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CLINICAL APPLICATION OF INFRARED-THERMOGRAPHY
IN INFLAMMATION DIAGNOSIS IN MEGA-HERBIVORES

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Abstract

After a brief introduction into the method of infrared-thermography, 11 case reports are presented. In these case reports a short history of the case, the infrared-thermographic findings, the procedures after evaluation and a short discussion. A general discussion rounds up the paper.

Zusammenfassung


Résumé

Après une brève introduction à la méthode de thermographie infrarouge, 11 rapports de cas sont présentés. Une brève histoire du cas, les résultats de la thermographie infrarouge, les mesures prises après évaluation, et un bref commentaire sont fournis. Une discussion générale conclut la totalité de l'article.

Key words: infrared-thermography, thermography, infrared, elephant, black rhinoceros, giraffe, hippopotamus, lameness, pododermatitis, traumatology.

Introduction

Infrared-thermography is a non-invasive method. With an infrared-camera, the body surface temperature of an animal is measured from a distance and the thermoprofile of this animal is then displayed as a thermogram. No direct animal contact or immobilisation is necessary. In this work a thermogram is defined as an infrared-picture of a detail or a whole animal with its surroundings. The thermoprofile of an animal can give information on or hints about certain aspects of the animal’s health status, e.g. on its current general thermoregulation or local temperature changes (2, 5, 6, 7, 15).

It is necessary for the investigator to have a clear understanding of the technical aspects of this method and its limitations (3, 7, 15). This, in order to make the application of infrared-thermography in veterinary medicine plausible, realise its potential, and discover pathological alterations while preventing inaccurate diagnosis and/or false interpretations of clinical findings. In addition, the investigator should have a modern working knowledge of thermoregulation, anatomy, morphology, physiology and pathological physiology of the animals to be investigated. Infrared-thermography is not a new form of colour photography. Technical information and many aspects of thermoregulation in zoo and wild animals are discussed in two Ph.D. theses (7, 10).
In megaherbivores such as giraffes, rhinos, elephants and hippos it is often very difficult to observe locations of injured areas on limbs, especially when no external lesions are visible. Here thermography can help locating the area and extend of the injury (7). As heat radiation is one of the five cardinal symptoms of an inflammation, infrared-detectors can localise this heat area, if the heat is radiated from close to the surface of the animal. This phenomenon has been intensely used in horse medicine (11, 12, 13, 16, 17, 18, 19, 20, 21, 22).

**Material and Methods**

For this investigation an infrared camera from the company FLIR (Forward looking Infrared) was used. The following models were used:

- Thermovision 470
- Thermovision 570
- Vetcam 695
- Thermacam 695 (includes a digital camera)

All images were captured during a standing or slow moving position of the animal. All animals were adapted to the ambient temperature for at least two hours, before image capture, unless specified otherwise. For each image a reference ambient temperature and relative humidity were recorded separately. If possible, the other animals of the zoo group were used as references. If this was not possible, reference images from the database were used.

The infrared images were then analysed using the computer program IRWin 522 or Reporter 2000, both specific software programs for analysing infrared images.

**Results**

In case reports the application of infrared-thermography in zoo animal medicine is demonstrated. As a non-invasive method infrared-thermography allows the investigating vet to get a quick picture of the location and extend of an alteration on the animals limbs. All colour infrared images for these case reports can be obtained from the author.

**Inflammation diagnosis in elephants**

**Case report 1**

Weaving in an Asian elephant

In this case, an Asian elephant *Elephas maximus* showed intense heat radiation over all joints in the hind and most joints of the front legs. This elephant had been chained over many years. In recent years it was only chained during the training sessions. The infrared-images revealed, however, the lasting damage done to the joints of this old elephant. As soon as the elephant starts to weave while chained, the heat radiation shows over these joints. It is interpreted as originating from the none physiological sideways movement of the joints, which in elephants is not normal to this extreme, as elephants normally move their limbs forward, and fare less often sideways. This heat radiation could be observed even four hours after the elephant was released from the chains and moving freely on the outside enclosure. Unfortunately chaining is still used in many institutions and in some zoos the only way to keep elephants (1). This case report indicates the necessity to investigate the effect of chaining in an intense study, as does also the next case report.

**Case report 2**

Overnight chaining in an African elephant

An African elephant *Loxodonta africana* was found to radiate more heat from the hip and knee joint of the right side as well al over the right carpal joint. From the investigation with a night vision video camera, it became clear, that this elephant, do to its size, could only sleep on his right body side, while being chained. Furthermore, there was no soft bedding material is this elephants stable for him to lei down on, so only bare concrete was available. The weight of the animal is therefore
placed on these specific joints during the whole night. Due to the chaining, there was no other way for this elephant to lie down. As the other side of the animal shows no signs of inflammation, the alterations on the right joints are most likely to be attributed to this inadequate holding facility. An enlargement of the stall should solve this problem at least partially, as it would allow the elephant to sleep on his other side as well.

**Lameness evaluation in elephants**

**Case report 3**  
Shoulder injury in an Asian elephant  
This Asian elephant *E. maximus* was observed walking in an abnormal way, but the origin of the lameness could not be found. The caretakers assumed that this elephant must have fallen while on the outside enclosure, but nobody observed this accident. Infrared-thermography revealed that the problem was in the right shoulder, exact in the elbow joint. The elephant was then treated on this joint by the local vet and the caretakers. In horse medicine thermography is often used in cases of unclear lameness (4, 20, 21, 22).

**Case report 4**  
Hip injury in an African elephant  
An African elephant *L. africana* was observe to move stiffly while walking on the outside enclosure. The senior caretaker assumed that the problem must come from the hip area. He had observed the mounting of this cow by the bull the day before. Infrared-thermography confirmed the assumption of the caretaker. The female elephant showed a localised intense heat radiation over the left ilio-sacral joint. The elephant was treated over several weeks by the local vet and followed-up with infrared-thermography for several weeks. A reduction of the heat area was found.

**Case report 5**  
Carpal joint injury in an Asian elephant  
During the establishment of the hierarchy in a group of female Asian elephants *E. maximus*, the elephants were separated through vertical bars for part of the day and during the night. A caretaker observed two elephants fighting through these bars. One of the elephants was found lame afterwards. The location of the injury, however, was not clear. Infrared-thermography detected the heat radiation area to be just above the carpal joint on the inside leg, hence most likely a soft tissue contusion was the cause. After the exact localisation the elephant was treated locally with good results.

**Case report 6**  
Hind leg injury in an African elephant  
An African elephant *L. africana* was found lame one day without anyone having observed an accident. This animal was the oldest individual of the group, with only young elephants below 6 years as companions. Hence, a hierarchical fight could be ruled out. Infrared-thermography revealed no changes in heat radiation over any joint, but a change in radiation over the skin connecting the left knee with the rump of the animal. This intensified heat radiation was visible from the side, the front and from under the animal. Hence it was inferred that the animal had no joint injuries but had acquired a muscle fibre rupture. As there was some ice on the ground of the outside enclosure it seemed most likely that the animal had slipped on this ice and overstretched and torn some of the adductor muscles in the left knee region. Local treatment improved the condition soon.

**Foot problems in elephants**

**Case report 7**  
Pododermatitis in an Asian elephant  
As is known from many elephants in captivity, they develop lesions on their toenails quite often (2, 8). There are multiple reasons for this. Often, however, the beginning of this problem goes by unnoticed, and hence treatment only starts at a late age of the lesions. In the following case, the b-
sions were observed with infrared at an early stage, and altering therapies were followed up closely over a two-year period. As can be demonstrated with the infrared images, the first therapy was not successful. The inflammation of the nails increases to include the leg fare beyond the carpal joint at the end of the first year of observations. Then the treatment was changed to a more radical surgical removal of altered nail tissue and included also the use of stronger antiseptic solutions. After the second year, the infection was reduced, but kept coming back periodically, as infrared further observations revealed. Here again a lot of basic studies are needed in the future to establish the use of infrared-thermography in observing pododermatitis in elephants and also to evaluate the current treatment used.

**Inflammation diagnosis in a rhino**

**Case report 8**  
**Lameness evaluation in a black rhino**  
A lameness in the front left leg was observed in an old hook-lipped rhinoceros. No thorough investigation was possible without immobilisation. As the risk of immobilisation in this old animal was too great, infrared-thermography was used as preliminary diagnostic tool. Thermography revealed the problem not to be in the front left foot, where there was a slight increase of heat radiation, but instead in the right knee area. A severe heat area was observed over the knee joint and the femoral bone. It was inferred that a muscle fibre rupture (*Musculus biceps femoris*) was the reason for the lameness. The observed lameness in the front left leg was compensatory. This case shows, that infrared-thermography is a valuable tool in the observation of the complete body surface of an animal and that hidden injuries can be detected, if they are located close to the surface (9, 17, 18).

**Inflammation diagnosis in giraffes**

**Case report 9**  
**Polyarthritis in giraffe**  
A giraffe was observed with severe joint problems over many years. The animal seemed stiff in the joints at times. During such times the crackling sounds in the joints were intensified. As the treatments did not improve the conditions, it was decided that euthanasia was the only solution. Two weeks before this took place it was possible to conduct an thermographic investigation on this animal. Thermography revealed severe inflammations over all joints in the front legs and in all joints of the back legs down to the hock joint. The joints distal from the hock showed no increased heat radiation. Necropsy confirmed the inflammations of the joints (23), as observed in infrared-thermography.

