of staples and transecting the tissue. The firing knob is pulled back to its original position and the halves of the instrument are separated. The seal should be inspected and reinforced with sutures where necessary. Especially for side-to-side intestinal anastomosis, the apices of the incisions may need to be reinforced with sutures.

These devices may be used singly or in combination to perform a variety of procedures. As an example, a side-to-side intestinal anastomosis may be performed by opposing the two transected portions of intestine to be anastomosed. The TLC is inserted with one half of

the instrument into the lumen of each segment of intestine. The instrument is discharged sealing the 2 segments of intestine and incising between them anastomosing the 2 lumina. The ends of both segments are then sealed with a TP instrument (figure 3).

The LDS stapler (United States Surgical Corporation, Norwalk, CT) is for vascular ligation. The hook shaped end of the instrument accepts a vascular pedicle. When the instrument is fired, it places two staple ligatures around the pedicle and transects between them. This device makes splenectomy simple.

The EEA stapler (United States Surgical Corporation) is used for end-to-end intestinal anastomosis. It is a long, tubular unit that accepts a cylindrical cartridge. It fires a double row of staples circumferentially connecting the lumina of two hollow organs to form an inverting anastomosis. A circular blade simultaneously cuts the redundant ring of intraluminal tissue to create a new lumen. It is available with cartridges of diameter of 31, 28, or 25 mm OD providing an internal diameter of 21, 18, 15 mm.

SUGGESTED READING

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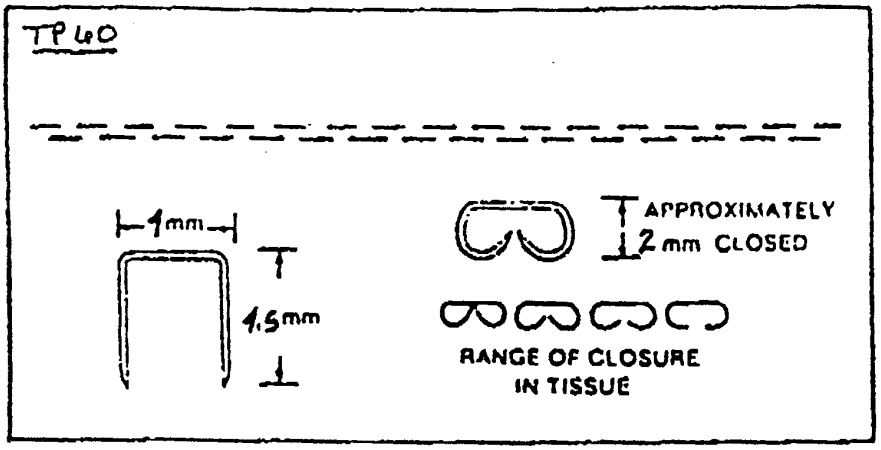

TP 60
1mm
1.5mm
APPROXIMATELY
2 mm CLOSED
RANGE OF CLOSURE
IN TISSUE

FIG 1

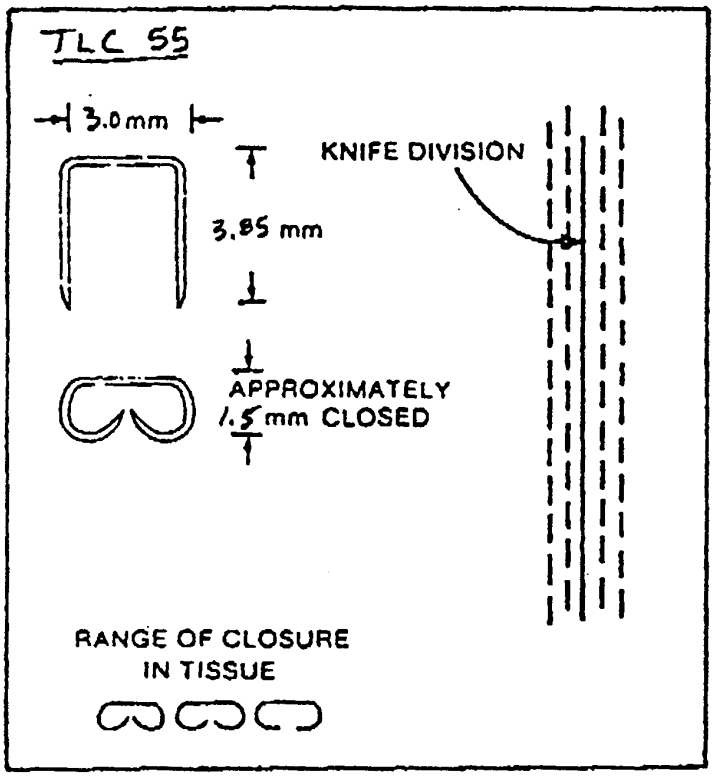

TLC 55
3.0mm
KNIFE DIVISION
3.85 mm
APPROXIMATELY
1.5mm CLOSED
RANGE OF CLOSURE
IN TISSUE

FIG 2

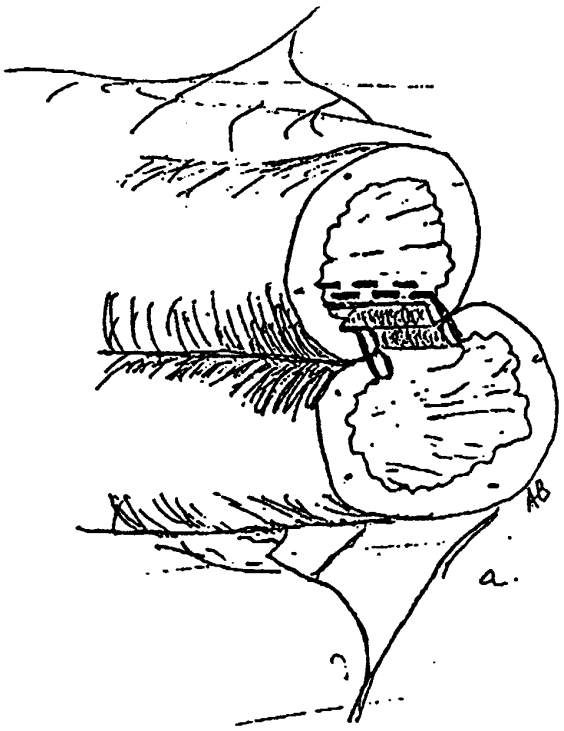
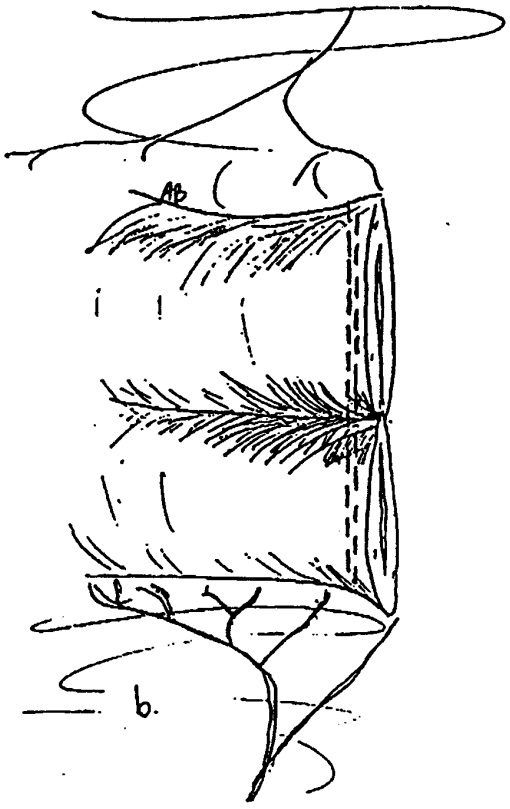


FIG 3

a. THE TLC IS USED TO CREATE A STOMA BETWEEN 2 SEGMENTS OF INTESTINE.



b. THE ENDS OF THE INTESTINES ARE SEALED WITH THE TP DEVICE.

Linear Staplers

Product Codes

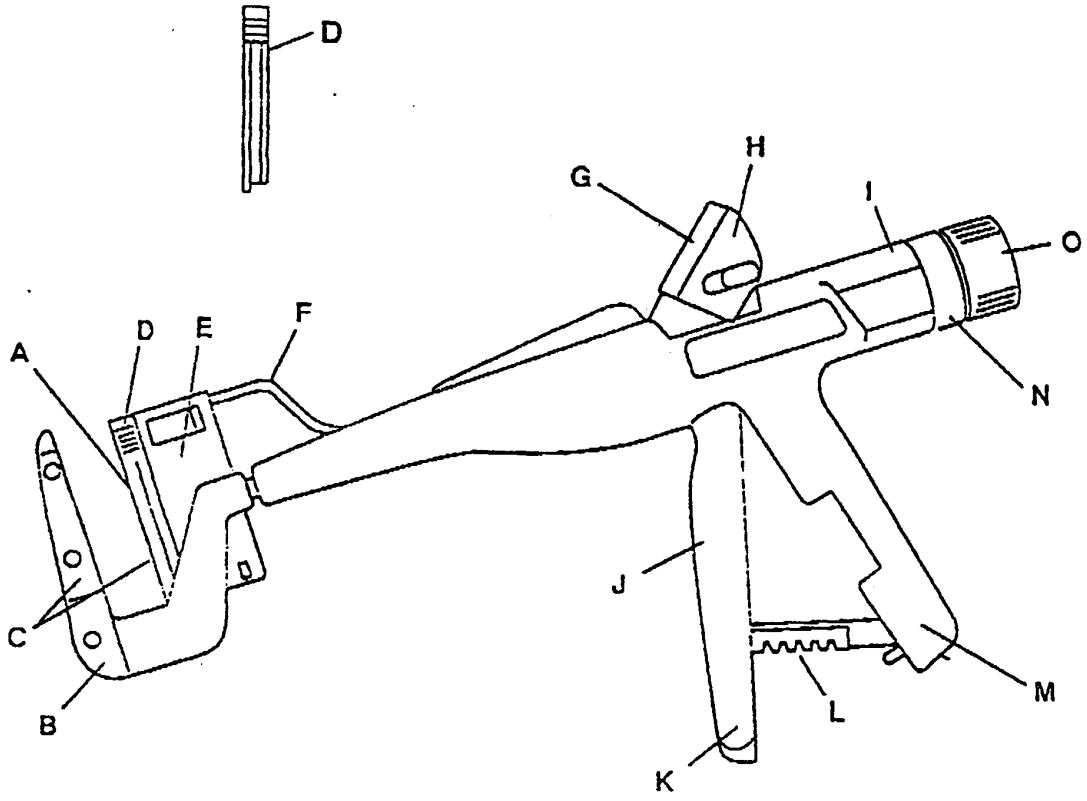
PROXIMATE* TL LINE (WITH TITANIUM STAPLES)

Product Code	Graphics	Staple Line	# of Staples	Crown Width	Leg Length	Wire Diam	Range of Closure	Reload
TL30	White	30mm	11	4.0mm	4.5mm	.23mm	1.0-2.5mm	TR30
TL60	White	60mm	21	4.0mm	4.5mm	.23mm	1.0-2.5mm	TR60
TL90	White	90mm	33	4.0mm	4.5mm	.23mm	1.0-2.5mm	TR90
TLV30	Red	30mm	15	3.0mm	2.5mm	.20mm	1.0mm	TRV30
TLH30	Yellow	30mm	11	4.0mm	4.8mm	.30mm	1.0-2.5mm	TRH30
TLH60	Yellow	60mm	21	4.0mm	5.5mm	.28mm	1.5-2.5mm	TRH60
TLH90	Yellow	90mm	33	4.0mm	5.5mm	.28mm	1.5-2.5mm	TRH90

PROXIMATE* TP (RL "PLUS") LINE WITH TITANIUM STAPLES

Product Code	Graphics	Staple Line	# of Staples	Crown Width	Leg Length	Wire Diam	Range of Closure	Reload
TP30	White	30mm	11	4.0mm	4.5mm	.23mm	1.0-2.5mm	TR30
TP60	White	60mm	21	4.0mm	4.5mm	.23mm	1.0-2.5mm	TR60
TPV30	Red	30mm	15	3.0mm	2.5mm	.20mm	1.0mm	TRV30
TPH30	Yellow	30mm	11	4.0mm	4.8mm	.30mm	1.0-2.5mm	TRH30
TPH60	Yellow	60mm	21	4.0mm	5.5mm	.28mm	1.5-2.5mm	TRH60
TPH90	Yellow	90mm	33	4.0mm	5.5mm	.28mm	1.5-2.5mm	TRH90

PROXIMATE* TP (RL PLUS)



A. Jaws

B. Anvil

C. Reloading Unit

D. Reloading Unit Gripping Surface

E. Cartridge Housing

F. Retaining Pin

G. Approximating Lever

H. Thumb Tabs

I. Alignment Arrow

J. Trigger

K. Trigger Release Button

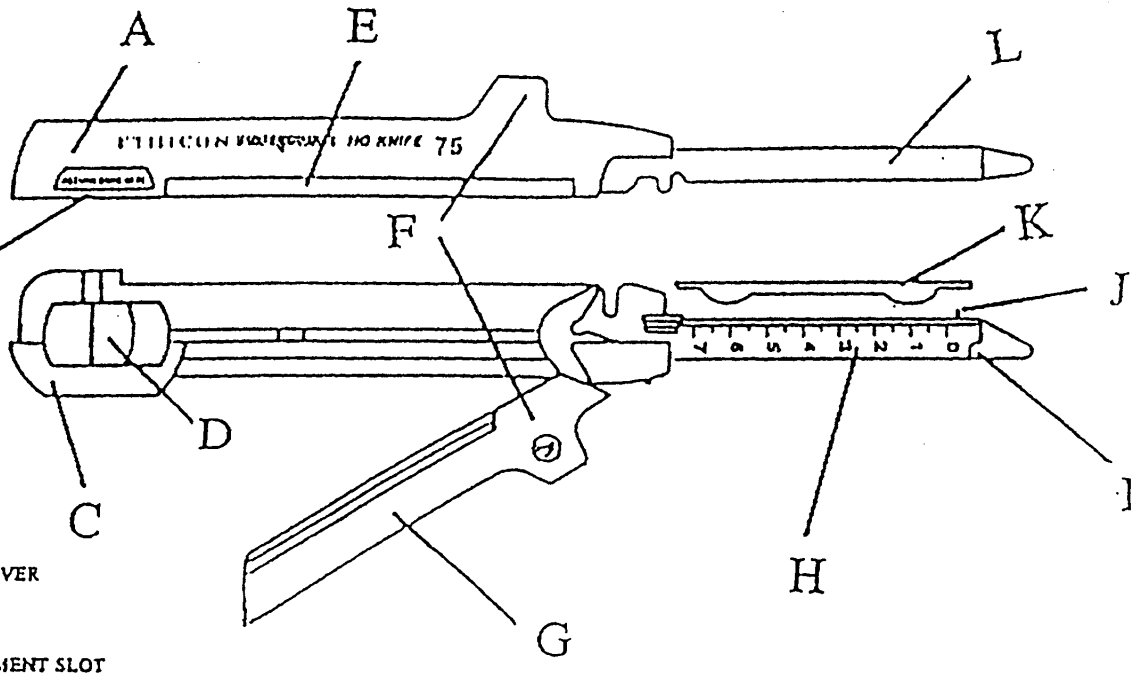
L. Safety

M. Handle

N. Gap Setting Scale

O. Adjusting Knob

PROXIMATE* LINEAR CUTTER 75 WITH LOCKOUT



A. ANVIL HALF
B. RETURN KNOB HERE
C. CARTRIDGE HALF
D. FORING KNOB
E. LOCKING RIB
F. SHOULDERS
G. ALIGNMENT/LOCKING LEVER
H. CARTRIDGE FORK
I. RELOADING UNIT ALIGNMENT SLOT
J. TISSUE RETAINING BUTTON
K. STAPLE RETAINING CAP
L. ANVIL FORK

ETHICON CUTTER vs. U.S.S.C. GIA's

PRODUCT SIZE		OPEN CROWN & LEG (mm)		CLOSED LEG (mm)		# STAPLES		WIRE DIAMETER		STAPLE LINE		CUT LINE	
Ethicon	USSC	Ethicon	USSC	Ethicon	USSC	Ethicon	USSC	Ethicon	USSC	Ethicon	USSC	Ethicon	USSC
Thin 55	Thin 60	3.0 x 2.5	3.0 x 2.6	1.0	1.0	50	60	.20	.21	57	60	53	54
Reg. 65	Std. 60	3.0 x 3.85	3.0 x 3.8	1.5	1.5	50	60	.20	.21	57	60	53	54
Reg. 75	Std. 60	3.0 x 3.85	3.0 x 3.8	1.5	1.5	70	84	.20	.21	77	80	73	74
Thick 55		3.0 x 4.5		2.0		50		.23		67		53	
Thick 75	Hvy 80	3.0 x 4.5	3.0 x 4.8	2.0	2.0	70	84	.23	.24	77	80	73	74

DISEASES OF ZOO MARMOSETS, TAMARINS AND GOELDI'S MONKEYS

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Introduction

Marmosets, tamarins and Goeldi's monkeys (*Callimico goeldii*) are small neotropical primates indigenous to Central and South America. The taxonomy of a number of these species and subspecies appears to be under continual revision but according to current ISIS usage, the three groups are classified as *Callitrichidae* (Calla-trik-id-eye). Earlier classification of marmosets and tamarins as *Callithricidae* (Calla-thris-id-eye) is no longer in vogue. Goeldi's monkeys have also been categorized as *Callimiconidae*. Genera of these families include *Callithrix* the marmosets, (pygmy marmosets have been classified also as *Cebuella*); *Saguinus and Leontopithicus* the tamarins, and *Callimico* the Goeldi's monkeys. Marmosets are smaller than tamarins and have specialized lower incisors for gathering tree sap (exudates).[8] Tamarins are a diverse group and contain the most species including the endangered lion tamarins (*Leontopithicus rosalia spp.)* and the cotton top tamarin, *(Saguinus oedipus*); Goeldi's monkeys are the more primitive of the 3 groups. [4,7,13]

Callitrichids usually live in family groups lending themselves to interesting exhibitry in zoo collections. Although adaptable, marmosets, tamarins and Goeldi's monkeys are delicate and require careful management for their successful display and propagation. Callitrichids are subject to a number of infectious and noninfectious diseases that need to be controlled or prevented. A recent comprehensive review by Potkay summarizes the world literature on diseases of Callitrichidae,[15] and includes descriptions of infectious, parasitic, nutritional/metabolic, tumors and diseases of special organ systems. Several recent additional disease surveys of various species of callitrichids have also been published.[3,5,9,17,22]

The purpose of this presentation will be to discuss and illustrate some published and unpublished conditions of marmosets, tamarins and Goeldi's monkeys that have occurred in callitrichids mainly from the collection at the National Zoological Park (NZP). Some of these cases were on loan to other zoo facilities at the time of their medical problem. Conditions improved by medical intervention or management changes will be emphasized. Selected references will be included when not cited by any of the above surveys.

Infectious Diseases

Viral diseases observed include herpesvirus infection and encephalomyocarditis virus in golden lion tamarins, and callitrichid hepatitis[12,19] and other hepatitis viruses[16] in Callithrix, *Saguinus* and *Leontopithicus* species from a number of sources. Some viral agents, including coronaviruses of questionable pathogenicity have been observed in callitrichids with enteric diseases.[18]

Of numerous bacterial diseases that have been described in callitrichids, *Streptococcus zooepidemicus* sepsis associated with horsemeat was observed in golden lion tamarins, red-bellied tamarins (*S. labiatus*) and Goeldii's monkeys,[20] *Yersinia pseudotuberculsis* and enterocolitica occurred in saddle-back tamarin (*S. fusicollis*), golden lion tamarin and Goeldi's monkey. *Pasteurella multocida* tooth infections and sepsis occurred in black-tailed marmoset (*C. argentata melanura*), golden lion tamarins and Goeldi's monkeys.[2] *Salmonella enteritidis*, and *Aeromonas hydrophila* infections occurred in lion tamarins. *Campylobacter* has been commonly isolated with from golden lion tamarins at NZP without clinical disease but has been associated with inflammatory bowl disease in other callitrichids colonies.[18] Enterohemorrhagic colitis occurred in common marmosets (*C. jacchus*) associated with enteroadherent *E. coli* (Dr. James Thompson, Wisconsin Regional Primate Center, unpublished observations).

Parasitisms

Toxoplasmosis occurs sporadically in callitrichids[15] and was observed in NZP golden lion tamarins on loan at 3 other zoos or destined for reintroduction to natural habitats in Brazil. Spirurid nematodes carried by cockroaches and copraphageous beetles represent an important group of helminths in callitrichids. *Pterygodermatites nycticeba* emerged as a significant spirurid in captive callitrichids primarily of golden lion tamarins[10] but has been found also in other tamarin species. *Trichospirura leptostoma* is a commonly found spirurid in the pancreatic duct of callitrichids that has been associated with "wasting disease" in common marmosets.[15] A previously innocuous spirurid of the oral cavity, *Gonylonema pulchrum*, recently emerged as an oral cavity pathogen in goeldi's monkeys.[2] The thorny-headed worm, *Prosthenorchis elegans*, also born by cockroaches remains a cause of fatal peritonitis from gastric penetration by the parasite in zoo callitrichid collections.[9]

Noninfectious Diseases

Several congenital anomalies and familial diseases were recognized in golden lion tamarins including fetal and neonatal malformations, retrosternal diaphragmatic defects,[11] and hyperbilirubinemia resembling the Dubin-Johnson syndrome in humans.[21] An unusually high prevalence of cystine choleliths and septate gallbladder occurs in lion tamarin species and other callitrichids,[14] with biliary obstruction an uncommon sequela.

Common organ-specific entities in callitrichids include a high incidence of glomerulonephritis in some species over six months of age attributed to IgM deposition in glomeruli via immune-mediated mechanisms;[1] a progressive nephropathy in Goeldi's monkeys may have a different pathogenesis. Gastrointestinal diseases including gastric ulcer, ulcerative and lymphoplasmacytic colitis, intussusception, and rectal prolapse were observed in different NZP callitrichid species. Peritonitis of unknown etiology was observed in NZP pygmy marmosets (*C. pygmyaea*).

A wasting syndrome of callitrichids derived from "wasting marmoset syndrome", appears to be a multifactorial condition related to any one of, or a combination of dietary factors, husbandry and chronic parasitic and enteric diseases;[5] it has not occurred as a problem in callitrichid breeding programs, particularly with lion tamarins raised in family groups at NZP.

Nutritional/metabolic, toxic and miscellaneous conditions observed include iron accumulation in callitrichid livers which is common and appears to be innocuous and probably related to dietary iron and lack of natural protective factors. Metabolic bone disease and other micronutrient deficiencies have been reported in callitrichids but appear to be less common today. As with other New World species, callitrichids require vitamin D_3 and need good sources of vitamin C, and E.

Toxicities are sporadic and uncommon; one significant event occurred at the NZP with inadvertent exposure to brodifcoum, a third generation anticoagulant rodenticide in which 4 golden lion tamarins were developed and died with a coagulopathy. Callitrichids are also subject to hypoglycemic episodes and bloat.[15]

A number of sporadic tumors are reported in callitrichids, but the most highly characterized tumor of callitrichids is colonic adenocarcinoma of cotton-top tamarins.[15] Tumors observed of two or more occurrences in golden lion tamarins included biliary adenocarcinomas and adrenal medullary tumors; lymphosarcoma was observed in a golden lion tamarin and a common marmoset, and an unusual melanotic ependymoma occurred in a Goeldi's monkey neonate. Hepatic myelolipomas have been observed in Goeldi's monkeys from NZP and from several other sources,[6] (Dr. L. Phillips, unpublished observations, Brookfield Zoo), and in adrenals of other callitrichid species.[5] The pathogenesis of these quasi-neoplasms is unknown.

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A CASE OF MYCOBACTERIOSIS IN A COMMON MARMOSET (*Callithrix jacchus*)

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Until the early 80's the cases of tuberculosis (TB) in human beings declined steadily. This trend underwent a dramatic reversal in 1986 and since then the percentage of new diseases has only been rising. This unprecedent resurgence of tuberculosis is largely related to the human immunodeficiency virus (HIV) epidemic and has had important consequences not only in human but also in veterinary medicine. A major cause of infection in AIDS patients are mycobacteria that belong to the *Mycobacterium avium* complex (MAC).[2] Under these circumstances the following case of a mycobacteriosis in a common marmoset (*Callithrix jacchus*) should not only be discussed because of the unusual host but also to draw attention to the unexpected circumstances under which this disease may appear.

A 5yr old male common marmoset (*Callithrix jacchus*) was presented with a history of lethargy and weakness of 4 days duration. The keeper had noticed that the animal had not eaten for several days and that the abdomen was distended. No connection to any husbandry change could be found.

The animal was an alpha male in a group of 14 animals. In two other separate rooms 13 more animals were kept for behavioral research. The cages were made out of galvanized-mesh and there was no direct contact to the outside or the observers, who were separated from the animals via a one way window. Feed consisted of fresh fruits, babyfood and a vitamin/mineral supplement.

Physical findings on initial examination were depression, a slightly bloated abdomen and emaciation, the weight of the animal being 240 gr. X-ray examination revealed a mild gaseous gastric distention. Feces were sent for parasitological and bacteriological examination. Neither parasites nor *Salmonella* spp., *Shigella* spp. or *Campylobacter jejuni/coli* were found. A first attempt at therapy consisted of 0.8 mg Butylscopolamin (BuscopanR, Boehringer) i.m., 0.15 ml Polyvitamin (ADE-Vitamin, Veterinaria) i.m. and 18 mg Calcium gluconate 10% (Calcium-SandozR) with 5 ml lactated Ringer's solution s.c..

The marmoset initially responded to the therapy and started to eat again. However ten days later the animal's health deteriorated acutely, showing paralysis of the extremities, and euthanasia was indicated. Necropsy findings included emaciation, some degree of anemia, slightly enlarged mesenteric lymph nodes and focal ulceration of the mucosa in the cranial part of the large bowel. No further changes were seen macroscopically in the intestinal mucosa; the rectum contained normal feces. Tissue specimens from the gut, mesenteric lymph nodes, liver, spleen, kidney, heart, lungs and brain were fixed in 10% buffered formalin and processed routinely for histopathologic examination. Paraffin sections were stained with hematoxylin and eosin as well as with Ziehl-Neelsen stains. Histopathologically a deep focal ulceration with granulation tissue formation was seen in the colon. In the areas

surrounding the ulceration, the submucosa and, to a lesser extent, the lamina propria were diffusely infiltrated with thick sheets of macrophages harbouring large numbers of acid-fast bacterial rods. Further locations involved included the gut wall and mesenterium underneath the ulceration, the capsule and the cortical sinus of the neighbouring mesenterial lymph nodes and, in decreasing amounts, the liver, mucosa of the small intestine, and spleen. Few multinucleated giant cells were obseved in the mesenterial lymph nodes, the macrophages were interspersed with some neutrophils in all organs.

Further histopathological changes observed included: moderate hemosiderin deposits in liver and spleen, a subacute cholangiohepatitis probably associated to an ascending bacterial infection and acute multifocal fibrinoid blood vessel degenerations with thrombosis in the brain. Moderate numbers of *Giardia* spp. trophozoites were seen in the lumen of the small intestine.

Mycobacteria could be cultivated from liver and spleen probes and they were classified as *M. avium-intracellulare*, belonging to the *Mycobacterium avium* complex (MAC). To that purpose the specimens were inoculated onto an agar-based medium (Middlebrook 7H11) and an egg-based medium (Lowenstein-Jensen). Furthermore the radiometric BACTEC method (BACTEC 12B, Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md.) was performed.

Because of the potential risk for human beings as well as for the other animals, it was decided to perform a tuberculin test on all the other remaining 26 marmosets. A PPD avian type tuberculin was used. Prior to the intradermal injection into the eyelid, the animals were anesthetized with ketamine HCl (10 mg/kg, i.m.). From each animal a blood sample was also taken from the vena femoralis. From four animals blood was submitted for CBC and serum chemistry evaluation. The results were within the normal range. From all the animals blood was also sent for an ELISA screening test on HIV1 and HIV2. All were negative. The reaction to the tuberculin test was checked after 24, 48 and 72 hours, but no animal showed any sign of erythema or edema.

Since no new animal had been introduced to the colony for at least three years and the marmoset that had died was born in the institute, it is supposed that the most likely source of infection were leaves from fresh branches, used for climbing, that had been contaminated with avian feces. This supposition is supported by Schröder (1990) who mentioned the role that birds can play in the transmission of *M. avium* to mammals.[3]

New World monkeys seem to be much less susceptible to mycobacterial infections than Old World monkeys. Brack showed in a literature review that between 1909-1991, 65 infections with *Mycobacterium* spp. in New World monkeys have been published.[1] From these 65 cases only 5 concerned *C. jacchus*. Urbain described a case of an *C. jacchus* that died of *M. avium*.[4] Although all New World monkeys are potentially susceptible to mycobacterial infections, because of endogenous and exogenous factors the disease is by far less frequent than in Old World primates. However, this case demostrates that even under laboratory circumstances the disease can develop and have fatal consequences.

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ENDOMETRIAL CARCINOMA IN TWO GOELDI'S MONKEYS (*Callimico goeldii*) IMPLANTED WITH MELENGESTROL ACETATE (MGA)

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Melengestrol acetate (MGA) is a commercially available, synthetic steroid employed widely in human and veterinary medicine for hormone replacement therapy, antineoplastic therapy, and as a contraceptive. The first Goeldi's monkey in this report was implanted with 0.16 gm MGA at 2.5 years of age for contraception. The monkey was then serially implanted after 2 years with an unknown dose, and 2.5 years later with 0.6 gm MGA. The animal presented at 8 years age depressed, febrile, and vomiting, and with labial swelling, and an enlarged uterus. Surgical excision of the uterus and ovaries was conducted one month later. The second Goeldi's monkey was implanted at 2 years of age with 0.16 gm MGA for contraception. Approximately 1.5 years later, ultrasonography disclosed an enlarged uterus with thickened walls. Ovariohysterectomy was conducted 26 months after the initial implant. Both Goeldi's monkeys are currently clinically normal.

Representative tissue from both surgeries were fixed in 10% neutral, buffered formalin, processed for routine light microscopy, sectioned at 3-6 μm, and stained with hematoxylin and eosin. Histologically, both uteri exhibited virtually identical changes. The uterine lumens were moderately expanded by amphophilic to eosinophilic, hypocellular, necrotic debris bound peripherally by moderately to markedly dense infiltrates of degenerate and necrotic neutrophils, with fewer macrophages and occasional lymphocytes. The endometrium was diffusely, moderately to markedly proliferative and consisted of dense, multinodular sheets, nests, cords, and rare papillae of variable sized though usually large, polygonal to fusiform cells with multifocal, intercellular bridges. The mucosal proliferations were often as thick or thicker than the entire myometrium. Rarely, the proliferative cells were covered superficially by normal appearing endometrium. Few to moderate numbers of occasionally bizarre mitotic figures were noted throughout the proliferative cells. The cells extended moderately and multifocally into the superficial myometrium, usually accompanied by the inflammatory infiltrate, and occasionally remote nests of cells were detected in the mid to outer myometrium. The cervix and ovaries were normal

histologically. Immunohistochemistry for vimentin, desmin, and cytokeratins in both cases revealed very slight, multifocal reactivity for desmin within the masses.

The histological findings were most consistent with an endometrial carcinoma. As the morphology of the lesions was virtually identical in both monkeys, and they both lacked significant reactivity to desmin, vimentin, and cytokeratin antibodies, a causative relationship between MGA implantation and formation of these lesions was concluded. Based on the development of identical endometrial neoplasia in two Goeldi's monkeys implanted long-term with MGA, this form of contraception is contraindicated in Goeldi's monkeys, and it should be used only when no other alternatives are available. Further, until detailed clinical and pathologic investigations are performed, MGA's use as a contraceptive in rare nonhuman primates should be minimized. Finally, nonsteroidal contraception should be developed for all exotic species.

AN EPIZOOTIC OF *Salmonella enteritidis* AT THE NATIONAL ZOOLOGICAL PARK

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Salmonellosis is caused by gram-negative bacteria, of the genus *Salmonella*, which has numerous subspecies and serotypes.[1,2] Transmission of infection is generally by the fecal-oral route, and the disease is primarily associated with enterocolitis.[1] However, when the organism enters the lymphatic and vascular circulation, septicemia results and multiple organ systems may be affected.[1] In chronic infections, shedding of the organism occurs and a carrier state may develop with sporadic shedding of the bacteria in feces; recrudescence of the disease may occur in times of stress.[1] *S. enteritidis* is most often associated with sporadic outbreaks in poultry,[3] and its significance has increased over the last five years due to its more frequent association with human food poisoning outbreaks, particularly in Europe.[3]

In early June 1993, two small Madagascar hedgehogs or tenrecs (*Echinops telfairi*) from the Small Mammal House (SMH) at the National Zoological Park, died after a short history of lethargy and respiratory distress. At necropsy, the main findings included: globose heart, recent depletion of adipose stores, splenomegaly, a markedly enlarged and pale yellow tan liver with an edematous thickened gall bladder. On histologic examination, cholecystitis, loss of hepatic lobular pattern with cholestasis, bacterial myocarditis, and splenomegaly with extramedullary hematopoiesis were seen. *S. enteritidis* was isolated from the lung and heart blood of both animals. Two more tenrecs died in mid-August and mid-September, respectively, after showing similar signs and despite antibiotic therapy (sulfatrimethoprim [Di-trim 48%, Syntex Animal Health, Inc., Syntex Agribusiness, Inc., West Des Moines, IA 50265, USA] and later Keflin [Cephalothin sodium, Eli Lilly & Co., Indianapolis, IN 46285, USA]). At necropsy, the same pattern of lesions was noted, although *S. enteritidis* was cultured only from the liver and gall bladder contents of the second animal.

Between 13 August and 14 September 1993, an outbreak of disease occurred in the Egyptian spiny mice (*Acomys dimidiatus*), from two separate locations in the SMH, in which 27 animals died. The majority of these were found dead without showing clinical signs, and often there were no specific findings at necropsy. Later, moribund animals were identified with conjunctivitis, a hunched posture, and in some cases dyspnea. Gross necropsy findings included: fibrinous peritonitis, pericarditis, or pleuritis. *S. enteritidis* was isolated from 11 out of 27 of these mice. Positive cultures grew from a variety of samples, e.g., liver, heart blood, thoracic fluid, intestinal loop, and uterine abscess. Septic thrombosis in multiple organs was

the main histological finding. All the remaining colony mice received seven treatments with chloramphenicol (Chloromycetin sodium succinate, Parke-Davis, Warner-Lambert Co., Morris Plains, NJ 07950, USA) on alternate days.

Due to the difficulty in assessing clearance of the disease in the spiny mouse colony and the possible zoonotic implications, the remaining 69 animals were euthanatized. Few gross lesions were seen on necropsy of these spiny mice. However, in seven mice, mild changes of peritonitis, pericarditis, pleuritis or lymphoid hyperplasia were evident. From 10 spiny mice samples of liver, and loops or swabs of ileum were collected for bacterial culture. *S. enteritidis* was cultured from six of the mice (7/19 samples).

Coincident with the tenrec and spiny mice cases, *S. enteritidis* was isolated from two animals in separate buildings in the Zoo. Salmonellosis was the cause of death in a lesser tree shrew (*Tupaia minor*) in the Zoo's behavioral research collection. The changes seen on histologic examination included multifocal bacterial endocarditis, endometritis, hepatitis, enteritis, myelitis, and widespread lymphoid depletion. *S. enteritidis* was isolated from the heart blood and uterus. In the Zoo's Great Ape House, a female Western lowland gorilla (*Gorilla gorilla gorilla*) was examined for a chronic gluteal abscess, which yielded *S. enteritidis*. In addition, *S. enteritidis* was isolated from one of four wild mice live-trapped in the SMH at the time of the spiny mouse colony euthanasias.

The outbreak of salmonellosis occurred at a time when the spiny mouse colony population and birth rate were high, which may have led to increased stress in the group. The tendency for the mice to cannibalize their dead is considered to be a further factor in perpetuating the infection in the spiny mouse colony. In 1981, when the spiny mouse colony was also at a population peak, a similar epizootic occurred, but on this occasion *Erysipelothrix rhusiopathiae* was isolated from the carcasses.

Salmonella phage types from the infected animals were assessed at the National Veterinary Services Laboratories, and were found to vary throughout the outbreak, however, they followed a clear pattern chronologically. Cultures from the first two tenrecs yielded phage types 14B and 8 respectively. Similarly, type 8 was isolated from the initial spiny mice that died, but from 23 August to 3 September 1993 there was a period when the samples did not conform to a particular phage type. After 4 September 1993 phage type 4 *variant* was cultured from the remaining cases. Phage type 4 *variant* was also found in the last tenrec mortality, the gorilla, the shrew, and the wild mouse. The variation in the phage types could have arisen from a number of phage types being present in the infection of an individual with dominant types developing on culture, or from different sources of infection. From 1980 until this epizootic, *S. enteritidis* has been isolated sporadically from 19 other cases at the Zoo. These findings suggest that spread of infection could have been through vermin movement or from fomites contaminated with feces. Subsequently, an effort was made to reduce wild mouse numbers through enhancement of the vermin control program.

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BENEFITS AND LIMITATIONS OF CLINICAL PATHOLOGY REFERENCE VALUES COMPILED FROM MULTIPLE INSTITUTIONS

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Introduction

The basis for diagnosis of an abnormal state of health rests on the concept of being able to define normal health. For clinical pathology, this requires developing for each species, a reference range of values that can be expected for each test. For certain species and tests, this process is further complicated by differences that are due to the sex, age or physiological status of the specimen. Clinical pathology reference ranges are statistical estimates of the true distribution of normal test results in the healthy population. In zoological medicine, the limited size of the population held within most individual zoo collections, imposes the additional problem of obtaining sufficient samples from an adequate number of individuals of normal health to generate a statistically valid estimate of these normal reference ranges.

One approach to solving the problem of a limited population for sampling has been to create a pooled, multi-institutional central data file for clinical pathology values. Multiple institutions each contribute information to the centralized file and reference values are calculated from the overall pool of data. While this strategy can provide reference ranges based on a larger number of samples and from a larger population, the centralized data file presents potential problems of a different nature that may reduce or even negate its' value. Concerns of potential importance, regarding pooled data, include sample quality problems, laboratory technique differences, sample collection methods, appropriate classification of the health status of the animal and transcription errors during the data pooling process. This paper attempts to examine the value of reference values obtained from pooled data when compared to reference values from a single institution for the same species and same tests.

Ideally, the reference range for a test should mirror the true distribution of the test results for the population (Figure 1). When this occurs, the clinician can be reasonably confident that significant test results will be correctly interpreted. However, when the reference range differs from the true population distribution, two types of error may occur. The first type of error (type A) results in the classification of a normal test result as abnormal based on the reference range (Figure 2). With the second type of error (type B), comparing the test result to the reference range causes an abnormal test result to be classified as normal (Figure 3). Both type A and type B errors can occur with a single reference range (Figure 4).

The goal of this project was to provide some initial estimates of the magnitude of type A and B errors that could be expected when using pooled data, to help assess the utility and validity of the pooled results. This goal was pursued by performing a systematic comparison of reference ranges obtained from a pooled data file maintained by the International Species Information System (ISIS) with reference ranges generated from a local data file maintained by the Milwaukee County Zoo (MCZ).

Methods

Data analysis was performed in May 1994. At this point in time, the ISIS pooled data file (excluding data contributed by Milwaukee County Zoo) contained more than 25,000 clinical pathology records from approximately 13,000 individual animals reported to have been in normal health at the time of sample collection. Records were contributed by 55 institutions in 4 countries, more than 1100 species were represented in the file, and slightly less than 200 species had 25 or more records in this file. The Milwaukee County Zoo had more than 5600 clinical pathology records in the MedARKS software system (ISIS) with 18 species having sample sizes of 25 or more records from animals of normal health. The MedARKS software routines were used to generate reference ranges from the MCZ data set. A program written by one author (JAT) was used to generate similar reference ranges from the pooled ISIS data set. Both programs use a recursive technique in which an initial estimate of the mean and standard deviation is used to identify outlying test values (those more than three standard deviations above or below the mean) which are then excluded from the final calculation of the reference range (mean and standard deviation information) for that test.

When analyzing the two sets of reference ranges, the MCZ reference range values functioned as the representative of the true population curve and estimates of type A and B errors were generated by comparison with the ISIS reference ranges. To decrease problems associated with small sample sizes, reference ranges had to meet stringent criteria for inclusion in the analysis: (1) only those species/test reference ranges calculated from a minimum of 25 records in the MCZ data set were selected for analysis, (2) the ISIS reference range for the same species/test had to be calculated from at least the same number of samples as found in the MCZ reference range. For each test, the type A and B errors were then calculated based on a normal Gaussian distribution. The true population curve was estimated from the MCZ mean $\pm$ 3.09 standard deviations (99.8% of the area under a normal Gaussian curve). The ISIS reference range was calculated as the mean $\pm$ 2.0 standard deviations (95.4% of the area under a normal Gaussian curve). Two standard deviations is commonly used by clinicians to establish reference ranges and, as mentioned, is the method used by the MedARKS software to flag suspect results.

Results

Of the 18 species in the MCZ data set that had a substantial number of records (at least 25) for at least one test, the analysis program identified a total of 216 tests in 11 of these species that met the criteria for inclusion (Table 1). The type A error estimates ranged from 0%

to 67.4%, with an overall mean of 8.4%. ISIS reference ranges calculated from a larger number of records (>250) tended to have slightly smaller estimated type A errors (mean = 8.8%), compared to those calculated from a smaller number of records (25-74; mean = 10.1%). Type B error estimates ranged from 0% to 38.6%, with an overall mean of 3.6%. Again, type B error estimates were lower for large sample ISIS reference ranges (2.2%) when compared to type B error estimates calculated from small sample ISIS reference ranges (4.7%). For both type A and B error estimates, there was extensive variation between tests and species.

Of the 11 species with tests that met the criteria for inclusion, 10 were mammals and 1 was avian. The average type A error estimated across tests for the bird species was 20.2% and the average type B error estimate was 1.3%. For mammals, the equivalent values found were 8.0% and 3.7%.

On a species level, the number of tests that had type A error estimates of <10% were identified, as were the number with type B error estimates <10% (Table 2). Finally, each test was examined in a similar manner to determine how many of the species had type A and type B error estimates that were <10% (Table 3).

Discussion

The single bird species (American flamingo) whose data qualified for this analysis had a higher overall average for the type A error estimates (20.2%), than did the mammal species (overall average = 8.0%). However, Table 2 shows that 4 of the 6 tests for the flamingo actually had type A error estimates of under 10%. The high overall average for this species then is due to extremely high type A errors in the remaining 2 tests (calcium and aspartate aminotransferase). With such a limited data set from a single avian species, it is not possible to make any generalizations regarding birds versus mammals, but the relatively low type A error estimates for 4 of the 6 tests is encouraging.

The preponderance of primate species within the mammal group (6 of 10 species) is probably a reflection of the fact that primates receive regular routine physical examinations and tuberculin testing at most zoos. As a result of this preventative health care, both the MCZ and ISIS data sets contain numerous records from this group of mammals. In general, primates tended to have low type A error estimates, with 98 of 122 tests (80%) having type A error estimates of <10%. For individual species, the percentage of tests with an estimated type A error of <10% ranged from 68.0% (orangutan) to 92.3% (gorilla). Type B error estimates were also quite low for primates, with the majority of tests having an estimated type B error of <10%.

The data for other groups of mammals is more limited, but overall there is a similar propensity for the majority of tests to have type A error estimates of <10%. The Asian elephant had the smallest number of tests (54.5%) with a type A error estimate of <10%, while the greater kudu had the highest number (94.7%). Type B error estimates for other mammals were also comparable to the values seen in primates.

The analysis by test is perhaps the most useful for the clinician. Several tests (cholesterol, phosphorus and direct bilirubin) had low type A error estimates (<10%) for all the species that had sufficient records for inclusion in the analysis. Another group of tests (calcium, glucose, hemoglobin and red blood cell count) had low type A error estimates (<10%) for 90% of the species. Finally, some tests showed very poor agreement between the ISIS and MCZ data sets (e.g. lactate dehydrogenase, and alanine aminotransferase) and as a result, the majority of type A error estimates were >10%. This analysis did not attempt to determine the basis for poor agreement between the ISIS and MCZ data sets, but it may be that these particular tests are more sensitive to the potentially complicating factors mentioned earlier (i.e. sex, age, physiological status, sample handling, laboratory techniques, etc.). Much larger pooled and single institution data sets would be needed to attempt to account for these factors in the analysis.

For the clinician, the usefulness of reference ranges derived from pooled data depends on agreement between the true population values and the reference range. The analysis presented here indicates that reference ranges from pooled data, in general, agree quite well with reference ranges established by a single institution. Species and sample size seemed to have less effect on calculated error estimates than did specific tests. Some tests (e.g. cholesterol) showed excellent agreement, while other tests (e.g. lactate dehydrogenase) showed poor agreement, and their estimated type A errors for pooled data reference ranges were quite high. Results should always be interpreted with caution when relying on pooled data reference ranges, but the information presented in Table 3 should assist the clinician in weighing the value of specific tests.

