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## **TOXOPLASMOSIS IN SCHEEPMAKER'S CROWNED PIGEONS (*Goura scheepmakeri sclaterii*): A HISTOPATHOLOGICAL EVALUATION OF THE LUNG LESIONS**

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### **Abstract**

This presentation will describe an acute outbreak of toxoplasmosis in crowned pigeons (*Goura scheepmakeri sclaterii*). The pneumonia is considered one of the key changes resulting in the acute mortality. In the discussion this hypothesis and its consequences are discussed.

### **Zusammenfassung**

Dieser Artikel beschreibt ein akuter Ausbruch von Toxoplasmose in rotbrust Krontauben (*Goura scheepmakeri sclaterii*). Die Pneumonie wird betrachtet als eine der Hauptveränderungen, die in akutem Tod resultieren. In der Diskussion wird diese Hypothese und ihre Konsequenzen besprochen.

### **Résumé**

Cette présentation décrit un cas aigu de toxoplasmose chez des goura couronné de Scheepmaker (*Goura scheepmakeri sclaterii*). La pneumonie est considérée comme étant l'un des facteurs résultant en mortalité subite. Dans la discussion, cette hypothèse ainsi que ses conséquences sont discutées.

**Key words:** *Goura scheepmakeri sclaterii*, crowned pigeon, toxoplasmosis, lungpathology

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### **Introduction**

*Toxoplasma gondii* has a worldwide distribution. Virtually all vertebrate species are susceptible to infection (3). Domestic and exotic felids are the only known definitive hosts of this parasite and as such are the only animals capable of completing the enteroepithelial life cycle of the parasite and shedding oocysts in their faeces.

Toxoplasmosis has been described in crowned pigeons earlier in different zoos: Philadelphia (7) Antwerp (4, 8) and Rotterdam (6).

The diagnosis is normally made at necropsy by demonstrating the *Toxoplasma*-cysts and-pseudocysts by light microscopy. In the past the diagnosis was confirmed by transmission to mice and pigeons (6, 8). Nowadays, immunohistochemical staining is more common (3). For research very specific and sensitive PCRs have been developed.

In acute cases lesions in crowned pigeons can be seen in many organs, including necrotic lesions in liver, spleen, intestines, kidneys and lungs (3, 6, 8).

In this presentation an acute outbreak will be described and special attention will be paid to the acute changes in the lungs. These changes may play a crucial role in the acute and high mortality.

## Case report

Unexpectedly four Scheepmaker's crowned pigeons (*Goura scheepmakeri sclaterii*) housed in the same enclosure died within 4 days. One morning the first bird (A) was found dead without preceding signs of illness. This female was aged 6 months. At the same time another 4 month old male (B) was found moribund. This male was born from the same parents but from a consecutive clutch. This bird was placed under a heat bulb after a blood sample was taken and treatment with dexamethason was started. It was found dead 3 hours later.

Four days later both parents (C, male and D, female) were found dead in the morning despite of a "preventive" treatment with enrofloxacin.

The birds were housed in a mixed exhibit with two yellow-tailed cockatoos (*Calyptorhynchus funereus funereus*), two blue-winged kookaburras (*Dacelo leachii*) and two wattled brush turkeys (*Aegopodius arfakianus*).

Three months earlier all the birds in the same building were treated with enrofloxacin because salmonella was isolated from a dead red-sided eclectes parrot (*Eclectes roratus polychloros*) and from a faecal sample from a conspecific.

## Blood sample

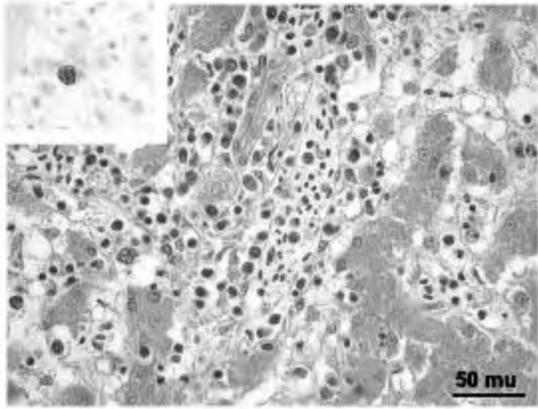
Test	Units	Value	ISIS ref. (n=1)
WBC	10 <sup>9</sup> /L	7,4	19,4
Hb	Mmol/L	5,70	7,82
PCV	%	25	42
Heterophils	%	81	37
Lymphocytes	%	18	56
Uric acid	Mmol/L	1,37	0,30
Ca	Mmol/L	2,39	
Cholesterol	Mmol/L	3,82	7,02
TP	G/L	34,6	
Alb	G/L	13,0	
AST	U/L	3105	39
T bill	μmol/L	<8,55	

## Post mortem

All birds were in good to excellent condition. Most birds still had food in the proventriculus.

The most prominent macroscopically recognisable alteration in all pigeons was seen in the lungs. All lungs showed dark red, marbled areas with oedema and a foamy content in the bronchi. Other areas of the lung were emphysematous. Changes of other organs were less consistent, but in general liver, spleen and kidney were degenerated (pale) and swollen.

*Toxoplasma*-like organisms were seen in the lungs, liver, spleen and intestines in impression smears stained by Hemacolor® (Merck, KgaA, Darmstadt Germany) (Fig. 1).

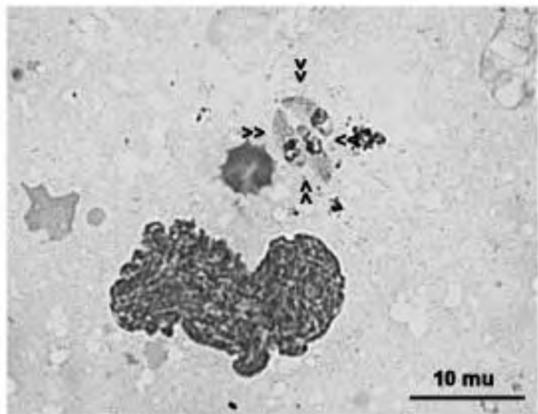


**Fig. 2:** liver with acute to subacute inflammations with necrosis. Insert: immunohistochemically positive organisms for antibodies against *Toxoplasma gondii*

### **Bacteriology**

Aerobic culturing from liver, lung, spleen and kidney was performed on Columbia agar supplemented with 5 % sheep blood (BioMerieux, 's-Hertogenbosch NL). *E.coli* was isolated from liver and lung (A), from spleen and lungs (B), from liver (C) and lung (D). From C in addition *Corynebacterium kitcheri* was grown from the lung.

No salmonella could be isolated from the intestines using the selective media Selenite enrichment broth (Oxoid, Haarlem NL) and Rappaport-Vassiliadis enrichment broth (Oxoid, Haarlem NL).



**Fig. 1:** *Toxoplasma* sporozoites in the lung in impression smears stained by Hemacolor®

### **Histology**

In liver, kidney and spleen acute to subacute inflammations with necrosis were seen. In the lungs peracute changes were noticed including hyperaemia and vascular damage leading to transudate and haemorrhages, which may have resulted in local hypoxia. Excessive cellular responses led to tissue damage in liver, kidney and spleen. This cellular component existed of lymphocytes, heterophils and macrophages. In the intestinal wall and pancreas a cellular infiltration without necrosis was seen. In the brain (D) no lesions were found. In all examined organs immunohistochemically positive organisms for antibodies against *Toxoplasma gondii* were demonstrated (Fig. 2)

### **Discussion**

#### **Infection route**

The first question of interest is how the birds could become infected. They are kept in an inside-building behind a glass barrier. Contact with cat faeces seems possible via the leaves collected in the zoo for the breeding purposes of the waddled brush turkeys. But also food (grains) polluted with cat faeces can often not be excluded (3, 6).

### **The acute reaction, with emphasis on the lungs**

The reaction-pattern as it is seen in the lungs is an excellent example to philosophise about what is going on in the body of the infected bird. It is a complex response to the invasion of a parasite, involving many systemic responses. It is an overlapping series of events that form a continuum (1). We see the typical local manifestations of an acute inflammation (Fig. 3):

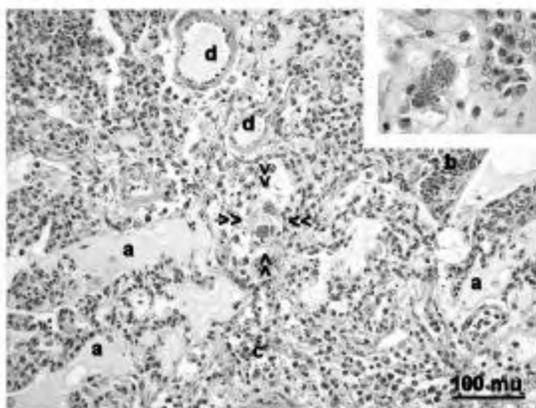
1. *Haemodynamic changes* including:
  - 1.1. Arteriolar dilatation resulting in hyperaemia.
  - 1.2. Increased permeability of the microvasculature with the outpouring fluid into the extravascular tissues resulting in oedema and fluid in the parabronchi.
  - 1.3. Peripheral margination of white blood cells in capillaries and venules.
2. *Permeability changes*
  - 2.1. The fluids change from watery transudate (oedema) to protein-rich, cell containing exudate.
  - 2.2. Increased vascular leakage induced by direct damage or a scala of mediators like vaso-kinins, complement fragments, leukotrienes, prostaglandins, some cytokines and platelet activating factors.
3. *Events involving leukocytes*

Although inflammation fundamentally is a defensive reaction, inflammations can be harmful as well. In many situations, and maybe also in this lung reaction, the host may suffer more from tissue damage as a result of an inflammatory reaction than from damage caused by the initiating stimulus had there been no inflammatory response at all.

An example is seen in calves: if neutropenia is induced by experimental means in calves with experimental induced pneumonic pasteurellosis, the calves are partially protected against the development of severe pneumonia and the resultant hypoxia (1).

The problem in the birds mentioned above is that an intensive inflammatory reaction occurs in the lung in which tissue damaging contributions of the inflammatory host-defence mechanisms (hyperaemia, fibrin, exudate and so forth) may be worse than tissue-damaging contributions of the original *Toxoplasma* infection.

All acute changes lead to local stasis of the circulation, increased distance between air (O<sub>2</sub>) and blood (CO<sub>2</sub>), resulting in a decreased supply of oxygen in all other tissues. This effect will decrease the potential for reaction and effective action in these organs. It will also support the development of local necrosis.