**Case report 10**  
**front leg injury in a giraffe**  
This very recent case concerns a giraffe with an increased heat radiation in the front left leg, but without an observed lameness. Periodically this animal shows this heat increase, sometimes emitted over the whole front left leg, sometimes only over the distal parts of the leg, sometimes disappearing altogether. This animal has scissor claws in the front left leg. Currently this reappearing increased heat radiation is followed using infrared-thermography and the debate continues whether or not to immobilise this animal in order to correct these claws. The newest findings will be discussed in the presentation.

**Inflammation diagnosis in a hippopotamus**

**Case report 11**  
**Front leg injury in a hippopotamus**  
One day the male hippopotamus suddenly showed a lameness of the front right leg. No accident was observed previously. The animal was so severely injured that he would not come out of the water at feeding times. In order to localise the problem infrared-thermography was used. The ani-
The animal was given a strong painkiller and later on allowed to come out of the water. The minute the animal came out of the water, the radiation over the right carpal joint started to increase disproportionally from that over his other legs or that of the females. Within minutes the carpal joint was “red-hot” compared with the “green” colour of the other legs. A temperature difference of more than 3.0 °C were measured 15 minutes after the hippo came out of the pool. In a horse a temperature difference of 3.0 °C or more is always considered pathological (12), even but differences of 1.0 °C are often considered pathological already (18). A second infrared investigation 11 days later revealed that the leg has not improved yet. The carpal joint still shows a tremendous increase of heat radiation beginning to show up the minute the animal comes out of the water and feeds on land. Here the discussion has to be continued whether or not another treatment should be applied to this animal, or whether the giving of painkillers actually hinders the healing of the leg. As the animal continues to utilise the leg, then without pain, maybe this hinders the healing process.

Discussion

The application of infrared-thermography has led to good results in detecting processes, which were associated with local or general changes in surface temperatures. For instance in the areas of joint inflammation, in traumatology, in localising the area of the origin of a lameness, or in the supervision of a healing process in an elephant with pododermatitis. An earlier investigation of an elephant with pododermatitis showed the same good results using infrared-thermography (2). Infrared-thermography enables the investigator to detect pathological processes in an early stage, and allows an evaluation of the applied therapy. This was already found to be very valuable in racehorse medicine in Great Britain (9, 16, 20), but also in pigs good results were achieved (14) or even in dogs (4). Even in companion animals, when other technique do not solve the case, infrared-thermography may help lead the investigator to investigate areas he/she missed before (4).

The great potential of using infrared-thermography in lameness evaluation was discovered in horse medicine in the United Kingdom a long time ago (11, 12, 13, 17, 18, 19). On racecourses this technique is often used as a preventative method for detecting of potential problems before they manifest themselves in form of a visible lameness. In lameness evaluation it is essential to evaluate both sides with the infrared-camera under the same conditions and within a close time frame. In horse medicine the legs of the animals are placed parallel to each other and then infrared-images are taken from the front an the back of the legs. This is the easiest way of investigation, as it allows direct comparison between both hind- and forelegs. In horse medicine it is easier to detect slight temperature changes then in zoo animals. Artefacts from uneven weight distribution over the legs can be minimised in horses by placing the legs exactly next to each other (17, 18, 19). This is not possible in zoo animals. Therefore temperature changes in zoo animals should only be evaluated if they are more then 1.0 °C different and continue to be there even if the animal shifts the weight from one lag to the other. Temperature differences of less then 1.0°C difference require a lot of experience from the investigator when interpreting infrared-images (7).

Infrared-thermography seems to be a valuable diagnostic tool in localising areas of tissue injuries or other forms of inflammations. Infrared-thermography can, however, not identify the cause of the injury, e.g. an infection or a simple distortion. If this information seems absolutely necessary than other conventional diagnostic tools have to be used. In case of lameness evaluation in Megaherbivores infrared-thermography seems to be an indispensable tool already today, as it can give information on the localisation of a problem that can be obtained with almost no other means (7).

References

A CONTRIBUTION TO THE PHYSIOLOGICAL BLOOD PARAMETERS AND INTESTINAL FLORA WITH COATIS (Nasua nasua)

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Abstract
A group of 9 South American coatis (Nasua nasua) had to be immobilised when being placed into a new enclosure. They were immobilised with the Hellabrunner mixture. As there are no facts concerning the physiological blood parameters and intestinal flora this operation was used to collect the corresponding data. The blood parameters were determined with the Vettest 8008 and the VetLyte by Idexx as well as a haematocrit centrifuge. The faeces samples were incubated aerobically and anaerobically and the germs were determined.

Zusammenfassung

Résumé
Un groupe de 9 coatis (Nasua nasua) a dû être endormi dans le cadre d’un déplacement. On a employé le mélange d’Hellabrunn. N’ayant pas de données concernant les paramètres sanguins physiologiques et la flore bactérienne fécale, on a profité de l’action pour obtenir des renseignements à ce sujet. Pour déterminer les paramètres sanguins sérologiques, on a employé le Vettest 8008 et le Vetlyte de la firme Idexx. Les prélèvements des excréments ont été cultivés en aérobie et anaérobie et on a déterminé les germes.

Key words: Nasua nasua, coati, blood parameters, physiological intestinal flora

Introduction
Zoos like to have coatis because of their permanent activity and sociability. They hardly ever become ill and are easy to keep. Because there is no information about the physiological blood parameters and the intestinal flora rectal swab and blood samples were taken from the immobilised animals when they were placed into their new enclosure.

Material and Method
It was a group of 9 clinically healthy South American coatis (Nasua nasua) consisting of 4 male and 5 female animals. The first two animals got the recommended dose of 0.2 ml Hellabrunner
mixture (5). This dose however only tranquillised them so that they needed more. The rest of the group – male and female adult animals – therefore got 0.4 ml Hellabrunner mixture. This dose caused a deep immobilisation within 5 to 10 minutes so that all manipulations (implanting chips, taking blood and rectal swab samples) were possible. After shaving the medial part of a front leg blood was taken from the V. cephalica antebrachii and collected in a Lithium-Heparin and an EDTA-Potassium coated Monovette® and brought to the laboratory immediately. There the EDTA-Potassium Monovette® was gently tossed for a couple of minutes in order to mingle the sample evenly. Then blood was put into a haematocrit capillary and centrifuged for 8 minutes in the haematocrit centrifuge and the result determined. The Lithium-Heparin Monovette® was centrifuged to extract the plasma which then was analysed in the Vettest 8008 and the VetLyte. Basis for the analysis was the great health profile supplemented by the creatinine kinase. The faeces was taken with a sterile Culturette® (Beckton Dickinson) and kept in the fridge until further use. The samples were then smeared out on the following culture media: E.coli-ident Agar, VRBD-Agar, XLD/BPLS-Agar, DCRM-Agar and the Slanetz & Bartley Agar. The incubation lasted 24 hours at 37° C aerobically, anaerobically with the DRCM-Agar and under COO-atmosphere with the VRBD-Agar.

Results

The results are presented in the following tables.

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+/- : Up to 10 colonies  
+ : 10-30 colonies  
++ : 30-100 colonies  
+++ : > 100 colonies  
++++ : Completely growth dense

**Discussion**

In this study only the haematocrit was determined as haematological parameter. Baronetzky-Mercier (2) has published 2 determinations of haematocrit of the small coati (N. olivacea) which both are a little bit higher. There were no serological blood parameters for coatis so far – only for the racoon. The cholesterol level in this study (2) seems to be rather high but also the racoon has levels between 3,26-7,06 mmol/l respectively 6,67 mmol/l. The CK-level is also very high. Baronetzky-Mercier (2) has no CK-level for racoons but an LDH-level, which also informs about the muscular exertion. This LDH-level of 1360-1630 U/l is also unexpectedly high compared to small pets like cats and dogs. This can be caused by the muscle activity during capture and sedation. The high blood sugar level too could have been caused by stress during immobilisation. These levels are not as high with racoons (2) where the levels are between 2,83 mmol/l respectively 3.50 mmol/l. The ALT-level too is considerably higher with racoons (2), but as the ALP-level is lower one can assume that the high ALT-level is physiological. The Ca:P-ratio is corresponding to the ratio with cats and dogs (1). The determination of the germs in the rectal swab samples showed nothing special. Although the animals had regularly been fed with whole chicken no salmonellas could be found. There were also no clostridia, whereas enterobacteriaceae, E. coli, coli-like germs and faecal streptococci were found in different frequencies.

**References**

NONOBSTRUCTIVE DYSTOCIA INVOLVING A COAGULASE E-POSITIVE STAPHYLOCOCCUS IN A MADAGASCAR GROUND BOA (Acantophis madagascariensis)

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Abstract
A coagulase-positive Staphylococcus has been cultured from the liver and lung of the foetus of a Madagascar ground Boa (Acantophis madagascariensis). The strain of the bacterium was described different from S. aureus but was not characterised further. This is the first reported case of dystocia in a reptile involving a pathogen Staphylococcus sp.