It is clear that the estimates of the type A and B errors presented here rest on a number of assumptions that might be challenged for specific species and/or tests. First, a normal Gaussian distribution was assumed; deviations from this distribution will impact the estimates of type A and B errors. However, for test result distributions with fewer test values in the tails of the curve, the true type A and B errors could actually be lower than those calculated under the assumption of a normal distribution. It is also possible, for a variety of reasons, that the MCZ reference ranges may not always accurately represent the true range of expected values for a particular species (when the entire captive population is considered). However, for the clinician working with a limited population at a single institution, reference values calculated from those individual animals will (by definition) represent the best estimate of the true population values (for that particular group of captive animals). We feel the analysis presented in this paper adequately simulates this situation.

Ideally, every institution should generate statistically sound reference ranges from their own records. However, in reality, limited populations and limited records from animals in normal health will always result in a role for reference ranges calculated from multi-institutional pooled data. For the clinician attempting to evaluate clinical pathology results using reference ranges calculated from pooled data, the analysis presented here should provide both guidance and reassurance.

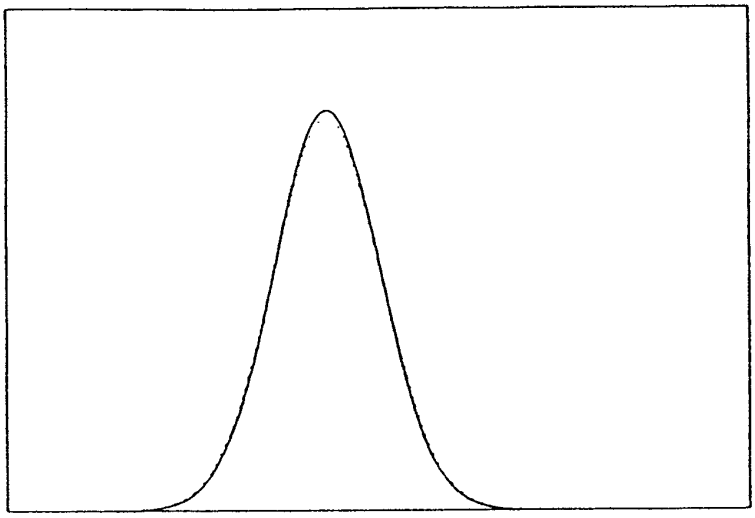


Figure 1 Demonstrates concordance between true population distribution (solid line) and population estimate from pooled data values (broken line).

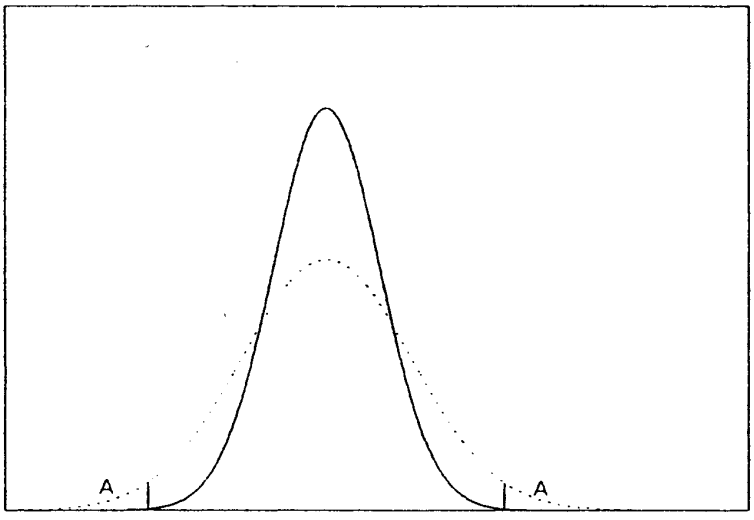


Figure 2 Demonstrates type A errors (classifying abnormal results as normal). Solid line represents true population distribution. Broken line represents population estimate from pooled data.

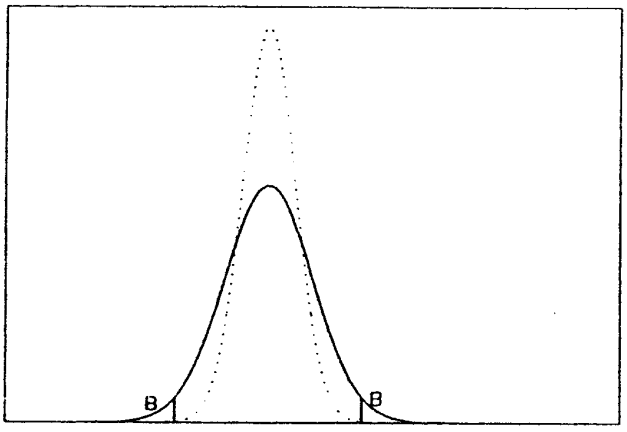


Figure 3 Demonstrates type B errors (classifying normal results as abnormal). Solid line represents true population distribution. Broken line represents population estimate from pooled data.

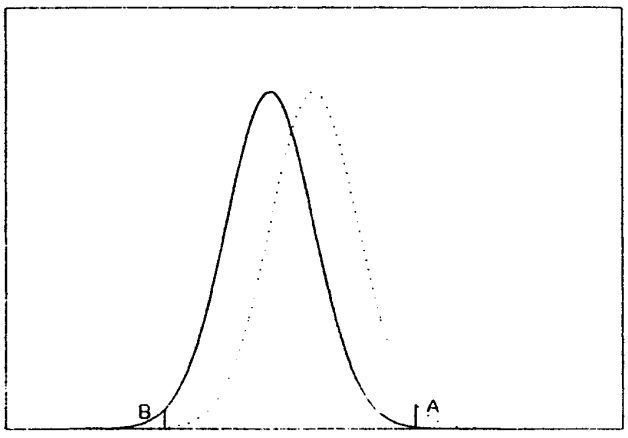


Figure 4 Demonstrates both type A and type B errors. Solid line represents true population distribution. Broken line represents population estimate from pooled data.

Species	Tests included
Phoenicopterus ruber (American flamingo)	6
Cercopithecus diana (Diana guenon)	4
Colobus guereza (Colobus monkey)	23
Papio sphinx (Mandrill baboon)	24
Gorilla gorilla (Gorilla)	26
Hylobates syndactylus (Siamang)	20
Pongo pygmaeus (Orangutan)	25
Elephas maximus (Asian elephant)	25
Aepyceros melampus (Impala)	22
Tragelaphus strepsiceros (Greater kudu)	19

Table 1: Species and number of tests that met selection criteria for analysis.

Species	Type A error <10%	Type B error <10%
Phoenicopterus ruber	4/6 (66.7%)	6/6 (100.%)
Cercopithecus diana	3/4 (75.0%)	4/4 (100.%)
Colobus guereza	21/23 (91.3%)	20/23 (87.0%)
Papio sphinx	18/24 (75.0%)	23/24 (95.8%)
Gorilla gorilla	24/26 (92.3%)	21/26 (80.8%)
Hylobates syndactylus	15/20 (75.0%)	17/20 (85.0%)
Pongo pygmaeus	17/25 (68.0%)	24/25 (96.0%)
Elephas maximus	15/25 (60.0%)	24/25 (96.0%)
Aepyceros melampus	17/22 (77.3%)	20/22 (90.9%)

Table 2: Number of tests for each species with an estimated error of <10%, the total tests included for that species and the percentage of tests in the <10% error category.

Clinical Pathology Test	Type A <10%		Type B <10%	
Cholesterol	9/9	(100.%)	8/9	(88.9%)
Phosphorus	9/9	(100.%)	7/9	(77.8%)
Direct Bilirubin	4/4	(100.%)	2/4	(50.0%)
Iron	1/1	(100.%)	1/1	(100.%)
Platelet count	1/1	(100.%)	1/1	(100.%)
Calcium	9/10	(90.0%)	9/10	(90.0%)
Glucose	9/10	(90.0%)	8/10	(100.%)
Hemoglobin	9/10	(90.0%)	10/10	(100.%)
Red blood cell count	9/10	(90.0%)	10/10	(100.%)
Blood urea nitrogen	8/9	(88.9%)	9/9	(100.%)
Chloride	8/9	(88.9%)	7/9	(77.8%)
Potassium	8/9	(88.9%)	8/9	(88.9%)
Sodium	8/9	(88.9%)	8/9	(88.9%)
Hematocrit	8/10	(80.0%)	9/10	(90.0%)
Total protein (colorimetry)	8/10	(80.0%)	8/10	(80.0%)
White blood cell count	8/10	(80.0%)	10/10	(100.%)
Alkaline phosphatase	7/9	(77.8%)	9/9	(100.%)
Globulin	3/4	(75.0%)	3/4	(75.0%)
Creatinine	6/9	(66.7%)	8/9	(88.9%)
Triglyceride	4/6	(66.7%)	4/6	(66.7%)
Uric acid	6/9	(66.7%)	9/9	(100.%)
Aspartate aminotransferase	6/10	(60.0%)	9/10	(90.0%)
Albumin (colorimetry)	4/7	(57.1%)	6/7	(85.7%)
Total bilirubin	5/9	(55.6%)	7/9	(77.8%)
Indirect Bilirubin	2/4	(50.0%)	4/4	(100.%)
Alanine aminotransferase	4/9	(44.4%)	9/9	(100.%)

Table 3: Number of species for each test with an estimated error of <10%, the total species for that test and the percentage of species in the <10% error category.

A NOVEL POX INFECTION IN PUDUS (*Pudu pudu*)

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Introduction

The poxviridae are a group of primarily epitheliotropic viruses which produce disease in a wide variety of mammalian, avian, and poikilothermic hosts.[5,8,9] The diseases they cause can be mild and self-limiting or of a generalized nature with high mortality and medical or economic importance.[7,8,9] Six genera have been described including Orthopoxvirus, Avipoxvirus, Capripoxvirus, Leporipoxvirus, Suipoxvirus, and Parapoxvirus. While many of these viruses have characteristic features, serologic procedures and viral isolation are required for definitive diagnosis of any given poxviral infection.[9]

The most extensively studied viruses are those known to cause disease in man or domestic animals. In cattle, sheep, and goats, the genera of concern are the ortho, capri, and parapoxes. While limited work on the poxviral infections of wildlife has been done, there are many reports of parapox infections and a few capripox infections in a variety of non-domestic species.

Contagious ecthyma (CE), a parapoxvirus, has spontaneously occurred in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*), mountain goats (*Oreamnos americanus*),[14] Dall sheep (*Ovis dalli dalli*),[16] muskoxen (*Ovibos moschatus*),[4] and others. Experimental transmission of CE has been reported in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), pronghorn (*Antilocapra americana*) and wapiti (*Cervus elaphus nelsoni*).[12] Of the capripoxes, Lumpy skin disease (LSD) has been detected serologically in African buffalo (*Syncerus caffer*)[3] and oryx (*Oryx leucoryx*) [6] and has been experimentally transmitted to giraffe (Giraffids) and impala (*Aepyceros melampus*).[17]

In the past decade, spontaneous outbreaks of unclassified pox infections have occurred in cervids.[1,16] In this paper, we report the first known occurrence of an unclassified poxvirus in pudus (*Pudu pudu*).

Materials and Methods

History:

All animals involved were housed in the same single species exhibit since 1983. They were maintained in family groups of breeding pairs with offspring. With the exception of "Pedro", all of the affected animals were born in the collection. "Pedro" (NYZS 83114) originated from Chile, S. America, but, at the time of the outbreak, had been in the collection for approximately 5 years.

Prior to 1983 the exhibit was unused for a number of years. Before that time, it housed red pandas (*Ailurus fulgens*) and muntjac (*Muntiacus reevesi*). The exhibit had been renovated before the pudu were moved into it in 1983.

Keeper staff caring for the pudu had no known contact with swine, cattle, sheep or goats. Possible contact with Bay duikers (*Cepholophus dorsalis*) may have occurred. However, there is no record of a similar disease problem in duikers at any time.

Necropsy: A complete necropsy was performed on "Vanessa". Samples of all organs were routinely examined by H + E. Sections of rumen, tongue, esophagus, liver, spleen and kidney were placed in sterile bags and frozen at -80°C. Oral and rumen lesions were swabbed for bacteriologic isolation.

Following microscopic review, a fresh full-thickness skin biopsy was collected from a live animal with active oral and cutaneous lesions This was shipped on ice packs to the F.A.D.D.L. along with a complete set of fixed tissues, frozen tissues, H & E stained glass slides, and kodachromes of lesions.

Virus isolation: Skin biopsy tissue from "Boo-Boo" was homogenized in 10 parts Eagles minimal essential medium (EMEM) and clarified by centrifugation at 3000 g for 15 min. supernatant was inoculated onto growing cell cultures of secondary fetal bovine lung (FBL), secondary lamb testicle (LT) and vero cells. Cells were grown with EMEM supplemented with 10% fetal bovine serum and 2% antibiotics in a CO_2 incubator at 37°C. With each passage, cells were frozen and thawed, centrifuged and supernatant fluid passed into freshly seeded cell cultures. Third passage virus made in vero cells was used for animal inoculation, neutralization studies, and stored at -70°C in EMEM with 20% fetal bovine serum.

Electron microscopy: For negative staining, virus was propagated in vero cells for 5 days when there was 50% cytopathic effect (CPE). The media and cells were frozen, thawed, and then clarified by centrifugation at 3000 g for 10 min. The supernatant was centrifuged at 40,000 g for 1 hour and the pellet was resuspended in 100 ul of media. Formvar-coated grids were floating on a drop of resuspended virus for 5 min. stained with 4% phosphotungstic acid (PTA), pH 7.4 and examined with a transmission electron microscope (Zeiss EM10).

An esophageal lesion from "Vanessa" was selected from formalin fixed tissues to embed for electron microscopy. The tissue was osmium post-fixed for 30 min, dehydrated in graded ethanol to 100% ethanol and embedded in LR White resin. Thin sections were stained with uranyl acetate and lead citrate.

Direct and Indirect fluorescent antibody (IFA) tests:

Monolayers of virus infected vero cells grown in 8 chamber slides were fixed in acetone at 3 days post-inoculation when there was 30% CPE. Serum diluted 1:10 in PBS was used for

the IFA test. For direct fluorescence, a fluorescein conjugate of convalescent goat pox antiserum was used which cross-reacts with all capripox viruses. This was diluted 1:5 in PBS.

Neutralization index (NI) test: The NI test using serum at a final 1:20 dilution and ten-fold dilutions of virus was performed as described (COTTRAL,1978) using 15,000 vero cells/microtiter well. Sera compared included bovine convalescent serum for lumpy skin disease (LSD), ovine convalescent serum for sheep pox (SP), mouse serum pool for vaccinia virus, mouse serum pool for canary pox, swine serum for swine pox (SwP), bovine serum for pseudocowpox, swine serum for African swine fever (ASF), and normal bovine serum. Two rabbit sera from rabbits hyperimmunized with the pudu isolate were also titered using the NI test against the pudu isolate, LSD virus and SwP virus. A swine testicle cell line, passage 145, was used to cultivate SwP virus for this test.

Animal inoculation: All animals were housed at FADDL in animal rooms in the biocontainment level-3 diagnostic laboratory building. Two adult sheep and two yearling steers were inoculated by various routes to determine pathogenicity of the viral isolate. One sheep was inoculated by the intradermal route in the lip in 2 sites with 0.2 ml of inoculum, titer of 10^5 plaque forming units (PFU)/ml. Another was inoculated with 5 ml IV and 0.2 ml dropped in each eye. One steer was inoculated with 10 ml IV, 0.2 ml intradermally in 2 sites in the lower lip, 1 site in the muzzle, and 0.2 ml dropped in each eye. A second steer was inoculated intradermally with 0.2 ml in 4 sites in the lower lip, 1 site in the muzzle, and 0.2 ml dropped in each eye. Sera were collected before inoculation and after 28 days.

Two SPF rabbits 2 months of age were inoculated at 2 week intervals with 10^6 PFU of purified virus in complete Freund's adjuvant initially and then twice with virus in incomplete Freund's adjuvant. At each inoculation, 5 subcutaneous sites were inoculated with 0.1 ml each. Serum samples were collected before inoculation and at 6 weeks.

Results

Necropsy Findings: Only one animal died during the course of the outbreak. A 2 year old adult female pudu developed lethargy and vague neurologic signs. She was in poor condition and weighed only 6.15 kg. No cutaneous lesions were seen.

The oral lesions consisted of many 3-5 mm yellow-white papules on the oropharynx and epiglottis and 5-10 mm umbilicated ulcers involving the tongue, buccal mucosa, and hard and soft palates. The ulcers had slightly raised yellowish borders. Fifteen to twenty ulcers were found in the mucosa of the cranial rumen and ruminal pillars. These were visible from the serosal surface. There were miliary erosions on the tips of rumen papillae. Numerous 5-20 mm erosive lesions were also seen in the mucosa of the reticulum and omasum. The remainder of the gastrointestinal tract was unremarkable. All lymph nodes were mildly enlarged and hyperemic. Additional findings included moderate hepatic and marked pulmonary congestion. A focal, 3 cm in diameter, fibrous white lesion was found on the pleural surface of the right lung.

Bacteriology: Many *Peptostreptococcus spp.* and many *Bacteroides spp.* were isolated from the rumen. No pathogenic organisms were isolated from the oral cavity.

Microscopic Findings:

GI tract: Mucosal lesions were well demarcated and appeared to progress from ballooning degeneration to epithelial erosion and ulceration. Mild lesions had 2-3 x thickening of the stratum spinosum due to marked cytoplasmic swelling and granularity with accompanying nuclear vesiculation and chromatin margination. There was also marked hyperkeratosis in all forestomachs and many bacterial colonies in the keratin of the omasum.

Individual epithelial cells contained single to multiple irregularly shaped, eosinophilic or basophilic intracytoplasmic inclusion bodies. These ranged from 2 to 5 μm in diameter and sometimes displaced a crescent-shaped nucleus.

More advanced lesions exhibited central erosions and intraepithelial neutrophilic infiltrates of varying severity in the stratum granulosum and/or stratum spinosum. Moderate to large numbers of a mixed population of lymphocytes, plasma cells, histiocytes and neutrophils were present in the propria and submucosa.

The most advanced lesions were severely ulcerative. The centrally ulcerated areas contained a coagulum of amorphous cellular debris, degenerate neutrophils, desquamated epithelial cells and bacterial colonies. Bordering cells exhibited severe ballooning degeneration but intracytoplasmic inclusion bodies were rarely found.

All stages of the mucosal lesions were accompanied by submucosal vascular congestion. There was no evidence of a vasculitis or lymphangitis.

The only skin lesions available for review were those in the mucocutaneous junction of the lip. Epidermal changes were similar to those described in the gastrointestinal tract. Ballooning degeneration was prominent in the stratum spinosum including that of follicular epithelium. Sebaceous glands were unaffected. There was a mixed dermal inflammatory infiltrate of varying severity in affected areas.

Lymph Nodes: The retropharyngeal and ruminal lymph nodes had marked medullary congestion and hemorrhage. There was multifocal acute coagulative necrosis of both cortical and medullary tissue and marked sinus histiocytosis. Intracytoplasmic inclusion bodies were occasionally found. There was moderate to severe cortical atrophy with no distinct follicles in most nodes.

Lung: There was diffuse, severe, alveolar congestion and multifocal edema. A focal mycotic granuloma was found adjacent to the bronchus.
Heart: Multifocal fragmented, hypereosinophilic myofibers with hyperchromatic, pyknotic nuclei were found throughout the myocardium.

Adrenal: There was focal medullary hemorrhage.
Brain, pituitary, kidney, liver, and thymus were unremarkable.

Virus cultivation: Cytopathic effect (CPE) consisting of cell rounding and plaque formation was observed first in FBL and LT cells at 2-3 days. Vero cells had similar CPE at 3 days and 90% CPE on day 4. Intracytoplasmic inclusion bodies were found in all cultures using H&E stain.

Electron microscopy of virus isolate: Negative stained preparations of virus grown in vero cells had numerous slightly pleomorphic virions measuring 200 x 245 nm. The virions were "brick" shaped with rounded edges typical of pox virions. The surface of the virus had a distinctive reticular pattern of tuft-like filaments unlike the usual basket weave pattern typical of other pox viruses. There was no helical pattern typical of parapox virions.

Electron microscopy of pudu tissues: Numerous pox-like virions were found in thin sections of the tongue and esophageal lesions from "Vanessa". These were found in the cytoplasm of keratinocytes. The intracellular virions were usually found singly or in small groups in the cytoplasm in areas free of cytokeratin filaments and often associated with vacuoles. The virions were found in various stages of assembly. Usually electron-dense oval particles with biconcave central nucleoids were found free in the cytoplasm or were sometimes membrane-bound.

Fluorescent Studies:

Using goat and sheep pox antisera for indirect FA studies of viral infected vero cells revealed a weak positive fluorescence in the cytoplasm of cells surrounding plaques. The LSD and contagious ecthyma antisera did not fluoresce. The direct FA conjugate for capripoxes gave diffuse cytoplasmic staining of infected vero cells and strong staining of intracytoplasmic inclusion bodies.

Animal inoculation: None of the sheep, cattle, or rabbits developed any lesions at the sites of inoculation, fever, or other signs of clinical disease. Sera from the 2 sheep and 2 steers inoculated with purified pox virus from the pudu had no neutralizing activity against the homologous virus. Both hyperimmunized rabbits had significant serologic titers against the pudu pox.

Table 1.
Serum neutralization index data comparing the pudu virus with other pox viruses.

Serum	NI vs pudu pox
Rabbit A anti-pudu pox	1.5*
Rabbit B anti-pudu pox	3.0*
Bovine anti-LSD	1.3
Swine anti-SwP	1.3
Sheep anti-SP	0.5
Mouse anti-vaccinia	0.2
Mouse anti-canary pox	0.9
Swine anti-ASF	0.4
Normal bovine	0.0
Bovine anti-pseudocowpox	0.0
	NI vs LSD virus
Rabbit B anti-pudu pox	1.0
Bovine anti-LSD	2.0
	NI vs SwP virus
Rabbit B anti-pudu pox	0.0
Swine anti-SwP	2.0
Rabbit B pre-vaccination	0.0
Normal swine serum pool	0.0

* Titers 1.5 or greater are considered positive.

Discussion

The results of the gross, microscopical, virological, and ultrastructural studies are consistent with the diagnosis of a pox infection in these pudus. The wide distribution of lesions with extension down the esophagus to the omasum with subsequent death of one pudu was similar to that seen with capripox viral infections, particularly GP and SP.[3] Since these diseases may cause high mortality in sheep and goats in Africa, the middle east, and much of Asia and are exotic to the Western hemisphere, diagnostic studies were carried out at the Foreign Animal Disease Diagnostic Laboratory at Plum Island to identify this virus. Susceptible sheep and cattle inoculated with the pox isolate failed to show disease and did not seroconvert to the homologous virus. This suggests that the virus failed to replicate in these species despite multiple routes of inoculation. Had this been one of the known capripox viruses (ie. SP, GP, or LSD), skin lesions, at least at the site of inoculation, and seroconversion would have been expected in both sheep and cattle, or goats had they been used. These viruses are so closely related that LSD is indistinguishable from isolates of SP and GP serologically, and even using restriction endonuclease methods, LSD can only be distinguished from some SP and GP isolates from Nigeria, Iraq, or India.[10]

Because pudus are small ruminants, it seems more likely that the pudu pox would be related to the other capripox viruses of ruminants. To help classify the pudu pox virus, the

neutralization index is often helpful. Since the NI with LSD and SwP sera were close to 1.5, additional tests were carried out with LSD and SwP viruses against rabbit B serum. However, these NI were also below 1.5. It must therefore be concluded that there is little serologic relationship between the pudu pox and LSD or SwP viruses.

Another pox virus has been described in reindeer[1] which causes keratoconjunctivitis and oral and cutaneous lesions. Though this has been less severe than the pudu outbreak and few internal lesions have been found, this virus has caused a similar and recurring disease in reindeer. The reindeer pox has yet to be cultivated *in vitro*, but is larger than the pudu pox measuring 250 x 350 nm.

The pudu lesions share some features of known capripox infections but differ in significant ways. Like SP and GP the pudu lesions extended from the skin and mucous membranes down the esophagus as far as the omasum with associated lymphadenitis in draining lymph nodes. However, the capripoxes,including LSD virus, readily spread and infect adjacent vascular endothelial cells, fibroblasts, skeletal muscle, and even superficial unmyelinated nerves [13] resulting in much deeper lesions that those seen in the pudu. Also, there was no viral pneumonia found in this pudu though a multifocal granulomatous pneumonia is commonly seen in cases of generalized SP and GP and is often the cause of death.[7]

It is likely that this pox virus is not closely related to either the capripoxes or swine pox, but a new pox virus. In fact, the ultrastructural surface features and size of this virus seem to be unique and unlike SP, GP, LSD,[10] swine pox,[2] vaccinia, canary pox, or the parapox viruses. The lack of significant neutralization by antisera to these viral diseases also suggest that this virus is at best a distant relative of known pox viruses of ruminants. Further studies will be required to characterize this seemingly novel pox virus of pudus.

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METASTATIC MINERALIZATION IN PACAS (*Cuniculus paca*)

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Death in pacas and agoutis in multiple zoological parks has been recently reported, and linked to a suspected dietary vitamin D3 toxicity.[1] We report on five additional cases, spotted pacas (Cuniculus paca) which died at the Pittsburgh Zoo between October 1990 and May 1991. Gross and microscopic lesions are illustrated. Five spotted pacas were received at the Pittsburgh Zoo from Lincoln Park Zoo, Chicago, Illinois between october 1990 and February 1991. One animal died during anesthesia for routine handling and physical examination, two had acute clinical deterioration an death, and two had a longer history of weight loss, dehydration, and wasting. Gross and histopathological evaluation of all animals revealed prominent mineralization in multiple soft tissues, especially kidney and heart. Clinical chemistries demonstrated increased serum inorganic phosphate and elevated blood urea nitrogen (BUN) and creatinine in all animals. Serum calcium levels were within normal established rodent (rat) limits. One animal were severely anemic (PCV=10%).

Their diet at both Lincoln Park and Pittsburgh Zoos consisted of a combination of rodent and monkey chow, and supplemental, fruits and vegetables. As previously suggested, the high vitamin D3 levels in various primate diets was suspected to have create a calcium - phosphorous imbalance in the pacas leading to the lesions observed.

Tissue mineralization (calcification) is historically divided into dystrophic and metastatic varieties. Dystrophic calcification is associated with the deposition of calcium, and trace amounts of iron, magnesium, and other mineral salts in degenerating or necrotic tissues. The metastatic form occurs in tissues which appear otherwise normal microscopically, and is generally associated with a derangement in calcium metabolism leading to hypercalcemia.[2] Differential considerations when evaluating mineralization of soft tissues include renal disease (Uremic mineralization), primary hyperparathyroidism, and pseudohyperparathyroidism. Hypervitaminosis D stemming from dietary excess, calcinogenic plant toxins, or certain rodenticides must also be entertained. Less common explanations are inflammation associated with calcium sequestering bacteria, species and strain specific idiopathic organ mineralization (e.g., spontaneous cardia mineralization of DBA mice), and a phenomenon known as calciphylaxis.[3] The specificity of nutritional requirements in various groups is easy to disregard, because the detrimental effects of dietary variation is often unnoticed or subclinical. Occasionally, however, disastrous consequences of either purposeful or inadvertent (as in mixed species exhibits) inappropriate feedings are seen.

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FATAL INFECTIONS WITH *Balamuthia mandrillaris* AMOEBAE IN GORILLAS AND OTHER OLD WORLD PRIMATES

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A 13-year old male Western Lowland gorilla (*Gorilla gorilla gorilla*) presented with a two-month history of progressive lethargy and reduced appetite, which had been attributed to depression resulting from the recent loss of a troop conspecific. Physical examination revealed only suboptimal body weight. Results of extensive hematological, biochemical and serologic testing were negative. Additional diagnostic testing was performed 30 days later due to continued deterioration. In addition to 8% weight loss since the previous exam, chest radiographs revealed an infiltrative disease process. Abdominal and hepatic masses were identified by ultrasound examination. Ultrasound guided biopsies of the abdominal mass revealed granulomatous inflammation and fibrosis, but no organisms were seen.

Exploratory celiotomy confirmed an inoperable disseminated nodular disease. Wedge biopsies of liver obtained during celiotomy exhibited marked granulomatous inflammation and fibroplasia with occasional intra- and extracellular amoebic trophozoites. These amoebae were round to irregular, 40 microns in greatest width, with eosinophilic granular to vacuolated cytoplasm and a single, usually eccentric nucleus. The morphology of the organisms was unusual in that the nucleoli were sometimes multilobulated (2-4 lobes), horseshoe shaped or multiple. Rare binucleate amoebae were also seen. The gorilla remained comatose after surgery and was euthanatized on the second postoperative day for humane reasons.

Postmortem examination revealed a large (15 cm x 10 cm), firm, fibrous, multinodular mass that surrounded the abdominal aorta and extended into the thoracic cavity. There were also confluent firm, tan, infiltrative nodules over the thoracic surface of the diaphragm. The liver contained similar nodules throughout the parenchyma, most prominently near the hilus. There was less extensive involvement of the kidneys, lungs and intercostal muscles. After fixation, the cut surface of the right occipital lobe of the brain exhibited scattered pinpoint to-1 mm diameter dark red foci.

Histologically, the extracerebral lesions consisted of extensive fibrogranulomatous inflammation with amoebae, as described above. The cerebral lesions were necrotizing and granulomatous and contained similar amoebae. A prominent vascular orientation to the lesions was noted in many tissues.

A similar granulomatous amoebic encephalitis with disseminated fibrogranulomatous lesions had been seen in a 5-year-old female Kikuyu colobus monkey (*Colobus guereza kikuyuensis*) that died one month prior to the gorilla. A retrospective search of the pathology files revealed three additional cases of granulomatous amoebic encephalitis in primates since 1973 ---a mandrill (*Papio sphinx*), another Western Lowland gorilla,[1] and a white-cheeked gibbon (*Hylobates concolor leucogenys*). A new genus and species of free-living Leptomyxid amoeba had previously been isolated from frozen brain specimens from the mandrill in this series.[2,3] This organism was subsequently named *Balamuthia mandrillaris*. Indirect immunofluorescence staining of brain sections from all five of these cases with a Balamuthia-specific antibody were positive, while staining with antibodies against five species of *Acanthamoeba* and *Naegleria* were negative. Electron microscopic examination of amoebae in the colobus monkey and the mandrill revealed similar ultrastructural features.

Examination of environmental water sources in the gorilla, colobus and mandrill exhibits revealed several different types of amoebae in 10/27 samples. Some amoebae had spiked pseudopodia, as would be expected for *Balamuthia mandrillaris*, but this finding is not specific. Unfortunately, attempts to isolate these amoebae *in vitro* failed due to overgrowth of the media by bacteria and other contaminants. Based on life cycles of other free-living amoeba, however, soil and standing water are the most likely sources of the organism in the environment.

Conclusions

1. All known cases of amoebic encephalitis at the San Diego Zoo and Wild Animal Park in the past 20 years have been infections with *Balamuthia mandrillaris* free-living amoeba.

2. Infection of extraneural tissues by *Balamuthia mandrillaris* may result in a protracted disease course (months to one year or more); but in all cases so far, central nervous system involvement eventually occurs, resulting in rapid decline and death.

3. Extraneural disease grossly and clinically resembles a malignant neoplasm; histologic examination is required to make the diagnosis.

4. The organism can be morphologically distinguished from *Acanthamoeba* and *Naegleria* by its slightly larger size, the presence of multiple nucleoli and by the fibrogranulomatous inflammatory response induced by organisms in extraneural locations.

5. Natural disease has so far been identified in four species of Old World primates (all from the San Diego Zoo and Wild Animal Park), humans (35 cases) and sheep (three cases). There is no evidence to date of horizontal transmission between animals or humans.

6. There is no known effective treatment in humans or animals, but Pentamidine is recommended at the highest dose the patient will tolerate.

7. Circumstantial evidence implicates soil and standing water in the environment as the most likely sources of the organism.

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LIONS, TIGERS AND BEARS: THE ROAD TO ENRICHMENT

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As zoo veterinarians become more adept at providing for the physical and medical needs of the animals under our care, we can turn our attention toward their psychological needs. Environmental enrichment (EE) should be recognized by animal caretakers as an important tool to promote both the mental and physical health of zoo animals. The basis of EE is that knowledge of a species' natural habitat, physiology, and wild behavior is combined to create a captive environment that is functionally more natural. Ideally this environment should encourage behavioral diversity, challenge animals intellectually and provide them with more choices and control of their lives. Veterinarians may have concerns about adding complexity and unpredictability to animal environments. However, providing surroundings that stimulate a greater range of natural behaviors, lessen stress and increase physical activity is the next step toward optimizing the health and reproductive capacity of captive animals.

EE is by no means a new idea. Zoo keepers have been providing objects and foods to stimulate activity in certain animals for years. The early focus has been on primates. Primates respond well to enrichment efforts; it is relatively easy to anticipate activities that will capture their attention. Only recently have zoos started to expand their efforts to provide regular enrichment to more animals, including reptiles, birds, marine mammals and carnivores.

Carnivore enrichment has its own challenges and limitations. Within the zoo setting it is difficult to provide carnivores with opportunities to locate and catch prey. Many carnivores are geriatric or have become accustomed to their sedentary lifestyles. Enclosures for carnivores are usually designed to house large dangerous animals safely, rather than to mimic their natural habitats. Ultimately, carnivore exhibits should be redesigned with enrichment in mind. Exhibits need to be constructed so that enhanced environments can be created and altered regularly and with reasonable effort. In the meantime, there is much we can do to expand the psychological space of carnivore exhibits, recognizing these limitations.

There is need for more field study and captive animal research to support carnivore enrichment. Knowledge gained from this research allows us to better evaluate captive behaviors and provide stimuli that will encourage natural behaviors to develop. Of the carnivores, bears and cats have received the most attention, with a focus on reducing stereotypic behaviors. Many of the enrichment methods chosen in the research projects discussed in this paper are relatively easy to implement and inexpensive. Significant, positive behavioral effects can be achieved if appropriate enrichment strategies are developed.

Ursidae

Bears are intelligent, curious animals that, like primates, spend much of their time in the wild foraging for a variety of food items. Bears tear apart trees, rake through dirt and carefully manipulate objects in search of fruits, small mammals, roots and other food items. Therefore, bears are a good target for enrichment strategies focusing on food presentation. A 1991 survey of 58 zoos exhibiting bears showed that 83% of respondents fed bears only one meal each day. Half of these zoos fed their bears in off-exhibit den areas.[15] Studies on feeding methods in bears reveal that offering food in ways that more closely mimic their feeding strategies in the wild stimulates natural foraging behaviors.[2,3,6] Providing simple and inexpensive enrichment items such as ice blocks with treats inside and browse has been found to increase overall activity and decrease abnormal behaviors.[6] Offering feeding devices that require manipulation, such as honey-filled feeder logs, can replace pacing and walking activities with more functional, goal-oriented behaviors. Providing several of these devices simultaneously and refilling them frequently reduces habituation to the objects.[2] Hiding food on exhibit in manipulatable objects has been shown to be a very effective technique, reducing stereotypic pacing from an average of 150 min/day to 20 min/day in one bear studied at the National Zoo.[2] In contrast, a mechanical feeding device that delivered food to a predictable location at varying intervals was not an effective means to decrease pacing behaviors.[2] These studies suggest that stereotypical behaviors in bears are the result of husbandry methods that do not provide adequate foraging and food handling opportunities.

Seasonal variations may be noticed when assessing the effectiveness of different enrichment methods in bears. An understanding of natural history and wild behaviors will allow enrichment strategies to be chosen that are appropriate to the time of year. Wild bears are usually occupied with mate seeking behaviors in the spring and foraging behaviors in the late summer and fall. When bears with a history of pacing were provided with bear odors in the spring, exploratory behaviors significantly increased. Hiding food items in the fall almost completely replaced pacing behaviors with foraging behaviors.[3] The olfactory sense of bears is very acute and they respond well to various enrichment techniques utilizing scents.

Bears have traditionally been exhibited in cages that do not lend themselves well to EE. Many bears are still exhibited in barren, concrete moated areas providing little or no exposure to plants or natural substrates. At most zoos, bear exhibits have not been the focus of recent renovation or redesign; as new exhibits have been constructed they have not been made larger than older exhibits.[15] In polar bears, fewer stereotypical behaviors are seen in exhibits that are larger.[15] However, size is not be the only important factor to a successful exhibit. Bears need to be provided with an exhibit that is as complex as possible. Concrete areas should be replaced with natural substrates that will encourage digging, nesting, burrowing, hiding, swimming, scratching, and foraging.[13] Climbing bears should be provided with structures that lead to opportunities such as food or a view of the distance. These structures should provide variable heights, textures, climbing challenges and exposure to the weather and visitors. Pools of water make good enrichment areas and do not have to be deep to be effective.[15] If possible manipulatable food hiding areas should be incorporated

into the design of the exhibit. Enrichment features may be considered an unnecessary expense when construction costs are being considered. Therefore, it is important for veterinarians to support enrichment during all discussions of exhibit design.

Felidae

Wild cats spend an enormous amount of energy locating, capturing, killing and processing the prey that they consume. Bengal tigers cover 10 to 20 miles searching for prey some nights and it is estimated they make a successful kill once in 20 attempts.[9] Cheetahs expend so much energy chasing prey that they have been seen to rest up to a half hour before feeding.[9] Leopards routinely carry carcasses weighing up to 70 kg into the safety of trees.[9] Removing these activities in the captive environment can lead to boredom, poor body condition, stereotypical behaviors and health problems. Although it is not possible to provide hunting opportunities to zoo cats, more naturalistic feeding is possible and can improve health and breeding.

Most zoos use commercially prepared soft food as the basis of their feline diets. Although these diets are thought to be nutritionally balanced and are convenient, they do not provide the physical and psychological benefits gained from carcass consumption. Focal palatine erosions, caused by molars contacting the palatal mucosa, have been found to occur much more commonly in cheetahs fed commercial diets than in cheetahs fed carcasses.[5] A likely explanation for these lesions is that reduced use of the masticatory apparatus leads to changes in the skull and dentition that cause malocclusion or insufficient wear of the first molars.[5] Feeding tigers large bones twice weekly has been shown to improve periodontal health and reduce plaque formation.[7] For large cats it seems beneficial to provide whole carcasses when possible. Even small carcasses like rabbits and chickens provide an opportunity to pluck, tear and make choices while consuming food. At the Potawatomi Zoo, a female leopard has been alleviated of a chronic hair plucking problem by providing whole chicken carcasses on a regular basis. Cats fed by hiding food items in piles of sticks or hanging food from elevated areas spend more energy acquiring food and are generally more active.

It has been shown that providing EE can reduce levels of stress in small cats. Measurements of urinary cortisol levels in leopard cats are a good indicator of stress and were used to monitor the effects of providing an enriched environment to cats previously housed in aversive conditions. Simultaneous behavior observations revealed that cats subjected to a stressful environment respond by becoming less active. When this same environment was made more complex by the addition of "furniture," these cats were better able to adapt and showed increased exploratory behavior, decreased pacing, and had decreased urinary cortisol concentrations.[1] Cat exhibits also need to be rendered more complex. Hiding areas, elevated resting sites, caves, pools, scratching trees, plants, and variable substrates should be provided. At the Glasgow Zoo cheetahs spend much of their time on a large wooden platform that provides a view of a nearby motorway.[14]

Unlike large cats, small cats consume several small meals throughout the day in the wild. Feeding small felids in a manner that more closely mimics their behaviors in the wild can be an effective enrichment strategy. Presenting a fishing cat with live fish caused an increase in activity level, behavioral diversity and enclosure utilization. A 60% reduction in the amount of time spent sleeping was seen, with most of this time replaced by a variety of hunting behaviors. Perhaps most impressive, is that these effects lasted from 48 hours to 8 days after fish were provided.[11] Feeding leopard cats by hiding food on exhibit several times each day increased exploring from 5.5% to over 14% and caused new behaviors to develop. Time spent stereotypically pacing reduced from 18% to less than 9%.[11] These techniques are effective because they decrease the predictability of feeding and provide an appropriate consequence for foraging behaviors. Providing multiple feedings will also increase keeper interaction, which has been shown to improve the reproductive success of small cats.[10]

Canidae

Very little research on enrichment in canine species is available. Many zoo canids exhibit stereotypical behaviors, particularly fixed running patterns. In fennec foxes it was demonstrated that stereotypic running is stimulated by several different environmental stimuli that evoke a flight response. Although offering enrichment items to these foxes improved behavioral diversity, providing large enclosures with secure hiding places was the most effective method of decreasing stereotypic running.[4] As more research and anecdotal enrichment information becomes available, enrichment strategies for canids can be fine-tuned. In the meantime, utilizing many of the techniques described for bears and cats might be successful.

Other carnivores

Many of the principles discussed for cats and bears are also likely to apply to other carnivores. The smaller enrichment items given to small cats can also be tried with mustelids, viverrids, procyonids and hyaenids. The North American otter husbandry manual will include results of a survey of enrichment items used at various institutions. This will prove to be a very valuable resource; similar information could be included in other carnivore husbandry manuals in the future.

Conclusion

The barriers to enrichment are motivation, time, and money. EE requires the cooperation of zoo management, keepers, curators, researchers, and veterinarians. Architects or exhibit design personnel, horticulturists, dieticians, educators and public relations personnel also need to be involved. Enrichment should be more than a boomer ball provided at the end of the day if there's spare time; it needs to become part of daily husbandry routines. Results to enrichment with carnivores may not be as dramatic as those seen with primates. Some keepers may be resistant to change or skeptical of new ideas. (you can't teach an old bear new tricks.) When expanding your EE efforts to include carnivores, it is important to

set up a program to coordinate enrichment efforts, allow ideas to be shared and generated, and ensure EE remains effective over time. As with any aspect of husbandry, EE will not maintain itself without supervision, structure and encouragement.

The public is increasingly exposed through television to the behaviors that animals express in the wild. Along with more natural and spacious exhibits, zoo visitors have come to expect more normal and diverse behaviors. EE provides an opportunity to show the public and animal welfare organizations that we are addressing all of the needs of animals in captivity. Environmental enrichment combined with graphics will promote more appreciation and understanding of zoo animals as ambassadors for their wild counterparts.

Ideas to encourage enrichment

1. Include a space for EE on the daily keeper forms.
2. Include EE in job descriptions and make contribution to enrichment efforts part of the performance review of your keeper staff.
3. Include enrichment in the master plan of the zoo.
4. Develop a form for keeping track of EE ideas that keepers try including spaces for assessment and suggestions for future applications.
5. Provide resources for keepers to look through for ideas.
6. Help convince management that EE is important and to allow time for enrichment to be done on a regular basis.
7. Have monthly meetings between curators or head keepers etc. to discuss what has been tried. Many ideas will work in more than one area.
8. Invite lecturers to discuss techniques for making behavioral observations to encourage more formal evaluations of enrichment ideas.
9. Urge architects to include enrichment features into the design of new exhibits or renovations of old exhibits. This is especially important for carnivores since enrichment items need to be more indestructible and are usually larger.
10. Enrichment efforts should include studies of enclosure utilization and preference and exhibits should be changed to reflect the findings.

Sources for enrichment ideas

1. The Shape of Enrichment newsletter, 1650 Minden Drive, San Diego, CA. 92111.
2. AAZK's Animal Keepers Forum and the U.K.'s equivalent RATEL.
3. International Zoo News
4. Zoo Biology
5. International Zoo Yearbook
6. USDA has some resources.
7. Animal Welfare, published by the Universities Federation for Animal Welfare (UFAW).

The following list comes from many sources including ideas compiled by Marty Sevenich at the Brookfield Zoo, David Shepherdson from the Metro Washington Park Zoo, Dianna Frisch at the Columbus Zoo, The Shape of Enrichment, other publications and conversations with many keepers.