**Fig. 3:** Photomicrograph of the lung of a crowned pigeon with peracute changes including hyperemia (b) and vascular damage leading to transudate (a) and hemorrhages, resulting in local hypoxia. Notice pseudocysts of *Toxoplasma gondii* (arrows and insert) c. cellular infiltrate, d. blood vessels. H&E stain.

The inflammatory process itself is aimed at maximising normalcy as an endpoint. Ideally this can be accomplished by removal of the initiating stimulus (in this case: *Toxoplasma* organisms) and regenerative repair of the injured tissue. Because of the vital function of the lung for the O<sub>2</sub>/CO<sub>2</sub> exchanges all therapeutic measures should be aiming at:

1. Preventing free fluids to come into the respiratory tissue (drowning effect) by using anti-inflammatory drugs to limit, control or otherwise modify inflammation (NSAID's ?).
2. Remove extravascular fluids from the lung as

soon as possible to allow minimal distance between air- and blood capillaries by stimulating kidney activity (diuretics?). This will also reduce the development of permanent scar tissue after recovery.

3. When the diagnosis is made in time, even immunocompromised patients may benefit from treatment with chemotherapeutic drugs (such as

pyrimethamine plus sulfadoxine, or trimethoprim plus sulfamethoxazole) (2, 5).

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## MANAGEMENT AND GENERAL MEDICAL ISSUES OF A MIXED SPECIES PENGUIN COLLECTION IN A CLOSED ENVIRONMENT

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### Abstract

Penguins and other seabirds are becoming increasingly popular in zoos and bird-parks. These animals are particularly challenging for the keeping institution, since their special needs are not easily met and specific facilities have to be designed, in order to maintain these birds according to the highest standards. The Loro Parque hosts the largest European penguinarium. Four penguin species are kept in two different exhibits. Each exhibit has different air and water temperatures and a different light cycle. Birds are fed, managed and checked medically according to the species-specific needs. This paper gives a survey on the medical and non-medical aspects of Sphenisciformes in captivity, including some selected cases in penguin medicine.

### General issues

#### *Systematic*

Penguins belong to the family *Spheniscidae*, the only one in the order *Sphenisciformes*. There are 17 living species of penguins, divided in 6 genera. All the penguin species live in the southern hemisphere.

#### *Housed species*

The Loro Parque's Penguinarium hosts four different penguin species: King penguin (*Aptenodytes patagonicus*), Gentoo penguin (*Pygoscelis papua*), Rockhopper penguin [*Eudyptes chrysocome (crestatus)*] and Humboldt penguin (*Spheniscus humboldti*).

#### *Housing*

Birds are divided into two different exhibits, according to their environmental needs. Both exhibits are closed and the internal environment is carefully monitored. The smaller exhibit hosts 30 Humboldt penguins, while the larger one houses about 160 penguins of the three other species.

#### *Water and Air Quality*

The quality of air and water is one of the most important tasks when managing a penguinarium. Both are constantly filtered and monitored for the presence of possibly pathogenic agents, such as fungi and bacteria. Monitoring is done through periodical air and water analysis and cultures.

#### *Feeding*

Penguins are fed with several different fish and water organisms (herrings, squids, krill, etc.). The diet specifications depend on penguin species.

#### *Birds identification*

Keepers can tell the difference between some individuals, but generally speaking individual penguins are very difficult to recognise. Two means of ID are used at Loro Parque for each individual: a plastic numbered wing-band, for the easy identification of each animal and a microchip, in the case the wing-band breaks or becomes not-readable.

## Medical issues

### *Sex determination*

Due to the excessive perivisceral fat amount, penguins are difficult to be sexed endoscopically. DNA related techniques are currently used.

### *Physical examination of penguins*

Penguins are routinely examined. The physical examination goes through a check of the general body conditions, birds are weighed, eyes are controlled for lesions, beak and oral cavity are examined for colour changes, focal lesions or diffuse alteration. Wings are checked for presence of bruising or other lesions. Feet must be carefully examined and if there is a suspect of bumblefoot lesions starting, these must be addressed immediately.

### *Routine checks*

Penguins must be checked on a routine basis, so that every bird is carefully examined at least once per year. This is normally done out of the breeding season, preferably before it, in order to fix any problems before the eggs are laid. It is very important to determine an individual baseline for each individual, so that in case of any symptoms, the collected samples can be compared with the "normal values" for the specific animal. What we consider very important is to have, at least, the following base data:

CBC (WBC/EWBC, PCV, Diff. Count of WBC).

Blood chemistry (AST, AU, LDH, Chol, CK, Bile Acid, Tot. Prot, EPH)

Aspergillus titers

Choanal and Cloacal cultures.

### *Breeding*

It is very important to be ready with the appropriate nesting areas, different for each species, well before the breeding season. In our experience penguins don't have big problems with egg laying, but low fertility rates are often reported in literature.

### *Medical control of the chicks*

Chicks must be weighed at day one. The umbilicus must be disinfected and sealed if it is still open. Typical problems of penguin chicks are poor growing rate, often depending on parents' misbehaviour or inexperience. Also omphalitis and subsequent septicaemia may sometimes be encountered. The chick may be weak and not be able to hatch, so that assisted hatches are one of the typical emergencies in penguins' neonatology.

### *Viral diseases*

Very little is known on viral diseases specific of this avian order and only a few viral diseases are reported in penguins. Most of these reports involve captive birds. Although Paramixovirus 1 infections have been documented in *Sphenisciformes*, and cutaneous pox lesions have been reported in otherwise asymptomatic penguins, in the Loro Parque exhibits we never experienced viral outbreaks.

### *Bacterial pathogens*

Penguins are susceptible to the pathogenic bacteria that affect other birds. *E. coli*, *Pasteurella* spp. and *Klebsiella* spp. can affect adult penguins as well as chicks. *Clostridium perfringens* and *Pseudomonas* spp. are also reported to be possible pathogens for *Sphenisciformes*.

### *Fungal pathogens*

Fungal diseases, especially aspergillosis are very well known and serious diseases of penguins. These birds, as some other avian species from extremely cold climates, are particularly susceptible to *Aspergillus* infections. Environmental prophylactic measures, as well as early diagnosis, are the keys to successful *Aspergillus* management in the up to date penguin exhibit.

### Parasitology

Although some parasites have been reported in literature, they are not considered a typical penguins' problem. The most studied penguins' parasite is *Plasmodium*. Malaria, like aspergillosis, is a typical disease of captive penguins and also this disease can be controlled by strict management measures. This explains why a closed environment, like the one that houses the penguins in our institution, can be extremely helpful in controlling this disease.

### Environmental contaminants affecting penguins health

Penguins, like all the fish-eaters, are exposed to intoxication by pesticides accumulated in fish. This include polychlorinated biphenyls (PBCs) and 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). Mortality can be high and strict control measures are necessary. One important thing is the choice of the fish provider. This will preferably be able to provide tested and certified fish and be insured for diseases or deaths depending on poor fish quality.

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## FEATHER MITES OF PSITTACIDAE

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### Abstract

Feather mites of psittacine birds are often neglected ectoparasites by veterinarians and bird keepers. Only very little is known about their biology, behaviour and damaging effects on the host. Feather mites (*Sideroferus lunula*, *Dubininia melopsittaci* and *Psittophagus* sp.) were detected in 53 % of 83 investigated birds of 25 different species or subspecies in preliminary studies about distribution of feather mites among psittacines kept in Germany.

### Zusammenfassung

Federmilben von Psittaziden sind bei Tierärzten und Vogelbesitzern wenig bekannte Ektoparasiten. Nur wenig ist über ihre Biologie, Verhaltensweisen und Schädigung für den Wirtsvogel bekannt. In ersten orientierenden Untersuchungen zum Vorkommen verschiedener Federmilbenarten auf Psittaziden, die in Deutschland gehalten werden, wurden auf 53 % der untersuchten 83 Vögel aus 25 verschiedenen Arten bzw. Unterarten Federmilben (*Sideroferus lunula*, *Dubininia melopsittaci* und *Psittophagus* sp.) nachgewiesen.

### Résumé

Les acariens des plumes des psittacidés sont des parasites souvent négligés par les vétérinaires et les éleveurs. Leur biologie, leur comportement et les effets sur l'hôte sont très peu connus. Ces parasites (*Sideroferus lunula*, *Dubininia melopsittaci* et *Psittophagus* sp) ont été détectées chez 53% des 83 oiseaux examinés, issus de 25 espèces ou sous-espèces différentes, lors d'études préliminaires sur la distribution de ces acarioses chez les psittacidés en Allemagne.

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**Key words** feather mite, *Dubininia melopsittaci*, *Sideroferus lunula*, *Psittophagus* sp., ectoparasite, budgerigar

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### Introduction

According to current knowledge some of the dinosaurs from which the birds evolved had at least partially feathers on their body, for example the *Caudipteryx* excavated 1998 in China. It seems that the birds have inherited a part of the parasites from the dinosaurs because a 120-million-year-old fossil feather, coming from the Lower Cretaceous Crato Formation of Northeast Brazil, has been infested with the eggs of an antique variety of feather mites (11).

Feather mites are highly specialised plumage and skin ectoparasites that are adapted for inhabiting certain microhabitats on a bird's body, mainly on flight feathers and large coverts of wings (6).

At present about 2000 different species of feather mites are described, but this represents less than 20% of the extant species. The three superfamilies of feather mites (Analgoidea, Freyanoidea and Pterolichoidea) include 33 families with 444 genera (8). Almost all recent orders of birds, excluding penguins, cassowaries and emus, have their own specific feather mite fauna (6). Feather mites of the order Psittaciformes belong to the following families: Apionacaridae, Dermoglyphidae, Proctophyllodidae, Psoroptoididae, Ptyssalgidae, Pyroglyphidae, Xolalgidae, Ascouracaridae, Falculiferidae and Pterolichidae (8). Some of the New World parrots harbor more than 15 species of feather mites (11).

Only very little is known about their biology, behaviour and damaging effects on the host.

Feather mites are also common on Psittacidae kept in Germany. But in contrast to the scaly mite, *Knemidocoptes pilae*, and the chicken mite, *Dermanyssus gallinae*, both well known to also occur on psittacines, the feather mites of psittacids are poorly described in the literature. In this preliminary study the distribution of feather mites on psittacine birds in Germany was investigated.

## Material and methods

In 1999 we started our investigations into the occurrence of feather mites on Psittacidae. We examined both the skins of 48 dead psittacids and also feathers collected from 35 birds by use of a stereoscopic microscope. Altogether the birds compared 25 different species or subspecies and were kept in Zoological Gardens or as pets and originated mainly from Saxony (Table 1).