Zusammenfassung
Ein coagulase-positiver Staphylokokkus wurde aus sowohl der Leber als auch der Lunge eines Fetus einer Madagaskar-Boa (Acantophis madagascariensis), kultiviert. Der Stamm dieses Bakteriums wurde anders beschrieben als ein Staphylokokkus aureus, aber nicht näher karakterisiert. Dies ist der erste Fall in dem über Dystocia bei Reptilen gesprochen wird, wobei pathogene Staphylokokken sp. im Spiel sind.

Résumé
Un Staphylococcus coagulase positif a été isolé à partir du foie et du poumon d’un avorton de Boa de Madagascar (Acantophis madagascariensis). L’espèce, décrite comme différente de S. aureus n’a pas été définie plus précisément. Ce cas représente la première description d’une dystocie impliquant un staphylocoque pathogène chez un reptile.

Key words: Reptiles, Acantophis madagascariensis, dystocia, Staphylococcus, reproduction

The case

Commemorative data
These data concern a wild born Madagascar Ground Boa female given by Customs on the 19th December 1996. At its arrival, it has been sexed and received an anthelmintic treatment (levamisole, 7 mg/kg); it weighted 4.8 kg and was pregnant. On the 3rd February 1997, it laid 12 hatchlings. They have been each all bred. Although the female refused every feeding, its health condition was good. We could attribute its anorexia to several parameters: the captivity stress, the pregnancy and some bad atmospheric conditions. We also knew that ground boas could fast several months when weather is dry in Madagascar. So we increased temperature, moisture and artificial sun light duration. In September 97, to set off appetite, 2 injections of 0.5 ml of Trophobolene (nandrolon 6,67 mg (1,67 mg/kg) + hydroxyprogesteron 6,67 mg (1,67 mg/kg) and 1 injection of 2,5 ml of METHIO-B12ND (a complex mixed of acetyl methionin + Larginin + hydroxycobolamin acetate) were done. It was then regularly force-fed twice a month with about ten mice until the beginning of 1999. Finally, in April 1999, it ate a pigeon. Its weight reached 6 kg at the end of August 1999.
In October 2000, once again, it showed anorexia. After clinical examination a new pregnancy was supposed. The female weighted 5.6 kg. Radiographs and ultrasonography confirm the pregnancy. Unfortunately, at this time, it showed some respiratory disorders for which it received an antimicrobial treatment with 24 mg (4.36 mg/kg) trimethoprim and 120 mg (21.8 mg/kg) sulfadiazine, daily during 10 days, and 6 mg of flunixin meglumine (1,1 mg/kg) the first day and 2.5 ml of Methio-B12\textsuperscript{ND} at the end of the treatment. In spite of this medical care, it showed anaemia and important emaciation. At the end of January bad health condition increased, which obliged us to rehydrate it, after what, it ate finally one pigeon. After its sloughing a cutaneous septic blister was discovered, cured with local ointment with neomycin. The 30\textsuperscript{th} March it showed a dropping of one dead foetus, a radiography showed six eggs and perhaps another one. On the 7th April, it laid one lifeless underdeveloped hatchling, weighting 105 g but no more foeti were laid afterwards.

**Differential diagnostic**
Non-obstructive dystocia may have many different aetiologies, although very few have been shown to truly cause dystocia (4). However, for this case, we can suggest some hypothesis. The past underfeeding and underweight of the animal may have led to poor muscle tone and/or an imbalanced diet and a lack of essential nutrients, especially calcium disorders. The observation of the high decomposition state of the foeti and the past respiratory disorders suggest an infectious cause.

**Treatment**

**Non surgical attempts**
Injections with 4 UI (1 UI/kg) oxytocin and 860 mg (215 mg/kg) of calcium have been given. Because this treatment was not successful, the foetuses were surgically removed.

**Surgical treatment: caesarean**
The snake has been anaesthetised with 400 mg of ketamin intramuscularly (93 mg/kg). The amount was sufficient for the surgery. An incision of about 20 cm was made between the second and the third row of lateral scales: first the skin, the muscle, the coelomic sera and finally oviduct wall were incised. After removing five dead foetuses, the oviduct wall and the were closed with an absorbable suture; the skin was closed with non absorbable sutures. As soon as the foetuses were removed, they were sent entirely to a laboratory for bacterial examinations. An antibiotic was administrated in the oviduct prior to closing (marbofloxacin, 10 mg/kg) and followed by intramuscular injections of 10 mg/kg of marbofloxacin daily for 15 days. Besides, for one month the animal is regularly hydrated with a mixed 50/50 NaCl à 0,9% and Glucose 5% (10 ml/kg every 3 days) with subcutaneous injections, maintained hospitalised with a constant temperature fixed at 29°C and with a regular photoperiod.

**Evaluation post operative**
Six days later, the general condition was progressively deteriorating, the animal showed increasing hypotonia. At this point, the antimicrobial agent was changed: marbofloxacin was replaced by gentamycin at 10 mg/kg every 48h for 12 days. The animal restart to eat 38 days later and 42 days later, the cutaneous stitches were removed. No more complications were seen. Today, the animal is fine and healthy.

**Bacteriological results**
The foetuses arrived entirely at the laboratory and they were autopsied. Bacterial cultures were made from biopsies from the liver and the lung. A pure culture of coagulase positive *Staphylococcus* could was identified. According to the laboratory, the cultured bacterium was no *Staphylococcus aureus* But further determination was not done.
Conclusions

The conditions under which the cultures were made, may exclude any contamination. Therefore we suggest that the bacterium may be directly involved in the dystocia. In the mean time, the hypothesis involving the past underfeeding and underweight of the animal has not been confirmed. We may conclude that in this case, we may have found several causes for the dystocia but the contribution of each of them is uncertain.

Discussion

Dystocia

Dystocia is the most common reproductive disorder encountered in captive reptiles. The true prevalence in each species is unclear. Some references showed that dystocia occurs more in snakes, where other suggest that it touches more the chelions. (4) In oviparous species with recent history of oviposition and visual presence of a caudal located mass, the diagnosis of dystocia is usually not difficult. However, in large pythons and viviparous snakes, the presence of eggs or foetuses is often more difficult to diagnose. X-rays or ultrasonography can be used to confirm the presence of eggs or youngs.

Surgical management of dystocia is indicated when medical management has failed to relieve the dystocia. In this case, it was an emergency. We didn’t try to attempt manual removal of the young. The undelivered autolytic embryos may cause infection, uterine rupture and peritonitis. This condition usually is manifested by progressive abdominal distension and is often fatal. If the female survives, sterility can result from scarring of the oviduct. Death generally occurs one or more days after obstruction has become apparent. When abdominal distension is present, surgery should be performed promptly to avoid fatal peritonitis.

Bacterial diseases and antimicrobial Drug Selection in Reptiles

Bacterial diseases are an important cause of morbidity and mortality in captive reptiles. Although a wide variety of bacteria have been incriminated as either primary or secondary pathogens, infections caused by Gram-negative bacteria are most common: Aeromonas hydrophila, Klebsiella oxytoca, Morganella morganii, Providencia rettgeri, Pseudomonas aeruginosa, and Salmonella arizonae. Bacterial disease is almost always secondary to immunosuppression e.g. as a complication in viral infections (15). In a general study of infectious diseases in Reptile, Gram-negative bacteria represent 94% of identified bacterial pathogens, the Gram-positive 1,4 % and the rest are yeast and fungus (27). Culture from the affected organ may aid in the formulation of the diagnosis. Blood cultures are warranted if septicaemia is suspected.

Specific bacterial infections reported with dystocia include Salmonella arizonae, Monocercomonas sp., Aeromonas sp., Pseudomonas sp. and Proteus sp ...(4). This case is the first report of dystocia involving Staphylococcus sp.

Staphylococci are widespread in the environment as well as in the air, the soil or in water. Infections with Staphylococcus sp. are uncommon in reptiles. The bacterium has been found in some case of blister disease in snakes (14). Aeromonas hydrophila may some time be associated with Staphylococcus aureus in infectious stomatitis in snakes (2). Some case of enteritis involving Clostridium and Staphylococcus are described in iguanas (1). Staphylococcus aureus in association with Pasteurella multocida may cause pneumonia in snakes and tortoises (5).

The important variety of species within the class Reptilia (leading to a wide diversity of the behavioural and physiological features) make the selection of the appropriate antimicrobial agent more difficult than for mammals. Since limited pharmacokinetic studies of only some antimicrobial agents have been performed in only a small number of reptilian species, dosage is either extrapolated from one species or is empirical (15). Antimicrobial drugs for common infecting agents in lizards and snakes are listed in table 1.

In Hoskins et coll. publication, 18 antimicrobial agents have been tested to measure the sensibility of 71 strains of Staphylococci (13). The groups of agents the less regularly active included penicillin, ampicillin, tetracyclin and sulfamids. The most regularly active agents were meticillin, amoxycillin, cefalotin, carbenicillin, gentamycin and niftrofurans.
We may suggest here that for this case, gentamycin at 10 mg/kg every 48h seemed to show great efficacy to treat a *Staphylococcus sp.* infection.