60 ways to enrich your carnivores

1. Build elevated platforms for animals to get a distant view.
2. Hang meat in elevated places for small and medium cats.
3. Feed polar bears in the morning and several times throughout the day to reduce hunger and the resulting stereotypic behaviors.
4. Provide straw for polar bears to use for nesting. Polar bears like branches, veggies, and ice blocks to chew just like other bears.
5. Drag meat around large bear enclosures so that they follow scent. Occasionally leave meat for them to find.
6. Place live fish in pools or a large watering trough for all carnivores, including otters.
7. Provide melons, squash, pumpkins, and coconuts as play items.
8. Freeze food items in ice for all carnivores.
9. Whole carcass feeding: chickens, rabbits, etc.
10. Feeder logs for bears-holes drilled in sides filled with various treats like peanut butter, honey, jams, grapes, nuts, feline diet, hard boiled eggs, fruit or vegetable pieces.
11. Place sprinklers so that they partially cover exhibit and remain out of reach.
12. Freeze rodent in an ice block with tail sticking out (to be used as a handle).
13. Feed small cats several feedings each day to mimic natural eating patterns.
14. Provide fresh herbivore dung for large cats.
15. Provide otters with several sizes and shapes of rocks for manipulation and hollow logs both on land and in the water. Can hide food items in these logs.
16. Build cricket dispensers for bears and small carnivores-place crickets in a hollow log or PVC pipe filled with shredded newspaper. Drill holes in dispenser to allow crickets to escape.
17. Browse or whole cornstalks can be offered to bears, and other carnivores.
18. Provide cow hides for cats. Rabbit hides can be frozen so they last longer.
19. String cable and grow vines bearing vegetables in bear exhibits.
20. Give catnip or other herbs to large cats.
21. Secure battery operated moving ball in a plastic box-offer off exhibit so people don't think an animal is trapped.
22. Hide chunks of meat in piles of branches, logs or rocks.
23. Provide a box or concealed area with brush fringe over entrance to provide security for small cats especially.
24. Rawhide bones: can hang in enclosure or soak in water.
25. Artificial tree food dispenser can be built that contains a mechanism to dispense food in several locations around base.

26. Artificial honey tree-a pump delivers honey from a container to a bowl at the top of the tree.-for bears.
27. Sand barrel feeder-small barrel filled with sand and food items with an opening at the bottom at which animals can dig to encourage the flow of sand/food.
28. Boomer balls can be made more interesting by covering with smells (animal carcass), filling with food, liquid, small stones or catnip. Try warm water with a small amount of blood or frozen food chunks.
29. Pipes can be buried vertically underground and filled with food items.
30. Cut deep "V" grooves into logs and hide food items in them.
31. Wire mesh sleeping nests can be added to den areas for bears.
32. Freeze blood drippings from frozen carnivore diets or meat into various forms for cats especially.
33. Place smells around exhibits. Can use hunting lures, perfumes, materials from other animals, food additives, sardines, etc.
34. Freeze food items into a block or tube and place in a container with a chute so that melting food items fall out.
35. Old leaves, wood chips, sawdust, forest bark or other substrates can be provided.
36. Bury hollow pipes in the ground so that bear can hook food items out.
37. For smaller carnivores hang a plastic pipe that will tip or swing when they travel through it.
38. A wooden box with a hole in it can be attached to the cage roof or side so that cats can climb and retrieve food.
39. Peat filled trays can be provided to cats for elimination purposes.
40. Transmit the sound of lions roaring from a distance-promotes territoriality in lions.
41. Provide turned-over trees (roots exposed) in exhibit.
42. Put cat perfume (extract of cat mint) on branches. Allspice and mace are good spices to try with jaguars and snow leopards.
43. Plant fragrant flowering bushes and elephant grass in enclosures.
44. For otters stuff hay into box made with 2"x 2" vinyl coated wire. Otters have to work to get hay out for bedding.
45. Play bird sounds.
46. Fleece toys can be rubbed on goats, rabbits or other animals and offered to small cats.
47. Offer empty beer kegs, traffic cones, or hanging car tires to cats or bears.
48. Freeze globs of peanut butter and jelly into ice for bears.
49. Offer food in burlap bags, boxes or large plastic drums: meat or bones for cats, other for bears.
50. Provide polar bears with mealworms, rats, bones, mustard, eggplants, watermelon, crickets, tomatoes, fish, blackberries, squid.
51. Provide jet of water from hose for small cats to play in.
52. Grow grass in small cat enclosures for eating.
53. Offer crabs or sea urchins to polar bears.
54. Hang logs on exhibit or in holding areas for cats.
55. Provide novel food items to bears: ketchup, chili peppers, garlic, BBQ sauce, salsa, sugarcane.

56. Sloth bears enjoy crickets, meal worms and rotten logs full of insects.
57. Sun bears particularly enjoy food hidden in elevated areas and hard-to-process fruits like. coconuts.
58. Offer carnivores hair from camels, sheep, etc.
59. Make a paste of flour, water, dog chow, and blood or honey or other materials and spread on walls.
60. Make paper mache balls using balloons and fill with food for bears.

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ENRICHMENT OF CAPTIVE NON-HUMAN PRIMATE ENVIRONMENTS, ONE CLINICAL VETERINARIANS PERSPECTIVE

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Introduction

Over the past decade zoo staff, visitors and federal regulators have been changing how they envision their responsibilities toward those members of the order primates that zoos house for public education, SSP research and propagation.

These concerns are reflected in the geometric increase in the number of articles in scientific and lay publications as well as entire books devoted to this issue.[1] This abstract does not pretend to present a complete summary of all the data and opinions expressed to date. Zoos house species of primates that are behaviorally, biologically, and ecologically diverse. Some examples of successful strategies will be given in hopes of stimulating new innovations that will fit your setting.

Methods

First, define the parameters that best assess the psychological and physiological well-being of each species in the collection. No universal definition of well-being exists.[2,3,4,5,6] List what is known about wild environment, social behavior, and diet when planning a program. Second, objectively and subjectively document the effect of any change. Controlled studies with comparisons between individual or groups held in different settings or of the same individual/groups before and after a change will do much to avoid criticism of zoos for change based on public pressure or unsupported opinion. Appropriate enrichment will vary with different species, age, sex, group composition and individual temperament.[7] Re-evaluation of established procedures is the final step in providing "optimal enrichment" for a setting. Limit the number of assessment parameters that will be evaluated. Too few may bias the results but too many make other results uninterpretable (given the way parameters may interact).[5]

The following is a list of categories of enrichment, methods to monitor success and costs to consider when formulating a plan. Some examples of each category are also given.

I. Categories of Enrichment
    A. Social
        1. Companionship
            a. same species
                1. pair
                2. single sex/age group
                3. family
                4. mixed age and mixed family groups - as appropriate to this species in the wild.

5. changes in other pairs or groups, (proximity to estrus females may affect stability or fight incidence).
1-6. Part or full-time

b. other species - mixed exhibit or dog as surrogate

2. Training to alter behavioral repertoire

3. Visual, auditory, olfactory exposure to animals/humans
   a. direct
   b. indirect - video of another exhibit of same species in main exhibit by holding area

B. Physical Features
1. Cage - size vertical and horizontal complexity
   Plantings, vine tangles, grapevines, bamboo, honeysuckle, kudzu, maple, pine, oak, cedar branches
   Rotate primates out of outdoor exhibit periodically to allow plantings to regrow
   Windows - to other animals/exhibits/outdoors
   Visual barriers to allow escape from view
   Tunnels to connect cages and allow migration or escape interactions with other species in exhibit
   Indoor/outdoor components
   Bedding material
   Perch, rope, garden hose, branches of various sizes and flexibility
   Nest areas - places to hide or sleep away from viewing

2. Diet - variability of composition, when and how offered
   Broadcast to allow foraging in hay or wood chips
   Crickets, mealworms, lizard, mice from murine viral free research vendors (or extras from local university).
   Seasonally rotating available fruit and vegetables, including beans, corn on cob
   Feed at differing locations within cage at heights suitable for species housed

3. Occupational - within and outside cage
   Objects to manipulate, i.e., toys, foraging, mirrors, T.V., tires, innertubes, radio, fishing in bowl of water or pool for food or toys.
   Stick retrieval of food outside cage, food puzzles - many shapes
   Games played against the public
   Freezing food or toy in block of ice
   Burlap sacks, cardboard boxes placed inside one another, rolls of paper to spread around an exhibit.

4. Physical - temperature, humidity, light
    How often changed - daily vs.seasonal
    Rain inside the exhibit

C. Ultimate Goals

1. Give control over some part of their environment, i.e., ability to alter whether in a group, on exhibit or off, when and what eaten, etc.

2. To engage the primates most complex cognitive and affective skills, so that they will be free of distress most of the time, be in good physical health and exhibit a substantial range of species typical behaviors.

II. Examples of methods to monitor success of enrichment:

A. Serial recording of physical characteristics: hair/skin, eyes, gait, posture, facial expressions, presence of diarrhea/constipation

B. Non-invasive -

1. Weight gain/growth
2. Number of births/deaths/yr.
3. Disease patterns
4. Incidence of wounding
    Self
    Interactions with others
5. Vocalizations
    Number and type
    Percent of distress calls
6. Behaviors, species-specific, quantification, percent of aggressive-affiliative, other, quality of parental care.
7. Keeper/public interactions (we can not ignore opinions)
8. Ability to deal with stress
9. Incidence of stereotypic of other abnormal behaviors

C. Invasive -

1. Telemetry
    Circadian temp and ECG
2. Sequential hormone analysis
    Fecal/plasma
3. Responses to behavioral training
4. Immune function
    Globulin subsets and quant
5. T cell % and response to mitogens
6. Cortisol response to dexamethasone suppression test

III. Costs of Enrichment:

A. Additional staff trained to implement and monitor program - monitoring health of staff/volunteer working with non-human primates.
B. Added stress on some primates not accustomed to communal living.
C. May increase morbidity and mortality while changing environment or due to falls from trees or fights.
D. Exhibit and holding area redesign.
E. Purchase costs of toys and other apparatus.

Results - Some changes that have been noted in an enriched setting

Appropriate enrichment clearly provides increased opportunity to minimize boredom, obesity and pathologic behavior. Pair or group formation is frequently not a simple task. Short or long term group incompatibility is always possible. Adult female *Macaca* in an established group with resident males may have a xenophobic reaction to newly introduced females. The males rarely intervene but the group of established females frequently injure new arrivals. New arrivals seldom are seen to defend themselves against these group attacks.

Same sex adult pairing is more difficult than infant/adult pairing in many species of *Macaca*.[8]

Placement of a radio and feeder with levers that allowed *Macaca mulatta* to turn a radio on/off and eat when they desired, decreased cortisol levels and decreased abnormal behaviors.[9]

Stable pairing or group housing old *Macaca* with juveniles leads to decreased NK cell and lymphocyte proliferation in the old Macaca two months after group formation versus that of single housed old *Macaca*.[10] This unexpected finding has been noted repeatedly and shows another cost of social housing even though the older *Macaca* were more active and appeared to interact well with the juveniles.

Increased time is spent foraging, aggression and regurgitation are decreased when great apes are offered multiple small meals and supplemented with seeds, fruit or browse each day.

All male langur groups have few fights if they are composed of an even number of members. They form affiliative pairs. An odd man (out) is unlikely to be accepted by a group.

Ruffed lemur or *Saimiri sciureus* group housed males. Only dominants of group will show an increase in testicle size and serum testosterone during breeding season. Aggression toward submissive males increases if a female is introduced during breeding season.

S. oedipus female - only the dominant female will cycle and conceive.[11] Young endocrine suppressed female *S. oedipus* are more likely to leave an extended family group to enter plastic tunnels and migrate to empty cages. Perhaps tunnels could be used to determine when they are ready to leave their family and establish a new group.[12]

Cage design for arboreal primates requires structures of differing diameter elasticity and texture arranged at various heights and angles to facilitate movement via indirect routes. This maintains eye - limb coordination as well as providing "enrichment". Non-sanitizable structures may need to be replaced at regular intervals but they should not all be replaced at one time which would remove all territorial markings. Changing placement of structures within the cage at regular intervals also provides novelty.

Captive born primates must learn how to interact with other species in a mixed exhibit. I have handled multiple arm wounds from yearling marmosets learning not to steal food from the mouth of sloths.

Unlike marmosets, galago neonatal mortality increases if mothers are not caged alone. In the wild, pregnant females have been noted to leave the group prior to parturition and do not return for several weeks.[13]

Summary

"Distress must be separated from eustress or innocuous stress with which animals effectively cope and might even seek and enjoy. ...Most animals, including humans, seek some form of stress to relieve an otherwise sedentary life."[14]

"Psychological well-being is a subjective phenomenon related to the quality of one's life experiences."[6] Soumi has shown that major differences exist between individuals of comparable species, age, sex and in some cases rearing experience in terms of their response to stress. Some monkeys respond to mild environmental stressors with a marked behavioral catachamine, steroid, and immune response while other individuals have minimal response to the same stimuli. This further adds to the difficulty of designing enrichment plans that will suit all members of a group without boring most to avoid stressing some. To quote Soumi and Novak, "The wisdom of Solomon was not sufficient to provide an optimal environment for all of his subjects. It may also be insufficient to create an optimal environment for all captive non-human primates."[5]

None of the parameters listed directly asses well-being. When in doubt, listen to the animals. Try different combinations to see which work. Accept some risk/cost as an unavoidable part of increased social and physical complexity. This was not designed to be comprehensive review. Many of the authors or texts cited can provide additional suggestions.

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HUSBANDRY TRAINING AS A TECHNIQUE FOR BEHAVIORAL ENRICHMENT IN MARINE MAMMALS

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The use of operant conditioning as a behavioral enrichment device has been a vital component of Sea World's marine mammal program for many years. This training is not only used to develop the behaviors seen in the shows, but it is also an essential part of the preventive medicine program. Husbandry training has allowed the performance of routine physical examinations and biological sample collection on a variety of marine mammals with minimal or no physical restraint, which results in reduced stress to both the animals and personnel. Frequent examinations and sampling allows the detection of medical problems at an early stage, often before physical manifestations are evident. Common procedures include physical examination, blood sampling, urine and fecal collection, milk sampling, radiology and sonography, biopsies; cultures of the respiratory, gastrointestinal and urinary tracts, body weights and measurements, oral and ophthalmic exams, as well as administration of medication and topical treatments. Without this program the staff would eventually be forced into providing more treatment of disease than prevention. Through behavioral enrichment programs such as husbandry training, not only is the environment more stimulating for the animal, but the veterinarian also has the opportunity to perform more routine diagnostics with less stress and trauma to the animal.

AN INSPECTOR'S VIEW OF THE NEW ANIMAL WELFARE REGULATIONS CONCERNING ENVIRONMENTAL ENHANCEMENT

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Presently only primates have specific regulations for environmental enhancement. During an inspection I first review the written program for environmental enhancement for the following items:

1. Group vs. single housing
2. Can they see/hear other primates?
3. Compatibility of group housing, especially multiple-species exhibits
4. Isolation for medical and other reasons and special attentions to be provided
5. Types of environmental enhancements being provided and the frequency with which they are used
6. High-risk groups and special provisions for them
7. Exemptions, if any, and why granted
8. Involvement by the facility veterinarian

During the inspection of animals and facilities I observe the following:

1. Animal behaviors and interactions
2. Injuries, evidence of fights
3. Adequate cage sizes
4. Are enhancements being provided/do they match what was written in the plan?

For other species, there are no specific regulations addressing environmental enhancements. Some regulations do address animal needs beyond food, water and housing. One regulation requires all species to be housed in appropriate groups away from animals that could affect their health or cause them discomfort. For marine mammals there, again, are specifications for compatible groups away from other animals that could cause stress or discomfort. They are also required to have access to other animals, unless isolated for specific reasons.

The last group of regulations that concern animal well-being are the regulations governing handling. These cover the following topics:

1. Direct handling of the animals
2. Training techniques
3. Food and water deprivation
4. Barriers or facility employees to prevent injuries caused by the public
5. Use of drugs for animal training and control

PRIMATE CAGE SIZES
EFFECTIVE FEBRUARY 15, 1994

Group	Weight		Floor Area/Animal			Height
	lbs.	kg.	ft 2	(m 2)	(in)	(cm)
1.	under 2.2	under 1	1.6	(.15)	20	(50.8)
2.	2.2 - 6.6	(1-3)	3.0	(.28)	30	(76.2)
3.	6.6 - 22.0	(3-10)	4.3	(.40)	30	(76.2)
4.	22.0 - 33.0	(10-15)	6.0	(.56)	36	(81.28)
5.	33.0 - 55.0	(15-25)	8.0	(.74)	36	(91.44)
6.	over 55	over 25	25.1	(2.33)	84	(213.36)

Group 1 - Marmoset, tamarins, infants less than six months of age of various species

Group 2 - Capuchins, squirrel monkeys, juvenile species (six months to three years of age) of various species.

Group 3 - Macaques and African species

Group 4 - Male macaques and large African species

Group 5 - Baboon and nonbrachiating species larger than 33.0 lbs. (15 kg)

Group 6 - Great apes over 55 lbs. (25 kg) brachiating species

Groupings are based upon typical weights for each species. Changes for obesity, age or pregnancy will not require additional space unless the primate is unable to make normal postural adjustments and movements.

Large members of a given species whose normal weight exceeds the weight of its usual group requires the next higher group.

EXAMPLE: A non-obese 70 lb. baboon would move into Group 6.

ANEMIA IN A CHIMPANZEE (*Pan troglodytes*) ASSOCIATED WITH LEAD TOXICITY AND UTERINE LEIOMYOMA

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Severe anemia occurred in a 19-yr-old female chimpanzee with a history of excessive menstrual bleeding during the previous four months. On initial physical examination the animal was found to be lethargic, cachectic, and to have pale mucous membranes. Hematologic findings included a normocytic, normochromic anemia (PCV=8%) and reticulocytosis. Radiographic evaluation of the abdomen showed multiple metal-density foreign bodies within the gastrointestinal tract. An enlarged uterus with a thickened uterine wall was observed with abdominal ultrasonography. Serum lead levels were found to be elevated (103 ug/dl), and oral chelation therapy with 2,3-dimercaptosuccinic acid (10 mg/kg t.i.d. p.o. for 5 days, then 10 mg/kg b.i.d. p.o. for 2 weeks) was initiated. The chimpanzee's PCV increased to 23% by the fourteenth day of hospitalization and an exploratory laparotomy was performed to evaluate the abnormalities of the reproductive tract. The uterus was observed to be diffusely thickened and an ovariohysterectomy was performed. Histopathology of the reproductive tract showed locally extensive uterine leiomyoma. The chimpanzee responded well to treatment and no further problems were observed as of 18 months post treatment.

The anemia observed in this chimpanzee was associated with uterine leiomyoma and lead toxicity. Anemia secondary to lead toxicity has been documented in other nonhuman primates and is considered to be due to impaired heme synthesis.[1,2,6] Uterine leiomyomas are the most common cause of uterine masses in humans and have been reported in chimpanzees.[3,4] Prolonged, excessive menstrual bleeding due to these tumors can result in profound anemia in women.[6] Either of these diseases may have been primary causes of the anemia in this case. It is assumed that the two separate disease processes had an additive effect in this case.

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INDUCTION OF MYDRIASIS IN THREE PSITTACINE SPECIES

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Abstract

Three curariform drugs, *d*-tubocurarine, pancuronium and vecuronium, and a combination of atropine + phenylephrine, were evaluated for their ability to produce mydriasis when instilled into the eyes of cockatoos (sulfur-crested and citron-crested; *Cacatua galerita* and *Cacatua sulphurea*). These drugs were tested with and without the surface acting penetrating agents saponin and benzalkonium chloride. The agent which resulted in the most significant change in pupillary diameter in the cockatoos and was then tested on two African gray parrots (*Psittacus erithacus*) and three blue-fronted Amazon parrots (*Amazona aestiva*). For each drug trial, one eye was randomly selected as a control (0.12 ml 0.9% normal saline) and the test drug (0.12 ml) was instilled into the opposite eye. Each pupil was videotaped while animals were unrestrained in their cages, using ambient light, at 15, 30, 45, 60 and 75 minutes post treatment. A coaxial infrared video photoretinoscope provided optimal visualization of the pupil. Measurements of pupil diameters were made using a computerized image analysis system. In the cockatoos *d*-tubocurarine did not cause consistent mydriasis, with or without penetrating agents. Atropine + phenylephrine and pancuronium did produce mydriasis in the cockatoos, but also resulted in systemic effects of weakness and shallow breathing, and mild ataxia. Saponin resulted in signs of ocular irritation (blepharospasm, tearing). Unilateral application of vecuronium (0.096 mg/eye), without surface acting agents, produced consistent and statistically significant mydriasis in all three species with minimal systemic effects in two of eight birds in which it was tested. Our data suggest that of the agents tested, vecuronium is the mydriatic of choice for topical application, though caution should be exercised when using this drug, especially for bilateral application.

Table I
Drugs, concentrations, penetrating agents added and total doses received. Agents were used in commercially available concentrations, diluted to a consistent concentration to accommodate changes which occurred when penetrating agents were added.

Drug	Concentration	Agent added	Dose
d-tubocurarine	2.42 mg/ml	none	0.29 mg
d-tubocurarine	2.42 mg/ml	0.025% benzalkonium chloride	0.29 mg
d-tubocurarine	2.24 mg/ml	1% saponin	0.29 mg
d-tubocurarine	2.24 mg/ml	0.5% saponin	0.29 mg
d-tubocurarine	2.24 mg/ml	0.1% saponin	0.29 mg
pancuronium	1 mg/ml	none	0.12 mg
vecuronium	0.8 mg/ml	none	0.096 mg
vecuronium	0.8 mg/ml	1% saponin	0.096 mg
atropine and phenylephrine	5 mg/ml atropine and 50 mg/ml phenyleph.	none	0.6 mg atropine and 6 mg phenylephrine
atropine and phenylephrine	4 mg/ml atropine and 40 mg/ml phenyleph.	1% saponin	0.48 mg atropine and 4.8 mg phenylephrine

DIET, CAST COMPOSITION, AND ENERGY AND NUTRIENT INTAKE OF RED-TAILED HAWKS (*Buteo jamaicensis*), GREAT HORNED OWLS (*Bubo virginianus*), AND TURKEY VULTURES (*Cathartes aura*)

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Introduction

Red-tailed hawks (*Buteo jamaicensis*), great horned owls (*Bubo virginianus*), and turkey vultures (*Cathartes aura*) are raptors that are representative of approximately 292 extant species of diurnal birds of prey and 162 extant species of owls. Studies of their digestive systems[4,18,21] have established that falconiformes (eagles, hawks, and vultures) have a right lateral diverticulum of the caudal part of the cervical esophagus, known as a crop, in which food passage may be delayed. Strigiformes (owls) do not have a crop, but food passage may still be delayed in an expansible, undifferentiated portion of the esophagus. The stomach, composed of the proventriculus and ventriculus (or gizzard) is relatively undifferentiated, with both chambers being expansible, because most of the food is quite soft and requires little mechanical reduction. Gastric juice, containing hydrochloric acid, mucus, and pepsin, is produced in the proventriculus, but gastric proteolysis occurs principally in the gizzard. The gizzard of raptors also serves as a filter to inhibit further passage of many of the poorly digestible items in prey, such as bones, teeth, fur, feathers, beaks, nails, and claws. These are formed into pellets or casts and are regurgitated and egested via the mouth.

The juncture between the small intestine and rectum of hawks and vultures is marked only by paired vestigial ceca, while in owls there are two relatively long ceca that are greatly expanded in their distal third, more typical of some granivorous birds. Cecal feces are voided separately by the owl from other feces.

Red-tailed hawks and great horned owls generally pursue live prey in the wild, while turkey vultures specialize in carrion, although vultures are known to occasionally supplement their diet with live mammals and birds. In captivity, the variety of food choices found in the wild is seldom available, and captive raptors are commonly fed dead rats, mice, hamsters, rabbits, or chicks. While the nutrient composition of some of these choices has been partially determined, cast formation and egestion has the potential to influence the supply of nutrients available for absorption. The objectives of this study were to measure consumption of killed 1-day-old chicks (*Gallus gallus*) and 3-wk-old Syrian golden hamsters (*Mesocricetus auratus*) by red-tailed hawks, great horned owls, and turkey vultures, to determine the composition of prey, egested casts, and excreta, and to determine whether these prey are likely to meet the nutrient and energy needs of these raptors when maintained in captivity.

Materials and Methods

Four adult red-tailed hawks, four adult great horned owls, and four adult turkey vultures were fed 1-day-old chicks or 3-wk-old hamsters in a crossover design so that each bird was fed each prey item over the course of the study. The raptors were obtained from the Potter Park Zoo, Lansing, Michigan, the Kellogg Biological Station, Hickory Corners, Michigan, and the Michigan State University Veterinary Wildlife Rehabilitation Center, East Lansing, Michigan. All birds were clinically healthy, but two red-tailed hawks, three great horned owls, and one turkey vulture had old, improperly healed fractures that made them unsuitable for release into the wild. Two other turkey vultures were imprinted on humans, likewise making them unsuitable for release. All other birds were entirely normal and were released into the wild after the study.

The chicks were unsexed, white leghorns from the Michigan State University Poultry Teaching and Research Center, East Lansing, Michigan 48824, USA. The Syrian golden hamsters were an HSD:Syr strain from Harlan-Sprague-Dawley, Inc., Indianapolis, Indiana 46229, USA. Both chicks and hamsters were euthanized with carbon dioxide and kept frozen at -20^0 C up to 2 wk (chicks) or 2 mo (hamsters) until fed.

The raptors were individually housed in a Michigan State University animal room in stainless steel metabolism cages, 83 cm high, 117 cm wide, and 69 cm deep, arranged in two groups of six with three cages above and three cages below. Wooden perches were installed in each cage. Floors were woven stainless steel with 2.5-cm openings. Collection trays, 5 cm below the floors, were covered with waxed paper to facilitate quantitative collection of casts, excreta, and uneaten food.

The birds were randomly assigned to the cages to modulate position effects on treatments. Temperature and relative humidity throughout the study ranged from 21-27^0 C and 28-60%, respectively. Light was furnished by two ceiling fixtures, each containing two 60 W incandescent bulbs on a 12-hr light, 12-hr dark cycle.

The frozen chicks or hamsters were thawed at 4^0 C, and each type of prey was fed once each day to two hawks, owls, or vultures for a 7-day acclimation period followed by a 5- or 6-day collection period. After a 3-wk rest period, the prey items were switched for a second 7-day acclimation period followed by a 7-day collection period. The chicks and hamsters were weighed before feeding each day and were fed to satiation. The raptors were weighed before and after each of the two trials.

Casts, excreta (combined feces and urine), and uneaten prey were collected and weighed each day and frozen for later analysis. Casts and excreta were lyophilized and ground dry at high speed in a Waring™ blender (Fisher Scientific, Pittsburgh, Pennsylvania 15219, USA) until homogeneous. Whole prey items were prepared for composition analysis by lyophilizing two replicates of three chicks or three hamsters each, followed by grinding in a Wiley™ mill (Fisher Scientific) through a stainless steel screen with 2-mm openings. All samples were analyzed in duplicate by AOAC procedures[17] for dry matter, gross energy,

crude protein, ether extract, ash, and mineral elements. Following nitric acid and perchloric acid digestion, whole prey and casts (composited by raptor species and diet) were analyzed in duplicate by atomic absorption spectrophotometry for calcium, magnesium, iron, copper, manganese, and zinc, and by atomic emission spectrophotometry for sodium and potassium. Phosphorus concentrations were determined by the spectrophotometric procedure of Gomorri.[11]

Differences between prey and raptor species were determined by analysis of variance and Student's t test.[9]

Results and Discussion

Composition of the 1-day-old chicks and 3-wk-old hamsters fed to the raptors is shown in Table 1. Hamsters were significantly ($P<0.05$) higher than chicks in dry matter, gross energy, ether extract, calcium, phosphorus, magnesium, potassium, iron, manganese, and zinc, and significantly lower ($P<0.05$) in crude protein and sodium. However, nutrient concentrations in both prey species appeared adequate in relation to predicted need.[20]

Weights and weight changes in the raptors over the 33-day study, prey eaten, and casts egested are shown in Table 2. Body weights of red-tailed hawks have been reported[24] to be 1.0 kg for males and 1.2 kg for females, comparable to the mean of 1.1 kg found here. The mean body weight of the great horned owls (1.5 kg) was within the weight range of 1.4-1.8 kg reported for four captive specimens.[5] The body weight of wild, male black vultures (*Coragyps atratus*) was reported to be 1.6-2.2 kg[19] compared to the mean of 1.9 kg found for turkey vultures in this study and a published weight of 2.0 kg for turkey vultures.[4]

Red-tailed hawks and great horned owls gained weight while there was a small weight loss for the turkey vultures. Turkey vultures appeared to be stressed more than the other species by confinement and the disturbance of feeding and cleaning, but their total weight loss was only 0.5% of initial weight.

Since the raptors were fed to satiety, not all the prey offered was eaten. Much of the time, uneaten prey was left whole. Sometimes, however, only parts of prey items were left. When the composition of the dry matter of uneaten prey was determined by chemical analysis, it did not differ significantly from the composition of the whole prey that had been offered.

The mean number of casts regurgitated and egested per day ranged from 0.3-1.4, with cast dry matter comprising 2-8% of dry matter consumed. Cast composition (Tables 3 and 4) differed appreciably from the composition of prey. Casts egested by all raptors were significantly lower than prey in potassium. Casts egested by hawks and owls were markedly lower than prey in ether extract. Casts egested by vultures were very high in ether extract, with indications that much of this ether extract was contributed by secretions of the vulture digestive tract.

Casts egested by owls were higher in ash, calcium, phosphorus, and magnesium than casts

egested by hawks or vultures, apparently due to the predominance of bone in owl casts. It has been noted that casts of free-living short-eared owls (*Asio flammeus*) contained 44% bone (by weight) compared to 17% bone in casts of free-living marsh hawks (*Circus cyaneus hudsonius*), even though these two species were shown to share habitats and food preferences.[2,23] Of the prey species individually identified in casts of short-eared owls and marsh hawks, meadow voles (*Microtus pennsylvanicus*) comprised 95 and 100% of the total, respectively.[2] Field observations indicated that both the short-eared owls and marsh hawks consumed the entire skeleton. A theory proposing that there is more corrosion of bone in the digestive systems of hawks than in owls[7,10] was later confirmed.[3] This may be a consequence of the lower preprandial pH in the gastric juice of hawks compared to owls (1.6 vs 2.4, respectively)[6] and the higher concentrations of pepsin.[15,16] The difference in pH is equivalent to about six times more hydrogen ions per ml of gastric juice in falconiformes than in strigiformes.

The formation of a cast in a great horned owl fed laboratory mice was studied using radiographic techniques.[13] Following entry of the meal into the ventriculus, it was retained by closure of the sphincter between the ventriculus and proventriculus. Because the pyloric opening was small (1.5 mm) and arose superiorly, much of the meal remained in the ventriculus during most of the digestive process. As digestion proceeded, the liquified portions of the meal were pumped by ventricular contractions into the small intestine. Indigestible material, such as bones, fur, teeth, and nails, collected in the inferior ventriculus and were gradually forced into a tight pellet. Before regurgitation, the pellet (or cast) lay in the superior ventriculus, immediately below the sphincter. The sphincter relaxed, and ventricular contractions plus contractions of the abdominal wall and proventriculus forced the cast upward until it was egested. In this owl, formation of the cast took about 8 hr, and regurgitation and egestion about 4 min.

Despite the differences in composition between prey fed and casts egested, estimated nutrient concentrations in digesta dry matter (prey minus casts; Table 5) were very similar to nutrient levels in the dry matter of whole prey. This was due to the low mass of casts egested relative to that of prey consumed. The only effect cast egestion had on nutrients potentially available for digestion and absorption was a decline in digesta ash and calcium in owls. Nevertheless, calcium supplies in owl digesta were easily sufficient to meet calcium needs, and the percentage of ash in excreta of great horned owls was significantly ($P<0.05$) less than in excreta of red-tailed hawks (Table 6).

Collection and analysis of excreta, as well as casts, made it possible to calculate the metabolizability of prey dry matter, gross energy, and crude protein (Table 7). These values were all very high for red-tailed hawks (mean >92%) and tended to be lowest for great horned owls, although the metabolizability of prey gross energy was essentially the same for owls and turkey vultures. The metabolizability of gross energy for great horned owls (90%) and turkey vultures (88%) fed chicks was similar to values noted for great horned owls (85%) fed turkey poults[5] and for long-eared owls (88%) fed laboratory mice.[12] However, the metabolizability of gross energy in ground rats (*Rattus norvegicus*) was only 70% for the Eurasian kestrel (*Falco tinnunculus*) and 73% for the screech owl (*Otus kennicotti*).[1]

Dry matter intakes as a percent of body weight and metabolizable energy (ME) intakes per unit of body weight (BW_{kg}) or per unit of metabolic body size ($BW_{kg}^{0.73}$) are shown in Table 8. The expression of metabolic body size was that used by Robbins[20] for nonpasserines. Mean dry matter intakes ranged from 1.7-3.2% of body weight, with consumption of chicks consistently lower than consumption of hamsters. These intakes were comparable to mean dry matter intakes (2.6% of body weight) reported for great horned owls fed laboratory white mice (*Mus musculus*) or 1-day-old domestic turkey poults.[5] Smaller raptors, such as long-eared owls (*Asio otus*) weighing 252 g (male) or 310 g (female) required 32 or 34 g per day, respectively, of laboratory mice to sustain body weight.[12] Assuming that the laboratory mice were 70% water,[14] respective dry matter intakes were 3.8 and 3.3% of body weight. Six female barn owls (*Tyto alba*), with a mean weight of 561 g and which were fed laboratory mice at environmental temperatures of 25, 15, or 5 °C, consumed dry matter equivalent to 4.2, 5.3, or 6.3%, respectively, of their body weight daily.[14]

Differences in prey dry matter intake are reflected in ME intakes per unit of body weight or of metabolic body size. In our study, ME intakes per unit of body weight decreased across raptor species as body weight increased. ME intakes expressed in kcal/$BW_{kg}^{0.73}$ ranged from 96-184 and did not completely compensate for the body weight (or raptor species) effect. It is estimated that the daily energy expenditure of free-living nonpasserine birds ranging in weight from 0.004-8.40 kg would be about 222 kcal/$BW_{kg}^{0.73}$.[20] The captive raptors in this study did not have to search for food, were in a thermoneutral environment, and there was no energy expenditure for flying; thus, a lower energy requirement for maintenance would be expected. Great horned owls were found to consume 142 kcal ME/kg body weight per day when fed laboratory mice.[5] These owls averaged 1.62 kg in body weight, and mean ME intake per $BW_{kg}^{0.73}$ would be 162 kcal/day. This value is within the range for all raptor species we studied but is somewhat higher than the values we found for great horned owls fed either chicks (102 kcal ME/$BW_{kg}^{0.73}$) or hamsters (136 kcal ME/$BW_{kg}^{0.73}$).

Australasian harriers (*Circus approximans*), weighing about 600 g and fed laboratory mice, 1-day-old domestic chickens, or small (17 g) New Zealand fish, the common bully (*Gobiomorphus cotidianus*), consumed 10.9, 13.6, or 14.1 g of dry matter per day.[22] Dry matter intakes expressed as a percentage of body weight were 1.8, 2.3, or 2.4, respectively, and were inversely related to percent fat in the prey dry matter (mice, 22.9%; chicks, 20.8%; fish, 12.3%). Daily mean ME intakes were 74, 83, or 98 kcal/BW_{kg}, respectively, or 65, 72, or 85 kcal/$BW_{kg}^{0.73}$. These values were appreciably lower than those found for red-tailed hawks in our study (153-180 kcal ME/BW_{kg}; 156-184 kcal ME/$BW_{kg}^{0.73}$).

Oxygen consumption was used to estimate resting metabolism in a saw-whet owl (*Aegolius acadicus*, 83 g), screech owl (151 g), and three sparrow hawks (*Falco sparverius*, 96-114 g).[8] The caloric equivalent of the oxygen used was 164, 132, and 157 kcal/BW_{kg}, respectively, or 84, 79, and 84 kcal/$BW_{kg}^{0.73}$.

Previously published data on ME intakes of turkey vultures were not found.

Conclusions

1. Daily consumption of 2.6 to 2.8 1-day-old chicks (@ 45 g) or 2.9 to 3.3 3-wk-old hamsters (@ 38 g) was sufficient to maintain body weight in adult red-tailed hawks, great horned owls, or turkey vultures when held captive in a thermoneutral environment for 1 mo.
2. Chicks contained 24% dry matter, 67% crude protein, 21% ether extract, 5% ash, 2.1% calcium, and 1.5% phosphorus, while hamsters contained 30% dry matter, 50% crude protein, 35% ether extract, 8% ash, 2.5% calcium, and 2.0% phosphorus.
3. Daily dry matter intakes were equivalent to 1.7-3.2% of raptor body weight.
4. Cast dry matter regurgitated and egested comprised 2-8% of dry matter consumed.
5. Daily ME intakes were 96-184 kcal/$BW_{kg}^{0.73}$.
6. Protein (essential amino acids), fat (essential fatty acids), and mineral intakes were sufficient to meet needs.
7. Vitamin intakes were not measured, but consumption of well-nourished whole prey would be expected to meet vitamin needs.
8. Feeding 1-day-old chicks or 3-wk-old hamsters to satiety should meet energy and nutrient needs of captive adult red-tailed hawks, great horned owls, and turkey vultures without further supplementation.

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Table 1. Composition of whole prey (1-day-old chicks and 3-wk-old hamsters) on a dry basis.[a]

Item	Chicks		Hamsters	
	Mean	SD	Mean	SD
Weight (g)	44.6	3.92	37.9	3.92
Dry matter (%)[b]	24.2	1.72	30.3	1.17
Gross energy (kcal/g)[b]	5.54	0.035	5.98	0.007
Crude protein (%)[b]	67.4	1.34	49.8	0.49
Ether extract (%)[b]	21.0	0.14	34.7	1.10
Ash (%)	5.4	0.45	7.5	1.29
Calcium (%)[b]	2.08	0.057	2.51	0.035
Phosphorus (%)[b]	1.47	0.028	2.03	0.014
Ca:P ratio	1.42	0.007	1.24	0.007
Magnesium (%)[b]	0.05	0.008	0.12	0.004
Sodium (%)[b]	0.71	0.006	0.46	0.008
Potassium (%)[b]	0.80	0.017	0.88	0.016
Iron (ppm)[b]	118	11.6	237	0.7
Copper (ppm)	7	1.2	12	1.9
Manganese (ppm)[b]	5	0.4	45	3.3
Zinc (ppm)[b]	72	0.04	94	2.5

[a]$n=2$ (two replicates containing three of each prey). Each replicate analyzed in duplicate.
[b]Chicks and hamsters significantly ($P<0.05$) different.

Table 2. Raptor weights, prey consumed, casts regurgitated and egested, and excreta (feces/urine) voided when red-tailed hawks, great horned owls, and turkey vultures were fed chicks or hamsters.

Item	Red-tailed hawks		Great horned owls		Turkey vultures	
	Mean	SD	Mean	SD	Mean	SD
Intial weight (g)	1,050	224	1,508	83	1,874	
	230					
Final weight (g)	1,106	210	1,548	118	1,864	
	100					
Weight change (g/d)	1.7	1.48	1.2	1.61	-0.3	4.96
Chicks eaten (no./d)	2.8	0.41	2.6	0.23	2.8	0.37
Chicks eaten (g/d)	129	26.0	115	16.9	126	21.0
DM intake (g/d)	31	6.3	28	4.1	31	5.1
Casts egested (no./d)	0.7	0.36	0.3	0.23	1.1	0.56
DM egested (g/d)	1.1	0.78	0.6	0.38	2.5	1.05
Excr. DM voided (g/d)	0.9	0.42	4.3	1.39	1.3	0.21
Hamsters eaten (no./d)	2.9	0.18	3.0	0.37	3.3	0.14
Hamsters eaten (g/d)	113	16.4	114	21.4	124	6.9
DM intake (g/d)	34	5.0	34	6.5	38	2.1
Casts egested (no./d)	0.9	0.25	1.4	0.26	0.9	0.51
DM egested (g/d)	1.0	0.46	2.8	1.21	1.5	0.59
Excr. DM voided (g/d)	1.5	0.31	3.4	0.95	3.1	0.75

Table 3. Composition (dry basis) of casts regurgitated and egested when chicks were consumed.[a]

Item	Red-tailed hawks		Great horned owls		Turkey vultures	
	Mean	SD	Mean	SD	Mean	SD
Dry matter (%)	34.4	4.72	54.5	16.91	46.0	5.99
Gross energy (kcal/g)	5.32	0.387	3.51	1.320	6.16	0.441
Crude protein (%)	78.5	2.76	62.7	14.17	62.2	6.74
Ether extract (%)	4.8	1.80	2.6	1.00	25.6	5.48
Ash (%)	2.3	1.63	15.4	11.03	1.1	0.57
Calcium (%)	0.39		7.46		0.18	
Phosphorus (%)	0.10		2.54		0.21	
Magnesium (%)	0.026		0.086		0.017	
Sodium (%)	0.477		0.607		0.135	
Potassium (%)	0.068		0.082		0.071	
Iron (ppm)	87		168		164	
Copper (ppm)	13		21		25	
Manganese (ppm)	3		17		8	
Zinc (ppm)	24		310		42	

[a] $n=3$ for numbers of red-tailed hawks and great horned owls whose casts were composited per bird and separately analyzed for dry matter, gross energy, crude protein, ether extract, and ash. $n=4$ for turkey vultures. After the above analyses were completed, the residual cast material was composited by species, since amounts were too small to permit separate analyses per bird. Thus, $n=1$ within raptor species for mineral values.

Table 4. Composition (dry basis) of casts regurgitated and egested when hamsters were consumed.[a]

Item	Red-tailed hawks Mean	SD	Great horned owls Mean	SD	Turkey vultures Mean	SD
Dry matter (%)	34.8	4.45	50.2	5.88	41.4	5.96
Gross energy (kcal/g)	4.73	0.236	3.51	0.188	6.37	0.284
Crude protein (%)	59.4	8.64	38.0	3.29	38.0	3.52
Ether extract (%)	2.0	0.65	2.4	1.68	31.7	7.31
Ash (%)	4.8	2.64	27.9	4.84	1.1	0.81
Calcium (%)	1.04		8.58		0.32	
Phosphorus (%)	0.41		2.45		0.39	
Magnesium (%)	0.029		0.157		0.021	
Sodium (%)	0.656		0.636		0.132	
Potassium (%)	0.093		0.080		0.092	
Iron (ppm)	203		389		294	
Copper (ppm)	24		28		25	
Manganese (ppm)	9		95		10	
Zinc (ppm)	64		297		18	

[a] $n=4$ for numbers of red-tailed hawks and great horned owls whose casts were composited per bird and separately analyzed for dry matter, gross energy, crude protein, ether extract, and ash. $n=3$ for turkey vultures. After the above analyses were completed, the residual cast material was composited by species, since amounts were too small to permit separate analyses per bird. Thus, $n=1$ within raptor species for mineral values.

Table 5. Gross energy and nutrient concentrations (dry basis) in digesta (prey fed minus casts egested) that were potentially available for digestion and absorption compared to prey fed.

Item	Prey	Digesta Red-tailed Hawk	Great horned owl	Turkey vulture
Chicks				
Gross energy (kcal/g)	5.54	5.55	5.58	5.49
Crude protein (%)	67.4	67.0	67.5	67.9
Ether extract (%)	21.0	21.6	20.9	20.6
Ash (%)	5.4	5.5	5.2	5.8
Calcium (%)	2.08	2.14	1.96	2.25
Phosphorus (%)	1.47	1.52	1.45	1.58
Hamsters				
Gross energy (kcal/g)	5.98	6.02	6.20	5.96
Crude protein (%)	49.8	49.5	50.8	50.3
Ether extract (%)	34.7	35.7	37.6	34.8
Ash (%)	7.5	7.6	5.7	7.8
Calcium (%)	2.51	2.55	1.96	2.60
Phosphorus (%)	2.03	2.08	1.99	2.10

Table 6. Composition of excreta (feces/urine, dry basis) voided when red-tailed hawks, great horned owls, and turkey vultures were fed chicks or hamsters.

Item	Red-tailed hawks			Great horned owls			Turkey vultures		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Chicks									
Dry matter (%)	2	54.6	13.58	4	34.7	6.87	4	44.2	6.09
GE (kcal/g)	2	4.13	0.665	4	3.80	0.202	4	3.64	1.109
Nitrogen (%)	2	8.5	2.67	4	14.3	2.66	4	5.4	1.48
Ether extract (%)	2	7.2	1.56	4	7.8	1.31	4	13.9	5.96
Ash (%)	2	26.8	8.84	4	18.4	0.70	4	36.2	11.19
Hamsters									
Dry matter (%)	4	62.3	10.53	4	39.9	15.29	4	41.9	6.31
GE (kcal/g)	4	2.56	0.202	4	3.12	0.253	4	3.26	0.637
Nitrogen (%)	4	5.0	1.26	4	14.2	3.08	4	5.0	0.90
Ether extract (%)	4	1.6	0.36	4	4.9	1.56	4	8.1	4.22
Ash (%)	4	49.1	3.36	4	23.5	5.66	4	41.8	6.55

Table 7. Metabolizability of dry matter, gross energy, and nitrogen in chicks or hamsters when fed to satiation to red-tailed hawks, great horned owls, and turkey vultures.[a]

Item	Red-tailed hawks			Great horned owls			Turkey vultures		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Chicks									
Dry matter (%)	2	95.2[b]	0.95	3	85.3[c]	2.84	4	87.7[c]	2.50
GE (%)	2	95.8[b]	0.99	3	89.8[c]	2.15	4	88.4[c]	2.85
Nitrogen (%)	2	94.0[b]	2.50	3	80.7[c]	5.20	4	90.5[b]	2.62
Hamsters									
Dry matter (%)	4	92.5[b]	1.89	3	83.5[c]	1.71	3	88.2[b]	1.51
GE (%)	4	95.7[b]	1.47	3	91.0[c]	1.19	3	91.9[c]	0.86
Nitrogen (%)	4	93.8[b]	1.28	3	79.3[c]	2.89	3	92.3[b]	0.25

[a]Calculated by subtracting the amount egested in casts and excreted in feces/urine from that ingested, dividing by the amount ingested, and multiplying by 100.
[b,c]Means on the same line with different superscripts are significantly ($P<0.05$) different.