For examination the mites were picked up with a needle and placed for permanent preparation in Berlese's medium. The mites were determined according to the keys proposed by Atyeo and Gaud, 1987 (2) and Gaud and Atyeo, 1996 (8).

**Table 1:** Number and species of psittacine birds investigated for the occurrence of feather mites

Bird species	Number of investigated birds	Number of birds with feather mites
Kea ( <i>Nestor notabilis</i> )	1	0
Leadbeater's Cockatoo ( <i>Cacatua leadbeateri</i> )	2	2 <sup>1)</sup>
Moluccan Cockatoo ( <i>Cacatua mollicensis</i> )	1	0
Galah ( <i>Eolophus roseicapillus</i> )	1	0
Black-winged Lovebird ( <i>Agapornis taranta</i> )	3	3 <sup>2)</sup>
Black-cheeked Lovebird ( <i>A. personata nigrigenis</i> )	2	2 <sup>2)</sup>
Red-fronted Parakeet ( <i>Cyanoramphus novaezelandiae</i> )	1	1 <sup>2)</sup>
Budgerigar ( <i>Melopsittacus undulatus</i> )	45	25 <sup>5)</sup>
Bourke's Parakeet ( <i>Neopsephotus bourkii</i> )	2	2 <sup>2)</sup>
Many-colored Parakeet ( <i>Psephotus varius</i> )	2	1 <sup>2)</sup>
Cockatiel ( <i>Nymphicus hollandicus</i> )	1	0
Crimson-winged Parakeet ( <i>Aprosmictus erythropterus</i> )	3	1 <sup>4)</sup>
Turquoise Grass Parakeet ( <i>Neophema pulchella</i> )	1	1 <sup>2)</sup>
Splendid Grass Parakeet ( <i>Neophema splendida</i> )	1	1 <sup>2)</sup>
Eastern Rosella ( <i>Platycercus eximius</i> )	1	0
Stanley Parakeet ( <i>Platycercus icterotis</i> )	2	1 <sup>2)</sup>
White-eared Conure ( <i>Pyrrhura leucotis</i> )	1	0
Brazilian grey-breasted Conure ( <i>P. l. griseipectus</i> )	1	1 <sup>2)</sup>
Demerara Conure ( <i>Pyrrhura leucotis</i> )	4	1 <sup>2)</sup>
Blue-fronted Amazon ( <i>Amazona a. aestiva</i> )	1	0
Orange-winged Amazon ( <i>Amazona a. amazonica</i> )	1	0
Yellow-headed Amazon ( <i>Amazona oratrix</i> )	1	0
Grey Parrot ( <i>Psittacus erithacus</i> )	2	0
Senegal Parrot ( <i>Poicephalus s. senegalus</i> )	2	2 <sup>2)</sup>
Blue and Yellow Macaw ( <i>Ara ararauna</i> )	1	0
Total number:	83	44

<sup>1)</sup> *Psittophagus* sp. <sup>2)</sup> *Dubininia melopsittaci* <sup>3)</sup> *Sideroferus lunula* <sup>4)</sup> eggs of feather mites

<sup>5)</sup> 2x *Dubininia melopsittaci*; 15x *Sideroferus lunula*, 8x both species

## Results

Altogether we detected feather mites in 53 % of the 83 investigated birds. Of 25 different psittacine species or subspecies 14 were infested with feather mites. Three different species were identified, the most frequent being *Sideroferus lunula* and *Dubininia melopsittaci*. The only host of *S. lunula* is the budgerigar. Of 45 investigated budgerigars 56% were infected with feather mites, 15 birds harboured *S. lunula*, 2 *D. melopsittaci* and 8 were infested with both species.

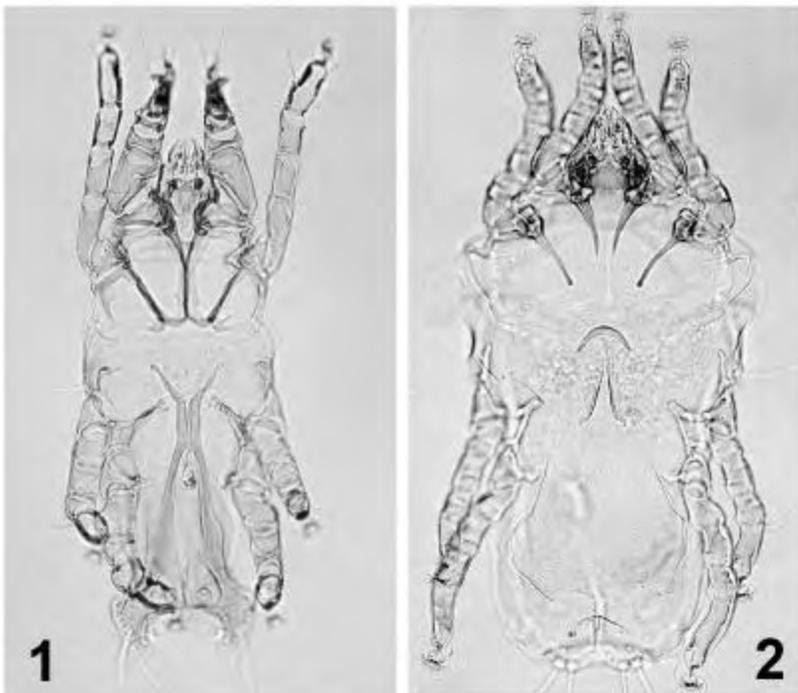
The other species of the budgerigar, *D. melopsittaci*, has a distinctly wider spectrum of hosts than *S. lunula*. We have found this mite also on the agapornids Black-cheeked Lovebird (*Agapornis personata nigrigenis*) and Black-winged Lovebird (*A. taranta*). And the mite was present as well as on the Red-fronted Parakeet (*Cyanoramphus novaezelandiae*), the Demerara Conure (*Pyrrhura egregia*), the Brazilian grey-breasted Conure (*Pyrrhura l. griseipectus*), the Many-coloured Parakeet (*Psephotus varius*), the Turquoise Parakeet (*Neophema pulchella*), the Splendid Grass Parakeet (*Neophema splendida*), the Bourke's Parakeet (*Neopsephotus bourkii*), the Stanley Parakeet (*Platycercus icterotis*) and the Senegal Parrot (*Poicephalus senegalus*).

The third species, *Psittophagus* sp., we have found only on two Leadbeater's Cockatoos (*Cacatua leadbeateri*).

On two feathers of the Crimson-winged Parakeet (*Aprosmictus erythropterus*) we have found only eggs of feather mites.

## Discussion

The most common psittacine in Germany, the budgerigar (*Melo-psittacus undulatus*), hosts two species of feather mites, *Sideroferus lunula* and *Dubininia melopsittaci*. Both species have been collected in Australia from caged as well as free living budgerigars and also from budgerigars in many other parts of the world (2, 7, 10).



**Fig. 1:** Male feather mite (*Sideroferus lunula*) from Budgerigar

**Fig. 2:** Female feather mites (*Sideroferus lunula*) from Budgerigar

### ***Sideroferus lunula* (Robin, 1877)**

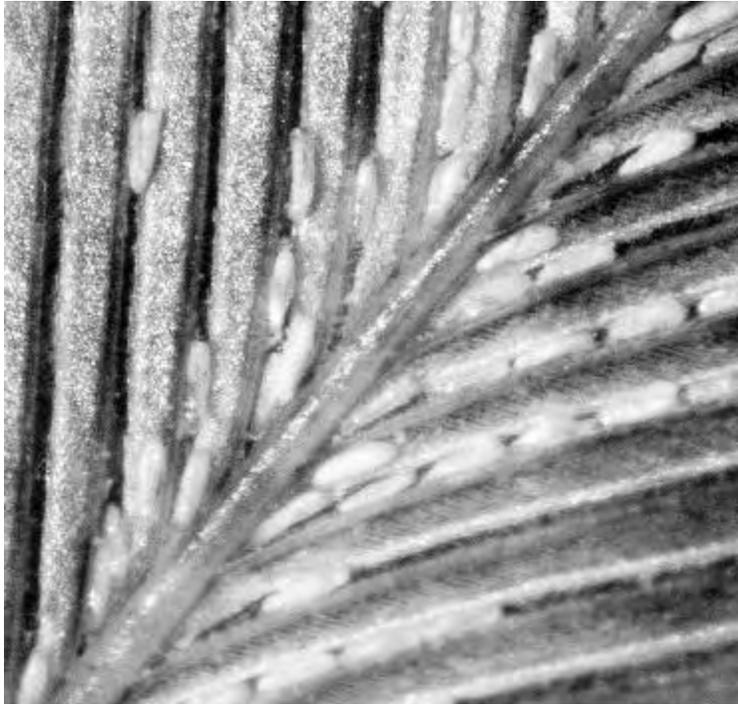
Already in 1877 Robin originally described *S. lunula* (Pterolichidae) as *Pterolichus lunula* (4), other known synonyma are *Protolichus lunula* and *Megninia lunula*. Both sexes are strongly sclerotised and the males (500 – 600 µm long) are the only mite stage living on parrots

having bifurcated tarsi on the first pair of legs (Fig 1). Because of this fact Atyeo and Gaud (2) placed this species in the new genus *Sideroferus*, from sideros (Gr., iron implement) and phero (Gr. carry, bear) referring to the modified tarsus I of the male, within the family Pterolichidae. In addition the males have two long paragenital apodemes extending from the genital region to the posterolateral margins of the body (Fig. 1). Females of this species are smaller than males (400 –

500 µm) and can be distinguished from females of *D. melopsittaci* by the posterior ends of the epimerites I that are not connected (Fig. 2).

This mite lives on the exposed surface of wing and tail feathers and is considered to be only mildly pathogenic (2, 10). We have found the mites in the same location on the feathers of the budgerigar. The eggs were laid on the barbs some distance away from the rachis, particularly on the inner ventral small feathers of the wing (Fig. 3).

The only known host for this mite is the budgerigar (*Melopsittacus undulatus*).