**Reptiles, abortion and zoonosis**

Captive reptiles are routinely identified as reservoirs of *Salmonella spp.* and reports of reptile-associated salmonellosis are increasing (24). Other zoonotic agents from Reptiles may be: *Mycobacterium sp.*, *Campylobacter sp.*, *Enterobacter sp.*, *Klebsiella sp.*, *Pasteurella sp.*

As a rule, when there is abortion, we first search for infectious pathological agents that could represent a zoonosis. The laboratory found a positive coagulase *Staphylococcus*. And the only thing that was important is to know whether it is or not a *S. aureus*. In this case it was not and the investigation has not gone further.

**Reproductive data for madagascar ground boa (Acrantophis madagascariensis)**

*Acrantophis madagascariensis* and *Acrantophis dumerili* occupy a unique position in reptile conservation. Both species of *Acrantophis* have been noted to be locally abundant in Madagascar. *Acrantophis dumerili* and *A. madagascariensis* are protected in Madagascar. There is some local exploitation of these boas for their skins. The reproductive husbandry for both species of *Acrantophis* is similar. *Acrantophis dumerili* has been bred regularly in recent years, and is easier to breed in captivity than *A. madagascariensis*. Gestation periods range from 8 to 9 months for *A. madagascariensis*. Litter sizes range from two to twenty-one, and one to eight for *A. madagascariensis*. *A. madagascariensis* neonates are much larger than *A. dumerili* neonates. Neonates feed readily on traditional boa foods.

Sexual maturity in *Acrantophis dumerili* occurs at about 4 to 5 years for captive-born females, and 3 to 4 years for captive-born males. Second-generation births are reported. A twenty-five year old female has been bred successfully, and a fifteen year old male also has continued to be fertile (28).

**Acknowledgements**

Thanks to Claire Réjaud, Jean Marc Martin, Pascal Tillaud and Gérard Dousseau

**References**

Table 1: Antimicrobial drug selection for common infecting agents in lizards and snakes (15)

<table>
<thead>
<tr>
<th>Infecting agent</th>
<th>Suggested drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>Amikacin/ceftazidime, enrofloxacin, piperacillin, Trimethoprim/sulfadiazine, ciprofloxacin</td>
</tr>
<tr>
<td><em>Bacteroïdes</em></td>
<td>Metronidazole</td>
</tr>
<tr>
<td><em>Citrobacter</em></td>
<td>Amikacin/ceftazidime, enrofloxacin</td>
</tr>
<tr>
<td><em>Clostridium</em></td>
<td>Metronidazole</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Amikacin/ceftazidime, enrofloxacin, Trimethoprim/sulfadiazine, ciprofloxacin</td>
</tr>
<tr>
<td><em>Fusobacterium</em></td>
<td>Metronidazole</td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>Amikacin/ceftazidime, enrofloxacin</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>Amikacin/ceftazidime, piperacillin, enrofloxacin</td>
</tr>
<tr>
<td><em>Neisseria</em></td>
<td>Amikacin/ceftazidime, enrofloxacin</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>Amikacin/ceftazidime, enrofloxacin</td>
</tr>
<tr>
<td><em>Providencia rettgeri</em></td>
<td>Amikacin/ceftazidime, piperacillin, enrofloxacin, gentamicin</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Amikacin/ceftazidime, enrofloxacin, gentamicin, Trimethoprim/sulfadiazine, ciprofloxacin</td>
</tr>
<tr>
<td><em>Salmonella arizona</em></td>
<td>Amikacin/ceftazidime, piperacillin, enrofloxacin, Trimethoprim/sulfadiazine, ciprofloxacin</td>
</tr>
<tr>
<td><em>Serrata</em></td>
<td>Metronidazole</td>
</tr>
</tbody>
</table>
Table 2: Dosage for antimicrobial drugs in some precise case

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Dose and route of administration</th>
<th>Once every</th>
<th>Studied reptiles</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>7 mg/kg, IM</td>
<td>24 h</td>
<td>Crotalus adamanteus</td>
<td>19</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>400 mg/kg, IM</td>
<td>24 h</td>
<td>Boiga dendrophila, Lampropeltis getulus, Python reticulatus, Elaphe guttata, Elaphe obsolete</td>
<td>21</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>80 – 100 mg/kg, IM</td>
<td>48 to 72 h</td>
<td>Python curtis</td>
<td>12</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>20 mg/kg, IM</td>
<td>72 h</td>
<td>Elaphe sp., Boiga sp., Python reticularis, Python m. bivittatus</td>
<td>1, 3, 20</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>6 mg/kg, IM</td>
<td>72 to 96 h</td>
<td>Trachemys scripta elegans</td>
<td>25</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10 mg/kg, IM</td>
<td>48 h</td>
<td>Chrysemis picta belli, Pseudemys scripta elegans</td>
<td>6, 16, 19, 25</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>2 – 5 mg/kg</td>
<td>72 h</td>
<td>Pituophis melanoleucus</td>
<td>19</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>2,5 mg/kg, IM &amp; SC</td>
<td>72 h</td>
<td>Neroida sp., Pituophis sp.</td>
<td>3, 8, 9, 22</td>
</tr>
<tr>
<td>Amikacin</td>
<td>5 mg/kg, IM</td>
<td>48 h</td>
<td>Gopherus sp.</td>
<td>10</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1 mg/kg</td>
<td>48 h</td>
<td>Python curtis</td>
<td>17</td>
</tr>
<tr>
<td>Amikacin</td>
<td>5 mg/kg then 2,5 mg/kg, IM</td>
<td>72 h</td>
<td>Pituophis sp.</td>
<td>23</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>40 mg/kg, SC</td>
<td>24 h</td>
<td>Pituophis melanoleucus</td>
<td>7, 19</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>50 mg/kg, SC</td>
<td>12 h</td>
<td>Drymarchon couperi, Elaphe sp., Lampropeltis sp.</td>
<td>11</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>50 mg/kg, SC</td>
<td>24 h</td>
<td>Boa constrictor, Python m. bivittatus</td>
<td>11</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>50 mg/kg, SC</td>
<td>36 h</td>
<td>Heterodon sp., Agkistrodon contortrix</td>
<td>11</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>50 mg/kg, SC</td>
<td>48 h</td>
<td>Crotolid sp., Agkistrodon piscivorus</td>
<td>11</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>50 mg/kg, SC</td>
<td>72 h</td>
<td>Neroida sp.</td>
<td>11</td>
</tr>
<tr>
<td>Enrofloxacine</td>
<td>5 mg/kg, IM</td>
<td>12 h</td>
<td>Geochelone elegans</td>
<td>26</td>
</tr>
<tr>
<td>Enrofloxacine</td>
<td>10 mg/kg, IM</td>
<td>24 h</td>
<td>Testudo hermanni</td>
<td>29</td>
</tr>
<tr>
<td>Enrofloxacine</td>
<td>6,6 mg/kg, IM</td>
<td>24 h</td>
<td>Python reticulatus</td>
<td>18</td>
</tr>
<tr>
<td>Enrofloxacine</td>
<td>11 mg/kg, IM</td>
<td>48 h</td>
<td>Python reticulatus</td>
<td>18</td>
</tr>
</tbody>
</table>
UNUSUAL FINDINGS IN A COTTON-HEADED TAMARIN \textit{(Saguinus oedipus)} WITH CHRONIC INTERSTITIAL NEPHRITIS: A CASE REPORT

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Abstract

A description of the clinical and post-mortem findings in a cotton-headed tamarin \textit{(Saguinus oedipus)} with chronic interstitial nephritis is given. In this case unusual locations of metastastic calcifications adjacent to the large bones were observed.

Zusammenfassung

Hier wird eine Beschreibung von den klinischen und pathologischen Befunde in einem Lisztäffchen \textit{(Saguinus oedipus)} mit chronischer interstitieller Nephritis gegeben. In diesem Fall wurden ungewöhnlich, nämlich neben den Röhrenknochen localisierten metastatischen Verkalkungen beobachtet.

Résumé

Une description des symptômes cliniques ainsi que la pathologie d’un pinché \textit{(Saguinus oedipus)} avec une néphrite chronique interstitielle est donnée ici. Dans ce cas, une localisation inhabituelle des calcifications metastatiques, à proximité des os longs, fut remarquée.

Key words: callitrichidae, cotton-headed tamarin, \textit{Saguinus oedipus}, chronic interstitial nephritis, metastatic calcifications.

Introduction

Chronic interstitial nephritis (CIN) is principally known as a disease of dogs and cats (1) but can also affect other animals like birds (5), primates (3) and reptiles (2). It is suggested that frequently an acute form of the disease precedes the fatal chronic diffuse phase and the acute form is probably always a complication of some systemic infection (7). In CIN the kidneys are usually shrunken, pale and firm. The capsule is usually thickened and adherent, cysts may be present anywhere in the organ (1). The alterations of the kidneys finally result in chronic renal failure (CRF). Hyperphosphataemia and renal secondary hyperparathyroidism (RSH) are commonly observed consequences of CRF due to a reduced excretion (8). The lowering of the serum calcium concentration is not only the result of the increase of phosphates but also the inability of the damaged kidney to produce the active metabolite of vitamin D (6). Soft tissue calcification is a regular feature and appears to most commonly affect the lungs, kidneys, arteries, stomach and myocardium. Other laboratory findings in CRF are acidosis, anaemia, azotaemia, hypokalaemia, and proteinuria (8).