Table 8. Dry matter (DM) and metabolizable energy (ME) intake from chicks or hamsters per unit of body weight (BW_{kg}) or metabolic body size ($BW_{kg}^{0.73}$) of red-tailed hawks, great horned owls, and turkey vultures.

Item	Red-tailed hawks	Great horned owls	Turkey vultures
Chicks			
ME concentration (kcal/g DM)	5.31	4.98	4.90
DM intake (% of BW)	2.9	1.8	1.7
ME intake (kcal/BW_{kg})	153	91	81
ME intake (kcal/$BW_{kg}^{0.73}$)	156	102	96
Hamsters			
ME concentration (kcal/g DM)	5.72	5.44	5.50
DM intake (% of BW)	3.2	2.2	2.0
ME intake (kcal/BW_{kg})	180	121	112
ME intake (kcal/$BW_{kg}^{0.73}$)	184	136	132

NUTRIENT COMPOSITION OF CROP MILK AND GROWTH OF SQUABS: NEW NON-INVASIVE TECHNIQUES FOR MILK COLLECTION AND BODY COMPOSITION DETERMINATION IN COLUMBIDAE (*Columba livia*)

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The increasingly low fledgling success rate of endangered Columbidae has prompted caretakers and conservationists to investigate alternative methods of raising these birds in captivity. Options explored have included foster raising and hand rearing of squabs. Despite implementing alternative means of rearing young, the fledgling success rate is alarmingly low (less than 10% for several endangered species of pigeons in the United States in 1993). These electives have provided a mode of propagation that is certainly preferable over having valuable squabs die from parental neglect, but are far from adequate for raising birds in significant numbers to preserve the species. Domestic Columbidae used as foster parents may be able to raise some proportion of exotic Columbidae squabs, but a very important difference exists between domestic and exotic species that may account for the low rate of fledgling success. Most domestic species used as foster parents are seed-eating birds as opposed to their exotic counterparts, which are primarily fruit eaters.[1] Thus, a significant difference may be reflected in the composition of nutritive crop milk of exotic versus domestic Columbidae. Crop milk is a holocrine prolactin-mediated secretion produced by both male and female parent pigeons and some species of flamingos and penguins. The milk is fed exclusively to squabs during the first 10 days of life.

An area of nutrition lacking pertinent information involves nourishment and growth of squabs. Various procedures used to investigate squab nutrition have been explored in a few species of pigeons and doves. To date, all approaches used to examine crop milk and body composition have been invasive, including surgical techniques and sacrifice of birds. Consequently, these procedures have been used only in domestic species. These methods prohibit analysis of crop milk and body composition in endangered species.

New non-invasive techniques have been developed for investigation of crop milk composition, squab growth, and body composition in pigeons. Through the use of non-invasive techniques, consecutive milk samples may be collected from the same bird for analysis, and body composition may be predicted without detrimental or terminal results. Therefore, an avenue for evaluation of squab nutrition and growth in endangered avian species now exists.

A method of milk collection has been developed whereby, after the squab was fed by the adult bird, milk was mixed in the squab crop by gentle massage and was manually expressed from the crop of the squab. Proximate composition of milk was determined using standard

laboratory techniques. Dry matter was determined by drying samples to a constant weight in a convection oven. Fat was determined by ether extraction. Protein was determined by the Nesslerization method, and carbohydrate was measured using a modified method of Marier and Boulet. Fatty acid analysis was performed by capillary gas chromatography.

Growth rates were determined by measuring live body weight after crop contents were emptied. Wing length measurements were taken of the radial-ulnar region on the same day as body composition measurements. Body composition measurements were taken by measuring changes in electromagnetic conductance of the whole body (EM Scan).

Histologic changes in the lactating crop gland were described by contrasting lactating and nonlactating tissue. Tissues were trimmed, processed, and stained with hematoxylin and eosin for microscopic examination.

Results obtained from the use of non-invasive techniques revealed that crop milk is comprised primarily of fat and protein. Dry matter ranges from 37% (37 ± 2.1; $n=16$) on day 1 of hatching to 24% (24 ± 1.8; $n=16$) on day 4 post hatching. On a dry matter basis, milk is 53% fat (53 ± 6.8; $n=16$) on day 1 of hatching and decreases to 38% (38 ± 3.6; $n=16$) on day 4. Crude protein content of milk on day 1 is 58% (58 ± 2.6) and increases to 65% (65 ± 3.4) by day 4. Only trace amounts of carbohydrate are detectable in samples of milk from days 1 through 4. Fatty acid composition of crop milk is similar to most mammalian milks, with similar proportions of total saturated fatty acids, oleic, linoleic, and linolenic acids. Oleic acid, the prominent fatty acid in most mammalian milks, is also the prominent fatty acid ($37.5\pm.8$ wt%; $n=12$) in pigeon milk.

Squab growth rates are rapid. Body weights on day 1 of hatching are 18 ± 5.2 g ($n=40$) and increase to 300 ± 12.8 g ($n=40$) by day 10 post hatching. Wing length measurements on day 1 of hatching are $1.2\ \pm.3$ cm ($n=5$) and increase to $5.5\pm.2$ cm ($n=6$) on day 14 post hatching. EM Scan body composition index measurements range from 38 for a day-old squab to 747 for a 2-wk-old squab. Body weight and wing length measurements can be used with body composition indexes to develop a regression equation for prediction of body composition. This information can be confirmed by validation studies involving direct chemical carcass analysis.

Increased vascularization, hyperplasia, and thickened crop mucosal epithelium are the primary histologic changes in lactating crop tissue. Intracellular lipid droplets are apparent within the crop mucosal epithelium and fill the crop lumen as epithelial desquamation occurs during lactation.

Energy dense, protein-rich crop milk supports the rapid growth squabs undergo during the first few weeks of life. Advances have been made in techniques used to collect milk and measure growth and body composition in domestic pigeons. New techniques may now be applied to gather urgently needed biological information on rare and endangered species to improve reproduction and growth in captivity. Appeals for research initiatives have been made to help ensure the existence of the crowned pigeon, which has experienced negative

population growth in captivity over the past 3 yr.[2] Use of these new non-invasive techniques in captive exotic species will provide a portion of the answer to those pleas and will greatly augment our knowledge and ability to care for this distinctive group of birds.

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USE OF NATURAL AND PROCESSED DIETS FOR SMALL-CLAWED OTTERS (*Aonyx cinerea*)

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Previously reported urinary tract problems of small-clawed otters may be associated with dietary management and may be ameliorated by dietary changes. There are several diets available for use with otters and other small carnivores. These diets include fish, commercially available dry, canned, and frozen diets. However, there are few baseline data on nutrient utilization of these different types of diets. A feeding trial designed to measure nutrient digestibility is one of the few noninvasive, in situ, techniques that can provide information on digestion and absorption. Percent apparent nutrient digestibility is defined as:

$$\frac{\textit{Nutrient intake} - \textit{Nutrient excretion}}{\textit{Nutrient intake}} \times 100$$

Furthermore, feeding trials can be conducted under many circumstances, including the use of exhibit animals. The objective of this study was to measure nutrient and energy digestibility of natural and processed diets fed to small-clawed otters.

Three diets were fed to a pair of Asian small-clawed otters at the Santa Barbara Zoo during the summer of 1988. The three diets fed were thawed smelt (supplemented with thiamin and vitamin E), canned ZuPreem Feline diet (Hill's Pet Products, Topeka, KS), and dry Feline k/d diet (Hill's Pet Products, Topeka, KS). The otters were fed each diet for an 8- to 10-day adaptation period and ad libitum intake was measured. Subsequently, the otters were fed 95% of measured ad libitum intake for 10 days and all feces were collected. Samples of diet and feces were analyzed for dry matter (DM), crude protein (CP), gross energy (GE), crude fat (CF), and ash. Carbohydrate was determined as the difference between 100 and the sum of water, CP, CF, and ash content. Data were analyzed using Bonferroni *t*-tests.

Nutrient content of the diets is presented in Table 1, daily dry matter intake is presented in Table 2, and apparent nutrient digestibilities are presented in Table 3. There was a wide range in nutrient content of these three diets (Table 1). The two commercial products also contained a variety of nutrient sources, including animal and plant ingredients. Daily dry matter intake for the pair of otters was lower when they were fed the canned ZuPreem Feline diet than the smelt ($P<0.05$) but there was no difference in daily dry matter intake between the canned ZuPreem Feline and dry Feline k/d or between the dry Feline k/d and

were fed the smelt than the processed products. Crude fat digestibility was similar for all three diets. Dry matter, carbohydrate, and energy digestibility were higher on the dry Feline k/d diet than the canned ZuPreem Feline diet, but crude protein digestibility was not different between the two processed diets. Differences in digestibility may be related to differences in ingredient composition and the processing associated with the commercial diets.

Differences in apparent digestibility also may be a consequence of the small sample size, seasonal effects during the feeding study, or the sequential feeding of the three diets. Potential advantages of commercial products may be the reduced variability between different lots of production. Decreased energy digestibility of these processed products may help control obesity. For growing young or lactating females, when protein requirements presumably are high, the higher digestibility of frozen fish protein may be advantageous, particularly in comparison to the lower protein Dry Feline k/d. However, the nutrient content of fish may be more variable and also requires vitamin supplementation. Feeding fish to captive animals may stimulate more natural feeding behaviors than feeding processed feeds.

Table 1. Chemical composition of diets (dry matter basis).

Nutrient	Canned ZuPreem Feline	Dry Feline k/d	Smelt
Dry matter (%)	38.6	93.0	23.4
Ash (%)	7.1	4.3	15.2
Crude protein (%)	40.2	26.6	54.6
Crude fat (%)	37.4	24.6	16.8
Carbohydrate (%)	15.4	44.5	13.4
Gross energy (kcal/g)	6.33	5.81	5.22
Calcium (%)	0.48	0.61	1.82
Phosphorus (%)	0.45	0.48	1.49
Sodium (%)	0.21	0.29	3.15
Potassium (%)	0.33	0.75	0.76
Magnesium (%)	0.02	0.05	0.08
Iron (ppm)	484	221	33
Copper (ppm)	10	23	5
Zinc (ppm)	63	135	83
Manganese (ppm)	7	21	7
Selenium (ppm)	0.82	0.67	0.65

Table 2. Daily dry matter intake (g).

Trial	Number of days	Daily dry matter intake (g, mean±SE)
Canned ZuPreem Feline	3	122.4±4.8[a]
Dry Feline k/d	4	158.2±5.9[a,b]
Smelt	6	165.3±9.5[b]

[a,b]Means with different superscripts are significantly different ($P<0.05$).

Table 3. Apparent digestibility (%) of dietary dry matter, nutrients, and gross energy (mean±SE, $n=10$).

Nutrient	Canned ZuPreem Feline	Dry Feline k/d	Smelt
Dry matter	75.9±0.95[a]	80.9±0.92[b]	89.7±0.27[c]
Crude protein	73.2±0.95[a]	76.1±1.26[a]	92.2±0.27[b]
Crude fat	96.0±0.52[a]	95.2±0.40[a]	96.4±0.23[a]
Carbohydrate	56.5±0.44[a]	80.4±1.09[b]	94.0±0.33[c]
Gross energy	82.6±0.72[a]	85.0±0.72[b]	94.8±0.14[c]

[a,b,c]Means within each row with different superscripts are significantly different ($P<0.05$).

NUTRITION, URATES, AND DESERT SURVIVAL: POTASSIUM AND THE DESERT TORTOISE (*Gopherus agassizii*)

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Abstract

Desert tortoises (*Gopherus agassizii*) fed high potassium diets restrict food intake, increase water intake, and excrete higher concentrations of potassium in urine and urates. When maintained at constant nitrogen intakes, tortoises on high potassium diets use most of the ingested nitrogen to excrete excess potassium as potassium urates. Thus, even a high nitrogen diet (equivalent to 20% protein) did not allow net retention of nitrogen. Animal nutritionists need to pay more attention to the interactions of potassium and nitrogen in reptile diets, as these interactions may have important ramifications for the conservation of threatened species.

Introduction

In the desert, rainfall is typically minimal in amount, local in distribution, and variable from year to year. Desert animals have evolved a variety of behavioral and physiologic means of minimizing water losses, and hence of surviving on very low water intakes. The simple structure of the reptilian nephron does not permit urine of high osmotic concentration to be produced.[2] Thus, alternative mechanisms of reducing urine volume are required. For example, desert reptiles convert nitrogenous wastes to highly insoluble salts of uric acid that can be voided as semisolid precipitates with little loss of water.

Herbivorous reptiles, such as the desert tortoise (*Gopherus agassizii*), are confronted with the additional problem that most desert plants contain very high potassium (K) concentrations. Even the lush growth that follows rains typically contains a higher concentration of K, relative to plant water content, than does tortoise urine,[4,5] indicating how wasteful of water it would be to excrete K via liquid urine. Some reptiles, such as iguanine lizards (including chuckwallas, *Sauromalus*; desert iguanas, *Dipsosaurus* and Galapagos land iguanas, *Conolophus*), have developed salt glands that excrete K salts directly.[3,6] However, desert tortoises do not have salt glands and thus do not have this option.

We hypothesized that desert tortoises are obliged to excrete excess K as K urates, but that this involves a substantial nitrogen (N) cost to the tortoises since uric acid is nearly one-third N. If so, tortoises pay a high price to eat K-rich plants, since N used for K excretion may need to be diverted from productive functions such as growth and reproduction.

We conducted two studies, one in which diets of varying K concentrations were fed on an ad libitum basis to determine the effects of dietary K on intake and excretion of K, and the other in which the same diets were fed at a restricted level to determine the effect of dietary K on N balance when N intake is constant.

Methods

Animals: Studies were conducted in 1993 with individually-housed desert tortoises at the Desert Tortoise Conservation Center (DTCC) near Las Vegas, Nevada. The animals had been wild-caught as hatchlings or young juveniles by the Nevada Department of Wildlife in 1990, and had been maintained at the DTCC on pelleted diets since 1991. Research was conducted under authority of 10(a) 1(a) research permit PRT-747182 issued to the Nevada Department of Wildlife, the Bureau of Land Management, and The Nature Conservancy.

Study 1: Twenty-four juvenile tortoises (body mass [BM] range 187-815 g) were blocked by mass into six blocks, and one animal in each block was assigned a pelleted diet containing either 0.5%, 1.6%, 2.7%, or 3.8% K (dry matter [DM] basis). These K levels were achieved by replacing sucrose in the pelleted diet by a mixture of K salts (potassium chloride, potassium bicarbonate, and potassium citrate in a fixed ratio). All other nutrients (including protein at about 20% of the diet) were held constant. Fresh food and distilled water were provided twice per week; uneaten food was removed, dried, and weighed to determine food intake. Animals were weighed at the beginning and end of the study. After a 3-wk adaptation period, animals were administered deuterium oxide by gastric intubation to determine water turnover, and total collections of all excreta (urine, urates, and feces) were made. The cages and tortoises were cleaned fastidiously with distilled water and a 1 g/L solution of lithium carbonate (to solubilize urates) at the beginning and end of the total collection period to account for residual excreta. The total collection period lasted 3 wk. Clean urine samples were collected for an additional 5 wk for deuterium analyses.

Study 2: The same animals, diets, and experimental protocols were used except that the amount of food offered was restricted in order that food intake (and hence N intake) would not differ among treatments. The amount of food offered was restricted to the level of intake of tortoises fed the 3.8% K diet in Study 1, i.e., about 2.9 g/kg BM/d (equal to 2.7 g/kg BM/d on a DM basis; Table 1).

Laboratory analyses: All samples of food and excreta (including cage washes) were frozen at -20^0 C until assayed. Fecal samples were dried at 60^0 C and any visible contaminants removed manually. Urine samples were centrifuged to remove suspended urates prior to analysis. Urate samples were dried, and the consequent moisture losses were assumed to be liquid urine; the N and K attributable to this urine contamination were calculated and subtracted. Any samples that were too mixed to permit separation of components were considered as "other" (along with cage washes). Samples for each animal were pooled by excreta category prior to analysis. Food and excreta samples were assayed for N concentration by a macro-Kjeldahl procedure using cupric sulfate as catalyst and 0.1 N hydrochloric acid to titrate the

ammonia generated.[1] Potassium was assayed by atomic emission spectroscopy after digestion of samples with perchloric and nitric acids. Deuterium concentrations of urinary water were analyzed by quantitative infrared spectophotometry after heat distillation.[7]

Results

In study 1, tortoises decreased voluntary food intake significantly as dietary K increased. Expressed per unit body mass, daily intake of food (dry matter basis) dropped from 6.8 g/kg BM/d on the low K diet to 2.7 g/kg BM/d on the high K diet (Table 1). Although the intake of K increased significantly from the 0.5% K diet to the 1.6% K diet, there was no significant difference in K intake among the 1.6, 2.7, and 3.8% K diets. Tortoises appeared to limit K loading by reducing food intake at high dietary K levels. A complication in this study was that intakes of N and other nutrients decreased as food intake declined. Thus, less N was available to form K urates when high K diets were eaten. Tortoises exhibited several compensatory responses. Water intake and excretion increased (as measured by isotope dilution; Table 1). The average K concentration in fluid urine also increased, from 0.25% (fresh weight basis) on the 0.5% K diet, to 0.51%, 0.58%, and 0.68% on the 1.6%, 2.7%, and 3.8% K diets, respectively. Urate K concentration increased from 6.1% on the 0.5% K diet to 15%, 17%, and 19% (dry matter basis) on the 1.6%, 2.7%, and 3.8% K diets, respectively. Thus, tortoises compensated for the increase in dietary K by (1) limiting K loads by decreasing food consumption, (2) increasing water intake and urinary K excretion, and (3) increasing K concentration in urates such that less N was required per g K excreted.

In study 2, the effects of dietary K level on N excretion and balance were examined (Fig. 1). In this figure the total height of the bars represents N intake, which did not differ significantly among the four dietary treatments. Total fecal N losses remained relatively constant, and there was only a small increase in urinary N losses with an increase in dietary K (in spite of an increase in urinary volume and urinary K output). However, urate N excretion increased dramatically with the increase in dietary K, as expected. The net effect was a pronounced decrease in retained N at high levels of K intake. At 3.8% dietary K, N balance was not significantly different from zero. In other words, even though the 3.8% K diet contained a high level of N (3.3% or 20.6% protein), tortoises were unable to retain any of it for growth. Relatively few desert plants (other than legumes and a few annuals) contain this much protein.

Discussion

These studies illustrate that dietary K can have a major effect on the amount of N that tortoises can use for productive functions such as growth and reproduction. It appears that a key indicator of the nutritional quality of forage plants may therefore be the N to K ratio (N:K). On high K diets, urates contain a N:K ratio of about 1.3:1. If all N and K in ingested food were absorbed during digestion, and if all K was excreted as urates, each g dietary K would require 1.3 g dietary N for excretion. However, only a relatively small number of desert plants have a N:K ratio of 1.3 or higher. These include plants which tortoises have been reported to select preferentially in nature, such as legumes (*Lotus, Lupinus*), filaree

(*Erodium*), globe mallow leaves (*Sphaeralcea*), seed heads of woolly plantain (*Plantago*), and Mediterranean grass (*Schismus*).

Given the ubiquity of K in most foods, and the ease with which it is absorbed and excreted by mammals, animal nutritionists usually pay little attention to the K concentration in diets. However, it is now evident that dietary K concentration, and especially its relation to dietary N concentration, may be of critical importance for tortoises in desert environments. If livestock grazing or other human activities influence the distribution or abundance of plants that have high N:K ratios, the long-term viability of tortoise populations may be affected. Given the threatened status of desert tortoises and other tortoise species, further research into the nutritional requirements of tortoises is clearly needed.

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Table 1. Dry matter, potassium, and water intakes of captive juvenile tortoises.[a]

Item	Dietary K concentration (%)			
	0.5	1.5	2.5	3.6
No. of animals	6	6	6	6
Intial mass (g) ±78	549 ±109	539 ±85	521 ±76	527
Dry matter intake (g/kg BM/d)	6.83 ±0.31	5.85 ±0.42	3.60 ±0.79	2.70 ±0.73
Water intake (g/kg BM/d)[b]	11.7 ±1.4	19.4 ±0.5	17.7 ±3.5	16.8 ±3.7
Potassium intake (mg/kg BM/d)	37 ±1.7	96 ±6.9	98 ±21.6	120 ±28.7
Water intake:DM intake (g/g)	1.7 ±0.16	3.4 ±0.15	5.2 ±0.41	5.7 ±0.44
Potassium intake:water intake (mg/g)	3.3 ±0.35	4.9 ±0.23	5.4 ±0.37	6.9 ±0.56

[a]Means±SE.
[b]Water intake estimated from isotope dilution; includes liquid water, food water, and metabolic water.

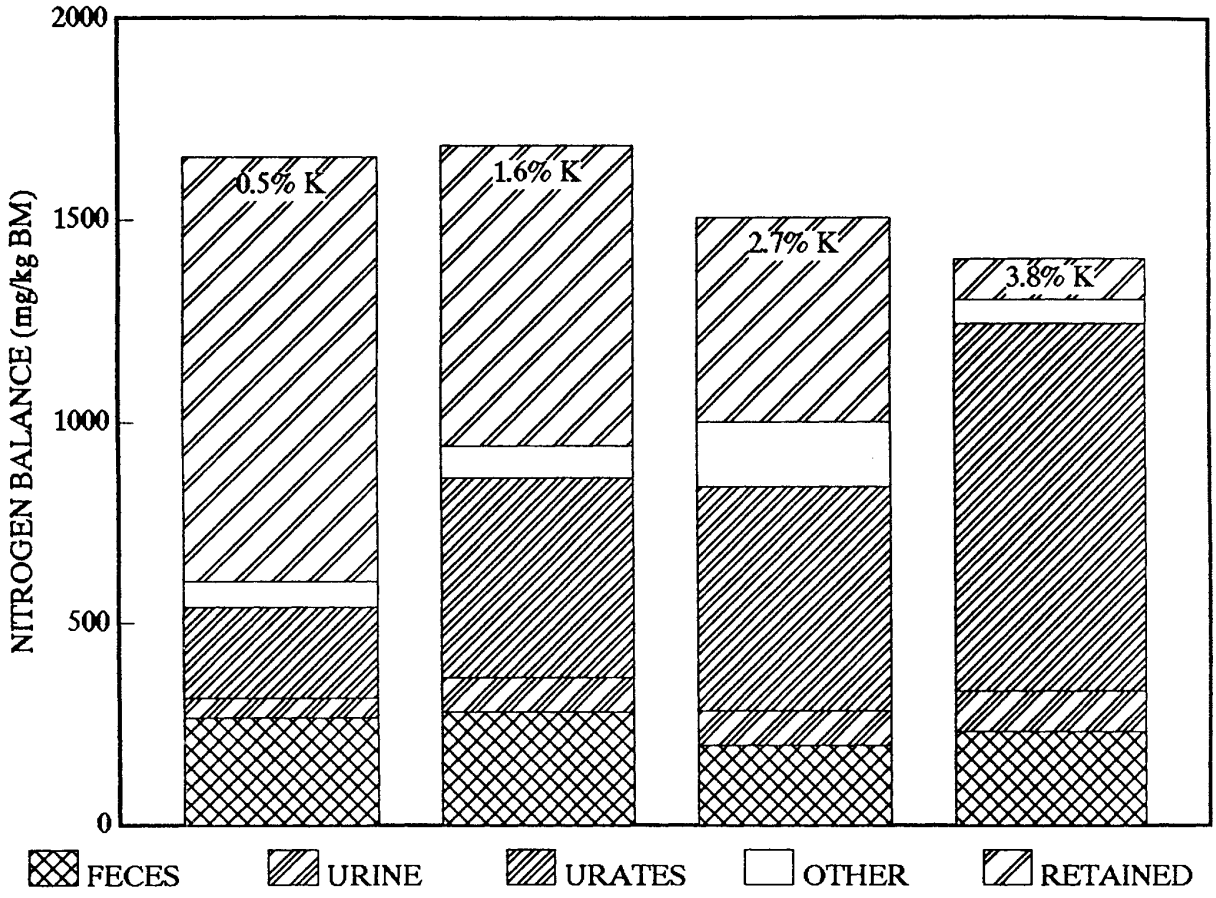


Figure 1. Intake and excretion of nitrogen (N) by desert tortoises fed four diets varying in potassium (K) concentration. The total height of each stacked bar represents the mean N intake of six tortoises. Routes of excretion are indicated by designated shading patterns; "other" refers to mixed excreta that could not be separated plus cage washes at the end of the trial. Retained N was the difference between intake and excretion.

UPDATE ON VITAMIN D AND ULTRAVIOLET LIGHT IN BASKING LIZARDS

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In nature many lizard species spend considerable time basking. While basking is important for thermoregulation, ultraviolet light B (UVB) from 290 to 315 nanometers (nm) provided by the sun also stimulates the cutaneous synthesis of vitamin D. When we place animals in captivity, we prevent them from using their natural source of both heat and UV light. In captivity, lizards housed without access to natural sunlight are typically provided with a source of heat such as an incandescent lamp or "hot-rock". Some species such as *Phelsuma* spp., *Chameleon* spp., and *Iguana* spp. are often provided with a source of UVB if housed indoors. Despite our recent gains in knowledge of proper husbandry and diets for captive lizards, veterinarians in zoos and exotic pet clinicians still observe rickets and osteomalacia in many species of lizards. There is undoubtedly species variation in dietary vitamin D requirements and the need for UVB.[3] However, we believe that UVB irradiation may be important for many species of lizards and should be considered an essential part of lizard husbandry until more is known about requirements of particular species.

A primary function of vitamin D is the regulation of calcium and phosphorus homeostasis. Vitamin D is essential for the uptake of calcium from the gastrointestinal tract and for adequate mineralization of bone. Many species, including humans, can utilize a dietary source of vitamin D. In the absence of sunlight, dietary vitamin D, at appropriate levels, should satisfy requirements. However, there may be species differences in the ability to utilize dietary vitamin D. Domestic birds and some platyrrhines have been reported to preferentially recognize and use cholecalciferol (vitamin D_3) rather than ergocalciferol (vitamin D_2).

Vitamin D metabolism in reptiles is poorly understood. In captivity many species of basking lizards are maintained under fluorescent lights, some of which may provide a source of UVB. Concentrations of serum or plasma 25OH-D are considered useful is assessing the vitamin D status of animals and humans.[4] For example, humans normally circulate between 8 - 60 ng 25OH-D per ml, although after several months of exposure to summer sun levels might reach 100 ng/ml.[4] The results of UVB studies with the green iguana (*Iguana iguana*) suggest that high circulating concentrations of 25OH-D are present after exposure to artificial UVB.[2] Although we do not know what normal levels are for any reptile, green iguanas housed outdoors circulate very high concentrations of this metabolite (> 400 ng/ml, $n = 8$). We believe that the green iguana may need either extraordinarily high dietary concentrations of vitamin D or daily exposure to a source of UVB.[2]

Results of our recent work with Komodo dragons (*Varanus komodoensis*) suggest that this species may also benefit from a source of UVB. Out of 13 Komodo dragons hatched at NZP, nine had long bone fractures discovered at about 2 mo of age. While the frequent handling of these young lizards may have contributed to the fractures, circulating levels of 25OH-D were low (13.8 ng/ml) after no exposure to UVB for 2 mo. Approximately 1 mo later, after exposure to UVB emitting bulbs (GE Chroma 50, Sylvania Design 50 and/or GE BL 40 black lights), six animals had 25OH-D serum levels of 45.8 ng/ml, whereas the remaining seven hatchlings that were not exposed to a UVB source had 25OH-D levels of 17.1 ng/ml. We presume that rapid growth increases requirements for calcium (Ca), phosphorus (P), and vitamin D and that non-reproductively active adults may be more tolerant of low dietary levels of Ca, P, and/or vitamin D, or of low exposure to UVB. Although we do not know what represents normal vitamin D levels for Komodo dragons, the National Zoo's adult female Komodo dragon had serum levels of over 90 ng 25OH-D per ml after being exposed to the summer sun in an outdoor enclosure for about 3 mo.

Factors which affect the usefulness of artificial light sources include UVB intensity, distance from animal, time of exposure, and temperature. Some fluorescent bulbs on the market apparently emit energy in the UVB range but are very weak emitters compared to the sun or to bulbs with high-energy output. Bulbs that emit weak energy in the UVB range may need to be on for relatively long periods of time and may need to be placed as close to the animal as practically possible if they are to provide sufficient irradiation for adequate stimulation of vitamin D production. We have produced circulating levels of 25OH-D in 24 green iguanas of over 225 ng/ml in healthy green iguanas exposed for 18 weeks to GE Chroma 50 bulbs or a combination of Chroma 50 and GE BL 40 black lights (M.E. Allen et al., in prep.). These bulbs were on a 12:12 cycle and were no more than 18 inches from the cage bottom.[1] Bulbs designed to emit higher energy in the UVB range have been used with green iguanas and also produced circulating levels of 25OH-D of over 200 ng/ml.[2] Many bulbs that are marketed as full-spectrum bulbs for the reptile trade may provide little, if any, UVB. In large, multi-species exhibits, irradiation of animals may be difficult with bulbs that are weak UVB emitters.

The cutaneous synthesis of the vitamin D precursor, 7-dehydrocholesterol, to pre-vitamin D (pre-D_3) occurs during exposure to UVB. However, the subsequent conversion of pre-D_3 to vitamin D in the skin is temperature dependent. Thus, not only is UVB exposure critical to the synthesis of vitamin D, poikilothermic animals must be sufficiently warm to allow this thermal isomerization to occur. This reaction is being studied in savanna monitors (*Varanus exanthomaticus*). In savanna monitor skin the $t_{1/2}$ of pre-D_3 to vitamin D was only 8 hr at 25°C.[5] In a control solution of hexane at the same temperature the $t_{1/2}$ was 91 hr.[5]

Exposure to the sun may be the best way to satisfy an animal's requirement for vitamin D. In zoos, direct exposure is rarely practical since many reptile exhibits are in temperate climates and reptiles are housed indoors year-round. In some large exhibits UVB emitting bulbs may not be practical. However, installation of UVB transmitting skylight materials may be possible in exhibit areas. While window glass and most plastics do not transmit UVB, an acrylic, Solacryl SUVT (1/4") (Polycast, Stamford, Connecticut, USA) transmits

UVB (295 nm) at 80% compared to air.[6] Zoo managers and exhibit design specialists should consider the use of such UVB transmitting acrylics when planning or renovating exhibits.

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HANDLING WAPITI ON A GAME FARM

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The following text comprises the script of a video presentation produced jointly by the Division of Audio-Visual Services, University of Saskatchewan, and Marimba Farm Inc, of Saskatchewan. The film depicts the handling of wapiti on a commercial game farm. The principles of handling are discussed, and in some cases specific reference is made to other species of deer that may be handled in a similar fashion.

Introduction

Wapiti, otherwise known as North American elk are a deer species well suited to the agricultural diversification movement that has taken place in the last 20 years.

There are several basic principles for handling deer in a farm situation. The importance of training cannot be overemphasized. This type of livestock should be given every opportunity to learn their way around the farm. Gates can be left open for them to investigate alleyways and new paddocks in their own time. When it comes time to handle them their knowledge of the layout will prove invaluable.

From time to time a deer species will need to be handled for routine farming procedures. These may range from paddock changes to more complex affairs such as weaning, pregnancy checking, de-worming, antler removal or assistance at calving time.

Paddocks

Paddock shapes that work best are those that are rectangular in shape with a gate near one corner, or alternatively, triangular, with the gate at the apex.

The deer are best moved from a paddock by a small number of people with whom they are familiar. The sudden invasion of a large number of strangers, especially people unfamiliar with stock, is not advised and may upset the animals. If the entire group move together they will remain as a unit. If for some reason a single animals gets separated, or even a small group, it is usually better to let the mob group together again so that they will move in concert. The exception would be animals that are thoroughly familiar with both the set-up and the handlers and that require individual treatment.

Gates

Gates should be wide enough to permit the passage of the group, and at the same time should be easily closed. Another consideration for gates is that they must be wide enough to permit the access of farm machinery.

Alleyways

All paddocks should connect to alleyways down which the animals can readily be moved. The width varies slightly according to the species of deer, but again farm machinery access may be a governing factor. Ten meters is a convenient width for wapiti. Any segment of an alleyway over 100 meters in length should probably be built with a corner or baffle so that animals do not break back.

Corners

One of the basic principles of deer handling is that they will readily move around corners. Even in alleyways corners are important, as the deer will seldom break back once the group has rounded a corner. Corners also serve to bunch up the herd as they hold together at the turn and then move through as a mob.

Visual barriers

Once the animals begin to approach the handling yards some pressure may be applied to them to persuade them to enter. At this time shade netting or boards will prove invaluable as a reminder of the barrier. If wire alone is used there is a potential for trauma, especially among younger stock who do not know their way around, and who may run fences, almost appearing to forget the presence of the wire.

Sorting yard

A sorting yard before the pens is extremely valuable. Larger mobs can readily be broken down into smaller groups and moved into the shed. Confident body language and eye contact can be used to separate a group and move them into another section of the system.

When males are in hard antler, there is an increased risk of trauma due to fighting. If wapiti have to be handed at this time it is advisable to get them into the sorting yards quickly an efficiently, and then to get them out and separated in the pens as soon as possible. This applies particularly to mature animals, which may inflict heavy trauma to one another, or to younger subordinates.

Pens

There are many designs of pens and chutes that work well. If deer are new to a property it is an excellent idea to allow them access to pens over several days and nights. This can readily be done by leaving feed in such areas and allowing the animals a chance to explore. This will pay dividends when the time for handling arrives.

In this set-up a series of sliding and swinging gates allows the handler to sort animals even further, even down to the individual animal. Here are a group of undergraduate students

sorting animals for weaning, vaccination and tick treatment. Using both sliding and swinging gates there is no need for anyone to enter the pens directly at any time, with one exception. Occasionally, if an animals balks, an experienced handler may enter with a shield. This serves not only to protect the handler, but increases body profile and confidence. This system is particularly valuable for the handling of large wapiti, and even has a useful role in the handling of other deer species.

Tub & half tub

From sorting pens the animals can be moved into a tub or half tub. This set-up shows a half tub that can readily be converted into a handling chute. The tub posts are set about 55 cm (22 inches) apart and as one gates swings around to meet the other several handling possibilities emerge.

It is in the tub that weaned calves are best handled. They can be crowded sufficiently to prevent them from running around personnel, and they will readily submit to procedures at this time. This technique is not generally safe for handling adult wapiti, but groups of adult red deer are often handled in similar situations.

Scale

A scale is an essential part of the farming operation. It can readily be incorporated into some part of the handling system.

Squeezes

If close handling is required some sort of squeeze or chute may also be a part of the system, although some farmers choose to do most of their handing in the tub. Several sorts of chute exist. They generally fall into one of three categories, and all three have their proponents, and specific advantages. Drop floor chutes are popular, especially for red deer and or fallow deer. They allow access to the head, back, hind end, and little else. Hydraulic chutes are seen on many farms, and have the advantage that stags in full antler can be handled with safety. This is particularly an asset at velveting time. The third type of squeeze is of a box design, and allows access to almost any part of the animal's body.

A halter may be used to control the head. In this case a homemade halter is particularly valuable as it allows a quick release once the procedure is finished.

Exit

The last part of the handling system is often ignored. It must be designed with some sort of ability to draft animals after they have been through. Gates and yards can be set up so that animals can be directed to specific areas of the farm. Again, as animals leave the

squeeze it is important for them to know their way around. If they have been given the chance to familiarize themselves with the set-up they will move unconcernedly through gates and along alleyways.

Conclusion

The basic tenets of handling consist of an understanding of the importance of training for both the animals and the personnel. It is probably true to say that these components have about equal value.

Alleyways, corners, visual reminders, dividing gates, tubs, chutes, and squeezes make up the physical structures. People and animals familiar with their surroundings, and knowing their objectives, make up the active parts. Routine handling of deer species should be an integral part of the farming operation.

INTRAVENOUS ANTIBIOTIC THERAPY IN HOOFSTOCK USING A PORTABLE, BATTERY-POWERED INFUSION PUMP

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Introduction

Several factors limit successful antimicrobial therapy in exotic hoofstock. Relatively few antibacterial and antifungal drugs are available for oral or intramuscular administration in ruminants, and delivery is often challenging. Frequent problems with oral antibiotic therapy include poor palatability, variable absorption, floral changes, and rumen degradation (e.g. trimethoprim). Intramuscular administration usually requires remote delivery. Drug volume and dosing interval must be adjusted to available dart sizes and the tolerance of the animal for frequent darting. In many species, the additional stress of the treatment regimen may be life-threatening.

An outbreak of interdigital necrobacillosis, associated with *Fusobacterium necrophorum*, occurred in impala (*Aepyceros melampus*) and springbok (*Antidorcas marsupialis*) at the North Carolina Zoological Park in 1993. Several cases progressed to osteomyelitis involving one or more digits despite repeated debridement and intramuscular administration of either procaine-benzathine penicillin G (Ambi-pen™, Butler Company, Columbus, Ohio, 43228, USA) or trimethoprim sulfadiazine (Di-trim®, Syntex Animal Health, West Des Moines, Iowa, 50265, USA). A method of intravenous antibiotic delivery was established using a portable, battery-powered infusion pump attached to the neck of the animal and programmed to deliver up to 5 days of continuous therapy.

Materials and Methods

A Provider® 5500 portable infusion system (Abbott Laboratories, Hospital Products Division, North Chicago, Illinois, 60064) powered by two 12 volt batteries was used for antibiotic delivery. Initially, the pump was programmed in the intermittent delivery mode which delivered a bolus of antibiotic at selected intervals. Between boluses, the pump operated at a minimum rate, or KVO rate (keep vein open), of 0.2 ml/hour. The KVO rate proved insufficient to maintain catheter patency, and the continuous delivery mode was used thereafter. The hourly rate of delivery during continuous therapy ranged from 2-5 ml/hour and was determined based upon the calculated daily dose and volume of antibiotic(s), the duration of therapy (3-5 days), and the total available fluid reservoir. A fluid bag of appropriate size (100-500 ml) and content (lactated ringers or 5% dextrose) was chosen to serve as the reservoir. Alkaline batteries were replaced every 3-5 days.

The following antibiotic solutions were administered intravenously in selected cases. 1) Vancomycin (vancomycin HCL, 500 mg/10 ml, Schein Pharmaceutical, Inc., Port Washington, NY, 11050, USA) at 20 mg/kg per day delivered as a 5% solution in LRS. 2) Clindamycin (Cleocin Phosphate^{R}, 600 mg/4 ml, The Upjohn Company, Kalamzoo, MI, 49001, USA) at 20 mg/kg per day combined with amikacin (Amiglyde-V^{R}, 250 mg/ml, Aveco Co., Inc., Fort Dodge Laboratories, Fort Dodge, IO, 50501, USA) at 15 mg/kg per day diluted in lactated ringers. 3) Trimethoprim sulfamethoxazole (trimethoprim 160 mg/10 ml and sulfamethozazole 800 mg/10 ml, Elkins-Sinn, Inc., Cherry Hill, NJ, 08003, USA) at 60 mg/kg per day diluted 1:5 in 5% dextrose.

For initiation of therapy, a 16 to 18 gauge jugular catheter was placed using sterile technique and sutured in place using a T-port extension (Abbott Laboratories, North Chicago, IL, 60064, USA). Two styles of intravenous catheter were used: an Intracath^{R} 6" intravenous catheter (Becton Dickinson Vascular Access, Sandy, Utah, 84070) or a Landmark^{R} 6" midline catheter (Menlo Care Inc., Menlo Park, CA, 94025). The fluid bag (reservoir) and pump were attached to either side of the neck using skin glue or umbilical tape stints. The fluid line, pump, and bag were wrapped with VetWrap^{R}.

Results

Impala and springbok instrumented with the infusion pump were surprisingly tolerant of the neck wrap and of the various noises emitted by the pump. During infusion, the pump clicked softly at regular intervals. If delivery was interrupted due to catheter or fluid line occlusion, low battery, or air-in-line, a continuous high-pitched alarm sounds. Animals acted normally and continued to eat despite the alarm which sounded overnight in some instances.

The first animal treated with the pump was an adult male impala with septic pedal arthritis. Minimal improvement was noted after 10 days of intravenous vancomycin therapy, and antibiotic solution was switched to amikacin/clindamycin. Within 3 days, marked improvement was evident, and therapy was continued for 7 weeks until the infection resolved. Mild, transient (5 days) diarrhea was associated with clindamycin. Weekly serum biochemical panels revealed no elevation in BUN or creatinine during the course of therapy in the impala. In springbok, intravenous antibiotic therapy with trimethoprim sulfadiazine or clindamycin/amikacin has been used to treat interdigital necrobacillosis and pneumonia.

Problems with the pump were minor, and were readily solved with experience. The infusion stopped with kinking of the catheter or fluid lines (in some instances due to bandage slippage), hair or dust covering the pump sensors, and clotting within the catheter during intermittent therapy. Corrective steps included the use of the continuous delivery mode, smaller and more pliable catheters (e.g. Landmark^{R} midline catheter), securing the pump and bag with stints, and regular cleansing of the pump sensors with 70% alcohol.

Discussion

Intravenous antibiotic therapy has been successfully delivered in impala and springbok with severe infections using a portable, battery-powered infusion pump. Portable infusion pumps are currently used in home health care for antineoplastic and analgesic therapy in people. Prices range from approximately $3,000 for the Abbott Provider^{R} 5500 pump to $5,000 for the more versatile CADD-PLUS^{TM} pump (Pharmacia Deltec, Inc., St. Paul, MN 55112, USA). The CADD-PLUS pump can be programmed to use either a 50 or 100 ml medication cassette attached to the pump itself, or a fluid bag reservoir as with the Provider pump. The portable pumps can also be used to deliver intravenous fluid therapy or total parenteral nutrition, similar to larger models. As an alternative to direct purchase, pump rental may be possible via a home health care company.

Problems encountered with the infusion pump were surprisingly minimal, and were mostly attributed to human error in programming and attaching the pump. No problems with phlebitis were seen with intravenous catheters maintained for up to 2 weeks. This technology provides the clinician with a greater selection of chemotherapeutic agents for use in exotic ruminants. Intravenous antibiotic therapy is possible using a portable infusion pump system and should be considered for severe infections in valuable hoofstock.

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EASTERN EQUINE ENCEPHALOMYELITIS IN A GERENUK, (*Litocranius walleri*)

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A 2.5 year-old male gerenuk at the White Oak Conservation Center presented with acute onset of bilateral thoracic limb paralysis, pelvic limb paresis, and a decreased panniculus reflex bilaterally in the thoracic region. There was no external evidence of trauma. Cervical and thoracic radiographs and a myelogram were normal. Treatment with dexamethasone and maintenance fluids was initiated and the gerenuk was transported to the Veterinary Medical Teaching Hospital (VMTH) at the University of Florida.

A CBC revealed leukocytosis (WBC - 8100/mm^3, normal < 5000) with a left shift. Biochemical profile was normal. Results of the cerebrospinal fluid analysis were protein = 87 mg/dl, and WBC = 40/mm^3, with lymphocytes as the predominant cell type (88%). Treatment was initiated with trimethoprim/sulfadiazine, pyrimethamine, and methylprednisolone sodium succinate. Maintenance fluid therapy was continued. The animal's condition continued to degenerate and euthanasia was performed within 30 hours after the onset of clinical signs.

No lesions were evident during gross necropsy. Meningoencephalomyelitis involving the brain, cervical, thoracic and lumbar spinal cord, and meninges was present on histologic evaluation of tissues. Mixed inflammatory cell infiltrates with perivascular-cuffing were typical of these lesions. Fluorescent antibody testing of brain tissues for rabies and BVD was negative. Serum antibody titers for *Toxoplasma* antibody were also negative.

Eastern, Western, Venezuelan, and St. Louis Encephalitis serum antibody titers were performed with an EEE titer of > 1:640 (positive = 1:80 and >) observed. There was no history of vaccination for EEE in this animal. Titers for the other encephalitis viruses were negative. Immunohistochemical staining for EEE antibody yielded positive intracytoplasmic staining of neurons and glial cells in the pons, medulla and spinal cord. Diagnosis of EEE was confirmed by isolation of the virus from a tissue homogenate of brain and spleen. EEE has been infrequently reported as a cause of encephalitis in goats and cattle, but has not been reported in exotic ruminants. EEE should be considered as a sporadic cause of neurologic disease in these species.