**Fig. 3:** Eggs from feather mite (*Sideroferus lunula*) on a feather from Budgerigar

***Dubininia melopsittaci* Atyeo and Gaud, 1987**



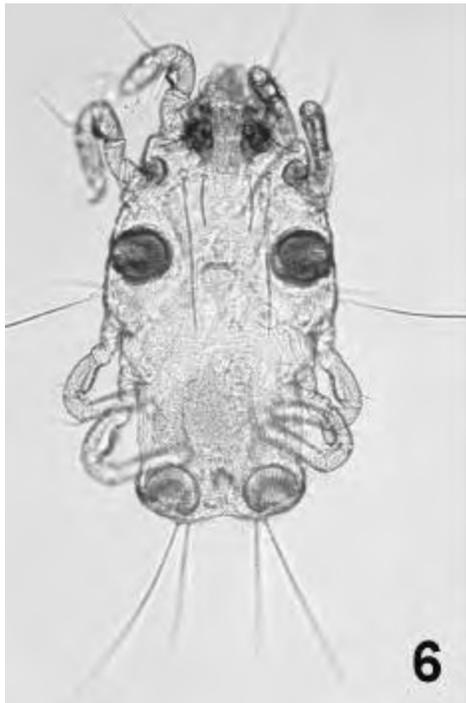
**Fig. 4:** Male feather mite (*Dubininia melopsittaci*) from Budgerigar

**Fig. 5:** Female feather mite (*Dubininia melopsittaci*) from Budgerigar

*D. melopsittaci* has been collected 1947 in South -Africa and was named first as *Megrinia* sp. (10). But not until 1987 Atyeo and Gaud redescribed this second species (Xolalgidae) as *Dubininia melopsittaci* (2). The males of this species are somewhat smaller (385  $\mu\text{m}$ ) than the males of *Sideroferus* and have not the bifurcated tarsi on the first pair of legs and lack the two long paragenital apodemes of *Sideroferus* (Fig. 4). The distinction of the females is more difficult. The epimerites I are in a V-configuration with the posterior ends of this epimerites being connected. Furthermore there are distinctions in the morphology of the genital region (Fig. 5). With a body length of 370  $\mu\text{m}$  the females of this mite are smaller than the females of *Sideroferus* (2). *D. melopsittaci* lives primarily on small feathers, especially anterior to the upper tail coverts (2, 10). This mite seems to be more pathogenic than *Sideroferus*. Because of the irritations caused by this mite the birds bite their plumage and destroy the feathers particularly of the tail and the wings (2, 3). Probably *D. melopsittaci* has a distinctly wider spectrum of hosts than *S. lunula*. We have found *D. melopsittaci* not only on the budgerigar but also on other Psittacidae (Table 1).

### ***Psittophagus* sp.**

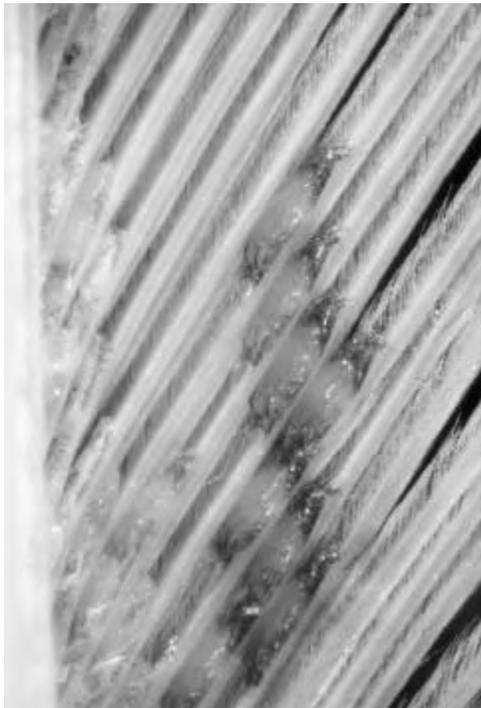
This new genus was proposed by Gaud and Atyeo, 1996 (Ficuliferidae). The name of the genus is derived from *Psittacus* (Gr., parrot) and *phagos* (Gr., eating) to refer to the commensal – host association (8). Male mites are 300 - 325  $\mu\text{m}$  long, female mites measure 425 – 470  $\mu\text{m}$ . *Psittophagus* species have one pair of lateral “glands”, each with radiating striae and one pair of posterolateral “glands” may be present, especially in females (Fig. 6). Species of this genus are known to occur on various parrots in Australia, particularly Cacatuidae (8). However, they were not described in detail and thus it is not possible to state the exact species of the mites collected in the present study from the Leadbeater’s Cockatoo (*Cacatua leadbeateri*).



**Fig. 6:** Female feather mite (*Psittophagus* sp.) from Leadbeater’s Cockatoo

An experienced investigator can detect the larger feather mites, like *S. lunula*, with the naked eye as tiny grey specks on the feather. For the smaller mites, like *D. melopsittaci*, microscopic examination is necessary. The feather mites are living particularly on the underside of the large feathers of the wing and tail and are mostly found in the grooves between the barbs, the head directed towards the rachis of the wing feathers (Fig. 7).

Reports on damaging effects caused by feather mites are contradictory. The mites are commensals and normally do not induce disease. The name “*Analges*” for a genus of feather mites meaning “w



**Fig. 7:** Feather mite (*Sideroferus lunula*) on a feather from Budgerigar

without pain", reflects the obvious tolerance of these mites by the host. However, heavy infestations may cause host reactions. Under such conditions the mites are not only found on feather but migrate to the skin. The irritating presence of thousands of mites may cause the bird to pull wing, tail, and body feathers. This may also cause skin lesions by the bill (2, 8). A survey of 198 exhibition budgerigars in the United Kingdom in 1996 showed that feather mites caused 18,2 % of feather diseases. Of 37 respective aetiologies feather mites were in position three (3).

Depluming itch in fowl caused by *Megninia ginglymura* was reported from India. The symptoms were manifest initially at the region of the tail with loss of barbules, breakage of feathers and feathers loss. Areas of the skin at the tail and ventral regions became bare and the lesions spread forwards including the back and sides giving the typical appearance of depluming itch (1). Feather

mites of the genus *Megninia* also caused considerable damages of plumage, disorder of swimming and adverse effects in the breeding productivity in ducks (9).

Another aspect of damage caused by feather mites is that they are potentially an important source of allergens for the bird keepers (5).

In the available literature there is no information on the control of feather mites of psittacine birds. From our personal experience sprays with pyrethrum or pyrethroids are suitable for control of feather mites in psittacids. The treatment must be carried out two times at an interval of 8 – 10 days, because these acaricids have no effect against the eggs of feather mites. The eyes of the bird have to be protected during treatment. In addition a cleaning with hot water and a subsequent disinfection of cages and equipment with an acaricid chemical is recommended.

### Acknowledgments

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European Association of Zoo- and Wildlife Veterinarians (EAZWV) 4<sup>th</sup> scientific meeting,  
joint with the annual meeting of the European Wildlife Disease Association (EWDA)  
May 8-12, 2002, Heidelberg, Germany.

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## **EXAMINATION OF REPTILES - AN INTERACTIVE LEARNING PROGRAM FOR STUDENTS AND PRACTITIONERS**

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### **Abstract**

In February 2001 an ICT project (Information and Communication Technology) was started at the Division of Zoo Animals and Exotic Pets of the University of Zurich to create an interactive learning program on the subject "Examination of exotic pets". The first CD-ROM will be on the examination of reptiles, the second on the examination of exotic mammals and the third CD will present the examination of birds. Together with a questionnaire, that may be completed over the Internet, the CD will be a part of the curriculum of the veterinary medicine.

### **Zusammenfassung**

An der Abteilung für Zoo-, Heim- und Wildtiere der veterinärmedizinischen Fakultät der Universität Zürich wird seit Februar 2001 ein ICT-Projekt (Information and Communication Technology) zur Erstellung einer multimedialen Lernumgebung mit dem Thema "Untersuchung exotischer Heimtiere" erarbeitet. Es sollen drei interaktive CD-ROM entstehen. Die erste Lernumgebung befasst sich mit dem Untersuchungsgang bei Reptilien, die zweite mit der Untersuchung von Heimsäugetern und in der dritten CD-ROM soll die Untersuchung von Vögeln dargestellt werden. Zusammen mit einem Fragenkatalog, der von den Studenten im Internet bearbeitet werden kann, sollen diese CD-ROM in den Lehrplan der Veterinärmedizin einbezogen werden.

### **Résumé**

En février 2001, un projet d'Information et Technologies de Communication a été initié au département Animaux Exotiques et de Zoos de l'université de Zurich afin de créer un programme interactif d'apprentissage sur le thème « examen des animaux exotiques ». Le premier CD-ROM portera sur l'examen des reptiles, le second sur l'examen des mammifères exotiques, et le troisième sur l'examen des oiseaux. Associé à un questionnaire, disponible sur Internet, ce CD fera partie du programme des études vétérinaires.

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**Key words:** reptiles, examination, learning program, CD-ROM

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### **Extended abstract**

The field of exotic pets medicine covers a variety of species. In the everyday veterinary practice these pets are of increasing importance. In the curriculum of the division of Zoo Animals and Exotic Pets of the veterinary faculty of Zurich the emphasis lies in the correct examination. It would be ideal, if each student could learn this on a basis 1:1 with an instructor. In reality this is not possible because of personnel and welfare reasons.

With the present program we would like to achieve the best possible profit / cost ratio. These interactive learning programs allow it for students to learn the examination with the help of pictures, videos, sound and text according to their own speed. In human medicine this has been a success for quite a few years now. Nowadays it's safe to assume that almost every student has the needed computer equipment.

This learning program will be provided on CD for the third year students. An additional questionnaire will be offered on the Internet for self-evaluation. The main objective of this CD-ROM is the correct handling and examination of snakes, tortoises and lizards. In addition the taking of common samples (e.g. blood), the carrying out of special examinations (e.g. endoscopy) and frequent pathological findings will be addressed.

The program is based on a general and a special part. The general part consists of chapters on taxonomy, keeping and feeding of reptiles. The special part of the CD is divided into three parts. Snakes, tortoises and lizards. Each contains information on different species, the examination, diagnostics, the application of medicaments and the most common symptoms. In addition the user can solve a variety of quizzes on the CD, print out fact-sheets and connect to the Internet questionnaire. Furthermore the Internet may be used for question and answer sessions with the course organisers or for the access of a list of useful links

The CD will be put into action at the end of 2002 in addition to the lectures. The other two CD's on exotic mammals and birds are in preparation and should be finished by the middle of 2003.

European Association of Zoo- and Wildlife Veterinarians (EAZWV) 4<sup>th</sup> scientific meeting,  
joint with the annual meeting of the European Wildlife Disease Association (EWDA)  
May 8-12, 2002, Heidelberg, Germany.