The purpose of this report is to describe the unusual clinical presentation and pathologic findings associated with CIN in a cotton-headed tamarin \textit{(Saguinus oedipus)}.
Case Report

A 0.413 kg, 11 year old female cotton-headed tamarin was reported to be lethargic. On examination the animal was lean despite reported normal appetite, the coat was ruffled and not shiny. The animal was housed in a group of six tamarins in an inside enclosure with free access to an outdoor enclosure. The main part of the diet of the tamarins consists of a variety of fruit and vegetables. In addition crickets, mealworms, grasshoppers, boiled cow meat or eggs are given as well as a vitamin/mineral supplement. They are regularly vaccinated against *Yersinia pseudotuberculosis*.

The blood sample of the tamarin revealed anaemia (PCV 24 %), lymphocytopenia (7%), azotemia (BUN 88.9 mmol/L), an elevated creatinine level (233 µmol/L), hypoalbuminaemia (16.8 gm/L) and an increased level of alkaline phosphatase (1130 U/L). Liver enzymes ALAT and ASAT were within normal limits as well as total bilirubin (ISIS Reference Values). In the blood smear polychromatic erythrocytes and anisocytosis were the main findings.

Radiographs of the whole body revealed enlarged kidneys, increased visibility of the large arteries around the heart, and four large radio dense masses symmetrically adjacent to both humeri and femurs. Smaller radio dense masses were seen on the thoracic wall, adjacent to the ribs (Fig. 1). The results indicated arteriosclerosis and kidney disease. The masses adjacent to the bones could not be easily interpreted. Before further examination could be performed the animal died.

Pathology

Necropsy revealed pale, firm and enlarged kidneys disseminated with very small cysts with a maximum diameter of 1 mm. The lung had a normal consistency and showed many tiny black spots. The A. pulmonalis and the aorta were very firm, the latter had cream coloured circular stripes (distance 1-2 mm) till 2 cm cranial of the Aa. iliacae externae. Adjacent to both humeri and femurs white irregular firm masses were found with a maximum size of 2 x 1 cm (Fig. 2). Smaller quantities of the same material were present on the external side of the thorax. The liver was slightly enlarged and rather firm. Otherwise no significant findings were observed.

Histology

The heart showed no changes, but the large vessels showed a degenerative calcification of the media. The smooth muscle of the media is focally replaced by pale-staining, acellular, hyalinised fibrous tissue. The lungs showed fine lines of metastatic calcifications in the interstitium and some focal anthracosis.

Fig. 1: radio dense masse on the thoracic wall (a) and masses symmetrically adjacent to both femurs (b). Fig. 2: adjacent to the femur a white irregular firm masses with a maximum size of 2 x 1 cm (arrow s).
The liver had stored much iron in the hepatocytes. The hepatocytes showed anisokaryosis and many nuclei showed peripheral condensation of chromatin on the membrane. The kidneys showed chronic interstitial nephritis resulting in interstitial fibrosis, irregular loss of tubular tissue and cyst formation. In the interstitium, at the basal membranes of tubuli and in the tubular lumina often fine granular to amorphous or crystalline of von Kossa’s staining positive calcium deposits were observed (Fig. 3).

**Fig. 3:** The kidneys showing chronic interstitial nephritis resulting in interstitial fibrosis (left HE), at the basal membranes of tubuli a fine granular Kossa staining positive calcium deposits are present (right).

The white “masses” described in the macroscopy were histologically metastatic calcifications characterised as accumulations of fine Kossa positive granulae mixed with larger massive calcium crystals in connective tissue (Fig. 4).

**Fig. 4:** Metastatic calcifications characterised as accumulations of fine Kossa positive granulae mixed with larger massive calcium crystals in connective tissue (HE)

**Discussion**

The arteriosclerosis is most comparable to the Monckeberg medial sclerosis in man (4). This disorder occurs principally in older persons, and does not commonly lead to clinical disorder. The alterations in the vessels were not considered atherosclerosis because the fibrofatty plaques and necrosis were not present. Although the phosphorus level in the blood has not been determined it is assumed to be elevated because in general serum phosphorus concentrations parallel BUN concentrations (4,8). The macroscopy and histological findings indicate that this is a case of renal secondary hyperparathyroidism. Metastatic calcium deposits were as expected observed especially in the lung and kidney. In addition large calcium deposits were found adjacent to the femurs and humeri as well as the ribs. It seems likely that the large calcium deposits are also the result of renal secondary hyperparathyroidism. This unusual location has never been reported in literature as far as we know.

**References**

WASTING MARMOSET SYNDROME (WMS) IN A Callithrix geoffroyi MONKEY

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Abstract

Wasting Marmoset Syndrome (WMS) is a disease which appears very often in Callitrichidae kept in captivity. This syndrome shows a progressive development and a high mortality. The alterations in different organs are highly variable in degrees and severity. Nevertheless some special clinical symptoms and pathological alterations appear very often in this disease, therefore it is possible to define the WMS according to these symptoms. Clear causes for the appearance of WMS are not known. This report describes a fatal case of Wasting Marmoset Syndrome in a Callithrix geoffroyi monkey from the Zoological Garden of Magdeburg, its main symptoms were observed in the intestinal tract and in metabolic bone alterations. The case is discussed in connection with the definition of WMS.

Zusammenfassung


Résumé

Le syndrome de dépérissement du marmoset est une maladie survenant surtout auprès des marmosets vivants en captivité. Elle est accompagnée d’une aggravation progressive de l’état général, ici des différents systèmes d’organes sont atteints aux dimensions distinctes. Ce syndrome montre un grand taux de mortalité et est un problème sérieux pour des élevages divers. Ce rapport décrit un cas grave du syndrome de dépérissement du marmoset chez un singe (Callithrix geoffroyi) du jardin zoologique de Magdeburg. Les altérations principales se trouvaient dans la région des intestins (entérite catarrhale chroniquement active) et du système squelettique avec des fractures multiples. On discute ce cas en rapport avec la définition du syndrome de dépérissement du marmoset.

Key words: Wasting Marmoset Syndrome, Callithrix geoffroyi, metabolic bone disease, chronic active enteritis.

Case history and clinical features

In October 2001 a marmoset (Callithrix geoffroyi) aged 5,5 years housed at the Zoological Garden of Magdeburg was sent to the German Primate Center (DPZ). The animal was euthanised because of a poor prognosis. The monkey had a weight of 239 g and was announced with the following anamnesis: In July 2001 the animal's state of health was obvious for the first time. There it could hardly move and could not make any use of its left hindleg. It spent most of the time on the floor of
its cage. The animal steadily lost weight despite a consistently good appetite. Its coat appeared very unkempt and greasy and in some locations (especially on the tailbase) there were hairless areas. An x-ray of the left hindleg was done, which confirmed the provisional diagnosis of a fracture. This fracture was treated conservatively. The animal's state of health improved for a while, however, later its condition aggravated again. As a result of the long term deterioration of the general condition of this animal the euthanasia was decided.

Pathomorphological findings

Gross pathologic findings were a worse state of nutrition. The coat was very unkempt and greasy with hairless areas at the tail base. Both sides of the thigh muscle showed an advanced atrophy. Severe rachitic rosaries were observed in the costal arch region at the transition of bones and cartilages. Moreover the following multiple fractures in the region of the hind extremities were found: A completely covered older fracture proximal at the left femur with callus formation, lacking any stability. Another completely covered, older fracture was found in the region of the left tibia with callus formation distally to the knee-joint. In addition another fracture of the right femur above the knee-joint with callus formation was diagnosed, by that the fracture reached a certain limited stability. Moreover a severe callus formation in the area of the right tibia beneath the knee-joint was observed, here, too, an older fracture cannot be excluded. The other bones were in general very thin, fragile, transparent, soft and easy to cut. In the small intestine as well as in the large intestine there were signs of a chronic enteritis seen with moderate lymphatic hyperplasia of the mesenterial lymphnodes. The liver was enlarged. The histological examination showed a moderate chronic gastritis, aggregated bacteria in the superficial epithelium of the oesophagus as an expression of dysbacteria, and in the region of the large intestine a severe chronically active enteritis, a severe dysbacteria with severe aggregation of a mixed bacterial population. Furthermore a severe haemosiderosis of the liver with moderate hydropical degeneration of the hepatocytes was noticed. The microbiological examination showed Entamoeba sp. (+++), Giardia sp. (+++) and Trichuris eggs (+++) in the small intestine. In the large intestine there was only a mild verification of Entamoeba sp. (+). Also Escherichia coli were found in the small as well as in the large intestine.

Assessment

The given case history, especially the lost of weight despite of a normal food intake, the typical alterations of the coat and the macroscopical as well as the histological results are standing for the Wasting Marmoset Syndrome. Especially the main results in the intestinal tract in form of severe chronical active enteritis with hyperplasia of the mesenterial lymphnodes as well as the findings of the bones are often connected with a Wasting Marmoset Syndrome. Also the haemosiderosis of the liver, which is diagnosed in this animal, could be seen as an indicator for an haemolytic anaemia, which is very often seen in animals suffering from WMS.