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CHRONIC RECURRENT ANEMIA, MASSIVE PULMONARY AND SYSTEMIC MINERALIZATION, CHRONIC INTERSTITIAL NEPHRITIS AND MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS, AND HEMOSIDEROSIS WITH MYELOPHTHISIS IN A EUTHANATIZED BLACK RHINOCEROS

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Clinical presentation

On October 27, 1991 a 15 year old, male, black rhinoceros (*Diceros bicornis michaeli*) was found with approximately one third of its tail denuded of skin and hemorrhaging. No other animals shared the enclosure. The tail was observed for the next few days and apparently normal healing began. The animal, however, was reported to be somewhat lethargic. On November 1, 1991 the animal had developed multiple, 2 cm., raised areas within the dermis or subcutis, over the scapular regions. The areas were firm, with the overlying dermis able to slide easily over the subcutaneous lesions. The skin lesions were not warmer than the surrounding tissue and no erosion of epithelium was present. Blood was collected two days later with results presented in Table 1. Normal values for the Brookfield Zoo Clinical Pathology Laboratory are given in Table 4.

Due to the well documented black rhinoceros anemia syndrome and associated skin disease, a blood monitoring program was initiated to monitor the weekly progress of the animal (Tables 1-3). The animal had a history of becoming lethargic and/or lame each winter since the mid 1980's. Previous blood work had not been consistently performed. By February 27, 1992 the animal no longer showed signs of lethargy and skin lesions had recovered to minor scarred areas. The hematocrit had maintained a level of 31% (RBC > 3.0 x 10^6) for almost one month and there were no overt signs of illness.

For the next year and a half, the animal would have periods of normal activity alternating with bouts of lethargy. Periods of normal activity correlated directly with an increase in the hematocrit (RBC numbers). Episodes of apparent lameness which correlated with a low hematocrit were also observed. The typical hematocrit during bouts of anemia was a normocytic, normochromic, non-regenerative anemia. On only a few occasions were reticulocytes, basophilic stippling, or an increase in the MCV seen. The leukogram remained within normal limits. During the initial progression of this disease, the serum chemistries were within normal limits. As time passed however, there were indications of renal disease

with increasing BUN and creatinine, along with shifts in the Ca:P ratio (Tables 1-3). Multiple urinalyses showed no urinary blood loss, and direct fecal examination and fecal occult blood tests were uniformly negative.

With the onset of winter in 1992, the animal was lethargic and anorectic. On September 28, 1992 weekly blood analysis was again initiated (Table 2). Anemia had recurred, with a hematocrit of 27% (RBC = 2.89 x 10^6). Within one week, skin lesions reappeared. They consisted of hemorrhagic, open, moist, 1-2 cm erosions present on bony prominences such as the tuber coxae, trochanter major, carpus, and elbow. Cultures of the open wounds produced variable growth of bacteria such as *Aeromonas hydrophila*. Leptospirosis titers revealed a titer of 1:200 for *Leptospira bratislava* and negative titers for *canicola, grippotyphosa, hardjo, pomona,* and *icterohaemorrhagiae* (Table 5). Due to the positive titer from December 8, 1992 a titer from previous stored serum was evaluated and found to be 1:400 for *Leptospira bratislava* on June 05, 1992 (Table 5). The animal was started on tetracycline, 5 grams, po, q12h along with supplementation of phosphorus (Wayne P-15) at 50 gm/kg of alfalfa fed. The dietary change resulted in a Ca:P ration of 1.23:1.0 whereas the standard diet is a ratio of 3.73:1.0.

The rhinoceros' attitude continued to deteriorate and the skin condition worsened. Severe lameness developed in the right forelimb. A distended area on the caudal aspect of the right carpus appeared along with a 4/5 lameness. This swelling was sterilely tapped using a 14 gauge needle which yielded approximately 100 ml of a thick and clear, serosanguinous fluid. The fluid had few RBC's, rare lymphocytes, and scattered bacteria. The hematocrit continued to decrease with the initial hematocrit of 27% having dropped to 19% by March 23, 1993. Again, numerous attempts were made to determine the source of the blood loss by performing multiple urinalyses, occult blood tests on feces and direct examination of fecal material. The results were negative as with the previous bout of anemia the prior year.

Four, 6 mm, punch biopsies of the dermal swellings over scapular areas were obtained using local anesthesia with 20% lidocaine injected subcuticularly. Deep and superficial biopsies from each site of the raised skin plaques were obtained. The biopsies revealed that the deep dermis and subcutis contained regionally extensive areas of necrosis with collagen hyalinization surrounded by zones of mineralized cartilage. These lesions were not typical "ulcerative rhinoceros skin disease" but resembled equine nodular collagenolytic granuloma ("nodular necrobiosis").

On March 24, 1993 antifungal therapy was prophylactically initiated due to the history of fungal infections in compromised black rhinoceros'. Itraconizole was given, 1.5 gm, po, bid, for the duration of the tetracycline therapy. The animals condition continued to deteriorate and by May 17, he could no longer walk more than a few feet without resting or collapsing. The hematocrit had dropped to 12% (RBC = 1.21 x 10^6). Due to the poor quality of life, unfavorable prognosis and prolonged course of the disease, euthanasia was performed on May 18, 1994.

Necropsy results

Gross examination revealed a moderately severely emaciated animal, with healing, cutaneous, ulcerations over pressure point areas. Two, large, subcutaneous, fibrous encapsulated masses were present on the elbow which internally contained gritty, caseous material. The lungs had thickened septa, numerous, 1-2 cm and occasionally larger, cavitations, and the lungs were gritty on section. Cytological smears of a lung cavitation revealed numerous fungal hyphae and spores. Numerous, large vessels and the heart valves had multiple, firm, gritty, intimal to medial plaques, and the cardiac vasculature was enlarged and tortuous. The kidneys had numerous, usually miliary but up to 1 cm, cysts, and poor demarcation of corticomedullary junctions. There was generalized lymph node enlargement, and cystic thyroid glands. Mild gastric ulcerations were noted, and femoral, humeral, and ischial bone marrow was dark brown and gelatinous.

Histologically, the lungs had multifocal, moderate sized to massive areas of alveolar septal calcification, bronchiolar and alveolar duct, mucosal to sometimes mural, mineralization, and associated with scant to moderate granulomatous infiltrate. Numerous cavitations were also present which centrally contained abundant necrotic debris and degenerate inflammatory cells, mineralized debris, and outer fibrotic and calcified walls. One large area of acute necrosis of parenchyma, with calcification of large amounts of necrotic tissue, abundant deposition of fibrin, and severe infiltrate of neutrophils, macrophages, giant cells and lymphocytes was also present. Interlobular septa and pleura were moderately to markedly fibrotic. The glandular, gastric, mucosa had moderate to severe, multifocal calcification, and large regions of muscularis were effaced by irregular, disorganized, hypercellular, cavitated, and often calcified collagen. Vasculature throughout most organs often exhibited intimal to medial calcification. Heart valves were similarly mineralized, as were adjacent myocytes, and surrounding myocardium had moderate, interstitial, fibrosis. The subcutaneous masses consisted of multilobular, fibrous-encapsulated, necrotic and mineralized debris, with scant granulomatous infiltrate. Moderate to moderately severe, random, and often occlusive calcification of renal tubules was present, and the kidneys also exhibited moderately severe, fibrosing, granulomatous interstitial nephritis with moderate obliteration of parenchyma. Remaining glomeruli exhibited marked enlargement and increase in cellularity and mesangium, and also often were sclerotic or had adhesions to thickened Bowman's capsules. The hematopoietic component of the bone marrow was nearly obliterated by dense sheets of hemosiderin-laden macrophages. Remaining hematopoietic cells consisted primarily of progenitor and maturing erythroid cells, although myelopoiesis and thrombopoiesis was also present. There also was moderate fibrosis between the abundant, bony trabeculae. Moderate to large numbers of macrophages distended with hemosiderin were also present in the lamina propria of the small intestine and colon, the liver, adrenal glands, lymph nodes, and spleen. Also, lymph nodes were usually moderately reactive, there was moderate, diffuse, hyperplasia of adrenal cortical zonas fasiculata and reticularis, moderate, granulomatous hepatitis with biliary hyperplasia and centrilobular, intrahepatocellular, biliary stasis, cystic hyperplasia of the thyroid glands with a focal area of dysplasia, and moderate lipofuscin deposition in brain stem neurons. *Aspergillus* sp. was cultured from one of the pulmonary cavitations, and was considered to be an opportunistic invader.

Interpretations

The most profound histologic lesions were the massive pulmonary and systemic vascular mineralization, the obliteration of bone marrow hematopoietic cells by hemosiderin-laden macrophages and fibrosis, the iron storage in various organs, and the interstitial nephritis and membranoproliferative glomerulonephritis. The severe and recurrent anemia probably led to the iron deposition in multiple organs and bone marrow. The hematopoietic cells exhibited an erythroid "shift", but if the sections of marrow examined were representative, there was inadequate tissue remaining to respond to the high demand. Erythropoietin production by the kidney was also probably moderately to markedly reduced. Additionally, an iron storage disease or primary bone marrow abnormality cannot be entirely ruled out, but is less likely. The cause of the tissue mineralization is also speculative. However, as the BUN was chronically elevated, and there were severe, chronic renal lesions, the most likely cause was uremia-induced tissue damage with resultant dystrophic calcification. The cause of the chronic interstitial nephritis and membranoproliferative glomerulonephritis also was speculative, as is usually the case even in well-characterized domestic species. The positive titer to *Leptospira bratislava* suggests the possibility of primary renal leptospirosis, but the chronicity of the lesions obscured the primary pathogenesis. The adrenal gland hyperplasia was secondary to chronic disease, and other lesions were either secondary or clinically insignificant. In summary, the tissue calcification, iron storage, interstitial nephritis and membranoproliferative glomerulonephritis, and hemosiderosis with myelophthisis were the most significant changes. Further, the marrow changes, anemia, and iron storage were likely all related, and the mineralization was probably directly related to renal lesions with resultant uremia-induced tissue damage.

There are numerous hypotheses to explain the recurrent anemia syndrome of captive black rhinoceros', including erythrocyte ATP deficiency and acatalasemia, nutritional deficiencies, exposure to toxins, hemoglobinopathies, immune mediated conditions, and leptospirosis. Failure to consistently demonstrate any one of these changes in affected animals suggests a complex, multifactorial pathogenesis, or a yet undefined mechanism. Additionally, there is a lack of thorough ante- and postmortem descriptions of individuals succumbing to this condition. The complex and intriguing findings in this case underline the need for thorough examination of all organ systems in black rhinoceros' that die or are euthanized due to profound, recurrent anemia, and the need for in-depth, scientific investigation of this syndrome. Further, investigation as to the cause of death of free-ranging black rhinoceros', and the characterization of typical geriatric lesions in wild animals would also probably elucidate upon the pathogenesis of this important condition.

Table 1. Selected blood values*, 1991.

	11/3	11/7	11/11	11/23	11/30	12/8	12/14	12/26
WBC	9.0	8.6	8.2	8.8	8.2	9.0	8.5	9.5
RBC	2.83	2.98	3.05	2.79	2.58	2.76	3.15	2.87
HCT	27.0	25.0	26.0	26.0	26.0	24.0	30.0	26.0
MCV	95	84	85	93	100	87	95	90
MCHC	34	39	38	35	32	37	35	36
BUN	12	10	13	17	30	13	14	14
CRT	1.3	0.8	1.2	1.4	1.2	1.3	0.5	1.3

*WBC=white blood cells x 10^3/ul, RBC=red blood cells x 10^6/ul, HCT=hematocrit in volume percentage, MCV=mean corpuscular volume in femtoliters, MCHC=mean corpuscular hemoglobin concentration in g/dl, BUN=blood urea nitrogen in mg/dl, CRT=serum creatinine in mg/dl, CA=serum calcium in mg/dl, PHOS=serum phosphorus in mg/dl.

Table 2. Selected blood values*, 1992.

	1/10	1/28	2/6	2/13	2/26	6/5	9/28	10/9
WBC	9.1	8.7	9.3	9.7	9.5	2.2	9.0	9.0
RBC	2.86	2.92	3.33	3.08	3.11	3.43	2.89	2.56
HCT	27.0	29.0	31.0	31.0	31.0	34.0	27.0	25.0
MCV	94	99	93	100	100	99	93	98
MCHC	37	36	37	35	35	34	39	40
BUN	15	17	16	16	17	19		11
CRT	1.2	1.3	1.3	1.3	1.5	1.6		1.0
CA								8.7
PHOS								3.8

Table 2 (Continued). Selected blood values*, 1992

	11/17	12/8	12/27
WBC	5.7	9.1	15.5
RBC	2.58	2.75	2.82
HCT	25.0	25.0	26.0
MCV	97	90	92
MCHC	34	37	37
BUN	15	14	16
CRT	0.9	1.5	1.6
CA		12.3	12.1
PHOS		6.2	5.3

Table 3. Selected blood values*, 1993.

	1/11	2/4	3/8	3/21	3/23	4/5	4/15	5/3
WBC	3.1	8.5	13.1	8.4	8.1	7.4	5.7	6.9
RBC	2.90	2.51	2.37	2.34	2.04	1.88	1.63	1.50
HCT	27.0	22.0	22.0	18.0	19.0	18.0	17.0	13.0
MCV	93	88	92	77	93	96	104	87
MCHC	37	40	44	44	38	38	34	41
BUN	20	14	26	27	28	31	25	27
CRT	1.8	1.8	1.5	2.4	2.4	2.1	1.9	1.7
CA		12.6	11.7		11.4	8.9	11.6	14.2
PHOS		6.6	6.9		7.5	6.5	9.1	4.0

Table 4. Normal blood values* for the black rhinoceros.

	Mean	S.D.	Minimum	Maximum
WBC	8.677	2.836	6.200	15.50
RBC	4.735	0.979	2.510	6.130
HCT	37.75	7.85	22.00	55.00
MCV	80.96	11.97	46.32	95.51
MCHC	36.75	1.90	33.64	40.00
BUN	10	2.78	7.000	15.00
CRT	1.073	0.042	1.040	1.120
CA	12.80	0.42	12.37	13.60
PHOS	4.500	0.923	3.700	6.600

Table 5. Results of Leptospirosis titers, State of Illinois Department of Agriculture Laboratory.

	2\26\92	6/5/92	12/8/92
L. canicola	negative	negative	negative
L. grip.	negative	negative	negative
L. ictero.	negative	negative	negative
L. hardjo	negative	negative	negative
L. pomona	negative	negative	negative
L. bratislava	positive @ 1:200	positive @ 1:400	positive @ 1:200

TB OR NOT TB: THAT IS THE QUESTION - ELIMINATION OF TUBERCULOSIS FROM A HERD OF AMERICAN BISON

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Tuberculosis is an infectious disease caused by acid fast bacilli of the genus *Mycobacterium*. There are three major serovars; *M. bovis, M. tuberculosis,* and *M. avium*. Although the two mammalian types are the most closely related, all three can cause disease in various species. *M. bovis* and *M. tuberculosis* in cattle cause infection beginning at a primary focus which is usually within the respiratory system. Caseous lesions in the regional lymph nodes result from their being seeded by lymphatic drainage of the primary lesion. The lesions may become encapsulated and dormant causing nonclinical signs or spread systematically causing a potentially fatal miliary tuberculosis. Is during this stage that the disease is most likely to spread to other animals though shedding can occur at other times. The bacilli are shed in respiratory secretions that are coughed up and aerosolized or swallowed and passed out in the feces.

In 1946 bison housed in the Golden State Park of San Francisco (now known as Golden Gate Park) were reported to be infected with tuberculosis. This diagnosis is reported to have been confirmed by post mortem examination and isolation of the organism *M. bovis* at the Fresno State Veterinary Laboratory. It is believed, though not documented, that the herd became infected from manure that was spread on the pasture as fertilizer. This manure came from a dairy herd that was later shown to be infected with TB. It was also noted by zoo personnel at the time that the bison paddock was maintained in "a state of poor sanitation."

Various recommendations were made for handling the infected bison which included culling the entire herd, instituting a test and slaughter program, and improving the sanitation of the paddock. Due to the fear of bad publicity, euthanasia of the entire herd was not considered a viable option. The paddock was cleaned and some testing of animals was done. Though the test records are scant, in 1959, one animal was euthanized after a positive test and it also had tubercles at necropsy. The threat of zoonosis was not considered significant enough to warrant isolation of the herd from public display. In 1963 it was recorded that the TB was eradicated from the herd through systematic procedure of testing and eliminating the positive reactors. No records could be found documenting the details of the testing procedures.

The situation was not discussed again until 1977 when the record states that it was "widely known and accepted" that the bison herd in the park was infected with TB. It is not clear if this re-diagnosis was based on testing, necropsy, or history. A committee was formed to evaluate the situation. It was felt that since the animals were not clinically ill or suffering, it would be inhumane to destroy them and, instead it was suggested the animals be sterilized; however, this was not done.

Various reports of public concern were filed with the Zoo and City of San Francisco over the next seven years. In 1983 the chief veterinarian of the Zoo lead another committee and discussed possible options. Culling of the entire herd was again considered but deemed unfeasible due to the threat of public protests. Instead, the herd consisting of 16 animals was transferred to a paddock built for them at the County Jail in nearby San Bruno in order to eliminate the threat of zoonotic TB for the general public. The paddock in Golden Gate Park was reportedly decontaminated and left vacant for one year. A new herd was brought in which was tested free of tuberculosis and brucellosis. It was again suggested that males from the original herd now housed at the San Bruno Jail be castrated. This was not done.

During 1984-1991 six animals died at the San Bruno Jail, two of these animals were found to have tubercle lesions at necropsy. Five births were also recorded. Little more was accomplished until the spring of 1991 when Dr. Freeland Dunker of the San Francisco Zoo attempted to handle the bison TB situation systematically. He instituted a test and slaughter program that included an effort to culture and isolate the *M. bovis* organism from infected animals. The testing procedure was quite elaborate and expensive as a result of the involvement of the San Francisco Department of Public Health. They required that all individuals involved with the testing and daily care of the bison wear protective clothing and respirators in order to minimize the potential for human infection. Each individual involved (keepers and veterinary staff) had to be trained and certified in the use of respirators. The State Veterinarians's office which would normally perform the testing of a suspected TB herd, refused to be involved with this bison herd because of the consequences of adhering to such a rigid protocol. They did not want to set a precedent for testing that they would have to follow throughout the state.

Testing involved immobilizing the bison with a combination of carfentanil and xylazine. The animals received an intradermal infection of 0.1 ml of double concentration of PPD Bovis in the cervical skin and 0.1 ml of the standard concentration of PPD Bovis in the caudal fold and an eyelid. A double concentration of PPD Bovis is routinely used for testing a known TB herd in order to identify any immune suppressed animals. The decision to test in three different sites was made based on discussions with the State Veterinarian in an attempt to determine if results of an eyelid test could be read from a distance avoiding the need to immobilize the animals for reading the test. All males were epididymectomized at this time. Reversal was accomplished with naloxone and yohimbine. Renarcotization occurred in a few animals and one died following trauma sustained during renarcotization.

Four animals had positive reactions and were euthanized. A comparative cervical test was not done on these animals because of the TB positive status of the herd. At necropsy, only two infect mediastinal lymph nodes were found in one of the four animals. These cultured positive for *M. bovis*.

After this initial testing, animals were retested several times. Once two negative herd tests at least 60 days apart were obtained, the respirator requirement was removed by the San Francisco Department of Public Health.

In the spring of 1992, the State Veterinarian's office was again contacted and told that the respirator requirement had been lifted. They were then willing to oversee and actually perform the testing of the San Bruno Jail bison herd. However, after reviewing the testing protocol which had been used, the new veterinarian in charge of the state TB eradication program decided that it was necessary to start the testing over because of concern that multiple injections of tuberculin may have desensitized the animals risking false negative test results. He placed a quarantine order on the herd and decided that the animals were to be tested 60 days apart by the State Veterinarian until two negative herd results were obtained with another negative test 6 months later. The State of California would remove all restrictions of movement after the negative "six months test." Then State and Federal rules mandate testing the herd annually for 5 years followed by two tests once every 3 years. Quarantine of the herd was required but culling of the herd was only recommended.

Animals were immobilized, a cervical test using a PPD Bovis double strength was performed by the State Veterinarian, and reimmobilized 72 hours later for the State Veterinarian to read the tests. The use of a new antagonist - naltexone- made the recovery period smoother and no more animals have been lost because of anesthetic complications.

In May of 1992 a young male was euthanized for a chronic, immune-mediated skin disease. No evidence of tuberculosis was found at necropsy performed by the state diagnostic laboratory (CVDSL). In September of 1992 one animal had a suspicious positive tuberculin reaction. It was euthanized but no evidence of TB was found at necropsy at the state laboratory. Three negative tests have followed at two successive 60 day intervals and then 6 months later. In October of 1993 the quarantine and hold order were officially released from the herd. At present there are 5 sterilized males and 3 females ranging in age from 2.5 years to 17 years. It is now possible and legal to sell these animals; however, the new owner would be required by law to test them once every year for 5 years, then every 3 years for two more negative tests. If there were a reactor they would be required to quarantine their herd and follow a state approved protocol to again clear the herd. Obviously, this would not be an economically wise purchase. If the animals were to go to slaughter they would face the possibility of being condemned or being marked unfit for human consumption.

In summary, it is evident that the cost to the Zoo for the care of the bison herd has been substantial, especially in view of the fact that they have been off exhibit since the early 1980's. The wisest decision might have been to cull the herd in the 1940's or at least to have followed through with a castration or segregation protocol to prevent reproduction . A Zoo depends heavily on its public image and issues that involve euthanasia are never straight forward. Since many of the bison are still young, the Zoo will have to continue to care for and test the San Bruno Jail bison for many years.

DISEASE AS A FACTOR IN THE MANAGEMENT AND REINTRODUCTION OF WILD POPULATIONS: THE TUBERCULOSIS EXPERIENCE

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The increasing interest in wildlife ranching in parts of South Africa and the relatively recent aims of reintroducing wildlife populations in the Middle East, have exposed problems which are the result of the growing veterinary consciousness within conservation bodies. Where wildlife populations are subjected to intensive hands-on management, such as in South African game parks, the presence of a disease can have a major influence on the spread of game animals from one part of the country to another. The author illustrates how disease can influence wildlife management programs with examples from Saudi Arabia and South Africa.

The occurrence of tuberculosis has threatened conservation programs in a number of places, for example in Saudi Arabia and South Africa. The author describes tuberculosis outbreaks encountered in those two countries, the problems that these outbreaks can pose, their implications, and the measures that were or that can be taken to deal with the disease.

In Saudi Arabia, tuberculosis threatened an important Arabian oryx captive breeding scheme, whose end point was to be the reintroduction of the species into the wild. Problems were encountered with diagnosis and the identification of infected animals at the individual level. The two measures taken to deal with this outbreak and the success of these actions are described. These consisted of treating the entire founder group of oryx and handrearing calves away from their dams.

The problem of tuberculosis in Cape buffalo is a relatively new one in South Africa. It is infinitely more difficult to deal with tuberculosis in an established and large population of herbivores, than was the case in the outbreak in the Arabian oryx. It also has greater possible repercussions due to the potential of implicating other wild species. Some threatened species sharing their habitats with infected buffalo are described, and the means available to limit the spread of the disease are discussed.

CANINE DISTEMPER AND EMERGING MORBILLIVIRUS DISEASES IN EXOTIC SPECIES

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Canine distemper (CD) in dogs and other carnivores has been known for centuries worldwide and has been the infectious disease of dogs with the highest fatality rate besides rabies. The disease is caused by canine distemper virus (CDV) which was first isolated by Carré in 1905. The virus belongs to the Paramyxovirus family and more recently has been classified in the morbillivirus genus together with Measles virus (MV), Rinderpest virus (RV), Peste de petite ruminants (PPRV) and now Phocine distemper virus (PDV), and dolphin and porpoise morbilliviruses.

Canine distemper is an acute or subacute highly contagious febrile disease that may include respiratory, gastrointestinal, and central nervous system (CNS) disease. The CNS manifestation may occur during the acute phase of the disease or several weeks or even months later.

Aerosol of respiratory secretion is the main route of transmission. Virus shedding begins approximately 7 days post infection. Acutely infected carnivores shed virus in all body excretions, regardless of whether they show clinical signs or not. CDV affects susceptible animals of all ages, but puppies are most susceptible when maternal antibody is lost. Dogs that completely recover from CDV infection are immune for years and probably for life. They do not shed virus and they are not persistently infected. Outside the host, CDV becomes inactivated rapidly. All of the common disinfectants are effective against CDV.[1]

Many different species of the order *Carnivora* are susceptible to CD and the mortality rate varies greatly between species. *Ailuridae*, *Canidae*, *Hyaenidae*, *Mustelidae*, *Procyonidae*, *Viverridae*, and *Felidae* have been reported to be susceptible to CDV infection.[19] Although distemper outbreaks in dogs, fur farms and in zoo carnivores have been greatly reduced in recent years due to vaccination, there are still regular outbreaks in free-living foxes, skunks and especially raccoons throughout the U.S.

The diagnosis of CD in dogs, zoo or wildlife carnivores is sometimes difficult to make. It is initially based on clinical signs. Serology is often useless because animals that die from distemper may or may not have measurable antibody. If significant levels of anti CDV-IgM antibodies are present it may be meaningful unless the animals has been vaccinated recently. Demonstration of inclusion bodies and/or CDV antigen in conjunctival smears or buffy coat cells confirm the diagnosis. However, these tests are frequently negative. A negative test does not rule out CD. Inclusion bodies and CDV antigen demonstration in postmortem

tissues are more reliable. Virus isolation can best be made from buffy coat cells during the early phase of infection. The cells are co-cultivated with mitogen stimulated canine blood lymphocytes. Between 3 and 6 days later CDV can be demonstrated in these cells by immune fluorescence (IF).[2]

Several dramatic and unexpected distemper episodes have drawn attention in the last 2 decades. The latest was an outbreak of canine distemper in captive lions, tigers, leopards, and a jaguar. Seventeen animals succumbed in a wildlife park in California in the fall of 1992. Most animals died from CNS involvement (seizures) after episodes of respiratory or gastrointestinal disease. The isolated virus proved to be CDV and did not differ from dog or raccoon isolates by monoclonal antibody (Mab) testing. A serological survey revealed that CDV infection in exotic felids in a circus is much more common than in zoo animals, which by their isolation are better protected from infection.[5]

Domestic cats were known to be susceptible to CDV infection without the development of clinical diseases.[4] Multiple fatalities in exotic felids from CDV was unknown, although a few single cases in tigers were reported.[7,13] In an attempt to explain the epizootic in exotic felids, we have tested the possibility of immunosuppression due to a second agent such as feline immunodeficiency virus (FIV) with negative results. Greater exposure to CDV infected wildlife (e.g.. raccoons) in wildlife parks versus better protected zoo animals might be an explanation. However, the possibility of a slight mutation of the virus has not yet been ruled out.

Another unexpected episode of canine distemper occurred in 1989 in javelinas (collared peccaries) in Arizona. Signs of encephalitis (blindness, myoclonus, reluctance to move, circling) were observed with significant mortality. Histologic lesions were confined to the CNS. The isolated virus was identical to CDV when tested with Mabs.[3]

In an earlier study[9] javelinas, which belong to the *Tayassuidae* and not the *Suidae* family showed clinical signs when inoculated with Rinderpest virus (RPV), another morbillivirus. In contrast, domestic pigs (*Suidae*) were previously found to be susceptible to CDV[4] and RPV[10] without developing disease. Both viruses replicate in lymphatic tissues of pigs without causing clinical signs and virus shedding.

In the 1980's outbreaks of distemper in dogs occurred throughout Europe. Viruses isolated from infected dogs proved to be classical CDV. There are two possible explanations for the outbreaks. One may be a large number of unvaccinated and, therefore, susceptible dogs. The disease was almost absent for many years before the outbreaks occurred, and dog owners may have been negligent about vaccinations. The other explanation might be the frequent use of egg and avian cell adapted CDV vaccines in Europe. The percentage of dogs that become immune after egg adapted CDV vaccination is smaller than the percentage of dogs vaccinated with canine cell culture adapted CDV.

In 1988 alarming news came from Europe. Over 90% of the harbor seal population (about 20,000) in the North Sea died from a mysterious disease that involved predominantly the

respiratory tract and the CNS. Initially a picornavirus and a herpesvirus were isolated but soon it became known that a canine distemper like morbillivirus was involved.[22] It was later classified as Phocine distemper virus (PDV). Antibody to this virus was later found worldwide in seals, even in serum samples taken several years earlier. The virus is believed to be present in seals around the North American continent but has not yet been isolated.[12] An unrelated disease outbreak in fresh-water seals in the Baikal Lake in Russia was believed to be classical CDV.

In the years following the epizootic in 1988, morbillivirus related disease outbreaks were found in other marine mammals, especially in dolphins in the Mediterranean. Morbilliviruses genetically distinct from PDV and CDV have been isolated from porpoises and from dolphins.[6,22] Increased mortality in the gulf regions in Florida and Texas in recent years have been speculated to be caused by morbilliviruses. Although viral antigen and positive serology has been demonstrated in tissues, the virus has not yet been isolated.

Earlier this century inactivated CDV vaccines were widely used but distemper in dogs and in zoo carnivores was still very common. Modified live (ML) CDV vaccines became available in the early 1960's. They reduced the incidence of distemper in dogs dramatically. Two types of ML-CDV vaccines were introduced which are still commonly used today. One was a chicken embryo and later chicken cell culture adapted CDV strain developed at Onderstepoort, South Africa[14] and the other was dog cell culture adapted CDV first reported from Sweden.[20] Both vaccine types have their advantages and disadvantages. The canine cell culture adapted CDV induces immunity in virtually 100% of susceptible dogs. However, on rare occasions it may induce a post-vaccinal encephalitis in dogs 7 to 14 days post-vaccination.[16] This has never been reported in dogs vaccinated with the egg adapted CDV. However, after vaccination with this strain, the percentage of immunized dogs is lower. The difference in virulence of the 2 vaccine types for wildlife has been demonstrated by Halbrooks *et al.*[15] They found the canine cell culture adapted CDV to be fatal for gray foxes while it was well tolerated by red foxes. In contrast, the egg adapted CDV vaccine immunized both gray and red foxes without side effects. There are several additional species that can be vaccinated safely with the egg adapted, but not with the canine tissue culture adapted CDV, as for example, bush dogs, maned wolves, or fennec foxes.[19]

The potential virulence of ML-CDV virus in different carnivores became apparent in the mid-1970's. Almost simultaneously vaccine induced distemper was reported in red pandas[8] and in black-footed ferrets.[9] Similar episodes were later reported in kinkajous[20] and in African cape hunting dogs.[21] Even the egg adapted CDV vaccine may be fatal for these species.[17]

The killing of black-footed ferrets by CDV vaccine was especially traumatic. The colony in South Dakota was lost and it appeared that the species which was under the endangered species status since 1964 had disappeared. Fortunately, another colony was detected in Wyoming in 1981. An outbreak of natural distemper almost eliminated this colony in 1985.[24] Six animals could be trapped. They were kept in separate buildings to prevent cross-infection and they were vaccinated with inactivated CDV vaccine in adjuvant. A

successful breeding program produced hundreds of black-footed ferrets in the following years all vaccinated with inactivated CDV vaccine. They have been sent to different zoos in North America and some ferrets have been released into their habitat.[23]

Inactivated CDV is not commercially available in the U.S. because there is no demand for dogs and the market for zoo animals is too small to be profitable. Hopefully, recombinant or subunit vaccines will appear on the market that would be safe and efficacious for zoo animals and endangered species. The CDV-Iscom vaccine produced in Holland is a step in the right direction.[11]

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VACCINATION AGAINST CANINE DISTEMPER IN EXOTIC CARNIVORES: SUCCESSES AND FAILURES

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History of Canine Distemper Related to Vaccination in Exotic Carnivores

Since the simultaneous reporting in 1976 of canine distemper (CD) induced by vaccination with modified live vaccines in a red panda (*Ailurus fulgens*)[4] and black-footed ferrets (*Mustela nigripes*),[5] additional vaccine-induced or associated (i.e, strongly suspected but not always proven to be the vaccine strain) cases were cited in more red pandas, gray fox (*Urocyon cineroargenteus*), kinkajou (*Potus flavus*), fennec fox (*Fennecus zerda*) and African wild dogs (Cape hunting dogs, *Lycaon pictus*) throughout the latter 1970's and into the 1980's.[11,12] Over the next decade more cases of CD associated with vaccination were reported in wild dogs[6,18] and some new species were added to the list including maned wolf (*Chrysocyon brachyurus*),[17]and the bush dog (*Speothos venaticus*).[10]

In all of these vaccine-related cases, different brands of commercial CD vaccines attenuated in tissue culture cells of canine origin were used with the onset of clinical CD occuring between 9 and 21 days after inoculation. In some cases, multivalent vaccines were used containing other attenuated live vaccines including canine parvovirus, canine adenovirus and canine parainfluenza virus. Clinical signs usually consisted of anorexia, mucopurulent ocular and nasal discharge and hyperkeratotic and/or pustular skin lesions. These were usually acompanied by an array of paretic and/or hyperreactive CNS signs and commonly, seizuring followed by death. Laboratory and pathological findings were most often similar to those of CD in domestic canines with panorgan and CNS involvement associated with typical eosinophilic intracytoplasmic and intranuclear inclusion bodies.[1]

More recently, CD was induced also with an attenuated CD vaccine of avian origin in 4, 4 1/2 month old European mink (*Mustela lutreola*) given a multivalent vaccine (Galaxy 6-MPH-L, Solvay Animal Health, Inc., Mendota Heights, MN 55120) with similar clinical signs and pathologic findings as with the canine origin vaccines described above (Sutherland-Smith, unpublished data). The virus isolated was proven to be compatible with a vaccine strain of CD virus by cultural characteristics and monoclonal antibody studies (Appel,

unpublished data). Canine distemper also occurred in a red panda as part of a vaccine-trial with a new CD vaccine of avian origin for ferrets (Fervac-D, United Vaccines, Inc., Madison, WI 53744) (to be discussed further below).

Earlier Recommendations for Canine Distemper in Exotic Carnivores

Most of the current recommendations for CD vaccination in exotic carnivores were based on published vaccine trials in several species held at the National Zoological Park in 1983.[12] Although limited in scope, results of these trials indicated that Fromm D, (Solvay Animal Health, Inc., Mendota Heights, MN 55120) a live vaccine attenuated in avian cells labled for use in domestic canines and ferrets appeared to be safe and efficacious for extralable use in maned wolves, bush dogs and fennec foxes. However, it was not recommended for use in red pandas because of the loss of a red panda dying from *Pseudomonas* pneumonia 16 days after vaccination with Fromm D at the NZP.[12] Canine distemper was never proven either pathologically or culturally from this vaccinated red panda but because of the exquisite sensitivity to modified-live CD vaccines of canine origin and the threatened status of this species, it was recommended at the time that Fromm D or other modified-live vaccines not be used in red pandas.

Instead, an adjuvanted experimental killed CD vaccine developed by one of the authors (MJGA) has been used in NZP red pandas and its relative the giant panda (*Aleuropoda melanoleuka*) also susceptible to CD.[9,20] This vaccine has been selectivley distributed for experimental use in SSP pandas held at other zoos.

Subsequent evaluation of this killed CD vaccine in red pandas based on trials in 42 individual animals (Montali and Appel, unpublished data) indicated a range of titers above 100 (the gold standard for protection in domestic dogs) in 18 animals, and below 100 in the remaining 24 with 9 of these red pandas developing a titer of only 10 or less. The use of an adjuvant derived from a saponin (*Quillaja saponaria)* did appear to enhance this killed vaccine and its performance has also been quite good in black-footed ferrets.[20] However, this same adjuvanted vaccine in red pandas has shown some low titers and inadequate durability. Vaccination boosters must be given two to three times annually which is often disruptive to breeding and management practices for this species. Overall, however, this vaccine has afforded protection to SSP red pandas and has shown promise, but it has not been possible to develop it for commercial use and it will not be available in the near future.

Recent Vaccine Trials in Cusimanse, Dwarf Mongoose and Red Pandas

Further trials using the new avian-attenuated vaccine Fervac-D were undertaken first in cusimanse (*Crossarchus obscurus*) and dwarf mongoose (*Helogale parvula).* Three previously unvaccinated juvenile cusimanse and 2 unvaccinated dwarf mongoose from the NZP developed titers (> 1000) for 3 months after serial vaccinations. One of the dwarf mongoose had experienced a vague illness with some CNS signs but recovered fully after several days. Five red pandas were then vaccinated sequentially with Fervac-D. The first

was an adult NZP animal previously vaccinated with killed vaccine that when given Fervac-D had its titer boosted from 10 to >500. Similarly, 4 additional previously vaccinated red pandas (3 on loan from Woodland Park Zoo) with low or immeasurable titers also developed titers >1000. The Fervac was then tried in 3 red pandas (between 6-7 months old) of different ownership. Two were previously unvaccinated and were being hand-raised at the Denver Zoo, and 1 was at the Binder Park Zoo and had received killed CD vaccine 4 months earlier. One of the two animals at Denver developed severe CNS signs 13 days after receiving the Fervac-D and was euthanized 3 days later. The other red panda developed some mild cutaneous signs but recovered, and the Binder Park animal remained normal. Canine distemper virus was isolated from both Denver animals and was characterized as a vaccine strain by monoclonal antibody tests (Appel, unpublished data.) Follow-up titers against CD virus in the Denver survivor was just under 1000 and in the Binder Park animal, >1000. The nonsurviving red panda was underdeveloped at birth but improved while being hand-reared. There were no underlying pathologic changes to explain any enhanced susceptibility to the Fervac-D tried.

Discussion

The emergence of disease in exotic species caused by morbillivirus[2] has heightened the need for safe and efficacious CD vaccines in exotic carnivores. Several CD vaccines have recently surfaced from other parts of the world for use in exotic carnivores. One, a killed vaccine from Australia has been made commercially available but no data on efficacy is available. An experimental subunit distemper vaccine developed in the Netherlands by Osterhaus (ISCOM-vaccine, from which the infectious component is removed)[13] has given some good preliminary results in terrestrial exotic carnivores in some European zoos (Personal Communication, Dr. Willem Schaftenaar, Rotterdam Zoo). This vaccine, however, could not be lyophilized and importing vaccines for use in the U.S. can be a complicated procedure. The new vaccine trials in the SSP red pandas and viverrids were driven by a distinct need to identify a workable CD vaccine in these threatened species. Economics has dictated against the development of a killed commercial vaccine against CD in a relatively small pool of exotic species in the U.S. Calculated risks were taken but failed to safely adapt the commercially available Fervac-D to the red pandas with the loss of 1 of 8 pandas tried. Based on these preliminary trials, Fervac-D therefore, cannot be recommended for extralable use in red pandas and at this time for other exotic species. In addition, any CD modified-live vaccines recommended for extralable used in exotic carnivores should be given separately at reasonable intervals and not in multivalent forms since immunosuppressive and other untoward vaccine interactions might lead to disease expression.

It could be argued that vaccination against CD in exotic captive species may not be necessary and that the risk of vaccination is too great. In fact, with the exception of some earlier outbreaks of CD in zoo settings,[11] and the recent outbreak in exotic cats in California,[2] naturally occurring CD has been quite uncommon in susceptible zoo-exhibited species in the U.S. A natural case of CD was reported in a red panda that had been vaccinated with the experimental killed vaccine mentioned above, 10 months prior to the

onset of disease.[16] The source of the distemper virus was determined to be wild raccoons in which CD had been previously diagnosed.

The most significant outbreak of naturally occurring distemper of unknown source was reported in 5 previously unvaccinated red pandas at the Kyoto Municipal Zoo in Japan,[8] and an outbreak occurred in a pair of unvaccinated red pandas and their 2 offspring that was associated with free-living martens (*Martes foina)* at the Leipsig Zoo in Germany.[7] Singular natural cases of CD of unknown orign have been reported in red pandas from China[21] and the Delhi Zoological Park in India.[14] Therefore, there is a definite and continual threat of CD in zoo settings where stray domestic and wild carnivores can co-mingle with CD susceptible exotic inhabitants. Raccoons present one of the greatest threats as CD may be endemic in this species in some parts of the U.S.[15] Unless zoo animals susceptible to CD could be totally isolated, the threat of outbreaks in groups of imperiled species like red pandas still remains and could result in some catastrophic losses if these animals are not immunized against CD.

As pointed out, a great deal of work remains to be done on vaccine use in exotic species.[3] A search for new CD vaccines devoid of infectious components for use in red pandas and other exotic carnivores should continue, particularly in light of the new molecular biological techniques available today. In addition to the the subunit ISCOM vaccine mentioned, experimental recombinant vaccines encoding CD virus antigens have been developed and used in laboratory rodent models,[19] and they should be explored for use in exotic species.

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CLINICAL IMPLICATIONS OF FELINE IMMUNODEFICIENCY VIRUS INFECTION IN AFRICAN LIONS (*Panthera leo*): PRELIMINARY FINDINGS

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Seroprevalence to feline immunodeficiency virus (FIV) in captive African lions (*Panthera leo*) in United States zoos ranges from 12-14%.[2,3] The number of animals sampled were 48 and 168, respectively. For free-ranging lions in East and South Africa, the seroprevalence is greater than 80%.[2] The impact of the virus on infected animals is not known. Selecting criteria used to evaluate the effect of FIV on domestic cats, including CD4:CD8 ratios, globulin levels and retinal lesions, the following preliminary findings from 11 captive, FIV seropositive lions were noted.

Flow cytometry of peripheral blood leukocytes from eight, FIV seronegative lions (7 free-ranging and 1 captive) yielded CD4:CD8 ratios ranging from 1.35-2.50. The average ratio in the domestic cat is 1.74.[5] Six of 11 seropositive lions had inverted CD4:CD8 ratios ranging between 0.32 and 0.68. In four animals, lymphocyte numbers were within normal limits ($\geq$ 1500/mm^3), and the inversion was due to an increase in CD8+ cells which is characteristic of FIV+ domestic cats during the long asymptomatic period of infection.[1,4] The remaining two lions with inverted ratios were lymphopenic and had an absolute decrease of both CD4 and CD8 positive cells. One of these lions had periodic episodes of abnormal behavior and the other one was experiencing severe hematuria of unknown etiology when sampled. Five seropositive lions had CD4:CD8 ratios ranging from 1.41-2.46, but four of these animals were lymphopenic.

Hypergammaglobulinemia is associated with FIV infection in domestic felids.[5] Nine of 11 seropositive lions had marginally elevated globulin levels (> 4.3 g/dl). Serum electrophoresis of samples from the five seropositive animals with normal CD4:CD8 ratios (four with elevated globulin levels and one with 4.3 g/dl) demonstrated a polyclonal gammopathy in all these lions.

The most common ophthalmic manifestation of FIV infection in domestic cats is retinal degeneration (English, unpublished observations). Lesions are usually multifocal, irregularly shaped, and located peripherally. Only two FIV seropositive lions have had complete

ophthalmologic exams. One lion had such severe retinal degeneration as to preclude a definitive etiology, whereas the second lion had lesions consistent with FIV associated retinopathy.

Additional clinical signs compatible with FIV infection reported in seropositive lions, as a result of the survey conducted by the senior author in 1993, include chronic gingivitis (n=5), periodic behavior changes (n=6), and myeloproliferative disease (n=1). Periodic behavior changes, usually characterized by atypical aggression, is being recognized with increasing frequency in FIV positive domestic cats that are otherwise asymptomatic (English, unpublished observations). Histopathologic examinations of brains from two FIV seropositive lions, one with behavioral changes, were unremarkable as is the case with brains from FIV positive domestic cats with and without behavioral changes.

Based on these preliminary findings, the impact of FIV on non-domestic felids warrants further investigation. However, the existing evidence suggests that FIV in African lions is not benign. As the seroprevalence to FIV remains relatively low in captive lions, screening for antibodies is important and positive animals should be isolated from negative animals. Based on last year's survey, 61% of responding institutions screen all or some felids for antibodies to FIV. Of 16 zoos with FIV seropositive felids, 11 indicated some change in management to keep these animals isolated. Transmission of virus occurs by close direct contact. Four of the five lions that still have normal CD4:CD8 ratios seroconverted within two years after the introduction of an animal that was subsequently discovered to be seropositive. The failure of these animals to exhibit inverted CD4:CD8 ratios may be related to the length of time they have been infected.

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DIAGNOSIS OF *Clostridium perfringens* ENTEROTOXICOSIS IN A COLLECTION OF CHEETAHS (*Acinonyx jubatus jubatus*)

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During the summer of 1993, 6 of 22 (4 male, 2 female) adult cheetahs (*Acinonyx jubatus jubatus*) in a private animal collection in northeastern Florida were exhibiting chronic, intermittent to continuous bloody, mucoid diarrhea. Six (3 male, 3 female) other adult cheetahs showed occasional blood and mucous drops in their stool. This condition appeared to arise in the collection in 1991 and spread slowly through the collection. One importation of 3 (2 male, 1 female) cheetahs from a large, captive cheetah collection in South Africa occurred in 1990, just prior to the increased incidence of abnormal stools in the cheetah collection.