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## DIAGNOSIS OF SOME COMMON DISEASES IN SNAKES, WITH RESPECT TO THE UNDERSTANDING OF DISEASE-INDUCED BEHAVIORAL CHANGES

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### Abstract

For a better and more rapid diagnosis and treatment, this study summarizes some specific disease-related behavioral changes. Such abnormal behavior is divided into four groups: abnormal posture, abnormal mobility and movements, increased nervousness and aggressions as well as decreased activity. Especially abnormal behavior that is connected with convulsions and pain, or leads to motorical dysfunctions because of central nervous disorders, seems of importance. Additionally some examples of behavioral changes are given that are due to the normal biology of snakes and can be misjudged as abnormal.

### Zusammenfassung

Für eine schnellere und bessere Diagnosestellung und der damit verbundenen Behandlung von Erkrankungen bei Schlangen, werden in dieser Veröffentlichung wichtige krankheitsbedingte Verhaltensänderungen besprochen. Derartige Verhaltensstörungen sind in vier Gruppen zu unterteilen: abnormale Körperhaltung, abnormale Beweglichkeit bzw. Bewegungen, erhöhte Nervosität und Aggression, sowie verminderte Aktivität. Besondere Beobachtung finden dabei Verhaltensänderungen, die mit Krämpfen und Schmerzen einhergehen, wie auch diejenigen, die durch Erkrankung des ZNS hervorgerufen werden. Ferner werden Beispiele von solche Verhaltensänderungen aufgeführt, welche auf die normale Biologie der betroffenen Schlangenart zurückzuführen sind, und nicht als abnormal missverstanden werden dürfen.

### Résumé

Afin d'obtenir un diagnostic et un traitement meilleurs et plus rapides, cette étude résume quelques altérations comportementales spécifiques de maladies. De tels comportement anormaux sont divisés en 4 groupes: posture anormale, altérations de la mobilité et des mouvements, nervosité et agressivité accrues et chute d'activité. Plus particulièrement, les comportements anormaux associés à des convulsions et de la douleur, ou menant à des dis-fonctions motrices liées à des troubles nerveux centraux semblent primordiaux. De plus, des exemples de changements comportementaux normaux sont donnés car ils sont parfois pris pour anormaux.

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**Key words:** snakes, diseases, behavior

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### Introduction

Proper reptile medicine requires not only experience, but also a great deal of interest. The inexperienced practitioner often does not know what to observe and how to recognize abnormal manifestations. This is due to a lack of knowledge of what constitutes the normal body language of a snake. There are more than 20 species commonly encountered as pets, and one should also be aware that each species has its own behavioral characteristics. Restrain will in many cases precede examination. Familiarity with the behavior and normal responses to handling will allow to limit stress, to gather information on the alertness and temperament of the animal, and also to work safely, especially in the case of large or venomous snakes (6). Changes in normal behavior can be

traced back to a variety of stressors, which can be physical, nutritional or social in origin, and also be related to the species and the individual history of the animal (5). One will, additionally, find that captive born animals are more tolerant to the captive environment. Inappropriate stressors that induce changes in behavior are not necessarily associated with pain but they invariably affect the well-being of the animal, and therefore need to be identified and expunged. Despite much interest and many publications on reptile biology there seems to be a distinct lack of information on the behavior of captive snakes, and specifically on its relationship to health and disease. The present paper opposes normal behavior of snakes to disease-induced behavioral changes that can in some cases be recognized by careful observation. By learning such modes of behavior, the veterinarian can contribute to the restoration of health in the ophidian patient.

### Identifying and rating of behavioral changes

The external examination of a snake, like in any other animal, should always begin with a careful inspection. Ideally the snake is first observed in its living environment, e.g. the vivarium, where behavior and external appearance can be studied and differentiated according to normal biology. The Green tree python (*Morelia viridis*) normally rests bundled on a horizontal tree branch with its head resting in the center of the coils (Fig.1). If the animal is found on the ground, this could already indicate an indisposition. Furthermore, if the terrarium lacks horizontal branches, the veterinarian will strongly recommend that the environment is corrected to suit the physiological and ethobiological needs of the species. We will focus on those behaviors that are specific and useful to know when examining a snake, with regard to abnormal posture, abnormal mobility and movements, as well as increased and decreased nervousness and aggression.

### Abnormal posture

Different species have different resting positions but a healthy snake never lies on its back. It will show the so-called "righting reflex", and turns around as soon as it is forced to lie on its dorsal side. Sometimes animals can be seen resting with the posterior part of their body tilted upside down. That is a sign of abdominal discomfort and may be due to physiological causes such as a heavy gestation. More commonly this will be due to pathological factors such as dystocia, constipation, enteritis (amoebiasis). This is not to be confused with the normal defensive behavior shown by the African Ringhals Cobra, (*Hemachatus haemachatus*), or the Ringed snake (*Natrix natrix*) among others, wherein the snake feigns agony and death to deter predators (Fig.2). A typical abnormal body posture is seen in animals with respiratory problems. The behavioral changes are subtle and gradual and often overlooked. The animal will tend to spend more time basking, to keep its snout at an angle when coiled, to "yawn" more frequently (it is actually trying to clear the airways) and finally stretch out the first third of the body with the rest of the body coiled. Usually clients consult when the snake is already showing more severe signs like staying with mouth and glottis wide open for a long period of time and expel mucus (Fig.3). The Malayan pit viper (*Calloselasma rhodostoma*) and some South American crotalids, however, typically rest in this position even when perfectly healthy.

An important behavioral aspect in snakes is the "tongue-flicking behavior" which can and should be observed in non-moving and moving animals. Tongue movement plays a very important perceptive role in snakes, and is known to indicate an alert animal that actively explores its environment. When a snake is ill and the well-being disturbed, the tongue flicking behavior is suppressed as the animal shows less interest in its surroundings and less response to stimuli. Here also species and individuals do not behave in identical manner. Animals are sometimes brought to the veterinarian with the complaint that they flick the tongue for an unusual length of time. This can be a normal behavioral feature of certain species (e.g. *Ahaetulla* spp.), and may be due to the presence of prey items or sexually active mates in the vicinity.

## Abnormal mobility and movements

A snake showing abnormal posture can display abnormal movements, but the animal can also have abnormal postures and move normally and vice-versa. The muscular-skeletal system of a healthy snake is symmetrically and exhibits a strong muscular tone that allows the animal to carry and balance its body weight by smooth and well coordinated movements. In this connection, we distinguish between abnormal movements and convulsions. Certain species show light spasms that are not disease-related; e.g. pythons show these muscle contractions throughout the whole body in the last third of pregnancy as well as when incubating their eggs. In other snakes the spasms can be observed during mating behaviour. Shiver-like behaviours are seen in snakes that have been harassed continuously for a long period of time. The genus *Bitis*, and *Bitis nasicornis* in particular, show this symptom readily when stressed beyond reason without the opportunity to escape or retreat (e.g. in a hiding box).

### a. Convulsive and /or pain related movements

Reptiles have neural components similar to those known for mammals. Reptiles also possess anti-nociceptive mechanisms to modulate pain and to display appropriate behavior in response to stimuli that are known to be painful in other vertebrates (4).

Vertebral fractures are mostly related to severe injuries and trauma and have to be discussed here. Such fractures often go undetected because palpation and examination are not always sufficient, and therefore a roentgenological examination is indicated. Paralysis posterior to the fracture is obviously conceivable, yet does not often occur as is evident from the number of incidental radiographic findings. When it occurs it has the characteristics found in mammals: the body portion caudal of the fracture will not be sensitive to pain, the muscles are paralyzed, and the cloaca is flaccid (2). Nevertheless, in the absence of paralysis, prognosis of vertebral fractures is good.

Convulsions are commonly seen in snakes. Pre-mortem agony manifests itself in the form of convulsions. Convulsions can also be encountered in severe renal infections (kidney stones, gout in the final state, uremia etc.), intoxication and septicemia, colic- and pain-related convulsions. Respiratory distress causes movements that are identical to convulsions as mucus and pus obstruct the trachea of the snake. When suffering from acute pain, a snake appears restless; the body will turn and twist under strong muscular contractions whereas the mouth tries to bite into anything. The typical post-mortem findings after agonal convulsions include a cramped-up body position (Fig.4), and substrate in the mouth that results from biting the ground. Lighter paroxysmal spasms can be observed in animals with an unbalanced Ca:P level that may lead to the medical picture of rickets. In that case, the anamnesis will be very important since rickets in snakes is exceedingly rare. Only in cases where snakes are constantly fed with pieces of heart meat instead of whole animals, osteodystrophy can develop with the above symptoms (1).

### b. Motorical dysfunction

Diseases that affect the central nervous system cause more or less the same kinds of symptoms: head shaking, loss of righting reflex, tremor, loss of muscular tonus, loss of balance and coordination, regurgitation of food, anisocoria and sometimes blindness, torticollis and stargazing (Fig.5).

The species-specific viral disease IBD (inclusion body disease) is primarily seen in the *Boidae*, and is probably over-diagnosed. IBD is caused by a retrovirus and is believed to be contagious via oral or aerogen infection as well as mites. It is of interest that most of the symptoms listed above can be found in IBD infections. Furthermore general symptoms like stomatitis are often associated with this disease. The prognosis of true IBD is fatal and euthanasia is sooner or later indicated. A paramyxovirus infection described in the family Viperidae primarily causes respiratory symptoms, but sometimes opisthotonus, and head shaking can also be observed. Certain infections with parasites can also lead to infections of the central nervous system and cause the symptoms listed beforehand. Frye (3) describes a meningo-encephalitis caused by *Acanthamoeba spp.* in which the snakes showed central nervous symptoms like stargazing. During this behavior the head is held stiffly up into the air because of a spastic contraction of the neck muscles. Stargazing has also been associated with other diseases like terminal septicemia shortly before death (2).

Another cause for central nervous disorders may be vitamin B1 (thiamine) deficiency. Thiamine deficiency is hardly conceivable in snakes, yet has been reported in snakes fed a diet of frozen fish

(1). Cerebrocortical necrosis and neuritis may be responsible for symptoms like incoordination, tremor and signs of torticollis. The animal is unable to strike properly and shows pseudo-blindness.

### **Increased nervousness and aggressions**

Snakes can become restless for various reasons: hunger, lack of hiding places, breeding season, lack of human contact, pain, external parasites such as mites and ticks, etc. Mites are sometimes overlooked, since they are very small and, depending on the species, do not spend much time on the snake. The discerning veterinarian can diagnose mites by spotting “fuligine”-like material in the water and by the zigzag posture of the animals. Uneven and rough spectacles, because of rubbing, and swollen eyes, caused by the mites sitting around the eyeball, are additional symptoms of mite infections.