Discussion

The Wasting Marmoset Syndrome (WMS) is a very important disease in marmosets (predominantly in Callithrix jacchus) kept in captivity (18). This syndrome shows a very high mortality (1, 2, 5, 6, 15, 17). The main symptom is the drastic weightloss while the food intake is still normal (2, 4, 6, 9, 10, 11, 12, 14, 15, 16, 17, 18) as also seen in this monkey. Instead of the weight loss poor weight gain and growth disorders were observed in young animals (7, 8, 9, 17). These monkeys differ also in the external appearance from healthy monkeys, which is shown in the quiet behaviour and on the other hand in alterations of the coat. The coat seems to be wet and greasy and looks very unkempt (10, 13, 14, 15, 16, 17). The monkeys loose also a part of their coat. The hairless-locations are particularly on the tail and on the head. No hairloss is seen on other locations of the body (7, 9, 10, 11, 13, 14, 15, 17). In a few cases metabolic bone alterations were described. These bones are very thin and soft, especially these from the roof of skull (11, 16).
This case is characterised by an extremely severe bone metabolic disorder. In the course of the disease many monkeys show also an atrophy of the skeletal muscle, leading to dysfunctions of the leg movement and sometimes complete paralysis, especially of the hindlegs (1, 9, 10, 11, 14, 15, 16, 18). In general the animals are very inactive and depressed (2, 7).

The intestinal system of monkeys suffering from WMS is also very often concerned. Chronic or intermittent diarrhoea is frequently seen (1, 3, 10, 13, 14, 16, 18). In most cases the faeces have a yellow colour and the consistence is thin and foamy. In a few cases the faeces are also fatty. In the course of the disease alterations in the faecal microflora are seen. The concentration of putrefactive micro-organisms like clostridia increases and the concentration of lactobacillaceae decreases (8, 14).

The characteristic histological findings are alterations in the intestinal system, as also seen in this case. In most of the cases a chronic colitis is described but cases of general enteritis are also mentioned (5, 9, 15, 16, 19). The intestinal alterations, which are diagnosed in this case probably, have caused a maldigestion, which is the reason for a metabolic disorder of calcium, phosphor and vitamin D3. Because of this disorder the metabolic bone alterations can be explained.

Almost all monkeys with WMS have a haemolytic anaemia, which is usually normochrom/normocytic and is clinically shown in pale mucous membranes (9, 15). The haemosiderosic alterations of the liver in this case can be interpreted as a result of a haemolytic anaemia. The results of the blood check-up are hypoproteinemia (hypalbuminemia) and an increase in serum aspartate-aminotransferase as well as an increase in alcalic phosphatase (2, 9, 14, 15, 17). Further histopathological findings are for example nephritis, pancreatitis and fibroses of the pancreas, skeletal muscle degenerations and alterations of the liver (4, 11, 16, 19).

Clear causes for WMS are not known. Probably it is a multifactorial disease. The following possibilities are discussed: Infections with Trichospirura leptosoma (2, 12), deficiency situations (in most cases there is a deficiency of protein mentioned) (1, 4, 14, 16, 19), hypervitaminoses (7) and also stress is mentioned as a reason for WMS (13). External factors like deficiency of space, movement or possibilities to play are also discussed. A genetic disposition cannot be excluded either (7, 18).

As there is no known monocausal aetiology of WMS, it is very difficult to find a uniform therapy. Rather a special emphasis of the symptomatical treatment of the clinical symptoms and the improvement of the animal’s general condition must be given. In several cases possible causes of this disease, which should be treated, are known. There are also special clinical symptoms and pathological alterations, like already mentioned, which appear very often. Their treatment could be used as a basic therapy.

Concluding it can be said that WMS is an important disease in callitrichidae with a high clinical relevance. But so far there are still many aspects, which are not known. Further research is necessary to improve our knowledge in the diagnostic and therapeutic fields of the described syndrome. Our case of WMS is particularly worth mentioning because of its severe metabolic bone alterations.

References


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THREE CASES OF CYSTIC LIVER ALTERATIONS IN A COLONY OF COTTON TOP TAMARINS (*Saguinus oedipus*)

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**Abstract**
Within the cotton top tamarin breeding colony of the German Primate Center three cases of cystic alterations of the liver occurred during the last two years. In one case, the cysts replaced nearly the whole hepatic tissue, whereas in the other two cases the alterations were restricted to capsular areas. The clinical and pathomorphological findings are described and possible origins are discussed. The alterations are presumed to be malformations corresponding to the cystic liver and “von Meyenburg complex” known in humans.

**Zusammenfassung**

**Résumé**
Nous avons observé trois cas d’altérations kystiques de la foie dans l’élevage de singes Liszt du Centre Allemand de Primates. Dans un cas les kystes ont remplacés presque la totalité du tissu hépatique, tandis que les altérations étaient limitées sur la capsule hépatique dans les deux autres cas. Ici nous décrivons les altérations cliniques et pathomorphologiques et nous discutons les origines possibles. Nous conclusons qu’il s’agit ici de malformations semblables à la foie kystique et le complexe von Meyenburg de l’homme.

**Key Words:** Cotton top tamarin (*Saguinus oedipus*), cystic liver, von Meyenburg complex.

**Case report**
Cotton top tamarins (*Saguinus oedipus*) are a highly endangered South American primate species. At the German Primate Center a breeding colony of more than 40 animals is kept within the European endangered species program (EEP). Within this species spontaneous tumours of the gastrointestinal tract, especially of the colon occur (1, 4). For this reason the animals were regarded as an ideal animal model for spontaneous colon carcinomas in humans. In our colony three cases with proliferative cystic alterations within the liver or the bile duct system were observed. Two male animals of 12 and 13 years and a female animal of 22 years were concerned. The animals were of different sex and age and the clinical outcome was very different. Two animals were found dead in their cages and one animal had to be euthanasied after diagnostic
laparatomy because of the severe alterations of the liver. In none of the cases typical clinical signs were obvious. The alterations were not directly associated with the death of the animal. The most severe cystic alterations were found in case No. 1. During routine clinical examination a massive palpable mass appeared in the abdomen. Ultrasonographic examinations showed an enlarged liver with many different sized cysts. Laparatomy revealed that the liver structure was changed by numerous diffusely spread cysts and the animal was sacrificed. Macroscopically the cystic alterations had replaced almost the whole hepatic tissue (Fig. 1). Light microscopical examination showed diffuse cystic structures throughout the whole liver parenchyma. The cysts were covered by single layer cuboid epithelium and contained mucous substances together with shedded epithelial cells. A severe bileduct hyperplasia and fibrosis as well as mild to moderate mixed inflammatory cell infiltration were present. Electron microscopy revealed that the epithelium covering the cysts had microvilli similar to those seen in normal bileduct epithelium. The cysts were separated by small amounts of connective tissue.

In the other two cases the cystic alterations were found by chance during routine necropsy. Macroscopically small areas on the surface of the liver showed cystic structures. In light microscopical examination these structures appeared to be cystic dilatations of bileducts which were only found in capsular areas. Additionally a focal severe bileduct hyperplasia and fibrosis were seen. The alterations appeared to be more severe in case no. 3.

![Fig 1: Case No. 1. Macroscopic appearance: the whole liver parenchyma was diffusely infiltrated by cyst formation of different size.](image)

**Discussion**

These three cases reported were the first cystic alterations of the liver found in our colony and as far as we know comparative alterations have not been described in literature for this species. The origin of these cystic alterations may be infectious, parasitic or lymphatic. We could find no hints for one of those, like parasitic structures within the cysts. Other possibilities for the alterations described are either neoplasia of the bileduct system or malformation. Cotton top tamarins tend to have spontaneous tumours of the gastrointestinal tract, especially of the colon. But there are rather few reports on spontaneous neoplasms of the liver or biliary tract in nonhuman primates (2, 3). Searching the literature we could find no reports on cystic liver alterations in cotton top tamarins. Histological findings resembled the findings in human bile duct adenomas (5), a very rare
tumour. Electron microscopy revealed microvilli on the surface of the epithelial cells lining the cysts in case No. 1. In bile duct adenomas microvilli are missing. For this reason a neoplastic genesis in the described case was excluded.

The morphological findings in case No. 1 strongly resembled a malformation known in humans, called cystic liver. The cystic liver contains numerous cysts of different sizes, which occupy almost the whole organ. Half of the cases occur in combination with renal cysts or cystic kidneys. The cystic liver is clinically inapparent for a long time, it is usually only found by chance. Besides cystic alteration in the kidney all mentioned characteristics of cystic liver could be found in case No. 1 (6, 7).

In case No. 2 and 3 the histological findings corresponded to another malformation known in humans, the „von Meyenburg-Komplex“. These complexes are macroscopically found on the surface of the liver. Histologically, they appear as irregularly sized bileduct structures covered by cuboid or cylindric epithelium and surrounded by stroma, which is poor in cells and has thick fibers. The „von Meyenburg-Komplex“ can be seen as pre-stage of the cystic liver, as it is found in 60% of cystic liver in humans and shows fluent transition to the cystic liver (6, 7).