Affected cheetahs passed stools that ranged from fresh blood and mucous only, to cylindrical mucosal casts covered with fresh blood and mucous, to soft stool with fresh blood and mucous drops, to soft yellowish stool. Bouts of diarrhea were generally short and episodic, lasting a day or two, and were interspersed with a variable period of normal stool production. Bouts of diarrhea generally began immediately after a fast day with the worst stools generally seen on the day following a fast day. Sometimes affected cheetahs would pass both normal and abnormal stools on the same day. Occasionally, tenesmus was seen during episodes of diarrhea with production of numerous small drops of blood and mucous. Affected cheetahs had good appetites and showed no evidence of systemic illness or consistent weight loss during this time period. Except for varying degrees of lympoplasmocytic gastritis, all cheetahs were considered healthy on annual exam.

Results of hematology and serum chemistry panels on all cheetahs were within normal limits based on in-house normals and ISIS Normal Physiological Data (ISIS, 12101 Johnny Cake Ridge Rd, Apple Valley, MN 55124, USA). Results of serologic screening for FeLV, FIV, feline coronavirus, feline panleukopenia virus, feline herpesvirus, feline calicivirus and toxoplasma were unremarkable for all cheetahs in the collection. Three fresh fecal samples were collected for the 6 most severely affected cheetahs when abnormal stools were passed. All fecal samples were negative for protozoan and metazoan parasites. All fecal samples were negative on aerobic and *Campylobacter* sp. culture for enteric pathogens. A *Clostridium perfringens* isolate was anaerobically cultured from the feces of 5 out of 6 of the affected cheetahs. Mycobacterial cultures were negative on all fecal samples. Acid-fast stained fecal smears were negative for acid-fast bacteria and cryptosporidia cysts. Bacterial spores consistent with *Clostridium perfringens* spores were seen on modified Wright's stained fecal smears. Affected cheetahs had average spore counts of 1 to 3 spores/high-power oil immersion field. Fecal smears from 3 normal cheetahs averaged 0 to 1 spore/high-power oil immersion field. Fecal samples from 3 out of 6 affected cheetahs were positive for the presence of *Clostridium perfringens* enterotoxin by a commercially available, reverse passive latex-agglutination (RPLA) test (Oxoid PET-RPLA toxin detection kit, Unipath Limited,

Wade Road, Basingstroke, Hampshire RG24 OPW, England).[1] No aerobic enteric pathogenic bacteria were isolated from colon mucosal biopsies taken from affected cheetahs. Endoscopic colon biopsies showed histologic changes ranging from mild multifocal suppurative colitis, through moderate plasmocytic mucinous colitis, to multifocal necrotizing colitis in affected cheetahs. Silver stains on the colon biopsies showed the presence of numerous spiral bacteria, the significance of which is unknown.

All affected cheetahs were treated with a combination of tylosin tartrate (Tylan Soluble, Elanco Products Co., Division of Eli Lilly & Co., Indianapolis, IN 46285, USA) 568 mg, Metronidazole (Metronidazole Tablets, USP, Rugby Laboratories, Inc., Rockville Center, Long Island, NY 11570, USA) 500 mg and psyllium fiber (Equate Natural Vegetable Powder, Perrigo Co., Allegan, MI 49010, USA) 3.0 teaspoonfuls P.O. BID for 14 days followed by tylosin tartrate 58 mg P.O. BID for 14 more days. Stools reverted to normal in all affected cheetahs within a few days of treatment. Two out of 12 of the affected cheetahs had reappearance of blood and mucous in their stools 1 to 2 months after the treatment period. These 2 cheetahs were retreated with tylosin tartrate 568 mg P.O. BID for 14 days. From this time until present, there have been no further recurrences of bloody, mucoid stool in any of the cheetahs.

Clostridium perfringens type A is one of the most common causes of food poisoning in humans.[3] Recently, *Clostridium perfringens* type A has been associated with nosocomial and acquired, acute and chronic diarrhea in dogs.[2,4] The diarrhea is caused by an enterotoxin which is a component of the *Clostridium perfringens* spore coat and is released only during the sporulation process.[3] The isolation of *Clostridium perfringens* from fecal cultures in humans and dogs does not correlate well with *Clostridium perfringens* enterotoxicosis (CPE), since this organism can be normal flora. Definitive diagnosis of CPE in dogs requires the identification of *Clostridium perfringens* enterotoxin in feces.[5] The PET-RPLA test was developed for use on people with suspected food poisoning but is also considered accurate in dogs and cats, since enterotoxin produced by all strains of *Clostridium perfringens* is antigenically similar and not species specific.[4] The assay can be performed relatively easily on feces either in-house or at several veterinary or human diagnostic labs in the USA. The toxin is very stable so fecal samples can be refrigerated or frozen prior to testing. The sensitivity of this test in detecting enterotoxin is approximately 2.0 mg/ml.[1]

The presence of *Clostridium perfringens* enterotoxin in feces correlates well with fecal bacterial spore counts of 10^6 organisms/gm feces.[5] *Clostridium perfringens* spores are rarely present (10^3 organisms/gm) in feces of normal dogs or dogs with diarrheas of other causes.[4] Fecal cytology using modified Wright's stained (Diff-Quick Stain Set, American Scientific Products, McGaw Park, IL 60085, USA) fecal smears to perform bacterial spore counts is a quick screening test of CPE. The spores are larger than most bacteria and are relatively easy to identify (safety pin appearance) under high-power oil immersion light microscopy. Spore counts of 2 to 3 or higher/high-power oil immersion field are considered abnormal and suggest CPE.[4]

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DILATED CARDIOMYOPATHY IN A CAPTIVE COLLECTION OF FLYING-FOXES (*Pteropus sp*)

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Case Report

During a 6 month period in 1993, 5 adult male island flying-foxes (*Pteropus hypomelanus*) either died from or were euthanatized because of congestive heart failure secondary to dilated cardiomyopathy. The bats, along with 8 other adult males and 31 adult females, had been imported as wild-caught animals from Indonesia in June of 1990. All bats were housed in indoor/outdoor flight enclosures at the Lubee Foundation, a private breeding and conservation center in North Central Florida. The initial importation group had been broken into several breeding groups into which the affected males were distributed. Other species of flying-fox housed at the foundation in 1993 included Rodriguez (*P. rodricensis*), golden-mantled (*P. pumilus*), giant (*P. vampyrus*), dog-faced (*Cynopteris brachyotis*) and grey-headed (*P. poliocephalus*). Except for 1 Rodriquez island flying-fox, which had also died from dilated cardiomyopathy in 1992, there had been no other cases of cardiac disease in the collection. All bats were fed a mixture of fruits and vegetables supplemented with primate chows and a vitamin supplement.

Clinical Signs

Of the 5 affected island flying-foxes, 1 bat was found dead. Three of the remaining 4 bats were noticed during routine physical examination to have poor peripheral perfusion making venepuncture difficult, and causing pale blue mucous membranes. The last affected bat was detected during thoracic radiography and ultrasonography. Some bats had auscultable tachyarrhythmias but no murmurs. All bats were lethargic, reluctant to fly, showed dyspnea after exercise, pectoral muscle wasting, and in the latter stages of the disease visible and palpable hepatomegaly. Two bats developed cephalic edema.

Radiography and Ultrasonography

Thoracic radiographs showed generalized cardiomegaly, and interstitial pulmonary densities consistent with pulmonary edema. Ultrasonography revealed ventricular and atrial dilatation, myocardial wall thinning, and poor contractility.

Necropsy

Gross necropsy confirmed cardiac dilation and hepatomegaly. Histologic examination showed varying stages of myocarditis and myocardial fibrosis. In 4 of 5 bats, skeletal muscle was examined, and in 2 animals mild myositis and fibrosis were evident.

Diagnostic Tests

Plasma was collected from 3 of the affected males prior to euthanasia, as well as from 6 normal adult female and the remaining imported male island flying-foxes. This plasma was submitted for determination of taurine, carnitine, copper, cobalt, zinc, vitamin E, selenium levels. Additional plasma was also submitted to determine the presence of antibodies to the equine encephalitis viruses, as well as encephalomyocarditis virus. Liver and kidney samples were frozen at the time of necropsy, and will be used to determine tissue levels of vitamin E and selenium. The most significant finding from the samples submitted was undetectable blood levels of vitamin E in all affected males.

Treatment

Two of the bats in which dilated cardiomyopathy was detected ante-mortem were treated with the angiotensin converting enzyme inhibitor enalapril (Vasotec 2.5 mg, Merck & Co., Inc., West Point, PA 19486, USA, 0.5 mg PO every 72 hrs). It is difficult to assess the therapeutic effect of this drug. In one bat, dilated cardiomyopathy was detected early (i.e., before the onset of hepatomegaly and edema), and the animal survived almost 6 months before being euthanitized. In the other bat, disease was detected late and animal euthanitized after only a short course of enalapril.

At present, investigations are under way to determine an appropriate source of vitamin E supplementation, and subsequently to analyze and correct the diet.

Previous Reports

Congestive heart failure associated with a cardiomyopathy has previously been described in a 16-year-old Indian fruit bat (*Pteropus giganteus*).[1] Digoxin and furosemide therapy appeared to ameliorate the clinical signs of disease in this animal.

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TRICHINOSIS IN A POLAR BEAR (*Ursus maritimus*)

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A twenty-eight year old (estimated) female polar bear (*Ursus maritimus)* at the Knoxville Zoological Gardens presented with a two month history of partial anorexia, weight loss and a stiff hindleg gait. Initial physical examination revealed a moderate paronychia in the right forelimb and complete blood cell count and serum chemistry analysis revealed a moderate to severe non-regenerative anaemia and a hyperglobulinemia. Radiographs showed bilateral tarsal and carpal degenerative joint disease. She was treated with a course of antibiotics and appeared to improve slightly.

Eleven months later it was noticed that her appetite had worsened and she had lost weight. Blood work revealed a worsening of the anemia (normocytic, normochromic and nonregenerative), a leucopenia, profound hyperglobulinemia and a hyponatremia. Thoracic radiographs indicated a well circumscribed cavitary mass with a mineralized shell in the caudal left thoracic cavity. A bone marrow aspirate showed there to be megakaryocytic and myeloid hypoplasia with a marked plasmacytosis. Serum protein electrophoresis was performed and the hyperglobulinemia was found to be due to a polyclonal gammopathy. It was concluded that the changes were due to chronic inflammation but despite extensive testing the source of the inflammation could not be identified. She was treated with a two month course of antibiotics, anabolic steroids and aspirin for the osteoarthritis.

Her condition remained static for six months, when she became non weightbearing lame on her left hindleg. Due to her poor quality of life and the poor prognosis for recovery, she was euthanized.

The necropsy results showed a moderate, multifocal, granulomatous myositis with intralesional encysted *Trichinella sp*. The larvae were identified as most probably the strain *T. nativa* but could also be a type 6 strain. A chronic abscess was detected within the lung with an associated diffuse pleural fibrosis. A moderate plasmacytosis was seen within the spleen and bone marrow. Diffuse renal interstitial and glomerular amyloidosis was also identified.

Trichinosis has been previously reported in free-ranging polar bears, generally as an incidental finding at necropsy. This polar bear was wild caught, and *T. nativa* being the arctic strain of *Trichinella*, this was probably an old infection acquired in the wild. However, the possibility of this being a recent infection acquired during captivity does exist. Possible

sources for a recent infection include wild rodents, particularly mice and rats or, less likely, the bear's food source. *Trichinella sp* has been identified in one other polar bear at the Knoxville Zoological Gardens.

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FIBER UTILIZATION IN THE LARGER MALAYAN CHEVROTAIN *(Tragulus napu)*

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Introduction

The larger Malayan chevrotain *(Tragulus napu)* is among the smallest (2.5 - 3.9 kg) of the artiodactyls. While this deer-like animal ruminates, it does not have an omasum, and the rumen is a non-glandular blind sac folded into a sigmoid pouch. The esophagus enters a cranial diverticulum of this pouch with a reticular epithelial lining. Digesta leaving the reticulorumen passes directly into a glandular compartment, homologous to the abomasum of more advanced ruminants, with glandular mucosa in the gastric and pyloric regions but not in the cardia.[5] Due to these differences, and an apparently rapid digesta transit time, there is some debate concerning an appropriate diet for tragulids.

In captivity, tragulids are commonly offered a large array of foods from which they may choose. This array generally includes a number of produce items, such as fruits and vegetables, that are high in palatability and in readily fermentable starches and sugars. When these are chosen to the severe reduction or exclusion of hay or herbivore pellets, explosive fermentation may result, leading to inflammation of foregut mucosa, diarrhea, and disruption of acid/base balance. Nevertheless, the ability of a tragulid to ferment fiber may be more limited than that of a larger, more advanced ruminant. Thus, the food offered to chevrotains should be sufficiently digestible to meet their nutrient and energy needs, yet contain sufficient fiber to maintain proper gut microbial activity and normal gastrointestinal tract function.

The objectives of this study were to examine the ability and efficiency of adult chevrotains to utilize complete, pelleted diets with two different levels of fiber as their sole food.

Experimental Procedure

Eight adult chevrotains (3.5) were assigned to one of two experimental groups. Age and sex were considered to avoid confounding these factors with treatment. Animals were housed individually in concrete-floored enclosures and had ad libitum access to water and one of two complete, pelleted feeds. Four chevrotains were fed a lower fiber diet, formulated to contain 16% acid detergent fiber (ADF), while the four remaining animals were fed a higher fiber diet formulated to contain 25% ADF (Table 1). Nutrient content of the pellets other than fiber was essentially identical.

The experimental design was that of a double crossover. Thus, each animal received each of the treatments during two separate periods (a total of four experimental periods). The initial two periods (the first crossover) will be designated as *Experiment 1*, the latter two periods (the second crossover) as *Experiment 2*. Digestive parameters were determined by the utilization of two digesta markers, chromium mordant (Cr-M), and chromium ethylenediamine tetraacetic acid (Cr-EDTA).[3,6] The Cr-M was used during Experiment 1 and served to mark the particulate phase, while the Cr-EDTA was used during Experiment 2 and served to mark the liquid phase. The markers were administered as a pulse dose to each animal at the beginning of each experimental period at the rate of 15 mg Cr as Cr-M during Experiment 1, and 20 mg Cr as Cr-EDTA during Experiment 2. Markers were prepared in empty gelatin capsules (size 00) and individually orally dosed. The chromium data will not, however, be presented in this paper.

Each of the four experimental periods consisted of a minimum of 2 wk for diet adaptation, followed by a 7-day collection period. Systematic total fecal collections were made during this time. Samples were immediately weighed, and frozen. Following each collection period samples were shipped to the Comparative Nutrition Laboratory at Michigan State University. Fecal samples were freeze dried, ground, and analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash, gross energy (GE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid lignin (AL), and chromium. Feed intake was determined and recorded on a daily basis.

Table 1. Nutrient composition of ADF-16 and ADF-25 (% of DM).

Nutrient	ADF-16	ADF-25
Dry matter	91.0	89.3
Crude protein	19.4	20.4
Ether extract	4.2	3.9
Ash	7.9	8.1
Gross energy (kcal/g)	4.5	4.4
Neutral detergent fiber	27.4	27.8
Acid detergent fiber	15.7	19.7
Acid lignin	3.7	4.4

Results and Discussion

Throughout both Experiments 1 and 2, all animals remained healthy, and had normal fecal consistency. Both diets contained adequate levels of digestible energy, as indicated by maintenance of body weight (±4%). Although different batches of the same pelleted feeds were utilized during the two experiments, there was no difference in the list of ingredients. Likewise, no differences were observed in the composition of the diet offered as compared

to the composition of orts (feed refused). Mean dry matter intake as an absolute value and as a percentage of body weight tended to be higher in animals fed the ADF-25 (Table 2). Since dry matter intake is controlled primarily by gut fill and digestible energy density, it is reasonable that a higher fiber diet would be consumed in greater amounts.

Table 2. Daily dry matter intake (DMI) and body weight (BW) relationships.

Factor	Treatment mean			
	Experiment 1		*Experiment 2*	
	ADF-16	ADF-25	ADF-16	ADF-25
BW (kg)	3.28	3.28	3.31	3.31
DMI (g/d)	90.79	99.31	89.47	94.07
DMI (% BW)	2.77	3.03	2.70	2.84

Apparent digestibility of the lower fiber diet (ADF-16), with one exception, was consistently greater than that of the higher fiber diet (ADF-25) (Table 3). Particle size significantly affects the rate, but not the extent (given sufficient time), of fermentation.[2] Since the particle size of the two pelleted feeds was believed to be essentially identical, the primary effect on passage rate was presumably the difference in fiber content. Higher fiber diets should move more rapidly through the gastrointestinal tract.[7] Thus, the lower fiber diet theoretically has a longer retention time and, therefore, would be more thoroughly digested.

Table 3. Mean apparent digestibility (%) of diet constituents.

Factor	Treatment mean			
	Experiment 1		*Experiment 2*	
	ADF-16	ADF-25	ADF-16	ADF-25
Dry matter	71.29	68.11	69.47	68.76
Neutral detergent fiber	43.90	34.43	42.22	41.34
Acid detergent fiber	26.09	22.37	29.41	37.37
Acid lignin	0.00	0.00	0.05	0.01
Hemicellulose	63.63	58.81	61.12	53.39
Cellulose	34.32	29.25	40.42	47.81
Gross energy	71.75	68.97	69.40	67.35
Crude protein	74.39	74.73	74.00	74.14
Total carbohydrate[a]	72.36	65.29	66.79	64.21
Non-fiber carbohydrate[b]	99.97	98.14	85.14	82.83

[a] 100 - % Water - % CP - % EE - % Ash.
[b] 100 - % Water - % CP - % EE - % Ash - % NDF.

Cellulose, ADF, and NDF digestibilities for ADF-25 in Experiment 2 were greater than expected. It is unlikely that the difference in digesta markers used between the experiments influenced the digestibility, since doses were quite low and administered as boluses. Different batches of both ADF-16 and ADF-25 were used in each experiment, however, and although dehydrated alfalfa meal was the fiber source in each of the diets, it is possible that the digestibility of the alfalfa meal was different if different batches of alfalfa were used in its manufacture. Allen et al.[1] reported a wide range of in vitro ADF and NDF digestibilities of alfalfa with the same ADF and NDF content. In 1,280 samples of alfalfa, they reported that those samples at 40% NDF (DM basis) had NDF digestibilities ranging from 25 to 55%, likewise at 40% ADF (DM basis) ADF digestibilities ranged from 22 to 52%. It is difficult to know why there was a discrepancy in the ADF, NDF, and cellulose digestibilities. However, it is possible that the dehydrated alfalfa meal used to manufacture the second batch of ADF-25 was more digestible than that in any of the other diets even though the ADF and NDF content (as a % of DM) remained relatively constant.

Gut morphology, rumen motility, and body size limit the foods an animal can efficiently use.[4] This suggests that small ruminants may be limited in their ability to efficiently digest higher fiber diets. The chevrotains in this study, however, were able to extract sufficient energy not only from ADF-16 but also from ADF-25 to maintain their body weight.

ACKNOWLEDGMENTS

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CIRCULATING LEVELS OF ALPHA-TOCOPHEROL AND RETINOL IN CETACEANS: SAMPLING AND CLINICAL CONSIDERATIONS

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Serum levels of vitamin A (retinol), vitamin E (alpha and gamma-tocopherol), cholesterol, and triglycerides were determined in numerous cetaceans on vitamin supplementation including bottlenose dolphins (*Tursiops truncatus*, $n=18$), killer whales (*Orcinus orca*, $n=7$), false killer whales (*Pseudorca crassidens*, $n=6$), Pacific white-sided dolphin (*Lagenorhyncus obliquidens*, $n=1$) and a spotted dolphin (*Stenella frontalis*, $n=2$). Comparisons were made with serum levels of vitamin A and E in 12 wild *Tursiops* obtained from Sarasota, Florida.

All baseline samples taken at Sea World were spun to separate red cells within 1 hr. Often these samples were taken at the same time as routine samples for health evaluation. As a result, serum was frozen from 2-6 hr after initial sampling. Table 1 shows the mean ($\pm$SEM) serum concentrations of vitamin A and E in cetaceans. Gamma-tocopherol, predominantly associated with ingestion of vegetation, was detected in numerous samples, ranging from 0.08 to 0.59 μg/ml.

As a result of unexpectedly wide differences between serum vitamin levels in aquarium and wild *Tursiops*, serum from five animals at Sea World was handled by four different methods to clarify the effects of variables in time, temperature, and separation from red cells. Differences in blood handling techniques resulted in a 42-75% change in serum alpha-tocopherol levels in some individuals and a 7-21% change in serum levels of retinol.

It is suggested that serum vitamin A and E levels reported for cetaceans should include specific handling techniques used, including time from collection to freezing, room temperature before freezing, whether cells and serum were immediately separated, and shipping time if the samples are not sent on dry ice and don't remain frozen.

Table 1. Mean (± SEM) serum levels of vitamin E (α-tocopherol) and vitamin A (retinol) in cetaceans.

Species	*n*	α-Tocopherol (μg/ml)	Retinol (μg/ml)
Tursiops truncatus (wild)	12	6.63 ± 0.49	0.03 ± 0.01
Tursiops truncatus (Sea World)	59	13.24 ± 0.57	0.15 ± 0.02
Lagenorhynocus obliquidens	1	8.67	0.08
Stenella frontalis	2	9.41	0.09
Pseudorca crassidens	6	12.12 ± 1.35	0.07 ± 0.01
Orcinus orca	10	10.68 ± 1.64	0.10 ± 0.02

Table 2. Vitamin A and E levels (μg/ml) in *Tursiops truncatus* serum handled under different conditions prefreezing.

	T. trunc. 1		*T. trunc. 2*		*T. trunc. 3*		*T. trunc. 4*		*T. trunc. 5*	
Prefreezing treatment	**A[a]**	**E[b]**	**A**	**E**	**A**	**E**	**A**	**E**	**A**	**E**
Spun and frozen immediately after clotting	0.60	10.4	0.51	13.5	0.42	18.0	0.25	16.4	0.60	16.8
Spun, separated, refrigerated 9 hr, then frozen	0.19	9.8	0.20	12.5	0.25	16.5	0.20	12.1	0.23	11.9
Spun, held at 75°F for 9 hr, then frozen	0.26	10.6	0.17	12.2	0.35	16.3	0.26	12.7	0.25	13.6
Refrigerated with cells for 24 hr, then spun and frozen	0.21	9.6	0.13	12.5	0.24	15.9	0.14	14.0	0.21	13.2

[a]Retinol.
[b]Alpha-tocopherol.

VITAMIN E FORMS FOR ELEPHANTS

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Introduction

Blood serum levels of vitamin E have been a concern for exotic animals in captivity, especially elephants and rhinoceroses.[3] Specifically, vitamin E deficiency has been linked to cardiac and skeletal myopathies and neuronal degeneration in ungulates.[2] Supplementation with vitamin E has been shown to prevent clinical signs of deficiency in these species.

Prior Reports and Studies

Several forms of vitamin E are available for use in supplementation of diets. Although it is generally recognized that the principal natural form of vitamin E (d-alpha-tocopherol or *RRR*-alpha-tocopherol) and its acetate ester are more bioavailable to animals than the synthetic form (dl-alpha-tocopherol or *all-rac*-alpha-tocopherol) and its acetate ester, most feed manufacturers use the synthetic acetate ester as the source of vitamin E due to its lower cost. The acetate ester is more stable than the free alcohol form of either natural or synthetic vitamin E. Several studies have indicated that the utilization of different forms of vitamin E varies dramatically from species to species.[1,4,6]

TPGS (d-alpha-tocopheryl polyethylene glycol-1000 succinate) is derived from the natural form of vitamin E. By linking the tocopherol to polyethylene glycol, the molecule achieves greater stability and it becomes water miscible. This water miscibility enables the vitamin to be absorbed by different mechanisms than the fat-soluble forms.[9,10]

These properties have led to the use of TPGS in the treatment of children with cholestatic liver disease, which is characterized by an inadequate secretion of bile salts and results in malabsorption of fat and fat-soluble vitamins. Sokol et al.[8] have shown that TPGS is effective in providing vitamin E to children with this disorder.

Increased water miscibility of the vitamin E supplement apparently enhances absorption and utilization in elephants.[11] Prior studies in which TPGS in an aqueous suspension was added

as a supplement to the diets of elephants and rhinoceroses have suggested that this form of vitamin E was utilized much more effectively than other forms.[5,7] In the present study, elephants from three zoos were fed a manufactured diet that utilized a dry premix containing TPGS as the source of vitamin E.

Protocol

A total of 16 elephants from three different zoological parks were used in the study. Of these, seven were African elephants (*Loxodonta africana*) and nine were Asian elephants (*Elephas maximus*). Blood samples were taken to establish a baseline blood serum alpha-tocopherol level for each animal.

During the transitional phase (beginning with day 0 of the study), each animal was pulsed with TPGS by oral administration of 77.4 ml (6,000 IU) of supplemental aqueous vitamin E TPGS each day for 3 wk at the Indianapolis and St. Louis Zoos and for 6 wk at the Los Angeles Zoo. At the same time, the animals began a conversion from their current diets to a diet of hay and Mazuri Elephant Supplement manufactured with 200-800 IU/kg TPGS as the source of vitamin E. Diet conversion was complete by day 7 of the study. Table 4 shows a nutritional breakdown for the diets at each of the three zoos involved in this study.

During the study, blood samples were taken on days 0, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112, and every 2 wk, or at appropriate intervals thereafter. Each sample was analyzed by Eastman Chemical Products Company for blood serum alpha-tocopherol level.

Results

As shown in Table 1, every elephant in the study showed a positive response to the dietary change. The Asian elephants responded more dramatically than the African elephants, but both species exhibited increased blood serum alpha-tocopherol levels after being fed a diet utilizing TPGS as the source of vitamin E. The Asian elephants all achieved circulating levels of alpha-tocopherol at or above the level reported for free-ranging elephants of 0.8 mcg/ml.[7] Baseline circulating levels in some individuals were as low as 0.21 mcg/ml. These animals achieved greater than a four-fold increase in serum alpha-tocopherol levels with TPGS supplementation. Serum alpha-tocopherol levels in the African elephants increased from a baseline average of 0.23 mcg/ml to a final average value of 0.43 mcg/ml.

Statistical analyses showed that there were significant treatment ($P<0.01$; Table 2) and treatment x species ($P<0.05$; Table 3) effects of TPGS. Mean serum alpha-tocopherol values (Table 2) were significantly increased ($P<0.01$) with TPGS supplementation (mean=0.39 mcg/ml before treatment; mean=0.73 mcg/ml after treatment). Table 3 is a matrix comparing each of the sets of variables: African baseline, African after treatment, Asian baseline, and Asian after treatment. Differences between the baseline and after treatment samples were significant for each species (African elephants nos. 1 and 2; Asian elephants nos. 3 and 4). Additionally, the after-treatment values for the African elephants (no. 2) were significantly lower than the values for the Asian elephants after treatment (no. 4) ($P<0.01$).

The veterinarians and keepers of the animals involved in the study have not reported any deleterious effects attributable to the TPGS. The diet was palatable and consistently eaten throughout the study.

During the course of the study, one of the Asian elephants, Pearl, gave birth to a calf in December 1992. Blood samples were drawn from the calf, and milk samples were taken from the mother shortly after birth. Both sets of samples were analyzed for alpha-tocopherol levels. The analyzed level of alpha-tocopherol in the milk sample was 0.96 mcg/ml, indicating that the TPGS was being absorbed and utilized by the mother. In addition, the calf's blood sample contained between 1.06 and 2.27 mcg/ml alpha-tocopherol. The alpha-tocopherol levels in the calf's blood and mother's milk were considered to be reasonable (Dierenfeld, pers. com.). Previously observed blood levels for elephant calves have ranged from 0.06 to 3.18 mcg/ml, with a mean of 0.64 ± 0.57 mcg/ml (Dierenfeld, pers. com.). Previously observed alpha-tocopherol levels in milk samples ranged from 0.09 to 0.55 mcg/ml, with a mean of 0.33 ± 0.12 mcg/ml (Mainka et al., unpublished). The results from the calf indicate that TPGS was being absorbed by the mother and was capable of being passed on to a calf. Additionally, these results suggest that the level of alpha-tocopherol in the milk of a cow being fed TPGS is adequate to sustain a healthy blood serum alpha-tocopherol level in the calf.

Conclusions

Vitamin E was effectively utilized by elephants in the form of TPGS when added to the diet. The results of this study indicate that including TPGS as the vitamin E source in elephant diets increased the circulating blood serum alpha-tocopherol levels as compared to pretreatment levels.

This study suggests that Asian elephants are more responsive to dietary TPGS than African elephants. More data are needed to confirm this observation.

Table 1. Serum alpha-tocopherol levels in individual elephants before treatment (baseline) and after treatment.

Zoo	Elephant (name)	Species	Weight (kg)	Dosage (IU vit E/kg BW)	Baseline (mcg/ml)	After treatment (mcg/ml)
Indianapolis	Cita	African	3436	2.3	0.35	0.48
	Ivory	African	2152	3.7	0.21	0.52
	Kubwa	African	2671	3.0	0.20	0.54
	Sophie	African	4372	1.8	0.19	0.32
	Tombi	African	2442	3.3	0.20	0.38
Los Angeles	Ruby	African	3977	1.0	0.17	0.40
	Tara	African	3282	1.2	0.32	0.50
	Becky	Asian	1704	2.3	0.21	0.99
	Billy	Asian	2591	1.5	0.22	0.81
	Dixie	Asian	2227	1.8	0.22	0.85
	Ginny	Asian	1977	2.0	0.29	1.26
	Gita	Asian	4659	0.9	0.56	1.01
St. Louis	Carolyn	Asian	2625	1.5	0.72	0.81
	Donna	Asian	2827	1.4	0.83	1.17
	Marie	Asian	2833	1.4	1.05	1.42
	Pearl	Asian	3023	1.3	0.83	0.93

Table 2. Statistical comparison of baseline and after-treatment serum concentrations of alpha-tocopherol (mcg/ml).

Sample	Least squares mean
Baseline	0.39
After treatment	0.73[a]

[a]Significantly higher ($P<0.01$) than baseline value using the general linear models procedure for least squares means.

Table 3. Statistical analysis of sample x species interaction effects upon serum concentrations of alpha-tocopherol (mcg/ml).

Number	Species	Sample	Least squares mean
1	African	Baseline	0.23[a]
2	African	After treatment	0.43[b]
3	Asian	Baseline	0.55[c]
4	Asian	After treatment	1.03[c]

[a,b,c]Means followed by different superscripts were significantly different ($P<0.05$) using the general linear models procedure for least squares means.

Table 4. Composition (by analysis) of elephant supplement and hays fed to elephants at the Indianapolis, St. Louis, and Los Angeles Zoos.

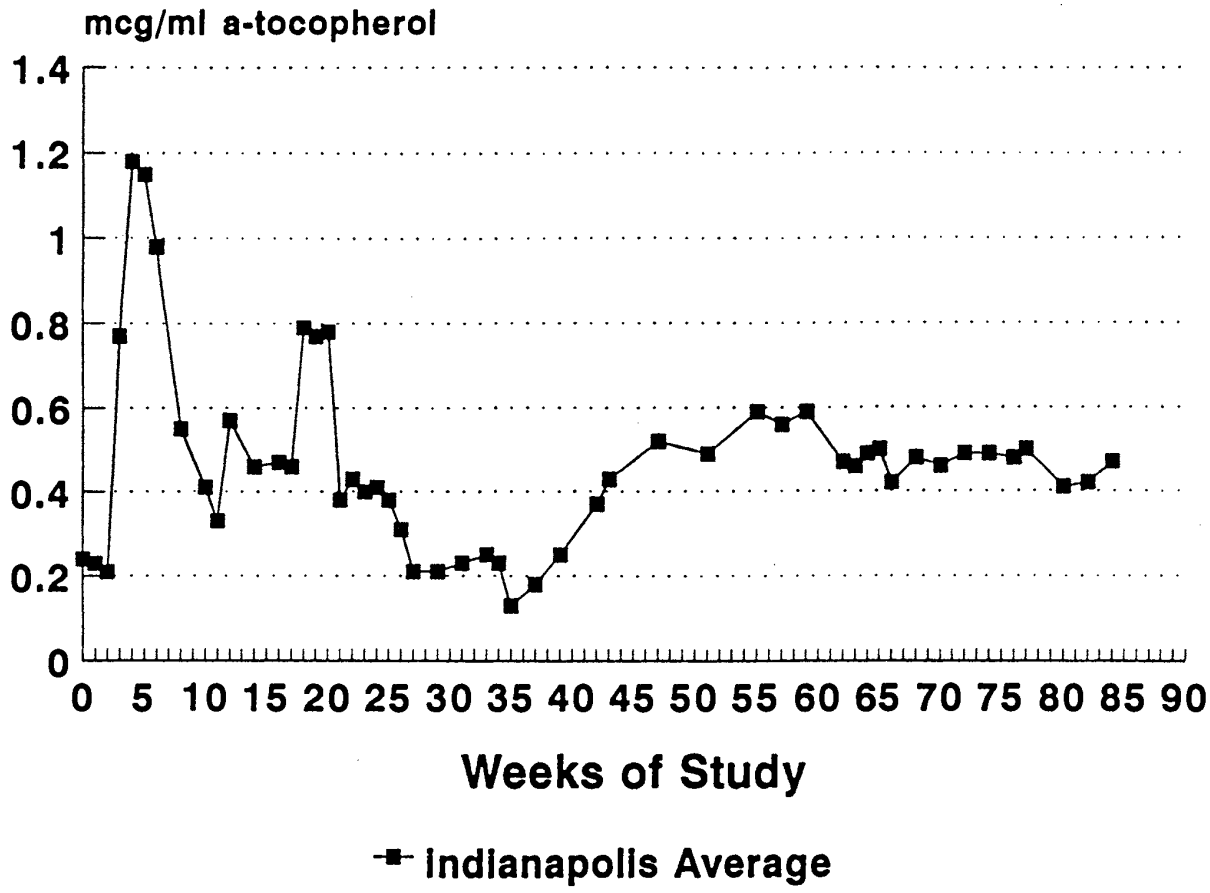
Zoo/ Analyte	Elephant supplement	Hay				
		Alfalfa	Bromegrass	Orchardgrass	Timothy	Undef[a]
Indianapolis						
Water (%)	12.2±0.5			15.7	10.5±2.7	14.6
CP (%)[b]	25.3±2.4			11.2	7.3±1.3	9.8
EE (%)[c]	5.8±0.5					
CF (%)[d]	9.9±0.7					
NDF (%)[e]				48.0	65.4±3.8	56.8
Vit E (IU/kg)[f]	853±89.8			62	34±19	59
Se (ppm)				0.06	0.04±0.03	0.06
St. Louis						
Water (%)	12.6	15.0	10.1			
CP (%)	22.1	15.5	7.3			
EE (%)	5.7					
CF (%)	10.0					
NDF (%)		38.9	64.6			
Vit E (IU/kg)	352	23	64			
Se (ppm)	0.58	0.05	0.06			
Los Angeles						
Water (%)	12.5					11.0
CP (%)	24.7					12.1
EE (%)	6.1					
CF (%)	10.3					
NDF (%)						61.0
Vit E (IU/kg)	828					192
Se (ppm)	0.81					0.10

[a]Type of hay undefined.
[b]Crude protein.
[c]Ether extract, preceded by acid hydrolysis (crude fat).
[d]Crude fiber.
[e]Neutral detergent fiber.
[f]Determined as alpha-tocopherol.

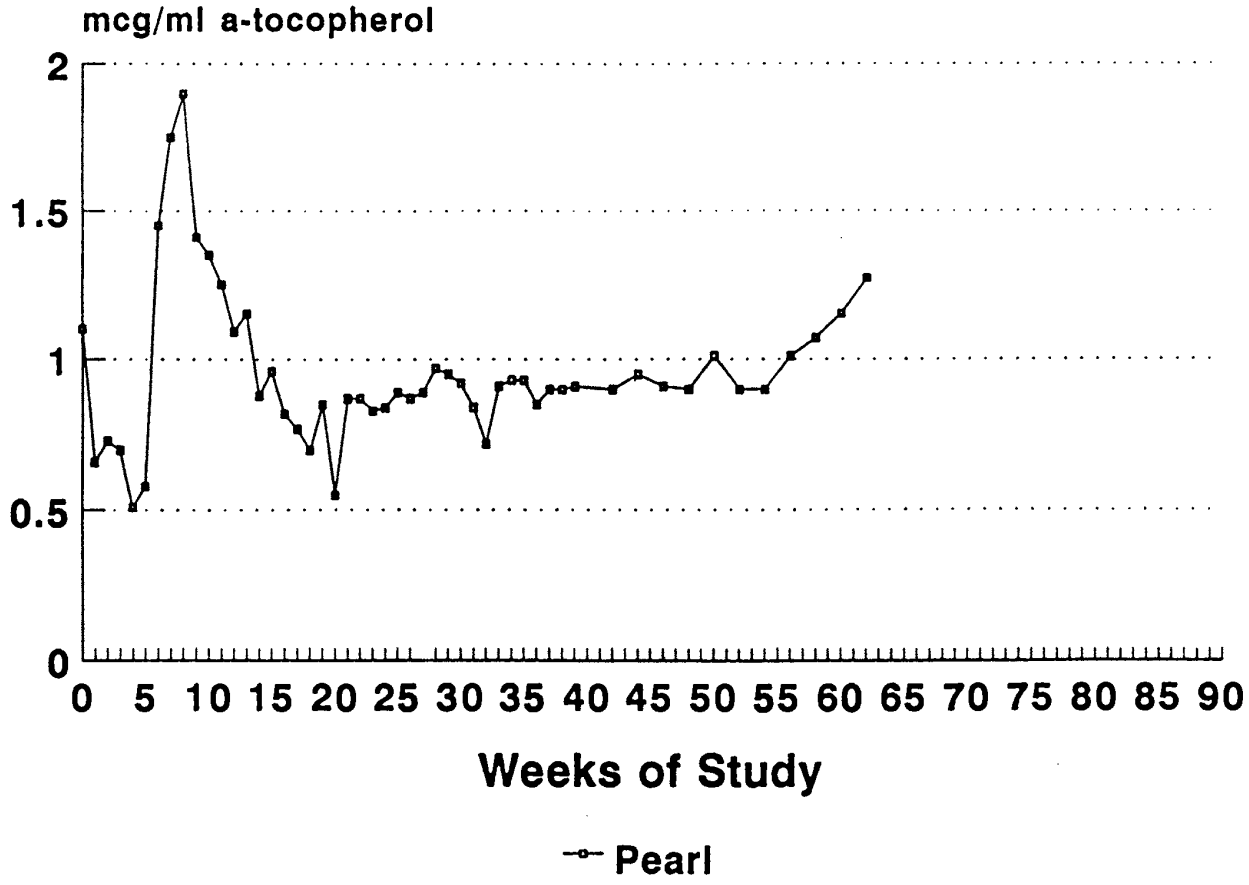
Average a-Tocopherol Values by Species

Species	Baseline	After TPGS
African	0.23	0.43
Asian	0.55	1.03

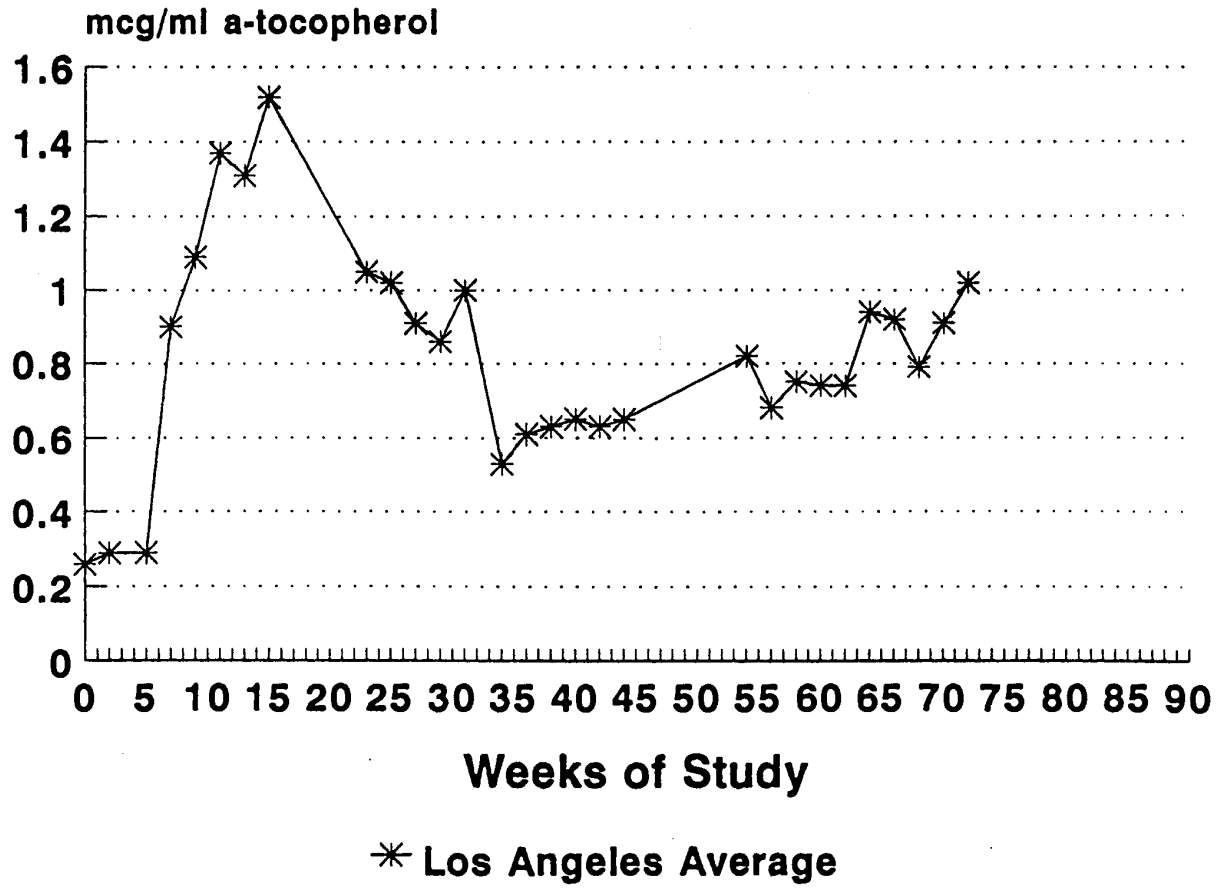
Circulating a-Tocopherol Levels



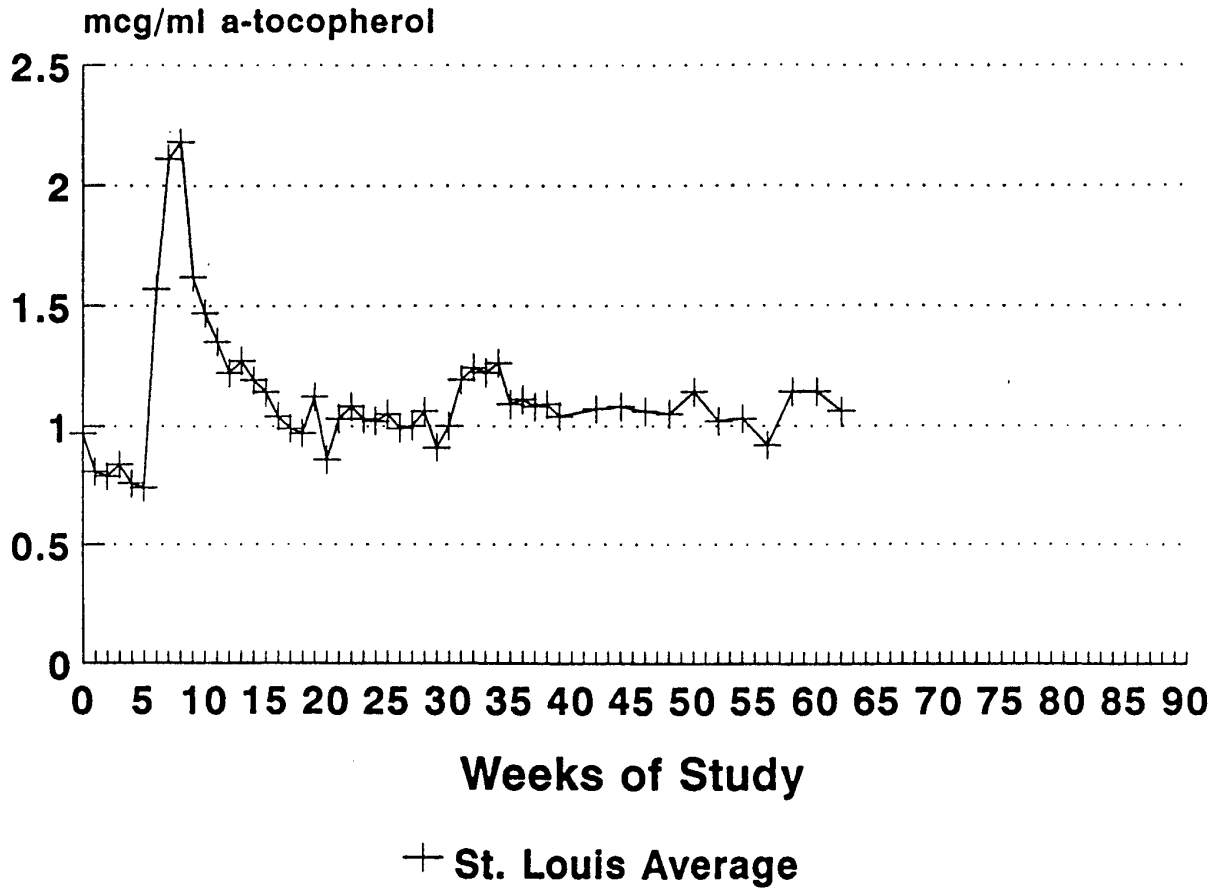
Circulating a-Tocopherol Levels



Circulating a-Tocopherol Levels



Circulating a-Tocopherol Levels



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FEEDING THE SUMATRAN RHINO (*Dicerorhinus sumatrensis*): DIET EVALUATION, ADAPTATION, AND SUITABILITY

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In Nature, the Sumatran rhino consumes up to 50 kg (fresh weight) of leaves and stems from broad-leaved herbs, shrubs, and trees daily; however, monocot grazing has also been observed. Ten species of native browses analyzed at the Nutrition Laboratory, Wildlife Health Center, Wildlife Conservation Society contained an average of 12.0% crude protein and 8.8% available protein. Total cell wall content (NDF) averaged 49.8%, ADF was 27.2%, and lignin, 13.3%. The hemicellulose content of these browses (22.7%) was more representative of monocot species than dicots. Of minerals quantified (Ca, Cu, Fe, K, Mg, Na, P, Zn), Na, P, and Zn levels may be limiting in native vegetation consumed by Sumatran rhinos compared to minimum requirements for the horse (NRC, 1984). Dietary dry matter consumed daily by Sumatran rhinos (n = 17) in four North American, two European, and one Malaysian zoo ranged from 15-20 kg (1-2% of body mass). Most of the dietary dry matter was provided by legume hays, formulated pellets provided 2-5 kg, and the remainder was provided by variable quantities of fresh browse and/or produce. Nutrient composition of these diets averaged 15% crude protein, <20% ADF, and were considered highly digestible. After prolonged diet refinement to improve diet palatability and fecal consistency, Bronx and Cincinnati Zoos feed an orchard grass/legume hay ad libitum (intake of approximately 10 kg), 3.2 kg of pellets developed for feeding moose, and honeysuckle, willow (*Salix* sp.), and mulberry (*Morus* sp.) browse. Mixed hay, rather than alfalfa, was chosen to more closely duplicate natural forage composition, while pellets based on aspen sawdust appeared to have a suitable carbohydrate content for this species.