### **Decreased activity**

Depressive snakes will tend to move less, however, there are large physiological variations in the level of activity. In captivity these are usually of seasonal (temperature / humidity / photoperiod) or hormonal causes. Many animals that hibernate in the wild but are not allowed to do so in captivity may show decreased activity during winter without going through a proper hibernation. A lack of adaptation to the terrarium, and a lack of retreat areas will make a snake shy to move about while causing chronic stress.

### **Conclusion**

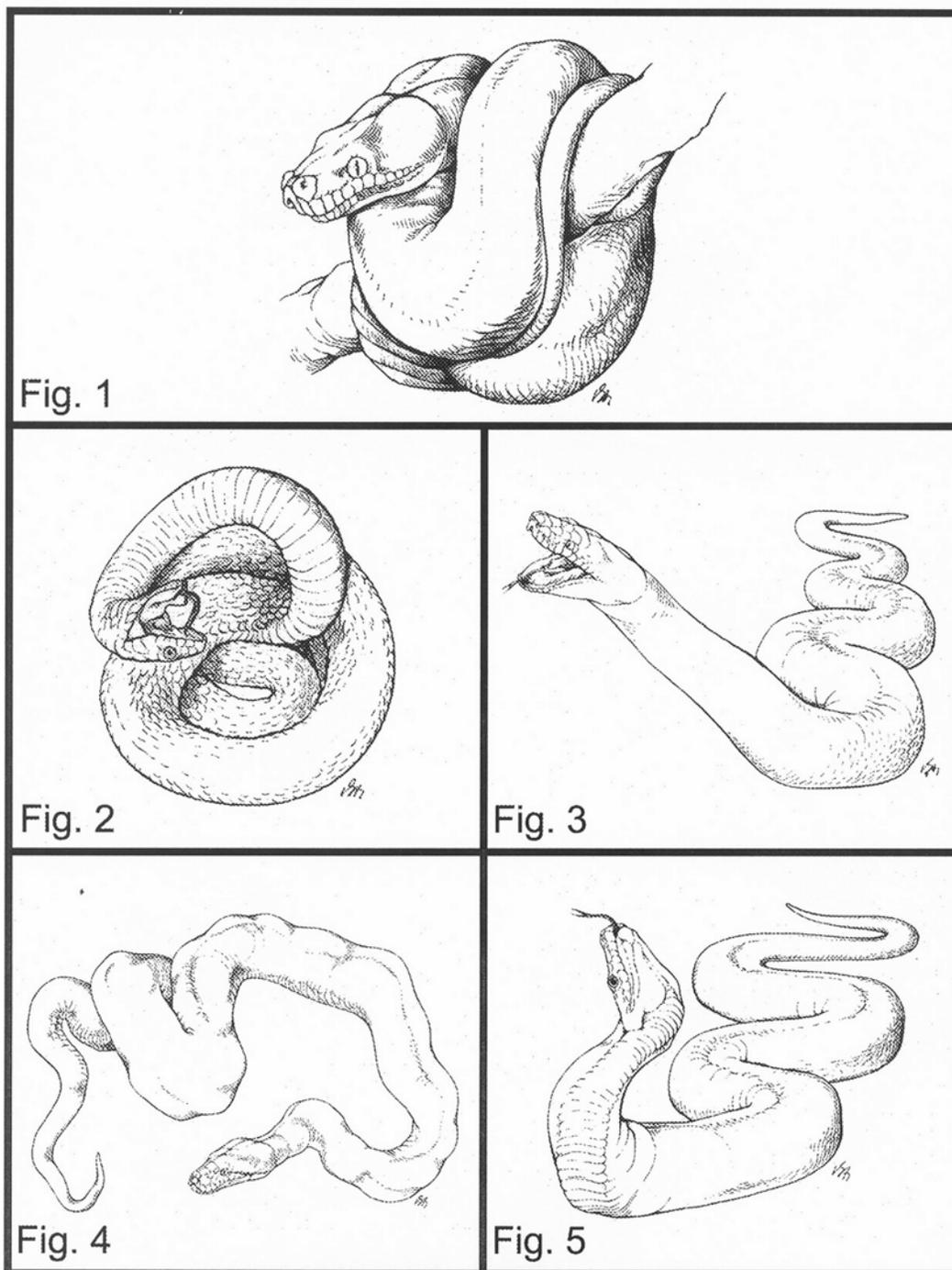
Practicing reptile medicine requires a good knowledge of reptilian biology, in particular of specific animal behavior. Only experience and true interest in this field will enable the examiner to identify behavioral changes that can lead to identify possible stressors and, thus, to proper diagnosis and treatment.

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**Legend:**

Normal and abnormal body postures of snakes;

Fig. 1: Normal posturing of the Green tree python (*Morelia viridis*) on a tree branch.

Fig. 2: Ringed snake (*Natrix natrix*) feigning death to deter predators.

Fig. 3: Body posture of a snake with breathing difficulties.

Fig. 4: Cramped-up body positioning after agonal convulsions.

Fig. 5: Loss of coordination and balance due to central nervous disorders.

(Drawings by C. I. von Stemm)

## RENAL FUNCTION IN GREEN IGUANAS WITH METABOLIC BONE DISEASE

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### Abstract

Twenty green iguanas (10 males, 10 females) suffering from metabolic bone disease were included in this project. The clinical symptoms included swelling of the hind legs with limited movement in all patients. Anorexia, constipation and lethargy have been observed in 90%, 55% and 55% patients, respectively. Typical picture of bone deformation consisted of marrow cavity expansion with extremely thinned cortex. Blood cytology revealed heterophilia, and eosinophilia (mean 71 and 6%, respectively). The mean percentage of basophils, monocytes and azurophils (mean 6, 19 and 11%, respectively) were in the physiological range, the number of lymphocytes (mean 23%) was lower than normal ranges for healthy iguanas. The mean values for total protein, ALT, GGT, glucose, cholesterol, potassium and calcium were within normal ranges for healthy green iguanas being 61.48 g/l, 0.70 gkat/l, 0.13 gkat/l, 9.07 mmol/l, 6.12 mmol/l, 4.00 mmol/l and 2.65 mmol/l, respectively. The mean concentrations of creatinine, uric acid, phosphorus and AST were significantly increased, being 58.12 µmol/l, 331.50 µmol/l, 5.01 mmol/l and 4.15 gkat/l, respectively. The mean plasma levels of ALP have decreased to 0.40 gkat/l. The phosphorus-calcium ratio has been altered significantly to 1.89. The post-mortem examination revealed renal oedema colon constipation, urinary bladder distension, tophi formation in pericardial sac and oophoritis. Microscopic examination of the kidneys has confirmed renal gout, renal tubular necrosis, tubulointerstitial nephritis, interstitial nephritis and renal cysts. We suggest that evaluation of the renal function is necessary for the prognostic purpose and effective treatment of green iguanas suffering from metabolic bone disease.

### Zusammenfassung

Im Rahmen dieser Studie wurden zwanzig an metabolischen Knochenerkrankungen leidende Grüne Leguane (10 männliche und 10 weibliche Tiere) untersucht. Als klinische Symptome traten bei allen Tieren Schwellungen der Hintergliedmaßen und erschwerte Bewegung auf. Anorexie wurde bei 90%, Verstopfung und Lethargie bei jeweils 55% der Patienten beobachtet. Das typische Bild der Knochenveränderungen zeigte eine Erweiterung der Markhöhle und einen extrem dünnen Cortex. Bei der Blutbilduntersuchung wurden Heterophilie (71%) und Eosinophilie (6%) ermittelt. Die relative Zahl der Basophilen (6%), Monozyten (19%) und Azurophilen (11%) war im physiologischen Bereich, die Lymphozytenzahl (durchschnittlich 23%) geringer als bei gesunden Leguanen. Die durchschnittlichen Werte für Gesamteiweiß (61,48 g/l), ALT (0,70 gkat/l), GGT (0,13 gkat/l), Glukose (9,07 mmol/l), Cholesterin (6,12 mmol/l), Kalium (4,00 mmol/l) und Kalzium (2,65 mmol/l) lagen im für gesunde Grüne Leguane normalen Bereich. Die durchschnittliche Kreatinin- (58,12 µmol/l), Harnsäure- (331,50 µmol/l), Phosphor- (5,01 mmol/l) und AST-Konzentrationen (4,15 gkat/l) waren signifikant erhöht. Die Plasmakonzentration von ALP war auf 0,40 gkat/l gesenkt. Das Phosphor-Kalzium-Verhältnis zeigte mit einem Wert von 1,89 eine signifikante Veränderung. Bei der postmortale Untersuchung wurden Nierenödem, Colonverstopfung, Harnblasendistension, Bildung von Gichttophi in der Perikardialhöhle sowie Oophoritis festgestellt. Die mikroskopische Untersuchung der Nieren zeigte Nierengicht, Tubulusnekrose, Tubulointerstitialnephritis, interstitielle Nephritis und Nierenzysten. Unseres Erachtens ist die Untersuchung der Nierenfunktion zur Prognosestellung und zur effektiven Behandlung von an metabolischen Knochenerkrankungen leidenden Grünen Leguanen unumgänglich.

### Résumé

Vingt iguanes vert (10 mâles, 10 femelles) atteints de MBD (Metabolic Bone Disease) ont été inclus dans l'étude. Les symptômes relevés comprenaient chez tous les patients une tuméfaction et une mobilité limitée des membres postérieurs. Anorexie, constipation et léthargie ont été observés chez respectivement 90%, 55% et

55% des patients. Des images typiques de déformation de l'os avec expansion de la cavité médullaire et un cortex extrêmement fin. L'hématologie a révélé une hétérophilie, une éosinophilie (moyennes respectives : 71% et 6%). Les pourcentages moyens de basophiles, monocytes et azurophiles (Moyennes respectives : 6%, 19%, 11%) étaient dans les valeurs usuelles. Le nombre de lymphocytes (moyenne 23%) était en dessous des valeurs usuelles chez l'iguane sain. Les valeurs moyennes de Protéines Totales, ALAT, gGT, Glucose, Cholestérol, Potassium et Calcium étaient dans les valeurs usuelles chez l'iguane sain, soit respectivement 61,48 g/L ; 0,70 gkat/L ; 0,13 gkat/L ; 9,07 mmol/L, 6,12 mmol/L, 4,00 mmol/L et 2,65 mmol/L. Les concentrations moyennes en créatinine, acide urique, phosphore et ASAT étaient significativement accrues, soit respectivement 58,12 mmol/L ; 331,50 mmol/L ; 5,01 mmol/L et 15,4 gkat/L. Les niveaux plasmatiques moyens de PAL étaient accrus (0,4 gkat/L). Le rapport phospho-calcique était significativement altéré (1,89). L'autopsie a révélé de l'œdème rénal, une constipation colique, une distension de la vessie, la formation de tophi dans le péricarde et une oophorite. L'examen microscopique des reins a confirmé la présence de goutte rénale, une nécrose rénale tubulaire, une néphrite tubulo-interstitielle, une néphrite interstitielle et des kystes rénaux. Nous suggérons donc une évaluation de la fonction rénale chez les iguanes verts souffrant de MBD dans l'intérêt du pronostic et de l'efficacité du traitement.