Cystic bile duct adenoma and cystic liver, respectively „von Meyenburg-Komplex“, are difficult to differentiate morphologically. The cases described here resemble malformations. In humans cystic liver and „von Meyenburg-Komplex“ are rare and mostly appear within polycystic diseases. No cystic alterations in other organs besides the liver were demonstrable in our cases. Apart from the morphological characteristics there is another hint at the theory that the cystic alterations emerged out of malformations. Animal no. 1 and 2 are close relatives, they have the same grandparents as shown in diagram 1 below. We suppose that the alterations might be a recessive defect, which would explain that the cysts appear only in old animals and without any cysts in other organs. At the moment the whole colony of cotton top tamarins is under investigation for further cystic alterations of the liver, which would support our theory.

**Diagram 1:**

```
  animal no. 63 (female)
   \      /   \
animal no. 1099 (male)  animal no. 62 (male)
   \   /    \
animal no. 1097 (male)
     |      |
    animal no. 1298 (male) case #1

  animal no. 408 (female)
     |      |
    animal no. 1455 (male) case #2

  animal no. 826 (female)
```

**References**

CASE REPORT OF A FATAL HERPES SIMPLEX INFECTION IN A GROUP OF COMMON MARMOSETS (Callithrix jacchus)

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Abstract
An outbreak of clinically obvious herpetic infection causing vesicoulcerative stomatitis in a family group of Callithrix jacchus is described. In all animals of the group Human herpesvirus 1 (Herpesvirus simplex) could be identified as causative agent as confirmed by histological, immunohistological, molecular biological investigations and virus isolation. The well known spontaneous interspecies transmissibility of Human herpesviruses results in fatal diseases in this highly susceptible New World monkey species. A clinicopathological description of spontaneous Human herpesvirus 1 infection is given. The significance of Human herpesvirus infection in new world monkeys is discussed and compared with other herpesvirus infections.

Zusammenfassung

Résumé

Key words: Callithrix jacchus, common marmosets, herpesvirus infection, Human herpesvirus 1, New World monkeys, vesicoulcerative glossitis, stomatitis
Introduction

Herpesviruses have been isolated from a variety of animal species ranging from invertebrates to primates. Nonhuman primates are primary hosts to quite a number of herpesviruses (4). Most of them are alpha-herpesviruses. The Human herpesvirus 1 and 2 (HHV-1 and HHV-2) are able to infect several nonhuman primate species but a different species susceptibility seems to exist. Four herpesviruses have been isolated from nonhuman primates which, based on their biological and biochemical properties, are similar to the Human herpesvirus 1 type 1 and 2 of humans (8, 20). The Cercopithecine herpesviruses 1 (Herpesvirus simiae, B-virus) naturally occur in many species of Asiatic macaques, producing clinical diseases similar to HHV in humans. Infection of macaques is usually mild and self limiting, leading to characteristic vesicular lesions of oral or genital mucous membranes (1,12). Zoonotic Herpes virus simiae infections in humans are severe and usually fatal. SA8, the Cercopithecine herpesvirus 2, occurs in African green monkeys (Cercopithecus aethiops). It leads to an asymptomatic infection of baboons in which it seems to be quite common. Two other simian viruses biologically similar to HHV have been isolated from some New World monkey species. One of these viruses, Herpesvirus tamarinus (HVT, Herpes T), was originally isolated from moribund marmosets (tamarins) and owl monkeys. Squirrel monkeys are the natural host for this virus (14), newly designated as Saaimiri herpesvirus 1 (SaHV-1). Herpesvirus ateleus (HVA-1), the second one, has been isolated from spider monkeys, which are the natural host for this virus. Both should not be confused with the lymphotropic Rhadinovirus, Saaimiri herpesvirus 2 (SaHV-2) and Herpesvirus ateleus (HVA-2), isolated from the same species and involved in tumorigenesis. HHV-1, HHV-2, SA8 and B-virus form a closely related sub-group of the primate herpesviruses, HVT-1 and HVA-1 are also closely related to the other four primate herpesviruses, albeit more distantly (8).

The mentioned viruses generally cause mild or inapparent infections in their natural host, but they remain latent, usually in neuronal ganglia, lifelong. Some are associated with severe infections when transmitted to other species than the natural host. The well known spontaneous interspecies transmissibility of herpesviruses is the base for a high anthropozoonotic risk and may result in fatal diseases of either man or animals. Human herpesvirus 1 (HHV-1,2) belongs to one of the better known human to nonhuman primate transmissible viruses, but spontaneous infections in monkeys appear to be rare. The original or reservoir hosts are humans, clinical symptoms were described as acute gingivo-stomatitis during the primary infection. Recurring oral or labial vesicles (HHV-1) as well as most genital vesicles and ulcers (HHV-2) in adults represent reactivation of latent herpes infection with virus shedding and viraemia. In Old World primates, natural HHV-1 infection has been described in gorillas (Gorilla gorilla) (7), chimpanzees (Pan troglodytes), bonobos (Pan paniscus) (17) and in white handed gibbons (Hylobates lars) (6,22,23). In these species the disease progression seems to be comparable to the human disease and the disease usually does not spread to a systemic infection. New World monkeys and prosimians seem to be of higher susceptibility, the disease course is severe and normally leads to death. In spontaneously infected owl monkeys (Aotus trivirgatus) the generalised febrile disease is characterised by erosions and ulcers on the oral mucous membranes and the mucocutaneous junctions of the lips (18). This is accompanied by focal necrosis and haemorrhages in all organs including the cerebral cortex (15) leading to death within 16 days after onset of the symptoms. Similar gross lesions are seen in naturally infected tree shrews (Tupaia glis) (16), lemurs (13) and experimentally infected marmoset and tamarin species (Callithrix sp., Saguinus sp.) (9,10). Spontaneous Human herpesvirus 1 infections were described as mortal cause of black-tufted-eared marmoset (Callithrix penicillata) in a conservation unit of State Park of Serra da Tiririca, Niteroi, in Brasil (5) as well as in four young pet marmosets (Callithrix jacchus) separately owned by private persons in Spain and Brasil (11,19).

This study is based on an HHV-1 outbreak in a group of Callithrix jacchus housed as a family group after suspected contact to an HHV-1 positive person. In this study, the clinical appearance and the pathomorphological alterations were described.
Case report

Animals

The infected animals belonged to a long existing colony consisting of up to 45 animals kept in a green house or in huts with access to outside. All animals were housed in their family groups (breeding couple with their offspring up to four generations). The infected family with their eight members was housed in mashed wire cages in the green house, with natural daylight and artificial light. The animals were fed a sap in the morning, vegetables, fruits and animal protein source in the evening, water ad libitum. In this green house there were up to 10 different families living without direct contact. Visual contact was prevented by long curtains, but indirect contact by caretakers, scientists, exchanged food or water bowls was possible.

Clinical findings

On the first day of the outbreak, the $\alpha$-male (animal no. 1) was found in a bad condition with apathy, anorexia, weakness and strong salivation in the morning and died the same afternoon. At this time all the other animals of the group showed no clinical symptoms of an infection. The following morning, one young adult (animal no. 2) and the $\alpha$-female (animal no. 3) developed strong salivation and apathy. Closer examination showed ulceration of the lips and the oral cavity. All seven animals were treated with Baypamun (Bayer, Leverkusen, Germany) 0.5ml/animal S.C. and 5 mg/kg Marboxyl (Vetoquinol, Oberursel, Germany). The next day, the young adult and the female were found dead. All other animals developed the same symptoms and were euthanasied. In summary, all eight animals had a 1-2 day's history of vesicular to ulcerative gingivitis and stomatitis accompanied by severe salivation, serous nasal discharges, anorexia and depression (see table 1). Three animals developed cutaneous lesions covered with fibronecrotic exudate at the mucocutaneous junctions (animal nos. 3,4,7).

Table 1: Animals and clinical signs of infection

<table>
<thead>
<tr>
<th>animal No.</th>
<th>age</th>
<th>sex</th>
<th>outbreak</th>
<th>death</th>
<th>oral ulcer</th>
<th>salivation</th>
<th>ano-rexia</th>
<th>mucocutaneous lesion</th>
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</table>
Fig. 1. Tongue; *Callithrix jacchus*, case No. 3. Electron microscopic demonstration of Herpes particles showing numbers of enveloped virions 140 nm in diameter in the cytoplasm and intercellular spaces. Transmission electron microscopy. 112500 fold.

**Pathology**

At necropsy all animals showed an acute gingivostomatitis. The tongues had multifocal single to coalescing, up to 0.5 to 1.0 cm diameter lingual erosions with rough borders. Some of these lesions progressed to real lingual or gingival ulcers (animal nos. 3,4,7) covered by a fibrinopurulent exudate. No other gross lesions were seen except a mild splenomegaly and a severe lymphadenopathy of the regionally lymph nodes.

Histopathological changes of the mucosal alterations were similar in all animals investigated. Light microscopy was performed on tissue specimens fixed in formalin and embedded in paraffin. Histology revealed a severe vesiculation and ulceration of squamous epithelia of the tongue due to acantholysis, parakeratosis, coagulation necrosis and polykaryocytosis forming necrotic plaques extending from the submucosa to the surface. Epithelial cells located at the ulcer margins showed various stages of degeneration and necrosis. Intranuclear inclusion bodies were seen particularly in border areas of the vesicles and ulcers. These inclusions filled the nucleus or were surrounded by a clear halo. A marked neutrophilic and lympho-histiocytic infiltration was evident in the underlying submucosa.