SURVEY OF SERUM CAROTENOIDS IN CAPTIVE EXOTIC ANIMALS

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Introduction

Carotenoids are a group of naturally occurring fat-soluble pigments. There are more than 500 carotenoids that exist in nature.[21] Some of these have provitamin A activity, the most common being β-carotene.[6,34] Plants are the primary carotenoid source in both human and animal diets. Roots, fruits, and vegetables also provide carotenoids. In general, herbivores obtain their pigments from ingested plants, and carnivores from prey animals which, in turn, obtained their carotenoids from their food. Most carnivores obtain vitamin A preformed,[25] but they may also obtain carotenoids from prey tissues and digesta. Carotenoids may be classified into two main groups: those based on carotenes, including α-,β- and ξ-carotene, and xanthophylls, which include oxygenated compounds. The xanthophylls include zeaxanthin, lutein, and astaxanthin.[21]

One of the most significant characteristics of some carotenoids is their transformation into vitamin A, an essential nutrient, present in nearly all animal species. Because animals are not capable of de novo synthesis of vitamin A-active substances, either retinol and its derivatives or retinol precursors must be provided in the diet.[9,21]

Vitamin A and carotenoids function as an integral part of many essential processes throughout the body. They are necessary, either directly or indirectly, for proper function of most organs. Recently, the human health benefits of carotenoids have received much attention. β-Carotene has been proposed as an important antioxidant and a potential cancer preventive[2,8,9,26,27,34] It has been suggested that dietary carotenoids influence longevity in man as well as in animals.[12,13] Carotenoids may be essential for proper immune function in humans and some species of animals.[10,11] Carotenoids are known to influence sexual dichroism and protective coloration in fish[21] and to accumulate in the plumage of birds. Color and plumage patterns play an important role in avian biology, used for both sexual attraction and visual communication.[4] Like vitamin A, carotenoids may play an important role in reproduction. Low β-carotene intakes by cattle have been reported to result in a higher incidence of silent estrus, decreased conception rates, and increased embryonic death.[34]

Much species specificity has been noted in the ability of animals to absorb dietary carotenoids.[6] Some animals, like humans, circulate carotenoids in their blood, while others appear not to accumulate any carotenoids in their tissues. Some birds accumulate xanthophylls almost to the exclusion of all other carotenoids.[7,20]

The extent to which fasting plasma or serum carotenoid composition and concentration reflects that of solid tissues is not known but is of importance in determining carotenoid "status" and, in turn, vitamin A status. It is assumed that high circulating levels of a carotenoid will also be reflected in high tissue levels. Many factors may influence this relationship. Tissues are likely to reflect years of consumption, whereas plasma levels may only reflect recent intakes.[29] In a study by Parker,[29] it appears that the predominant carotenoids in both tissue and plasma are the same; however, this may simply be a result of the equilibrium of tissue and circulating pools.

Materials and Methods

Blood samples were obtained opportunistically from Brookfield Zoo animals that were immobilized for routine physicals, diagnosis of a specific ailment, or prior to euthanasia. Animals were of both sexes and all ages.

Blood was drawn by veterinary staff in a nonheparinized tube and covered to protect it from light as soon as possible. Upon return to the hospital, the sample was centrifuged and serum separated. This serum was covered, labelled, and frozen for up to 5 yr at -80° C until thawed for analysis. Duplicate aliquots of thawed serum were extracted and analyzed by HPLC using the method described by Stacewicz-Sapuntzakis et al.[36] The carotenoids α- and β-carotene, lutein, lycopene, β-cryptoxanthin, and canthaxanthin were examined.

After chemical analyses were completed, data were arranged taxonomically by species and by individual sample. Means for individual species were then calculated. Multiple samples for a single individual were averaged to one value, then averaged with the other individual means for that species.

Results and Discussion

There was considerable variation in total carotenoid concentration among species of the same order as well as substantial individual variation. Some of the variation may have been due to extended storage times. In primates, which appear to absorb a wide range of carotenoids, variation included the type of carotenoid found in the serum as well as the concentration. Birds and felids appear to be selective responders, having primarily one carotenoid in their serum. Lutein was high in the majority of avian serum samples, while β-carotene was the only carotenoid found in feline samples. However, felines did not consume significant quantities of other carotenoids. Most of the hoofed mammals, marine mammals, and carnivores had no carotenoids present in their serum. Means and ranges by order are presented in Table 1.

MAMMALS

Marsupialia: The western grey kangaroo, the only marsupial sampled, had only low levels of β-carotene. Serum concentrations ranged from undetectable to 3.7 μg/dl for four animals.

Primates: New World monkeys, in general, had low carotenoid levels. Despite a diet moderate in carotenoid content, golden lion tamarins exhibited no measurable levels of carotenoids in their serum. Old World monkeys had a wide variety of both levels and types of carotenoids. The sooty mangabey had the highest mean total carotenoid concentration (368.6 μg/dl) of all mammals. Great apes ranged from 18.0-155.9 μg/dl. Serum levels of all carotenoids were much lower in the gorilla than in the orangutan, even though these species consumed a similar diet. Because of their close taxonomic relationship, one might expect a similar response to dietary carotenoids. Surprisingly, few data exist quantifying serum carotenoids in primates[24,28,35] and most of these involve supplementation with a particular carotenoid. Like humans, most primates examined seem able to absorb both xanthophylls and carotenes.

Cetacea: Free ranging dolphins had total carotenoids ranging from undetectable to 5.3 μg/dl, primarily lutein and β-carotene. Captive dolphin serum contained no measurable carotenoids. As in most primates, free-ranging dolphins show considerable individual variation. Reports were not found on carotenoids in cetaceans.

Carnivora: Canids, mustelids, ursids, and viverrids had, in general, no detectable carotenoids. Studies conducted with dogs have shown low to moderate levels of circulating β-carotene.[5,18,37,38] Felids, in contrast, had β-carotene levels ranging from 5.4 μg/dl in the bobcat to 56.8 μg/dl in the jaguar. The presence of β-carotene in feline blood is contradictory to previous research which indicated that domestic cats do not absorb oral β-carotene.[1,21] Only recently has there been mention of the domestic cat having β-carotene in its blood.[5] Studies by Lakshmanan et al.[24] suggested that domestic cats lack the necessary enzyme to convert β-carotene to vitamin A. The ability of exotic felids to convert β-carotene to vitamin A has not been studied. The lack of this ability in the domestic cat raises the question whether β-carotene in the liver of exotic felids can actually be used. Perhaps the high serum β-carotene levels in exotic cats is due to their inability to convert β-carotene to vitamin A.

Pinnipedia: Like the captive cetaceans, the sea lions, harbor seals, and walruses had no detectable serum carotenoids. This is consistent with data of Schweigert et al.[33] who found no detectable β-carotene in the serum of 65 free-ranging grey seals.

Proboscidae and Hyracoidae: Eleven samples from six elephants contained no detectable levels of any carotenoid. The same was true for both rock hyraxes sampled.

Perissodactyla: The perissodactyla had total carotenoids ranging from undetectable in the black rhinoceros to 12.5 μg/dl in the Grant's zebra. This latter concentration is similar to the 14.0 μg of β-carotene/dl reported in horses by Baker et al.,[5] but considerably lower than the 50.4 μg/dl carotene reported by Vander Noot et al.[39] Both cattle and horses show seasonal variations in plasma carotene, probably associated with the higher concentration of carotenes found in fresh summer pasture as compared to that in dormant, winter pasture or cured hay.[20,32]

Artiodactyla: Two bovids, the banteng and the wisent, had high levels of β-carotene (111.3 and 120.8 μg/dl, respectively). Studies by Anderson et al.[3] have demonstrated that domestic steers may have β-carotene levels of 297.8-464.1 μg/dl. It is interesting to note that the Congo buffalo, also a bovid, had no detectable levels of any carotenoid. In the warthog, α- and β-carotene were detected in the serum, along with β-carotene levels of 9.2 μg/dl. This is contrary to studies done by Poor et al.[31] that showed the domestic pig did not have detectable levels of serum β-carotene. No other ungulates, with the exception of one kudu (0.70 μg/dl lutein), had detectable levels of any carotenoid. Many of the exotic hoofstock sampled were antelope, and may be more closely related to sheep or goats than to cows. Goats do not have carotenoids in their blood or tissues,[22] and sheep usually have low levels only.[30] Goodwin and Gregory[22] suggested "carotene" is converted to vitamin A in sheep, not absorbed intact. This could explain why we see no detectable levels of carotenoids in these animals.

BIRDS

Total serum carotenoids of birds ranged from 3.9 μg/dl in the Humboldt's penguin to 1,996 μg/dl in the greater flamingo. The primary carotenoids identified were lutein and canthaxanthin. Since flamingo diets are supplemented with carotenoids to improve plumage coloration, these concentrations were similar to those reported by Fox and co-workers.[14-17] The mandarin duck, while receiving a low carotenoid diet had high levels of lutein. No published data were found for carotenoids in ducks.

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Table 1. Serum carotenoids in Brookfield Zoo animals.

Order (n)[a]	Mean total carotenoids (μg/dl)	Mean highest species (μg/dl)	Mean lowest species (μg/dl)	Predominant carotenoid
MAMMALS				
MARSUPIALIA (4)	1.6	W. grey kangaroo 1.6	----- ND[b]	β-carotene
PRIMATES				
New World carotene monkeys (7)	24.3	Spider monkey 78.0	G. lion tamarin ND	β
Old World monkeys (41)	77.4	Sooty mangabey 368.6	Mandrill 12.4	Lutein
Great apes (12)	67.0	Orangutan 155.9	Siamang 18.0	Lutein
CETACEA (43)	1.9	Wild dolphin 2.5	Captive dolphin ND	β-carotene
CARNIVORA				
Non-felids (16)	ND	Sloth bear 0.4	All other ND	Lutein
Felids (36)	24.2	Jaguar 56.8	Fishing cat 5.4	β-carotene
PINNIPEDIA (13)	ND	-----	-----	-----
PROBOSCIDAE (6)	ND	-----	-----	-----
HYRACOIDAE (2)	ND	-----	-----	-----
PERISSODACTYLA (13)	6.8	Grant's zebra 12.5	Black rhino ND	β-carotene
ARTIODACTYLA (27)	9.6	Wisent 129.3	Most others ND	β-carotene
BIRDS				
SPHENISCIFORMES (31)	3.9	H. penguin 3.9	-----	Lutein
PELECANIFORMES (2)	37.1	Brown pelican 37.1	-----	Lutein
CICONIIFORMES (16)	1,118	Grtr. flamingo 1,997	Sacred ibis 17.8	Canthaxanthin
ANSERIFORMES (8)	102.1	Mandarin duck 210.3	Whistling duck 39.6	Lutein
CHARADRIFORMES (14)	120.6	Grey gull 169.9	Inca tern 71.3	Lutein

[a]Number of individuals sampled.
[b] None detected.

FORMATION OF A NUTRITION ADVISORY GROUP TO THE AMERICAN ZOO AND AQUARIUM ASSOCIATION (AZA)

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Introduction

In January 1994 a meeting was held in Bethesda, Maryland to form a Nutrition Advisory Group (NAG) to be of service to the American Zoo and Aquarium Association (AZA). The purpose of this paper is to announce the formation of this group and to describe its mission and goals. As with all new organizations, the structure of NAG will undoubtedly undergo change.

Scientific Advisory Groups (SAGs) to AZA (which is what NAG is) are formed to:
1) Provide technical/scientific support to AZA SSPs, TAGs, SAGs, FIGs, WCMC, and the Conservation and Science Office and Board.
2) Form cooperative working relationships with university, nongovernment, and government scientists in areas of interest to AZA member institutions.
3) Coordinate certain research projects, especially those involving cooperation among a number of AZA institutions.
4) Liaise with appropriate professional scientific societies.

Since nutrition affects every facet of animal management, it is expected that there will be much interaction between NAG and other disciplines, SAGs, TAGs, SSPs, and related groups.

The need for a Nutrition Advisory Group relates to the importance of comparative nutrition as a science integral to good management of zoo animals. The formation of NAG recognizes the importance of this discipline and promotes better communication and coordination among nutritionists and those requiring nutrition information (zoos and aquariums). NAG also can help provide the leverage needed to accomplish special projects, conduct research, and encourage feed suppliers to provide needed products.

As discussions of NAG evolved, a community of comparative nutritionists was identified, having no direct connection with zoos or aquariums but representing an important resource of knowledge and research capability, that could undergird the efforts of practicing nutritionists in those institutions. To be most helpful, it was clear that comparative nutritionists involved in research and practicing nutritionists working in zoos and aquariums should interact with each other.

To promote this interaction, the formation of an association of comparative nutritionists is being considered. Hopefully, such an association would encourage the advancement of the science of comparative animal nutrition, facilitate the exchange of scientific information, and serve as an information resource of use to zoos and aquariums.

Members of such an association might be members or consultants to NAG with the responsibility to develop and advance rational feeding programs for captive animals, based on sound scientific principles, and in this way to serve AZA. A description of the mission and goals of NAG follows.

Mission

The mission of the Nutrition Advisory Group (NAG) is to promote the welfare of animals in captivity by incorporating the science of nutrition into their husbandry.

Goals

1) Identify nutritional and dietary problems in zoos and aquariums and facilitate their resolution.
   a) Gather information.
   b) Conduct studies.

2) Establish a mechanism for professional review of nutritional and dietary information provided by and to AZA committees and subgroups, including -
   a) NAG Technical Papers and NAG Newsletters.
   b) AZA publications such as SSP and TAG Management Guidelines, Nutrition Section.
   c) Nutrition-related projects proposed to SSPs and TAGs.

3) Coordinate acquisition and dissemination of nutritional information.
   a) Initiate and coordinate symposia, workshops, and training programs.
   b) Develop and publish technical papers, newsletters, and nutritional databases.

4) Encourage and coordinate nutrition-related investigations among zoos, aquariums, and collaborating institutions by endorsing, organizing, and supporting studies of -
   a) Nutrient requirements.
   b) Food composition.
   c) Dietary husbandry.

Membership

Membership in NAG is predicated on a desire for service to AZA and recognition that the principal clientele are zoos and aquariums.

Criteria for membership include -

1) Demonstrated interest and commitment to zoo and aquarium animal nutrition.

2) Advanced degree in nutrition or comparable experience in zoo or aquarium nutrition/dietary husbandry/feeding management.

3) Approval by the executive committee of NAG.
   a) In general, those with obvious conflicts of interest (e.g., majority of income from production or sale of feeds) would be ineligible.
   b) Those who consult for feed manufacturers but who do not derive a majority of their income from such activity would be considered on a case by case basis.
   c) Full disclosure of real or apparent conflicts of interest is required.

4) Associate membership may be awarded to students with an interest and commitment to zoo and aquarium animal nutrition but who do not otherwise meet the criteria for full membership. Participation in NAG activities is encouraged, but associate members will not have voting privileges.

Executive Committee

The founders of NAG constituted the initial Executive Committee, and all individuals satisfied the membership requirements given above. A chair (Crissey) and vice chair (Fulton) were elected. The chair and vice chair will represent the Executive Committee of NAG (after appropriate consultation) in contacts with other groups and organizations and, in concert with other Executive Committee members, will coordinate and disseminate information, facilitate action plans, appoint subcommittees and task forces, receive applications for NAG membership, and identify qualified scientists for professional reviews.

Five-Year Action Plan

The AZA has requested that each Scientific Advisory Group (SAG) prepare a 5-yr action plan and identify six priority projects, including one on site. Responses to this request will be formalized at NAG's second meeting. In addition, the following subcommittees have been formed:

1) Quality control, vendor compliance, and analytical assurance techniques.
2) Behavioral enrichment and food use.
3) Applied nutrition conference.
4) Handrearing of birds.
5) Technical papers.
6) Newsletter.
7) Coordination with CBSG.

VITAMIN D DEFICIENCY RICKETS IN COLOBINAE MONKEYS

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Vitamin D deficiency rickets was diagnosed in three juvenile colobus monkeys (*Colobus quereza*) at the Toledo Zoo from 1989 to 1991, and one juvenile Francois langur (*Presbytis francoisi*) at the San Diego Zoo in 1988.

Vitamin D is important in maintaining adequate serum calcium levels. The ultimate function of vitamin D is to enhance mineralization of bone, especially in young, growing animals. There are two forms of vitamin D, ergocalciferol (vitamin D_2), which is produced in plants, and cholecalciferol (vitamin D_3), which is produced in the skin of mammals and some other species. Rickets, the lack of mineralization of osteoid or cartilaginous bone, can result from a primary or secondary deficiency of vitamin D or its metabolites, or a calcium or phosphorus deficiency.

In 1989, a 10-mo-old colobus was presented with signs of an abnormal gait, difficulty climbing and walking, and a dome-shaped head. Physical examination revealed severe angular deformities of all long bones and swollen joints with increased laxity. Radiographs revealed cupping of the metaphyses, widening radiolucent epiphyseal plates, and bowing of the long bones. Abnormal serum chemistry findings included: calcium 8.1 mg/dl (ISIS reference range 8.8-10.8 mg/dl), phosphorus 2.7 mg/dl (ISIS reference range 3.6-7.4 mg/dl), and alkaline phosphatase 1,293 IU/L (ISIS reference range 36-924 IU/L). A vitamin D metabolite (25OH-D) level was determined to be <10 ng/ml (no reference range available for colobus; human reference range 25OH-D_3 27.6$\pm$9.2 ng/ml; 25OH-D_2 3.9$\pm$3.0 ng/ml).[5] The radiologic and biochemical findings were consistent with primary vitamin D deficiency. Because of the extent of the lesions, this animal was euthanized. Histologic findings, including diffuse dysplasia of the bone and cartilage along the physeal/metaphyseal junctions characterized by retention of poorly mineralized hypertrophic cartilage, lack of osteoid formation, and poorly mineralized trabeculae in the primary and secondary spongiosa, further supported the diagnosis of rickets.

In 1991, a 5-mo-old colobus monkey showed clinical signs of weakness, reluctance to move, and exhibited pain when handled by its mother. The physical examination revealed swollen limb joints and increased joint laxity. Radiologic findings were similar to those in the previous animal but were less pronounced. Serum biochemistry was unavailable. Levels of 25OH-D were determined to be <10 ng/ml. The animal was treated with 30,000 IU ergocalciferol i.m. in a slow release suspension weekly for 8 wk. Improvement of motor skills was noted with the first week of therapy. Radiographs taken 4 and 8 wk later showed marked improvement of bone density around the epiphyseal plates. Significant serum chemistry values at the 4-wk recheck included: alkaline phosphatase 6,831 IU/L, calcium 9.9 mg/dl, and phosphorus 5.0 mg/dl. Significant serum chemistry values at 8 wk included: alkaline phosphatase 3,762 IU/L, calcium 9.4 mg/dl, and phosphorus 8.4 mg/dl. The level of 25OH-D was determined to be 118 ng/ml at 4 wk. At a pre-shipment examination 1 yr later, the 25OH-D level was 13 ng/ml.

A 2-mo-old colobus monkey was also examined at this time, although it showed no clinical signs of rickets. Physical examination revealed mildly increased joint laxity. Radiographs showed no gross lesions, although there was equivocal loss of bone density. Serum chemistry values were normal other than an elevated alkaline phosphatase of 2,268 IU/L. The level of 25OH-D was determined to be <10 ng/ml. This animal was treated with 15,000 IU ergocalciferol i.m. at the time of initial examination and 1 wk later. The animal was then moved to an enclosure with access to unfiltered sunlight. Radiographic evaluation 4 wk later was normal. The level of 25OH-D 1 yr later was 19 ng/ml.

In 1988, a 10-mo-old Francois' langur was noted to have an abnormal gait. This monkey was the oldest of three juveniles in the enclosure. Physical examination revealed increased joint laxity and swelling and a mildly collapsed thoracic cavity. These findings were not present at an examination done 5 mo earlier. Radiographs showed classic lesions of rickets. Significant serum chemistry findings included: alkaline phosphatase 2,530 IU/L (ISIS reference range 162-804 IU/L), calcium 9.9 mg/dl (ISIS reference range 8.9-10.3 mg/dl), and phosphorus 1.7 mg/dl (ISIS reference range 2.9-6.7). The animal was treated with 2,000 IU vitamin A and 200 IU cholecalciferol, then given 10 IU cholecalciferol p.o. s.i.d. for 1 wk. The monkey was then switched to 4,000 IU ergocalciferol p.o. daily for the next 2 wk, decreased to 1,000 IU p.o. daily for 3 wk, and finally 400 IU ergocalciferol (human RDA) was given p.o. daily for 8 wk. Improvement was noted in radiographs taken 4 wk after treatment began. Vitamin D metabolite levels were not measured before treatment was initiated but 25OH-D was determined to be 64 ng/ml 2 d after treatment began.

All the animals in this report were housed in indoor exhibits with no exposure to ultraviolet (UV) radiation. Adult diets contained adequate vitamin D levels. None of the rachitic monkeys were completely weaned at the time of diagnosis. Clinical findings and response to treatment were indicative of a vitamin D deficiency in these animals. Vitamin D deficiency has been seen in human infants fed exclusively breast milk or nonfortified cows milk.[3] Vitamin D levels in human milk are inadequate to prevent rickets after 6 mo of age.[2] Cutaneous production of vitamin D by the child is required by this age to prevent rickets.[1] It would seem a similar situation exists in the Colobinae family, as the most severe signs

were seen in the older infants in the above cases. Conversely, infants born and raised in outdoor enclosures or enclosures with UV permeable skylights at both zoos did not show clinical signs of vitamin D deficiency. It could therefore be concluded that cutaneous production of vitamin D through exposure to ultraviolet radiation in the antirachitic range (285-315 nm)[4] is vital to the normal development of infants in the Colobinae family and should be considered for all primate species.

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EFFECT OF DIETARY PROTEIN LEVEL ON DRY MATTER INTAKE AND BODY MASS OF CAPTIVE ADULT, MALE FRUIT BATS (*Artibeus jamaicensis*)

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Introduction

Many zoological institutions maintain reproductive fruit bat colonies, both Megachiroptera and Microchiroptera; however, actual nutrient requirements and nutrient strategies remain largely unknown. Much of the literature on bat nutrition is a description of bat feeding or foraging habits in the wild. Some studies involved collecting and analyzing food items for various nutrients.[3,4,7] Others examined excreta or stomach contents of bats in the wild.[6,7] Each of these methods has its limitations and none quantitatively defines the daily nutrient requirements of bats. To date, the only fruit bats for which estimates of nutrient requirements have been made are in the suborder Megachiroptera.[6,7] Few, if any, quantitative nutrient requirement data for frugivorous Microchiroptera are available.

Objectives

Fruit bats appear to support maintenance, growth, and reproduction by consuming high quantities of low-protein, high-calorie foods. The objective of this study was to address the requirement of dietary protein for maintenance of adult frugivorous *Artibeus jamaicensis* bats. Information of use in the formulation of diets for *A. jamaicensis* bats in captivity has been generated, and preliminary data, supporting further studies on the protein requirements of growing *A. jamaicensis* bats, have been developed.

Materials and Methods

The study group consisted of 39 adult male fruit bats (*Artibeus jamaicensis*) chosen at random from the captive colony at the Brookfield Zoo. The bats were housed together in a walk-in flight cage. Temperature, humidity, and light cycle were controlled. The bats were fed three isocaloric diets differing in dry matter and protein concentration as shown in Table 1. The diets were each fed for 1 wk with a 1-wk adjustment period between treatment periods. Diet 2 was fed during the acclimation period prior to beginning the study and also during the adjustment periods between each treatment. Food intake was measured and recorded daily. The bats were weighed at the end of each week.

Table 1. Nutrient concentrations in the experimental diets.[a]

Diet no.	Protein (%)	Estimated ME[b] (kJ/g) [kcal/g]	Dry matter (%)
1	1.30	14.44 [3.45]	15.38
2	4.90	15.32 [3.66]	18.58
3	7.20	15.53 [3.71]	21.45

[a]Dry matter basis.
[b]Coefficient of variance 3.84%.

Results

The average body mass per bat was relatively stable throughout the study with respective means of: diet 1=37.42 $\pm$ 6.63 g, diet 2=37.62 $\pm$ 6.74 g, and diet 3=38.76 $\pm$ 6.78 g. Therefore, it appeared that the bats ate to maintain body mass on each dietary treatment. Figure 1 is a comparison of average body mass per bat on each of the dietary treatments.

The average daily dry matter intake per bat decreased as protein concentration in the diet increased, with respective means of: diet 1=8.61 g/day, diet 2=5.38 g/day, and diet 3=4.03 g/day. Figure 2 is a comparison of the average daily dry matter intake per bat on each of the dietary treatments. The bats consumed 60% more dry matter on diet 1 (the most protein-dilute) than on diet 2.

The bats consumed more energy on the diets lower in protein and dry matter concentration with respective means of: diet 1=124.33 kJ/day, diet 2=82.42 kJ/day, and diet 3=62.59 kJ/day. Figure 3 is a comparison of the average daily energy intake per bat on each of the dietary treatments. The bats consumed 51% more energy on diet 1 (the most protein dilute) than on diet 2.

Figure 4 is a comparison of the average daily protein intake per bat on each of the dietary treatments. As protein intake increased, dry matter, and energy intake decreased. Protein intake may have reached a plateau on diet 2 with an average protein intake per bat of 0.27 g/day for diets 2 and 3.

Discussion

Energy intake: Morrison[3] conducted a study on the foraging ecology and energetics of wild *A. jamaicensis* fruit bats on Barro Colorado Island, Panama. He reported the *A. jamaicensis* fruit bat requires 36.8 kJ/day (8.8 kcal/day) for basal metabolism and has a total daily energy expenditure of 43.9 kJ/day (10.5 kcal/day). He found that figs are the preferred food item, and one fruit bat carried away 50 $\pm$ 21 g figs/night, providing 61.3 kJ (14.6 kcal).

Another study conducted by Morrison[2] looked at the efficiency of food utilization by captive *A. jamaicensis* fruit bats on Barro Colorado Island, Panama. He found, based on their net utilization, that these bats must consume 66 g of figs (*Ficus insipidia*) per day to generate the 50.2 kJ (12 kcal) needed for basal metabolism (36.8 kJ/day, 8.8 kcal/day) and flight (13.4 kJ/day, 3.2 kcal/day). Morrison stated that a more reasonable estimate of consumption of figs by these fruit bats would be 85 g per night, providing 59.8 kJ/day (14.3 kcal/day).

The bats in the present study consumed food in amounts providing more estimated metabolizable energy than observed in either of the two previously discussed studies conducted by Morrison. Based on the more controlled circumstances of the present study, the estimate by Morrison of a BMR = 36.8 kJ/day (8.8 kcal/day) may be too low. On the other hand, energy expenditure in this study may not be representative of natural conditions. It is possible that ambient temperature and humidity affected the energy requirement of the bats in the present study since they were held in an environment where the temperature ranged from 20 to 30°C with an average of 27°C, and relative humidity was highly variable, ranging from 5 to 64 percent. This may have affected the bats ability to regulate body temperatures and, therefore, could have artificially elevated their BMR.

The fiber concentrations of the diets, although not reported here, were slightly higher on diet 1 than on diets 2 or 3. However, the differences were small and unlikely to have diluted the energy concentration of diet 1 sufficiently to increase food intake to meet energy needs. The bats may have dissipated the extra energy consumed on diet 1 by increasing their activity level, thereby maintaining, rather than gaining, body mass. However, activity levels were not monitored.

Protein intake: Total daily protein intake per bat ranged from 0.11 g to 0.28 g with an average intake of 0.27 g/day for diets 2 and 3 in this study. Morrison,[2] in his study on the efficiency of food utilization by captive *A. jamaicensis* fruit bats, determined these bats consumed 85 g of figs per day. This quantity of figs would provide 0.30 g of protein daily. Morrison also concluded that since most mammals require 37 mg protein per kcal of BMR, these bats would need to absorb 0.33 g of protein per day. Morrison proposed that since plant protein usually needs to be consumed in larger amounts than animal protein to provide essential amino acids in sufficient quantity, bats in the wild must have a supplemental source of protein in their diet, such as insects. However, the present study showed that captive, adult male *A. jamaicensis* bats maintained body weight on 0.27 g dietary protein per day, very close to the amount of protein provided by 85 g of figs (0.30 g/day) and to the protein requirement estimate based on BMR (0.33 g/day), although the latter refers to absorbed protein. The bats in the present study consumed only 0.11 g protein per day on the diet with the highest proportion of fruit (diet 1), clearly less protein than Morrison estimated was consumed in the wild. This is further evidence that the bats in the present study may not have met their protein requirements on the more protein-dilute diet.

A possible explanation for the maintenance of body mass on the protein-dilute diet (that did not appear to meet protein needs) could have been a change in body composition. The bats may have gained body fat due to increased consumption of the high energy diets with

a concomitant loss of body protein and possibly water. Alternatively, 1 wk on these dietary treatments may not have been sufficient to affect body mass.

Conclusion

The bats in this study were fed three isocaloric diets varying in protein and dry matter concentration. The average body mass per bat did not vary throughout the study, although each diet was fed for only 1 wk. Dry matter intake and estimated metabolizable energy intake decreased with increasing dietary dry matter and protein concentration, but daily protein intake increased until it reached a plateau. The apparent protein requirement for maintenance was 0.27 g protein/day. The bats appeared to have adjusted their daily dry matter intake, irrespective of energy intake, to meet their protein needs. The estimated metabolizable energy consumed by the bats at the protein intake plateau was 72.51 kJ/day. Since the *Artibeus jamaicensis* fruit bat naturally consumes a diet low in protein and relatively high in energy, these bats may regulate daily food intake to meet their protein rather than their energy requirements, a strategy that differs from that of many other herbivores.

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Figure 1. Mean body mass of adult male fruit bats.

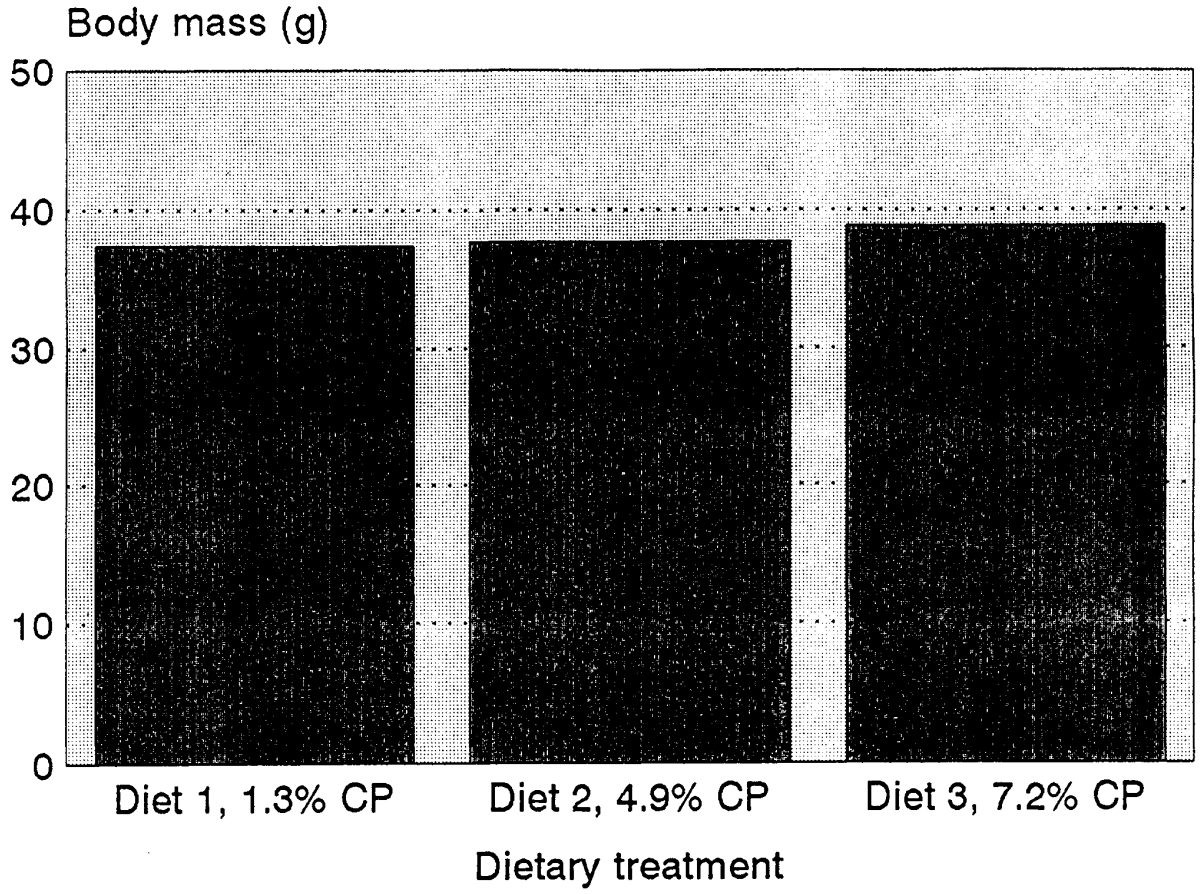


Figure 2. Mean daily DM intake of adult male fruit bats.

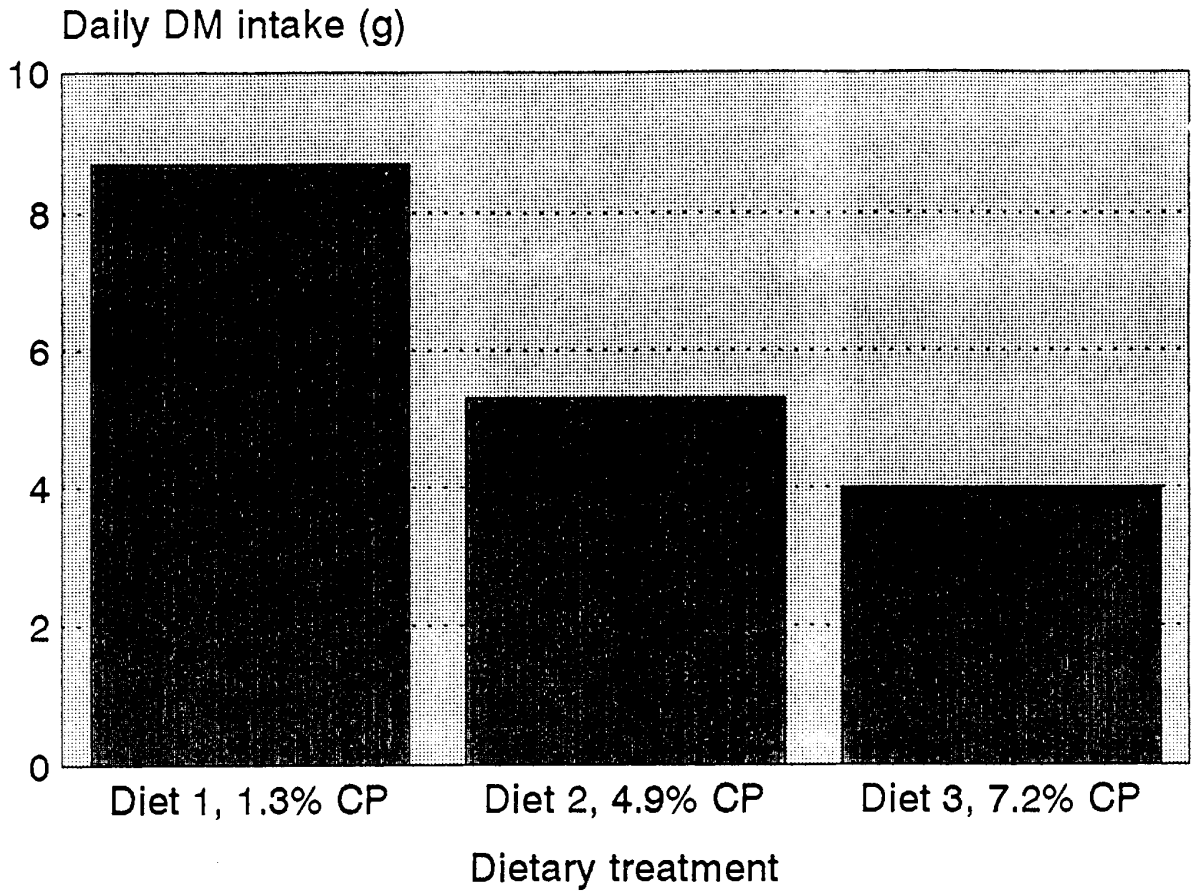


Figure 3. Mean daily ME intake of adult male fruit bats.

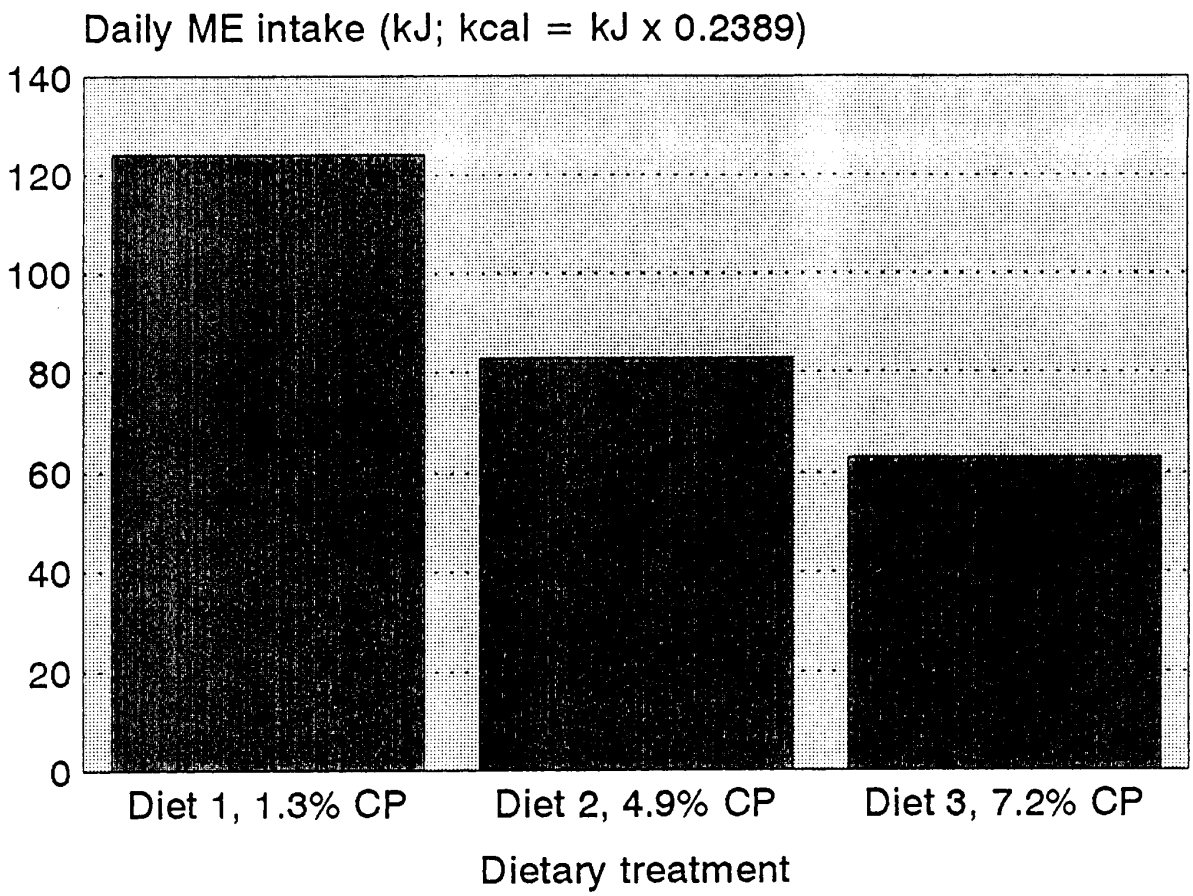
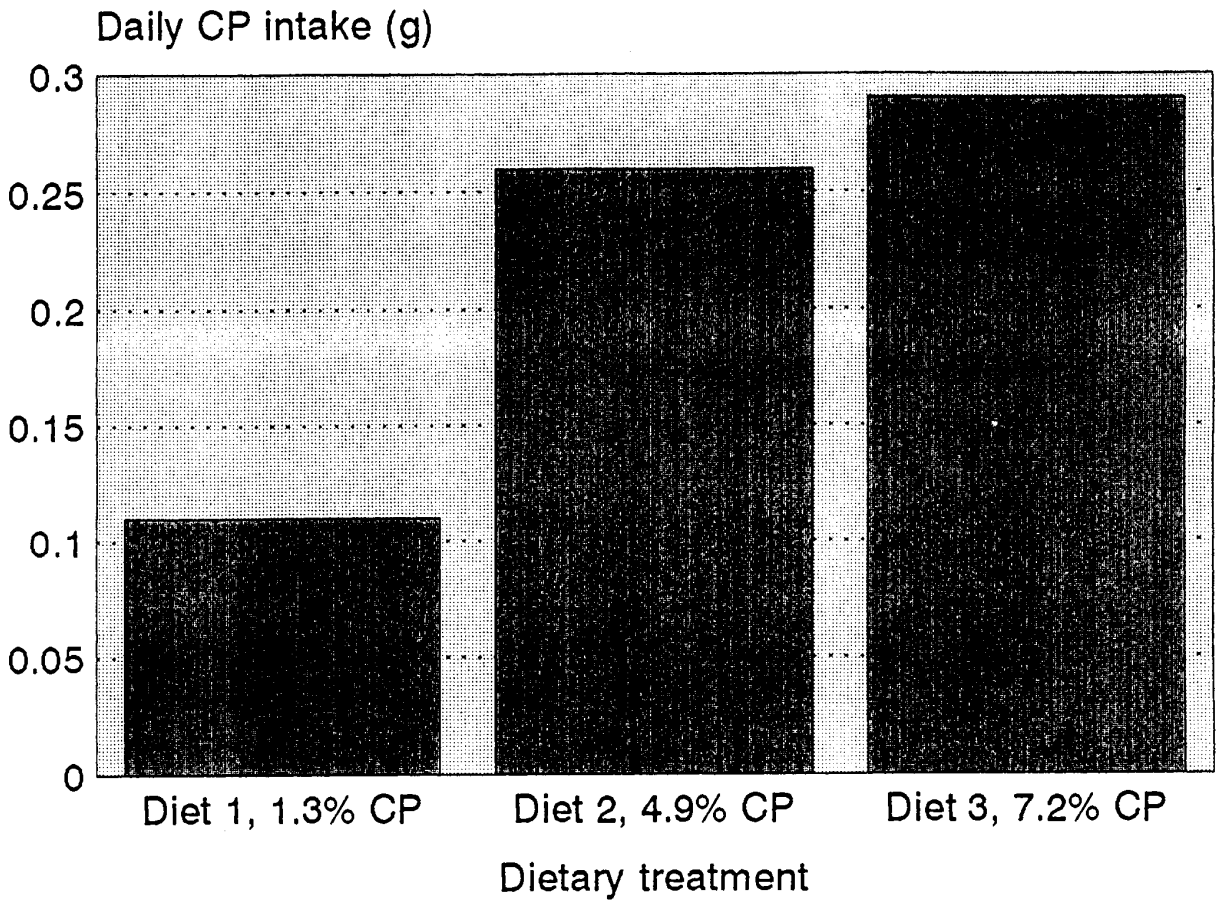


Figure 4. Mean daily CP intake of adult male fruit bats.