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**Key words** : plasma biochemistry, renal insufficiency, reptiles

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## Introduction

Metabolic bone disease is frequently observed in green iguanas kept in captivity. Different factors can predispose to this disease. Chronic metabolic problems caused by insufficient nutrition as well as poor husbandry which are frequently observed could result in different forms of bone diseases. Clinical signs associated with metabolic bone disease may vary, usually include lethargy, anorexia, general weakness, colon constipation, tremor and hind legs paresis. Metabolic bone disease may be associated with chronic renal insufficiency (1, 2). The aim of this study was to compare blood cytology and plasma biochemistry for the renal function monitoring in green iguanas with metabolic bone disease.

## Materials and methods

A total of 20 green iguanas suffering from metabolic bone diseases were included in this project. On the basis of anamnestic data, as well as the physical examination of the patient, iguanas with swelling of the hind legs and limited movement (SHL) were characterised individually as anorectic lizard (AN), patient with constipation (C) and/or lethargy (L). Blood samples for the determination of biochemical as well as cytological profiles were collected from the ventral coccygeal vein approximately one-third of the tail length from the cloaca. Blood smears were prepared using a coverslip technique and were air-dried. The Pappenheim method of biphasic staining with May-Grünwald and Giemsa-Romanowski stain was used. All plasma biochemical assays were performed by use of automated analysers. The concentration of total protein (TP), creatinine, glucose, uric acid, blood urea nitrogen (BUN), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST),  $\gamma$ -glutamyltransferase (GGT), cholesterol, calcium (Ca), phosphorus (P) were performed by CobasMira analyser (Roche). The plasma concentration of potassium (K) was performed by Atomspec analyser (Hilger & Watts). Biopsy specimens obtained during general necropsy from kidneys, liver and ovaries were preserved in 10% neutral buffered formalin for 12 to 24 hours and processed in a standard paraffin technique. Calcified tissues were demineralised in formic acid. Soft tissue sections of 4-6 $\mu$  were stained using hematoxylin-eosin. Microscopic pictures were evaluated using the magnification of 100 to 1000 with the Olympus BX-40 microscope equipped for photo-documentation by a digital camera Olympus 2000 Z. Statistical analyses of haematological and plasma biochemical values were performed by use of Stat Plus commercially available software (Stat Plus, version 1.01, 1990, VUvE Brno, Czech Republic).

## Results

Clinical signs associated with metabolic bone disease included swelling of the hind legs with limited movement in all patients (100 %, with the same frequency of manifestation in both sexes 10/10), anorexia in 18 (90 %, 9/9) iguanas, constipation in 11 patients (55 %, 5/6) and lethargy in 11 patients (55%, 6/5). We have revealed heterophilia, and eosinophilia (mean 71 and 6 %, respectively). The mean percentage of basophils, monocytes and azurophils (mean 6, 19 and 11 %, respectively) were in the physiological range, the number of lymphocytes (mean 23 %) was lower than normal ranges for healthy iguanas. The mean values for total protein, ALT, GGT, glucose, cholesterol, potassium and calcium were within normal ranges for healthy green iguanas being 61.48 g/l, 0.70  $\gamma$ kat/l, 0.13  $\gamma$ kat/l, 9.07 mmol/l, 6.12 mmol/l, 4.00 mmol/l and 2.65 mmol/l, respectively. The mean concentrations of creatinine, uric acid, phosphorus and AST were significantly increased, being 58.12  $\mu$ mol/l, 331.50  $\mu$ mol/l, 5.01 mmol/l and 4.15  $\gamma$ kat/l, respectively. The mean plasma levels of ALP have decreased to 0.40  $\gamma$ kat/l. The phosphorus-calcium ratio was altered significantly from the physiological range 0.4 – 1 to 1.89. The post-mortem examination revealed renal oedema in 4 patients, colon constipation in 3 patients, urinary bladder distension in 2 patients and tophi formation in pericardial sac of 1 patient. Oophoritis has been observed in one 4 years old female. Microscopic examination of the kidneys has confirmed renal gout, renal tubular necrosis, tubulointerstitial nephritis, interstitial nephritis and renal cysts. Hepatic lipidosis, tophi formation in liver parenchyma and acute hepatitis have been observed in 2, 2, and 1 iguanas, respectively. The femora surrounded by cuffs of cartilaginous tissue has been registered during necropsy in all 5 iguanas. Typical picture of bone deformation consisted of marrow cavity expansion with extremely thinned cortex.

## Discussion

The pathological condition of kidneys result frequently in profound calcium metabolism disturbance (2, 3, 4, 5). At the present study we have observed renal oedema with colon constipation and urinary bladder distension in patients with metabolic bone disease. Microscopic evaluation of the renal parenchyma revealed renal gout with tubular necrosis and tophi formation, tubulointerstitial nephritis, interstitial nephritis and renal cysts. Our findings are in accordance to Boyer et al. (6), even with some differences in the physiological range. The plasma levels of ALP have decreased; mean values for ALT, GGT, glucose, cholesterol and calcium were within normal ranges for healthy green iguanas (7). The normal phosphorus and calcium concentrations in the blood serum of iguanas are 1.4 – 3.1 mmol/l and 2.5 – 3.5 mmol/l, respectively (7). The phosphorus-calcium ratio is altered at an earlier stage of renal disease as compared to uric acid and seems to be a sensitive parameter for the kidneys function monitoring as well as for diagnosis of metabolic bone disease in reptiles (1, 8, 9). Stein (10) has published compilation of haematological values with broad range for specific parameters. The mean percentage for heterophils at our present study could be generally evaluated as significantly elevated. We have revealed heterophilia (71 %) and eosinophilia (6 %). The mean percentage of basophils (6 %), monocytes (19 %) and azurophils (11 %) were in the physiological range, the percentage of lymphocytes (23 %) was lower than normal ranges for healthy iguanas. We suppose that all green iguanas with clinical signs of metabolic bone disease have to be examined for their kidney function.

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## HEALTH MANAGEMENT OF A COLONY OF BREEDING GABOON VIPERS (*Bitis gabonica rhinoceros*)

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### Abstract

A retrospective study of a Gaboon viper breeding colony is presented. Two different groups were made for a better disease understanding. First group consisted of wild caught animals. Rhabdiform and Pentastomid parasites, *Salmonella* infections and necrotic stomatitis are the most representative diseases. The second group consisted of captive breeding animals and breeders. Diseases including respiratory infection (pneumonia) and renal disease (gout) were most commonly found. Treatments and new management recommendations are discussed. Use of a bacterin in a refractory pneumonia case is also discussed.

### Zusammenfassung

Eine retrospektive Studie über eine Gabon viper Brutkolonie wird präsentiert. Die Kolonie wurde in zwei Gruppen wurden eingeteilt, um die verschiedenen Krankheitsbilder besser zu verstehen. Die erste Gruppe bestand aus wilden, gefangenen Tieren. Rhabdiforme und Pentastomide Parasiten, *Salmonella* Infektionen und nekrotische Stomatitis waren die am häufigsten auftretenden Krankheiten. Die zweite Gruppe bestand in gefangenschaft lebenden Bruttieren und Züchtern. Krankheiten der Atemwege (Pneumonie) und Nierenerkrankungen (Gicht) wurden am häufigsten gefunden. Behandlungen und neue Managementvorschläge werden besprochen, sowie der Einsatz von Bacterin in Fällen von refraktärer Pneumonie.

### Résumé

Une étude rétrospective d'une colonie reproductrice de vipères du Gabon est ici présentée. Deux groupes différents ont été constitués afin de mieux comprendre la maladie. Le premier groupe était constitué d'animaux capturés à l'état sauvage. Les infestations parasitaires à Rhabdiformes et Pentastomidés, les infections à *Salmonelles* et les stomatites nécrotiques sont les maladies les plus représentées. Le second groupe était constitué d'animaux élevés en captivité et de reproducteurs. Des maladies telles que des infections respiratoires (pneumonie) ou des maladies rénales (goutte) ont été le plus souvent rencontrées. Les traitements, ainsi que de nouvelles recommandations pour la gestion de ces animaux en captivité sont aussi discutés.

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**Key words:** Gaboon viper, stomatitis, acclimatisation, management, parasites, pneumonia

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### Introduction

Establishing of new arrived reptiles is usually a hard task for the veterinarian. Captivity in origin countries increases stress and diseases are exacerbated, specially, in animals with a hiding habit. In July 1998, Naturaleza Misteriosa, the reptile exhibition at the Madrid Zoo received a shipment from Ghana to reinforce the *Bitis* viper breeding colony. Ten wild-caught adult Gaboon vipers were a part of the shipment.

Also we present in this report the diseases of the captive-bred animals and of the initial breeding colony, with a total of 23 specimens, 11 of them resident and the remaining itinerant.

Finding	Number
<b>BACTERIOLOGY</b>	
<i>Enterobacter sakazakii</i>	stomatitis n= 2
<i>Morganella morganii</i>	stomatitis n=2
<i>Pseudomonas aeurogino sa</i>	stomatitis n=2, respiratory tract n=4
<i>Salmonella spp.</i>	cloaca n=7.
<b>PARASITES</b>	
Protozoan infections	n=3.
Cestodes	n=5.
Nematodes	n=10.
Rhabdias infection	n=10.
Pentastomid infection	n=5.

**Table 1.** Findings in the laboratory samples of the wild Gaboon vipers (*Bitis gabonica rhinoceros*) ( n=10 ).

## Methods and Findings

### **Wild caught animals**

On arrival the animals were from very thin (six individuals) to fairly poor condition (4 animals), very dehydrated and with a very noticeable respiratory distress (4 animals). Two animals were presented necrotizing stomatitis. The animals were housed to the isolation area in individual enclosures and in a dry environment with pine wood shavings to provide hiding places and decrease the stress.

Before starting a treatment, samples were obtained from the gums and oral mucosa in the animals with necrotic stomatitis using standard procedures for microbiology and biopsy procedures. From the trachea in five of the animals samples were taken for cytology and microbiological examination and from the cloaca (five animals also) as cloacal washing, for parasitology.

Results from those exams are detailed in the Table 1.

Organ	Pathological findings
Gums	Chronic inflammatory reaction (nematode and bacterial); gingival hyperplasia; hyphae infiltration.
Lungs	Bacterial and nematode focal pneumonia ( <i>Rhabdias</i> spp).
Liver	Mild to severe hepatic lipidosis.
Kidneys	Severe interstitial nephritis; tubular degeneration and mineralization.
Intestine	Chronic nematode enteritis; fibrin deposits.
Fat bodies	Completely depleted.

**Table 2.** Results from the necropsy of two wild Gaboon vipers dead during acclimatation.

Results from gum biopsies revealed a chronic inflammatory infection with rhabditoid nematodes and bacterial colonies.

A initial therapy included:  
**Fluid therapy:** 5 animals received a single bolus of 25ml/kg IV of a Lactate - Physiologic 50-50% combination followed by a 50 ml/kg IC for three days of the same mixture. The other animals received 50 ml/kg IC for three days.

*Warm water baths* (30°C) were given for two hours to increase the BMR and to clean the animals. *Ceftazidime* in a dose of 20 mg/kg IC q 72h for seven treatments was given to the animals with necrotic stomatitis.

Also some hyphae were presented in the sample, so chlorhexidine was added to the treatment in the animals with stomatitis or positive to *Rhabdias*.

As can be seen in table 1, the results included the very heavy burden of rhabditoids and pentastomida. *Pseudomonas* and *Morganella* were isolated from the gums, trachea and lungs, so an aspiration pneumonia from the rhabditoids was suspected.

*Salmonella* was isolated from seven of the animals after processing only one sample as a possible result of the stress. With the three samples all the animals were carriers.

No isolation of the fungal hyphae was obtained.

Three days later the fluid therapy was discontinued and an antiparasitic therapy was initiated. The therapy included metronidazol (80mg/kg PO) and ivermectin (200 µg/kg IM) the first day and praziquantel (8 mg/kg IM) and fenbendazol (50 mg/kg by stomach gavage) two days later.

The treatment was repeated fifteen days later.

Ceftazidime (20 mg/kg IM q 72 h for seven treatments) was initiated in all the animals to prevent lung infection due to the heavy rhabditoid and pentastomida burden.

This same day one of the stomatitis individuals was found dead. Results from this necropsy and an other animal that died later, are presented in the table 2.

Amikacin (2.5 mg/kg IM q 72 h) was added to the ceftazidime in the other individual as a more aggressive therapy.

Seven days later remaining animals where considered to be stabilised and food was firstly offered. Recheck of the animals showed that three of them still had a significant pentastomida level, so additional ivermectin treatment was installed. However, final pentastomida removal is only possible by lung transcuteal endoscopy (2,3,5,6,7,8,11,13,14).

Species	Cases
<i>Pseudomonas fluorescens</i>	2
<i>Pseudomonas aeruginosa</i>	5
<i>Proteus vulgaris</i>	1
<i>Morganella morganii</i>	6
<i>Citrobacter freundii</i>	2
<i>Aeromonas hydrophila</i>	3

**Table 3:** respiratory findings in the captive-breeding colony

At this point a blood sample was taken from each animal from the coccygeal tail vein. This site is very commonly used in reptiles but blood from this site is usually contaminated with lymph fluid. Therefore a cardiocentesis were performed (10,15).

Three months later another of the specimens was found dead without symptoms (results are presented in the table 2). No biochemistry was performed until this point, but visceral gout was observed in this case, so a renal panel was performed to six animals of the total colony.

### **Captive breeding colony**

The most significant findings are respiratory problems that are usually due to a dry environment and stress conditions (such as the reproductive season, exhibition terrariums, change of terrariums and no deep substrate to hide). Eight cases are reported from 1996 to early 1999. There are some very common problems at this colony in a retrospective study.

Microbiology was performed in all the cases. Results are presented in the Table 3.

One case is especially interesting. The specimen was an adult six years old captive bred female. She is very little (85 cm long and only weighted 1.5 kg).

She was part of an experiment in 1993 to feed those animals with a "synthetic" food that contains beef heart and liver with egg and a multivitamin/ mineral supplement.

Grow rates were very low and all (5 animals) except this animal died from respiratory infections (suspected pneumonia) in that same year. No more information was available from those animals because we were not in the staff at this date.

This animal was from this year a positive carrier of *Pseudomonas aeruginosa* and *Morganella morganii*, and antibiotic treatment (with an antibiogram) was no successful, so a bacterin was elected to treat the animal (1,6,9).

*Pseudomonas aeruginosa* and *Morganella morganii* were isolated from the respiratory tract in pure culture. The autogenous bacterin was prepared by growing the isolate in 100ml of trypticase soy broth for 24 hours at 38° C, then adding 3 ml of 30% aluminium hydroxide and 0.3 ml of 37% formalin. The bacterin (0.1 ml) was administered IM, and bacterin increments (0.1 ml) were administered IM every other day for three additional treatments. Two months after the last bacterin injection, the snake was re-evaluated by another culture, as no signs were observed, and isolation revealed again the same pathogens.

Another bacterin was prepared and 0.5 ml of it were injected IM q 72 h for 18 treatments and then weekly for 12 treatments. Enclosure temperature was increased to 29°C to increase the BMR, and C vitamin was added to food (fresh killed rat) 1 hour before the snake meal, and to the animal on each bacterin treatment.

Also humidity was increased to 70-80% and deep pine shaving substrate was provided. Another culture two months later indicated that both bacteria had disappeared.

Visceral gout was the other of the most common problems in the colony (3,4,8,12,13,14,15). First cases were confirmed only in the necropsy, but after those, a renal biochemistry panel was performed to adult breeding individuals. Results are detailed in the Table 4 and 5.

	1	2	3	ISIS
Haematocrit ( % )	22.6	27.1	19.8	22.4±5.3
Total solids ( % )	7.0	9.4	12.3	4±1.1
WBC ( 103 /m l )	15.5	15.0	22.7	6.935±3.775
RBC (106 /m l )	0.65	0.87	0.68	1.46±0.94
Lymphocytes ( % )	55.0	57.0	51.0	32±26
Heterophils ( % )	26.0	39.0	21.0	21.3±21.4
Monocytes ( % )				31±0
Azurophils ( % )	18.0	4.0	26.0	15±12
Eosinophils ( % )	1.0	0.0	2.0	2.4±1.91
Calcium (mg/dl)	6.7	8.5	7.7	falta
Phosphorus ( mg/dl )	13.0	12.1	14.3	falta
Uric acid ( mg/dl )	14.2	9.3	15.4	falta

**Table 4a.** Haematology and selected plasma biochemistry of the diseased breeding Gaboon vipers (*Bitis gabonica rhinoceros*) Males (n=3).

	1	2	3	4	5	ISIS
Haematocrit ( % )	19.0	22.0	23.0	18.0	14.0	22.4±5.3
Total solids ( % )	7.8	9.0	6.7	8.1	6.3	4±1.1
WBC ( 103 /m l )	17.6	14.2	23.5	18.3	17.4	6.935±3.775
RBC (106 /m l )	1.1	0.8	0.6	0.78	0.97	1.46±0.94
Lymphocytes ( % )	57.0	62.0	59.0	65.0	79.0	32±26
Heterophils ( % )	23.0	21.0	27.0	11.0	7.0	21.3±21.4
Monocytes ( % )						31±0
Azurophils ( % )	19.0	17.0	22.0	23.0	14.0	15±12
Eosinophils ( % )	1.0	0.0	2.0	1.0	0.0	2.4±1.91
Calcium (mg/dl)	13.0	7.0	8.0	14.0	7.0	12.2 1.8
Phosphorus ( mg/dl )	12.0	12.0	6.0	6.0	16.0	6 0.52
Uric acid ( mg/dl )	1.3	2.1	3.2	0.4	4.8	0.7 0.52

**Table 4b.** Haematology and selected plasma biochemistry of the diseased breeding Gaboon vipers (*Bitis gabonica rhinoceros*) Females (n=5)

Two renal biopsies were performed in two animals with suspected renal disease .

Results revealed a mild glomerular degeneration, some nephrosis foci, and dystrophic, mineralisation in kidneys and liver.

Management of animals included a decrease of ambient temperature to 23-26 °C, heavy misting twice a day and ultrasound vaporisation two hours daily,

Fluid therapy was initiated at 50 ml / kg IC daily, to increase the renal clearance volume but was discontinued shortly after for the aggressive improvement of the animals.

## Discussion

Rhabditidae family is commonly isolated from amphibians and reptiles in captivity. They are especially common in animals from humid regions, from temperate and humid tropical regions. Usually these are incidental findings, but present health problems in immunodepressed and debilitated animals. More problematic are the secondary bacterial infections.

Pentastomida are difficult to eliminate from the hosts. Levamisol or ivermectin are sometimes effective against them, but endotoxin shock and associated pneumonia are worse than their effect. Endoscopy removal is one option, but not always performed due to the equipment cost and personnel training.

Nematodes are not usually a problem but may cause enteritis when proliferate.

For all this, antiparasitic treatments before coprological examination should not be performed, because the animals main problem could not be obvious anymore.

Bacterial isolations are not especially important as they are enteric flora, but contamination of the oral cavity in poor hygienic conditions can lead to stomatitis as the result of such contamination and the immunodepression.

*Salmonella* is a normal flora of reptile intestines and only some strains have clinical significance. Results from serotyping are still not available.

Elevation of the BMR in reptiles is usually less used than desirable, but now is more recommended, specially in therapeutics, but perhaps have more utility in feeding habits and immunostimulation.

Long term captivity is not always an indicator of successful captive management. Renal disease and visceral gout was an incidental necropsy finding, but showed the poor renal activity of the stabilised animals. Annual exams to all animals with should be performed, at least in valuable and aged specimens.

Bacterins are no common therapy in the reptile infection treatment. Only few records are reported in the past. Today, with the improvement in the management of the species and the antibioresistance, this could be very useful in the treatment of very chronic pathology.

With the change in management conditions, respiratory problems have almost completely disappeared (only one case in a juvenile in four years), biochemistry renal panels are within normal ranges and animals have more calm behaviour. Also they present more extended mating periods.

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