CARDIAC ULTRASOUND IN A HARBOR SEAL (*Phoca vitulina*) WITH CARDIAC FAILURE

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A 15 year old female harbor seal was examined at the Veterinary Medical Teaching Hospital, University of California, Davis in August 1993 for severe depression and inappetence of 5 days duration. The seal had a history of periodic episodes of anorexia for several years. Because of marked weakness and debilitation, all examinations were able to be performed without sedation. Physical examination failed to reveal auscultable evidence of cardiac disease. Diagnostic evaluation included a CBC and blood chemistry panel, thoracic and abdominal radiographs, an ECG, and abdominal and cardiac ultrasound examinations.

The CBC, chemistry panel, abdominal radiographs, and ECG were not diagnostic. Thoracic radiographs showed moderate cardiomegaly with bilateral atrial enlargement and possible enlargement of the pulmonary arteries. Abdominal radiographs were non-diagnostic. Abdominal ultrasound examination showed hepatic vein dilation and caudal vena caval dilation with swirling spontaneous contrast, indicative of sluggish flow. Echocardiography showed marked dilation of the left ventricle and atrium (LV diastolic diameter 7.8 cm, LA/Ao ratio 2.2), feeble mitral valve motion, markedly reduced LV systolic function (LV shortening fraction 13%), and marked spontaneous contrast formation in the left atrium and ventricle. Doppler echocardiography showed mild mitral valve regurgitation and physiologic pulmonary valve regurgitation. These findings indicated severe myocardial failure with signs of both left and right heart failure. The seal developed seizures and died approximately 8 hours after the ultrasound examinations. Necropsy examination showed meningoencephalitis, multifocal myocarditis (intralesional protozoa, most likely sarcosporidian, were identified in sections of brain and were assumed to be present in the heart) and hepatic and pulmonary evidence of venous congestion due to bilateral congestive heart failure.

The radiographic and ultrasound findings were characteristic of severe myocardial failure with markedly reduced stroke volume and clinical signs of low output failure. This case illustrates the application of 2D and Doppler echocardiography in cardiac evaluation and disease recognition in a marine mammal. Thoracic ultrasound has been used to help diagnose pulmonary problems in several marine species, and several species of marine mammals have been trained to tolerate diagnostic ultrasound examinations. Echocardiographic examination of normal animals during training, and the development of normal standards in these species, would greatly facilitate the examination of ill individuals and perhaps recognition of cardiac abnormalities at earlier stages when therapy could be attempted.

WINTER DYSENTERY: ROTAVIRUS AND CORONAVIRUS INFECTION IN HOOFSTOCK

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Introduction

Various agents have been incriminated in diarrhea outbreaks in ruminants in this country including bacteria such as *E. coli*, *Campylobacter* sp., *Salmonella* sp. and *Yersinia* sp.; viruses such as rotavirus, coronavirus, bovine virus diarrhea (BVD), infectious bovine rhinotracheitis (IBR), and others; protozoa such as *Eimeria* sp. and *Cryptosporidium*; and helminths. Rotavirus and coronavirus affect replicating intestinal epithelium causing absorption problems as well as fluid and electrolyte loss. Alone or in combination with other agents can cause significant morbidity and mortality particularly in young animals. Winter dysentery is a name given to a highly contagious outbreak of diarrhea seen in cattle usually during winter.[3] The diarrhea is profuse, foul-smelling, and often blood-tinged.[3] Originally *Camplyobacter jejuni* was proposed as the cause but it is now generally recognized to be associated with coronavirus.[3,8] This report describes a rotavirus diarrhea outbreak in a zoo as well as a coronavirus outbreak in another zoo where either virus was the main or only etiologic agent found.

Case report

In winter 1986-87 at a large midwestern zoo an outbreak of watery to semi-solid consistency, gray to brown color diarrhea occurred in two stalls housing three bongo (*Tragelaphus eurycerus*) indoors. Other bongo as well as yellow-backed duiker (*Cephalophus sylvicultor*) were housed on a lower level of a two floor indoor-holding facility. On the upper holding level giant eland (*Taurotragus derbianus*) as well as Grant's Zebra (*Equus burchelli*) were housed also. Very limited outdoor access in separate exhibits was permitted during this time of year. Immediately rigorous controls such as cleaning affected animals stalls last, footbaths outside each stall and facility entrance, as well as separate cleaning utensils and feeding dishes were applied. No other animals clinically showed diarrhea mainly due to these procedures.

No animals were immobilized for serology or other blood work but fecal samples were taken for parasite detection (direct, flotation) and bacterial cultures were negative for *Salmonella, Yersinia* and *Campylobacter*. Advice from an area veterinary school suggested treating for

Campylobacter sp. so a lincomycin-spectinomycin powder (LS-50 Water Soluble Powder, Upjohn) was added to the drinking water for seven days. An electolyte replacement oral solution (Eltradd, Haver) was also offered during this time. Both solutions were consumed in good quantity by the affected bongo.

Over a five to seven day period the feces became normal. Appetite ranged from fair to good during this time, and no significant weight loss occurred. No other symptoms such as coughing, eye lesions, or oral ulcers appeared in the affected bongo.

During the winter of 1987-1988 several stalls of bongo were again affected by a diarrhea outbreak similar to the previous year. Rigorous isolation and cleaning procedures were again instituted, and no other animals were affected. Again parasite identification and stool cultures revealed no bacterial pathogens, protozoa, or helminths. Treatment of the diarrhea was essentially the same as the 1986-1987 outbreak. Resolution of the diarrhea again occurred in five to seven days. Fecal samples were submitted to National Veterinary Services Laboratory, Ames, Iowa for virus isolation and electron microscopy. Rotavirus only was identified by ELISA testing.

At the Montgomery Zoo ruminant species housed in the African hoofstock area include slender-horned gazelle (*Gazella dama*), gemsbok (*Oryx gazella*) and greater kudu (*Trageluphus strepsiceros*); separated by a water moat and housed in an adjacent barn are 1.2 reticulated giraffe (*Giraffa camelopardalis*). During the day animals share an eight acre exhibit with irrigated Bermuda grass substrate. At night animals are kept in a stalled barn with access outside in small chain link paddocks. When temperatures dip below 20 F all animals are locked inside the heated barn. During winter 1993-1994 temperatures were consistently low enough to necessitate frequent indoor-only housing.

In January 1994 an adult, three year old female gemsbok exhibited clinical signs including partial anorexia, depression, and watery diarrhea. Stool culture was negative for *Salmonella, Yersinia,* and *Campylobacter. Cryptosporidium* check was negative. Fecal flotation revealed *Trichuris* eggs in low numbers. Virology was negative for rotavirus by ELISA but numerous corona-like viral particles were seen on electron microscopy.

Treatment included ivermectin (Ivomec, MSD Agvet) electolyte oral solution (Biolyte, Upjohn) as well as lincomycin-spectinomycin in drinking water. Consumption of oral solutions was poor due to habituation to the automatic waterer. Nevertheless, fecal consistency was significantly improved by day three and normal by day five. Food consumption followed the same pattern with first only hay being eaten followed by increased pelleted diet (Herbivore Diet, Mazuri, Purina Mills) consumption.

Within ten days of the onset of diarrhea in the gemsbok both bongo also started to have gray, loose stool. Within 24 hours the diarrhea had progressed to abundant frank blood mixed with a green-water component. Fecal direct and flotation tests revealed a background level of strongyles thought to be insignificant due to regular treatment. Stool cultures were

negative for bacterial pathogens. Several *Cryptosporidium* checks were negative. Virology was positive for coronavirus-like particles seen on electron microscopy (E.M.). ELISA testing for rotavirus was negative. Treatment included ivermectin, flunixin (1.1 mg/kg IM q24h X 5d), (Banamine, Schering-Plough) oral electolyte (Biolyte, Upjohn) solution, lincomycin-spectinomycin oral powder in drinking water, and ceftiofur (4.4 mg/kg IM q24h X 5d) (Naxcel, Upjohn). Consumption of oral solutions was poor as with the gemsbok. But by day 3 most of the blood had disappeared and by day 7 stools were normal.

The only other affected animals in the African area were Dama gazelle. These animals showed semi-solid stools for 4-5 days as the only clinical signs. Treatment was the same as for bongo. Slender-horned gazelle, greater kudu, and the Grant's zebra were unaffected. Giraffe in the adjacent barn and exhibit were normal also. Feces from dama gazelle was ELISA negative for rotavirus but positive for corona-like virus on E.M.

At day 3 of the bongo outbreak diarrhea started in the Asian hoofstock section with the banteng (*Bos javanicus*). Of the banteng only the mature female was affected by profuse, bloody diarrhea. The mature bull demonstrated moderate coughing during this time but had normal stool. The six month old young was clinically normal. Treatment for the mature female consisted of long-acting tetracycline (20 mg/kg q72h IM). (LA-200, Pfizer Co.), flunixin (1.1mg/kg im q24h X 3d), and oral electolyte solution. Fecal parasite checks were negative, and stool culture was negative for pathogens. Viral testing was not done due to loss of specimens in transport.

Several days after the diarrhea outbreak in banteng, loose stool with small amounts of blood present began in the adjacent stall where axis deer (*Cervus axis*) were held. Feces were submitted only for virology which was negative for rotavirus but positive for coronavirus-like particles. No treatment was done and all axis deer were normal in three to five days. A similar pattern was seen in the blackbuck with several mature animals affected. A young blackbuck less than two months old had normal stool while the dam had loose, bloody diarrhea. Again no treatment was done in this group, and all animals were normal by day five from onset. Other unaffected bovids in the Asian area included nilgai (*Boselaphus tragocamelus*). Unaffected cervids included sika deer (*Cervus nippon taiwanus*), Eld's deer (*Cervus eldis*), and Reeve's muntjac (*Muntiacus reevesi*).

Survey of unaffected ruminant species included giraffe and a number of ruminant species in an old zoo section. These included goats (*Capra hircus*), llama (*Llama glama*), dwarf zebu (*Bos primigenus indicus*), four-horned sheep (*Ovis aries*), fallow deer (*Cervus mesopotamicus*), and white tailed deer (*Odocoileus virginianus*). All species surveyed were negative ELISA for rotavirus and demonstrated corona-like viral particles on E.M.

Control measures included administration of an oral rota-coronavirus modified live vaccine (Calfguard, Smith-Kline Beecham) in the face of the outbreak to all unaffected bovid animals. These included slender-horned gazelle, greater kudu, blackbuck, nilgai, and American bison (*Bison bison*). No adverse reactions were seen in any vaccinated animals. Since modified-live virus was given to these animals no E.M. was attempted for virology on

their feces. Other measures included extensive disinfection of stalls and equipment as well as footbaths and separate boots for each hoofstock barn. However, necessary keeper rotation and coverage between these areas could not be restricted.

Approximately one month after the main outbreak a male pronghorn antelope demonstrated loose stools but no blood for three days. Feces were negative for rotavirus by ELISA but demonstrated corona-like particles by E.M. The only other possible positive samples for coronavirus seen at the Montgomery Zoo came from several slender-horned gazelles with a three day duration of loose stools. Although previously vaccinated the samples were negative by ELISA for rotavirus and positive for corona-like virus by E.M. This occurred several months after vaccination.

Discussion

Rotavirus infections have been reported in a variety of domestic animals, wild animals, birds, and man. It has been reported in a zoo nursery situation.[1] Diarrhea is most severe in young cattle less than three weeks of age, but adult cattle can be affected as well.[5] Cross species infection has occurred experimentally between species.[5] Diarrhea is not regarded as severe as with coronavirus infection in young calves.[6] This was certainly true in the comparison of diarrhea in bongos with each respective virus. Other factors such as stress, secondary infections, and antibody status may contribute to mortality.[5,6] Diagnosis of rotavirus can be accomplished by electron microscopy, fluorescent antibody technique, or ELISA. Of these, ELISA may be the most practical as well as accurate for the group A antigens which form most bovine isolates.[5]

Coronavirus has also been reported in a variety of animals but isolates are thought to be host specific. It has been postulated that bovine coronavirus can affect or exist in all ruminating animals which was demonstrated in most families from this report.[2] Diagnosis of coronavirus can be made by fluorescent antibody technique or electron microscopy.[6] As with rotavirus, mortality may occur due to rapid dehydration or be enhanced by stress and secondary infections. Although no mortality occurred in this outbreak, extremely cold weather was linked to mortalities as another zoo during a coronavirus outbreak.[7]

There appear to be certain conflicts in descriptions of coronavirus infection in literature. One current textbook describes the infection as occurring most severely in calves and mentions dysentery as not a characteristic of the infection.[4] Another textbook mentions outbreaks almost solely in mature animals and with dysentery as a prominent feature. The latter condition prevailed in our experience and may relate to virulence between strains of the virus. Mild respiratory disease is mentioned as a clinical feature but was not observed in any species except the wild bovine-type, the banteng.[6]

Control of the rotavirus and coronavirus infection is often difficult.[4,5] The efficacy of the oral modified-live vaccine has been questioned and is not recommended by many authorities.[4] Nevertheless, studies in Europe document success with the vaccine, and one

zoo in North America appears to have had success as well as safety in controlling neonatal diarrhea with this product.[4]

Vaccination in the face of this outbreak seemed to be ineffective at least in the case of the blackbuck. Since this outbreak seemed to be the "mature animal" version versus the "neonatal" version perhaps vaccine failure could be explained in terms of variation in vaccine production, variation in field strain virulence, or failure of vaccine to confer protection with some field strains.[4] A more recent killed vaccine (Scourguard 3, Norden Labs) for rotavirus, coronavirus, and entertoxigenic *E. coli* may yield better results but strict guidelines for booster timing may be difficult in many open, mixed zoological exhibits.[4]

Rotavirus and coronavirus are distributed worldwide and inapparent carriers at least for coronavirus exist.[6] Both are highly contagious by orofecal transmission especially when sick animals are not separated in a timely manner.[5,6] Feed, bedding, equipment, and people contaminated with virus spread the disease rapidly from one area to another.[5,6] Restricted stalls and mixing of species as well as restricted keeper movement between barns probably accounted for limited spread in the rotavirus outbreak versus the widespread coronavirus outbreak in the two zoos in this case report.

Treatment consists of controlling dehydration and secondary infections.[5] Oral electolyte replacement or intravenous fluid therapy is especially important in young animals.[5,6] Antibiotic therapy may be indicated in the face of specific culture results or dysentery which indicates significant intestinal mucosal damage. Systemic antibiotic therapy was undertaken in the coronavirus outbreak with bongo and banteng due to their value and severe dysentery. Mild or moderate cases may need no treatment as illustrated by axis deer, pronghorn, and blackbuck.[5]

As a final note *Campylobacter jejuni* is mentioned in texts as etiology for winter dysentery but was not found in multiple cultures at either zoo in many species. Due to various management considerations no immobilizations were conducted during the outbreaks to survey for other causes of viral diarrhea such as IBR or BVD. At least at the Montgomery Zoo, however, no IBR, BVD, or other diarrhea-associated viruses have been detected during routine necropsies, clinical cases, or opportunistic serology of various species.

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SEROLOGIC EVALUATION OF FREE-RANGING LIONS (*Panthera leo*), LEOPARDS (*Panthera pardus*) AND CHEETAHS (*Acinonyx jubatus*) FOR FELINE LENTIVIRUS AND FELINE LEUKEMIA VIRUS IN BOTSWANA

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Large felids have both negative and positive impacts on the economy of the southern African Republic of Botswana. Conflicts between livestock and wildlife, especially carnivores, are ever intensifying. At the same time, safari hunting and photographic tourism are major industries, with charismatic predators such as lions (*Panthera leo*), leopards (*Panthera pardus*) and cheetahs (*Acinonyx jubatus*) playing assorted important roles. The Botswana Department of Wildlife and National Parks, recognizing the economic importance and aesthetic value of these large cats, has thus designated them as priority species for the establishment of disease data bases.

Large felid populations in southern Africa have demonstrated varying degrees of lentivirus exposure, with some populations thus far showing no such evidence. Botswana lions, leopards and cheetahs were evaluated for evidence of exposure to feline lentivirus (n=53) and feline leukemia virus (n=52) using samples primarily collected via country-wide distribution of kits to safari hunters and Department of Wildlife and National Parks field officers involved in problem predator management. All sampling was opportunistic; no cats were captured, anesthetized or killed for the purposes of this project. Five different assays for antibodies to lentivirus (Table 1) were utilized on most samples (cougar lentivirus indirect immunofluorescence assay; cougar lentivirus western immunoblot; Petaluma domestic cat isolate-based enzyme-linked immunosorbent assay; Petaluma domestic cat isolate-based immunofluorescence assay; Petaluma domestic cat isolate-based western immunoblot), with one test being used for detection of feline leukemia virus antigen (domestic cat isolate-based enzyme-linked immunosorbent assay).

Almost all of the cats in this study appeared to be normal, free-ranging animals. None of the cats tested demonstrated evidence of feline leukemia infection. Significant evidence of lentivirus exposure, which was defined as a positive result on at least the cougar lentivirus western immunoblot, was found in cats of all three species: eight of 31 sampled lions (25.8%), three of 18 leopards (16.7%) and one of four cheetahs (25%) demonstrated evidence of exposure to a feline lentivirus. In domestic cats FIV seropositivity is strongly correlated with FIV infection. Exposed cats were found in geographically diverse parts of the country (Figure 1).

While this study is by no means a comprehensive evaluation of the lion, leopard and cheetah populations of Botswana, it does reveal evidence of large felid lentivirus exposure in a portion of sub-Saharan Africa previously unexamined. By providing information on lentivirus and feline leukemia virus exposure in at least a small sample of Botswana's lions, leopards and cheetahs, the findings outlined here will hopefully help scientists and managers in southern Africa to make more informed decisions when it comes to the movement of large cats locally or internationally for research, management or commercial purposes.

Table 1. Lentivirus status of all 53 lion, leopard, and cheetah samples screened using the combination of assays described in the text. Animals deemed positive, defined as having a positive result on at least the CLV WB, are grouped together within each species for ease of comparison.

ID#	CLV IFA	CLV WB	Pet FIV ELISA	Pet FIV IFA	Pet FIV WB	Age[a]	Sex
LIONS:							
15	+w	+	+2xs	-	-	5	M
25	-2xs	+2xs	+2xs	-2xs	+w	8	M
44	-2xs	+2xs	+2xs	+2xs	+w	8	M
57	-2xs	+2xs	+2xs	-2xs	NE	9	M
61	-2xs	+2xs	-2xs	-2xs	-2xs	Adult	M
135	+2xs	+2xs	+2xs	-2xs	NE	7	M
281	+2xs	+2xs	-2xs	-2xs	+2xs	9	M
301	-	+w	+	-	-	7	M
302	-	-	+2xs	-	-	8	M
1	-	-	-	-	NE	Adult	F
2	-	-	-	-	-	Adult	F
12	-	-	-	-	-	5	M
14	-	-	-	-	NE	4	M
36	-	-	-	-	-	8	M
39	-	-	-	-	-	Adult	M
152	-	-	-	-	-	Adult	M
154	-	-	-	-	-	Adult	M
227	-	-	-	-	-	1	M
237	-	-	-	-	-	<1	F
273	-	-	-	-	-	3	F
275	-	-	-	-	-	3	F
285	-	-	-	-	NE	9	M
287	-	-	-	-	NE	14	M
295	-	-	-	-	NE	Adult	F
340	-	-	-	-	-	3	M
341	-	-	-	-	-	3	M
353	-	-	-	-	-	<1	F
355	-	-	-	-	NE	4	M
360	-	-	-	-	NE	4	M
364	-	-	-	-	-	3	F
381	-	-	-	-	NE	Adult	M

Table 1. (continued)

ID#	CLV IFA	CLV WB	Pet FIV ELISA	Pet FIV IFA	Pet FIV WB	Age[a]	Sex
LEOPARDS:							
1	-	+	-	-	I	4	M
2	-	+	+	-	+	5	M
372	-	+2xs	-2xs	-2xs	-2xs	Adult	M
3	-	-	-	-	-	1	F
4	-	-	-	-	NE	3	M
11	-	-	-	-	-	Adult	M
13	-	-	-	-	-	6	M
35	-	-	-	-	-	Adult	M
41	-	-	-	-	-	8	M
42	-	-	-	-	-	10	M
43	-	-	-	-	NE	7	M
294	-	-	-	-	NE	1	M
310	-	-	-	-	NE	Adult	M
322	-	-	-	-	NE	<2	M
323	-	-	-	-	NE	6	M
326	-	-	-	-	-	5	M
407	-	-	-	-	NE	<2	F
480	-	-	-	-	NE	2	F
CHEETAHS:							
2	-	+	-	-	-	3	M
1	-	-	-	-	-	7	F
219	-	-	-	-	NE	Adult	M
315	-	-	-	-	NE	Adult	M
TOTALS							
53 CATS	3	12	8	1	4		41M/12F

[a]Ages in years, if provided, are very approximate based on assessments made in the field by safari hunters or wildlife officers with a diverse range of experience. Adult = fully grown animal but numerical age estimate not provided.

2xs = test repeated twice. +w = weak positive result. NE = not evaluated.

I = indeterminate.

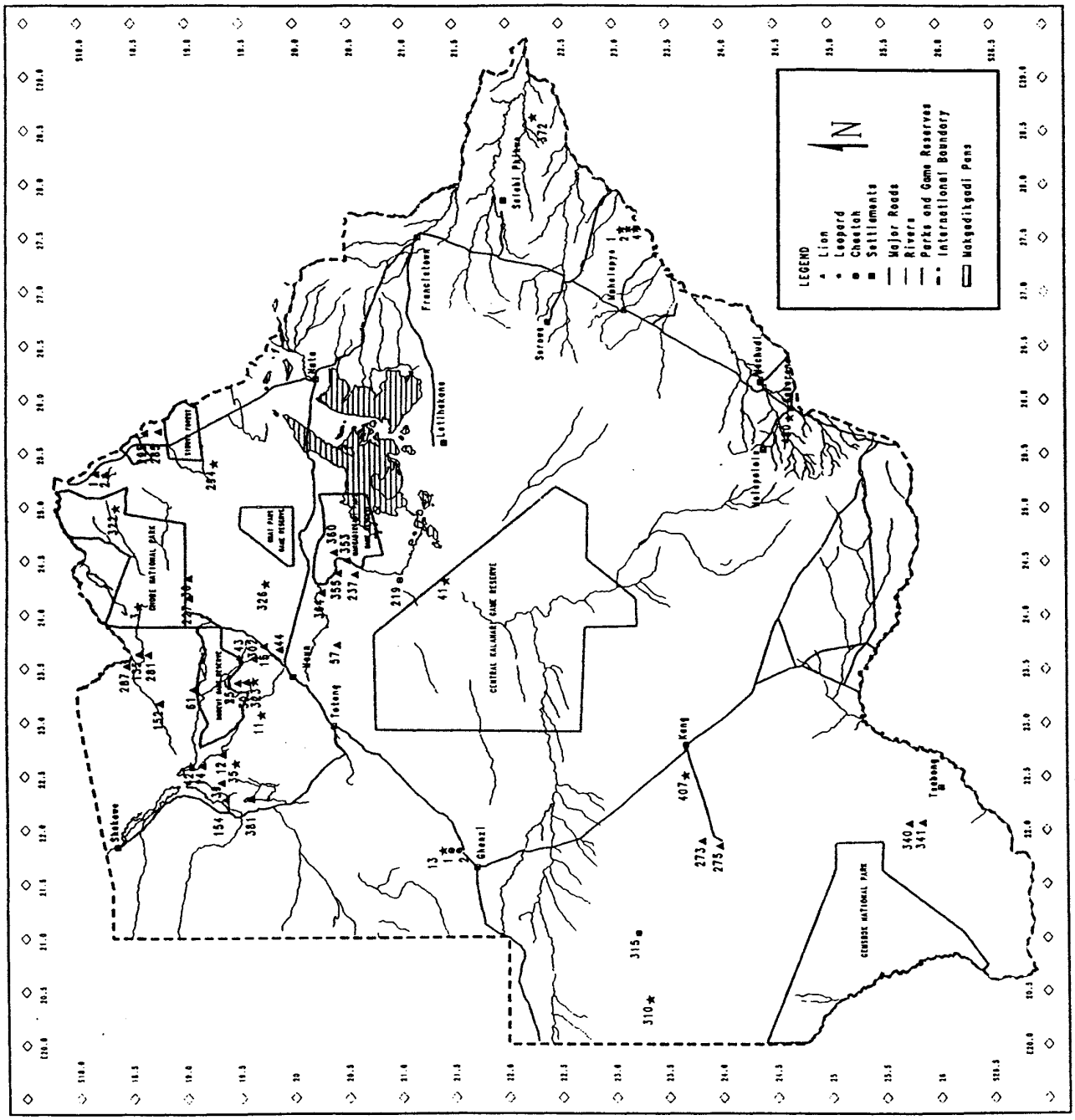


Figure 1. Geographic distribution of 53 Botswana lions, leopards, and cheetahs at the time they were sampled. Note legend in lower right hand corner. Scale: 1 cm = ~50 km. Cat locations are within an approximately 50 km radius of identified points.

INTRODUCTION OF TWO WILD MALE WHITE RHINOCEROS (*Ceratotherium simum*) TO FACILITATE BOMA ADAPTATION OF AN INJURED SUBADULT

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A rhino rescue operation in Botswana led to the capture and translocation of four white rhinoceros (*Ceratotherium simum*). At the time of capture, an approximately three- to four-year-old bull was found to have three bullet wounds estimated to be approximately seven to 10 days old. Internal injuries that may have resulted from the wounds could not be adequately assessed. All four animals, including the injured bull, were judged to be in good body condition at the time of capture and translocation to holding bomas. The subadult bull refused to eat any significant quantity of food offered, even after 10 days in captivity. It appeared to be seeking social interaction--vocalizing and rubbing against the fence between itself and the adult bull. Given the history, age and general behavior of this subadult bull, it was felt that the problem observed was most likely not the "classic" confinement-related inappetence of the white rhinoceros. The decision was made to not give the young bull access to a larger paddock, as it was felt this would only serve to further isolate it from the other animals. In addition, the rearrangements necessary to achieve this might have disrupted the otherwise smooth boma adaptation process in the other three animals. Thus, paddocks were kept closed off.

Post-capture inappetence is a potentially life-threatening problem for free-ranging white rhinos put into bomas, especially older bulls, with animals often not feeding for the first seven to 10 days (or longer) post-capture. Ideally, bomas should be situated so that anorectic animals can be released back into the field if no evidence of feeding is seen within this time period. Such animals often start grazing almost immediately after they are out of the boma. In the case of this project, security concerns precluded building holding facilities in actual rhino habitat in northern Botswana. In addition, the private sanctuary established to protect the rhinos was still in the process of trying to raise funds for a perimeter fence, so the only option for "release" from a boma would be to allow the animal into a slightly larger paddock.

The gate between the two bulls' bomas was opened 12 days after the young bull's capture. This was felt to be a novel but potentially dangerous approach to the inappetence problem. Normally, wild-caught white rhino believed to come from different social groups are not penned together, especially if they are of the same sex and of disparate sizes. The young bull was approximately 70 percent the size of the adult bull. No aggression was manifested

as the young bull crossed over into the adult bull's boma. The young bull's overall attitude improved, it charged the fence much less, and it followed the old bull around constantly. The two animals were frequently in bodily contact. The young animal watched the old bull eating, and then it too started to eat. The adult bull tolerated the young bull relatively well, only occasionally gently horning it in the axillary region.

Although short-term improvement was seen, the young rhino died six days after the introduction to the adult bull, despite ongoing veterinary intervention for suspected wound-related sepsis and secondary anorexia. Necropsy revealed that a bullet that had entered the right shoulder had gone through the thoracic and abdominal cavities, leading to diffuse pleuritis and peritonitis, which was confirmed histopathologically. Examination of the bullet recovered from the abdomen revealed that it was of a 7.62 mm caliber, fired from an AK-47 or an SKS semiautomatic weapon, according to the Botswana Department of Wildlife and National Parks Anti-poaching Unit.

The fact that this animal died from bullet wound-related injuries should not detract from the positive behavioral responses the companionship of the adult bull seems to have elicited. The authors were unable to find any similar cases of such an intentional introduction of two captive white rhino bulls documented in the literature.

ACKNOWLEDGMENTS

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TWO SUCCESSIVE CESAREAN DELIVERIES IN AN ELDERLY PRIMIGRAVID LOWLAND GORILLA *(Gorilla gorilla)* AND NEONATAL CARE OF THE OFFSPRING

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Introduction

In April, 1991, "Betsy", a 34 year old Lowland Gorilla at Melbourne Zoo, became pregnant for the first time. This unusual situation arose because she had not had access to a fertile, willing male until May 1990, when the sire, a newly imported seven year old male, was introduced to her group. For the previous 27 years she had lived with an infertile male. During that period, introductions and then artificial insemination were attempted using a fertile male, but both were unsuccessful.

First Pregnancy

As Betsy was an elderly primigravida, it was predicted that there was a reasonable probability of labor complications. Using the range of gestation periods recorded for gorillas (241 - 289 d[6]), the earliest expected parturition date was calculated and a 24 hour watch commenced seven days prior to that date.

Labor

Labor commenced at 18.50, December 30, 1991 (gestation = 264 d). It appeared to start normally with irregular contractions which gradually became more frequent and regular, reaching peak frequency (every 2-5 minutes) after five hours. Although most gorilla labors recorded have lasted less than three hours, [1,2,3,5,10] we were aware of at least two prolonged cases (9.5 and 18 hours [5,11]) which had resulted in successful, unassisted delivery of live infants. After 18 hours of established labor, Betsy still had frequent, regular contractions but no progress had been made. By 23 hours, she appeared to have entered a period of secondary inertia as the contractions became less frequent and regular. Anesthesia and examination were elected.

Anesthesia

After 26 hours of labor, Betsy was darted with 1200 mg ketamine (10 mg/kg), but required two further doses each of 500 mg IM ketamine at 14 and 33 minutes before she could be approached safely. She was transported to the surgery, masked with nitrous oxide and oxygen (4:2) and 5% isoflurane for two minutes, then intubated (10.0 mm cuffed tube) and maintained, with assisted manual ventilation, using the same gas mixture and 1.5% isoflurane. Respiratory rate (12-20 bpm) and pulse rate and strength (60-80 bpm) remained normal throughout the procedure. An intravenous catheter was placed for the delivery of warmed Hartmans solution (total : 2.2 L).

Surgery

On vaginal examination it was found that the cervix was only 2 cm dilated and not yet completely effaced. Cesarean section was performed through the lower segment of the uterus via a transverse suprapubic incision through well defined anatomical layers. It was known that in previously recorded cases of cesarean section and hysterectomy in gorillas [2,8], difficulty had been encountered in exposing the lower segment of the uterus because the bladder is adhered to its ventral wall by the utero-vesical peritoneum. However, in this case, a narrow (3 mm wide) strip of peritoneum was found to be non-adherent to the uterine muscle at a point just cranial to the bladder. This was incised, giving good visibility of, and access to, the lower segment. A transverse incision was made in the uterus and a live male fetus delivered in intact membranes. The fetus was bathed in meconium and very little normal foetal fluids; it had been in occipito-posterior position (ie. ventroventral with respect to the mother instead of the normal position of dorsoventral). Oxytocin (10 U) was given IV at the time of cord clamping to assist delivery of the placenta. After it was removed, the uterus was exteriorized and closed in two layers with 1 Vicryl (polyglactin 910). The abdominal incision was repaired with 1 Vicryl throughout and the skin closed using continuous subcuticular 3/0 Vicryl. Immediately after delivery, fentanyl (100 mcg) (for intra and post-operative analgesia) and ceftazidime (2 g) were given IV. After closure the wound margins were infiltrated with 15 ml 0.5% bupivicaine. Anaesthetic recovery was uneventful. Post operatively, Betsy was given oral cephlexin for seven days (500 mg q. 6 hours). She did not manipulate her suture line and had resumed normal activity and appetite by six days postpartum.

Care of the neonate

The passing of meconium in utero is most commonly caused by significant hypoxia in the fetus during labor. It is a potentially dangerous situation because meconium is usually aspirated as the hypoxic neonate takes its first gasping breaths, resulting in severe lung disease.[9] Within the first minute of delivery the infant showed weak body movements, including respiratory efforts. Oxygen was administered immediately at 2 L/min via a 5 Fr.G. catheter passed down one nostril. Under direct vision with a neonatal laryngoscope vigorous

attempts were made to suction out as much meconium as possible from the mouth, pharynx, larynx and trachea in an attempt to prevent its aspiration. However, as respiration became established, it was clearly distressed, indicating significant inhalation of meconium. Respiratory rate was rapid (50-80 bpm) with marked retraction of suprasternal, intercostal, and subcostal soft tissues, indicating very non-compliant lungs. A chest wall deformity, consisting of marked flaring of the lower ribs and forward protrusion of the lower sternum, was present with the first breaths, and rapidly worsened. It was so gross that it was unclear in the first few days whether it was a congenital malformation, or secondary to the lung disease, but as the lung disease resolved, so did the deformity. By five minutes of age, the infant was active with a heart rate of 144-168 bpm (high compared to healthy human neonate). There was a right ventricular "heave" on chest palpation and a very loud pulmonary second heart sound on auscultation, indicating probable pulmonary hypertension (a frequent concomitant of meconium aspiration in the human neonate). Oral mucous membranes were pink whilst receiving oxygen at 2 L/min but trial withdrawal of this resulted in immediate cyanosis. Body weight was 2.35kg (within "normal" weight range for gorilla neonates) but he appeared very scrawny with little subcutaneous tissue, suggesting weight loss from poor nutrition ("placental insufficiency") in the week or two before delivery. The infant was cleaned, dried and placed in a humidicrib. Feeding with Wyeth Low Birthweight formula for human infants commenced at five hours of age with a good suckling response.

Supplemental oxygen (delivered directly into the humidicrib as the cannula was poorly tolerated) continued to be required until 59 hours of age. Blood gases and pulse oximetry performed a short time after cessation of oxygen were normal, confirming that lung function was adequate. At seven hours of age, twitching eyelid movements were observed; three hours later there were frank multi-focal clonic seizures. The cause was found to be hypoglycemia (blood glucose : 27 mg/dL), probably as a result of the intra-uterine growth retardation and intra-partum hypoxia (both predisposing factors for neonatal hypoglycaemia in humans). Initially 10 ml 10% dextrose was given by orogastric tube, then an intravenous infusion of dextrose was established : 4 ml of 50% as a bolus, followed by 10% at 4 ml/hr. After three days, the rate of infusion was gradually reduced as blood glucose levels became stable and satisfactory (70-110 mg/dL); it was ceased on day 4. The infant's feeding response, although initially very strong, was very reduced by day 3 and it became necessary to feed entirely by orogastric tube until normal suckling resumed on day 8. This phenomenon is not uncommon in human infants with significant neonatal disease. From day 5-12, frank blood was found regularly in the faeces. Faecal culture, abdominal radiographs and vitamin K administration (0.5 mg IM) ruled out the involvement of enteric pathogens, neonatal necrotizing enterocolitis (seen in human neonates following perinatal hypoxia) and vitamin K deficiency. The cause was not determined but blood streaked-stools are not uncommon in formula-fed human babies. It resolved spontaneously. By day 9 the infant could maintain his body temperature at room temperature (26^0C) and was removed from the humidicrib.

Thereafter the infant had few problems. Reintroduction to Betsy was not attempted because by the time the infant's health had stabilized, she had no obvious mammary development. However, he had daily visual and tactile contact with her and all other

members of the group from day 5 until 8 months of age when he was introduced to Betsy. Over the next 12 months he was successively introduced to the remainder of the group.

Conclusion

The cause of Betsy's non-progressive labor is believed to be desultory incoordinate uterine activity probably as a result of her age, primigravidity and foetal malposition. Placental insufficiency in humans occurs in cases of maternal hypertension or other disease and in elderly primigravidae.[4] In this case, the latter was believed to be the cause. The intra-partum foetal hypoxia was most probably caused by the prolonged anaesthetic induction; the placental dysfunction may have also contributed.

Second Pregnancy

Betsy was diagnosed with her second pregnancy in October 1993. The decision was made in advance to perform another cesarean delivery because, given her age, there was a good chance that placental insufficiency would occur again and, as this not uncommonly causes neonatal asphyxia, there would be considerable risk if natural delivery occurred. Furthermore, it was considered most probable that she would have another incoordinate labor, given her age and the possibility that this fetus may also be in occipito-posterior position (over 50% of the cases of this in women are repeatable due to an abnormally shaped pelvis [4]). As the date of conception was not accurately known, it was not possible to predetermine a date for elective cesarean. Therefore it was planned to perform the delivery as soon as possible after labor commenced. A 24 hour watch commenced seven days prior to the estimated "due" date, calculated using the estimated conception date and a 264 d gestation.

Labor, Anesthesia and Surgery

Labor commenced at 17.45, April 23, 1994 (gestation = approx. 261 d). Peak frequency of contractions (regular, every 3-5 minutes) was reached after 1.75 hours, and continued until anaesthetic induction at 21.00. She was darted with 150 mg zolazepam and 150 mg tiletamine (Zoletil) (2.5 mg/kg total dose) and could be approached after 17 minutes. She was masked and intubated (10.5 mm tube) as on the first occasion but was additionally given alcuronium (10 mg IV) to permit control of ventilation and to effect better muscle relaxation to facilitate the surgery. She was hand-ventilated at 12 bpm throughout the procedure using nitrous oxide and oxygen (4:2) and 0.5% isoflurane. An intravenous infusion of Hartmans was given throughout the procedure (2.5 L). Heart rate (110-130), blood pressure (130/80 - 170/100) and SpO2 (90-98%) were monitored using a NIBP pulse oximeter. As good application of the finger probe was difficult, the lower SpO2 readings are questionable. Vaginal examination once again found an uneffaced, 2 cm dilated cervix. Cesarean delivery was performed using the same approach as previously. The wall of the lower uterine segment was very thin and tore on delivery causing troublesome hemorrhage, particularly at the right angle of the uterus. A live female fetus in normal position and normal fetal

fluid was delivered in intact membranes. Ergometrine (0.25 mg), fentanyl (100 mcg) and ceftazidime (2 g) were given IV immediately after delivery. After delivery of the placenta and wound closure, neostigmine (5 mg), atropine (1.2 mg) and a further 50 mcg fentanyl were given IV, and the wound margins were infiltrated with 0.5% bupivicaine. Spontaneous breathing commenced six minutes after the administration of neostigmine. Anaesthetic recovery was uneventful. Post-operative antibiotics were given as on the previous occasion.

Care of the neonate

At delivery the infant had poor muscle tone and no spontaneous movements except infrequent gasps, however the heart rate was > 100 bpm and her gasping respirations improved her color. During the first minute the mouth and pharynx were cleared of secretions by suction, oxygen was administered by face mask and she was dried to reduce evaporative heat loss. After one minute, respiratory effort ceased and heart rate fell. Artificial ventilation was commenced with a Laerdarl neonatal resuscitation bag and face mask; by four minutes she was still not breathing so was intubated with a 3.5 mm uncuffed e.t. tube and hand ventilated. By eight minutes she was breathing regularly, moving her limbs and gagging on the tube which was removed two minutes later. She then continued to breathe well with good color and heart rate. Body weight was 2.52 kg and there was no evidence of growth retardation as seen in the previous infant. She had become hypothermic (33^0C) during resuscitation and was placed in a humidicrib. At forty minutes of age the infant was placed on the anaesthetized Betsy's breasts and suckled quite well for 15 minutes. The poor condition at birth was most probably due to a combination of hypoxia during the long anaesthetic induction, and placental transfer of anaesthetic agents. Her rapid recovery suggests that anaesthetic agents were the main cause as recovery from hypoxia is usually slower. Because of her possible intra-partum hypoxia (which can cause subsequent hypoglycemia), blood glucose was measured at two hours of age : it was 18-36 mg/dL. Because this is low by human standards and within the range which caused convulsions in the previous infant, an intravenous infusion was commenced of 10% dextrose at 6 ml/min and formula feeding commenced a short while later. By 24 hours of age blood glucose levels had stabilized (85 mg/dL) and the rate of infusion was gradually decreased and then ceased at 35 hours. By 52 hours the infant could maintain body temperature at room temperature (24^0C) and was therefore introduced to Betsy nine hours later. Betsy was an inexperienced mother and had never observed natural rearing by a gorilla. For the first six days she displayed only a brief period of interest in the infant, eventually putting her down and leaving her. As a result the infant was left with her for only 2-5 hours each day. On the seventh day of introductions (nine days post partum) she suddenly showed intense interest in the infant and displayed very good mothering behavior, with suckling commencing that day. Further separation has not been necessary and introductions to the remainder of the group commenced six days later.

Discussion

There are few reports in the literature of obstetrical problems in gorillas. Mallinson *et al.* (1973) reported a case of occipito-posterior position which was delivered spontaneously after

a long nine hour labor, and Fullerton *et al.* (1990) reported a case of uterine rupture in a grand multiparous female during her eighth labor. This case is unusual because of Betsy's advanced age at the time of her first pregnancy, and this is believed to be the major cause of the obstetrical problems encountered. The method of cesarian section used in this case is preferred in humans over the alternative, a vertical incision through the upper portion of the uterus, because with the former there is a lower risk of scar rupture in subsequent pregnancies, the healing of the scar is better and infection is reduced as the wound is extraperitioneal.[4] The additional advantages of the lower segment approach in the gorilla are that it requires only a low abdominal clip and therefore preserves hair for the infant to hold onto, and there is minimal self-interference with the wound because discomfort is minimal and the incision is not visible below the protruding abdomen. Betsy's uterus, evidently thinned by the procedures, is now at risk of rupture if she enters spontaneous labor again. Therefore, if she has a third pregnancy, an elective cesarian will be performed at term. Of the two anesthetic induction agents used in these procedures, ketamine will be preferred in the future as it is most probable that it was the zolazepam which was the major cause of the initial hypotonia, hypothermia and hence hypoglycemia in the second infant because benzodiazepines rapidly cross the placenta and have these effects on the fetus.[7]

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A GLOBAL PERSPECTIVE OF VETERINARY MEDICINE: CAPTURE, MEDICAL MANAGEMENT AND REINTRODUCTION OF WILD POPULATIONS

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The Natal Parks Board in South Africa is responsible for the management of over 100 reserves of varying sizes within the Province of Natal/KwaZulu. As part of the management of many of these reserves, some 4,000 head of wild game are captured annually. These range in size from the suni to the elephant. The bulk of these wild animals are caught using the boma technique where they need have no physical human contact. The remainder are captured by chemical means or with nets. The reasons for capturing these animals include population regulation, conservation of endangered species, problem animal removal, research, and financial gain for other conservation projects.

The post capture care of wild ungulates presents specific problems which are addressed on a species basis, such as the use of long-acting tranquilizers in stress-prone ones such as nyala and reedbuck, or the training of rhinoceros or Cape buffalo to accept captivity prior to shipment.

The ultimate aim is to provide healthy animals for reintroduction to the wild or for export into captive situations. But healthy animals released into a new environment can be challenged by diseases or predators. Multiple steps are taken to assure minimal possibilities of transferring disease and parasites, along with assuring the animal is in its best condition to afford resistance to new disease challenges in its new environment and be able to cope with the stresses of a new environment. This new environment may challenge its immune system, as there is invariably an altered nutritional base, variations in topography and predator prey relationships, all of which can effect the animals ability to survive.

Steps for assurance of a successful translocation include an expedient, effective method of handling the animal, a physical examination, an acclimation period, and proper introduction into its new environment.