Furthermore, three animals (animal nos. 1,2,3) developed a mild interstitial hepatitis characterised by mild multifocal lympho-histiocytic cell infiltration. Inclusion bodies were not observed in these lesions. All other organs investigated were without special findings.

Immunohistochemistry for *Human herpesvirus 1* was performed on formalin-fixed, paraffin-embedded sections using a monoclonal antibody directed against *Human herpesvirus 1* and 2
antigen (Biogenex, Cat. No., MU086-UC). Immunohistochemistry revealed a specific antigen-antibody reaction in the lingual lesions of all animals, in particular within the ulcer regions and degenerated cells.

Electron microscopy of the ulcerative epithelial lesions revealed the presence of intranuclear viral nucleocapsids either empty or filled with electron dense material as well as large numbers of enveloped virions in the cytoplasm and intercellular spaces. The intranuclear particles measured 80-100 nm, the enveloped particles had an outside diameter of 150 nm and more (Fig. 1). Many of the virus particles had the hexagonal configuration characteristic for members of the herpesvirus group. Size, localisation and morphologic features were consistent with identification of the virus as a member of the herpesvirus family. Margination of the chromatin was a consistent finding in virus infected cells.

Virology

Herpesvirus was isolated from swap specimens of the oral cavity of six animals (animal no. 3 - 8), the remaining two other animals were not investigated. Growth characteristics of the isolated virus were consistent with those of α-herpesviruses. For further characterisation, PCR analysis was performed on tissue material obtained at necropsy and frozen at –80°C. A primer set designated B9/B10 was used to identify the virus. B9/B10 are located in the highly conserved C1 and C2 regions of the glycoprotein gB from HHV-1, HHV-2, B-virus, SA8 and HVP-2 and amplifies the highly divergent D2 region of gB. Restriction digestion of the PCR product with Hae III results in virus-specific restriction pattern (2). For further investigation DNA was isolated from blood and organ probes of the infected animals. The PCR with DNA of the tongue as template showed a strong amplification product of 360 kb like the HHV-1 positive control (Fig. 2). To identify the origin of the PCR amplification products, restriction digestion with Hae III was performed with the purified PCR products. The restriction fragments had the size specific for HHV-1: about 175 bp, 126 bp and minor bands in the range of 40 and 20 bp (computer predicted, 2). They comigrated with the restriction fragments of the HHV-1 control DNA indicating that the animals were infected with HHV-1.

Discussion

Reports on natural HHV-1 and -2 infections in Callithrichids are rare (21). Most available data are based on experimental infections. This fact, does not reflect the real situation in habusndry’s of Callithrichids and may be misinterpreted as a low risk for infection. For this reason this report of an HHV-1 infection in Callithrichids is important in several aspects. It clearly demonstrates the fulminant progression of herpesvirus infection in New World monkeys and shows the high susceptibility of this species for human herpesviruses. The animals developed classical clinical signs of a herpesvirus infection. The clinical course of this outbreak was dramatically leading to death of a whole Callithrix jacchus family within three days after onset of the first symptoms. Alterations of the oral mucous membranes were erosive to ulcerative. Histologically, various stages of massive epithelial damage were noted. Typical intranuclear inclusions were found. All investigations were indicative for a Human herpesvirus 1 infection as causative agent for the disease outbreak in this group of Callithrichids. Interestingly, none of the animals developed a severe necrotizing hepatitis or a multifocal nonsuppurative meningoencephalitis, alterations, which are commonly found in herpesvirus infections of other nonhuman primates (3,21). In the cases described by Juan-Salles (11) the animals developed alterations of the central nervous system but no necrotizing lesions in internal organs like in our cases. A possible explanation for this observation is the short period of the disease. All animals died in the acute phase of infection. The most characteristic gross findings in the reported cases of Human herpesvirus 1 infection were discrete vesicles, necrotic plaques and erosions or ulcers on the oral mucous membranes and mucocutaneous junctions. These lesions cannot be distinguished from Herpesvirus tamarinus-infection, which leads to a systemic infection with identical gross and microscopic lesions (9). Definitive diagnosis can only be made by virus isolation and identification, by immune staining
techniques and molecular investigations as demonstrated in the data presented. Unfortunately, the source of the virus in this outbreak could not be identified conclusively. The eight monkeys were born and reared at the same facility and had no known contact to any other animal species. It seems likely that close contact with persons suffering from oral herpesvirus infection were the source for transmission of virulent HHV-1. Most reported infections in various species of nonhuman primates have indicated that contact with man was the source of infection (6,13,16,18,23). Visitors, students or caretakers might be able to infect the animals, for example by passing partly eaten cookies or fruits into the cage. Most reported cases of herpesvirus infections in Callithrichids occurred in animals kept by private persons often in close association to the family (11). Close contact seems to be necessary for transmission of the infection. The route of transmission in spontaneous infections in nonhuman primates is unknown but direct contact or aerogen transmission seems to be the most important routes. Once brought into a colony the disease spreads rapidly with high morbidity and mortality (9). Since humans are the natural or reservoir host for the virus, the contact with symptomatically and subclinically HHV-1 infected human beings should always be avoided.

References


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A Rhesus Macaque (Macaca mulatta) Imounded by Custom Authorities: Infestation with Sucking Lice (Pedicinus obtusus)

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Abstract

A case of infestation with sucking lice (Pedicinus obtusus) was diagnosed in a juvenile, human-primed rhesus macaque (Macaca mulatta) which was illegally imported from Azerbaijan, custom-impounded and reached the Paul Ehrlich Institute. Ivermectin (two subcutaneous doses at 0.3 mg/kg with a three-week interval) successfully controlled the louse infestation.

Zusammenfassung

Es wird über den Befall mit Läusen der Art Pedicinus obtusus bei einem jungen, auf Menschen geprägten Rhesusaffen (Macaca mulatta) berichtet, der, ursprünglich aus Aserbaitschan kommend, vom Zoll beschlagnahmt worden war und das Paul-Ehrlich-Institut erreichte. Durch zwei Medikationen mit Ivermectin (3 mg/kg subkutan) in dreiwöchigem Abstand wurde der Lausbefall erfolgreich eliminiert.

Resumé

Le cas d’un jeune singe rhésus (Macaca mulatta) provenant d’Aserbaïdjan et ayant été gardé comme animal de compagnie est décrit. Cet animal avait été saisi par la douane et mis en quarantaine dans notre institut. Durant cette période il manifesta une infestation sévère avec le pou Pedicinus obtusus. Le traitement avec l’ivermectine fut efficace et la protection du reste de la colonie de primates ainsi assurée.

Key words: Rhesus macaque, Macaca mulatta, sucking lice, Pedicinus obtusus

Introduction

Infestations with ectoparasites including sucking lice have been reported to occur rarely in primates (1,2). Nevertheless, several species of sucking lice were found from a wide variety of non-human primates (5). For the detection of sucking lice in primates the absence of social grooming behaviour was thought to play a major role (6), since this behaviour is able to remove almost every visible louse and a major amount of eggs in group-housed non-human primates. There are, however, two groups of non-human primates in which it is more likely that a louse infestation can be detected:

1) individuals currently living without contact to other non-human primates,
2) individuals which do not groom (e.g. groups of very young individuals).

Here, a case of severe infestation with sucking lice is presented in a custom-impounded rhesus macaque.
Case report:

A young (<1 year) male rhesus macaque (*Macaca mulatta*) arrived at Frankfurt Airport in a transport-cage coming from Azerbaijan in transit to Slovenia. Since no transit documents were available for the animal, the German custom authorities impounded it. The animal was brought to the veterinary station at Frankfurt Airport, where it was kept for two days. Finally, the Federal Nature Conservation Agency referred the animal to the experimental facility at the Paul Ehrlich Institute (PEI) because no zoological garden could be found that wanted to take it. In the PEI facility, a six-week quarantine period was performed in order to detect any signs of infectious diseases. In this time it became clear that the animal was phenotypically of Chinese origin and was primed on humans.

During the quarantine period in the PEI facility the monkey was routinely checked for (1) virus infections (Herpes B-virus, Ebola-like viruses), (2) bacterial infections (palpebra-tuberkulin-test, microbiological faeces testing for pathogens) and (3) parasites (microscopical faecal examinations and clinical skin examinations). In addition, blood cell counts were done.

The rhesus macaque was free of detectable viral and bacterial pathogens. No pathological alterations were detected in the blood counts. However, it was clinically observed that the individual scratched itself frequently. In a narcosis, about 20 sucking lice and about 100 nits were found in the fur of the animal. Living lice were mainly seen in places where the skin was thin and the number of hairs reduced. Adult lice were approximately 1.5 mm long and brown in colour (Fig. 1). Nits appeared grey and were mainly seen in the upper part of the hairs located at the ventral abdomen. No signs of skin irritation could be detected. Specimens were collected in 70% alcohol and identified as *Pedicinus obtusus* by the presence of three pairs of paratergal plates at the abdomen (3).

Following two subcutaneous injections of 0.3 mg/kg ivermectin (Ivomec S®, Mérial, Hallbergmoss) in a three-week interval living lice were not detected (4).

![Adult female of Pedicinus obtusus (x 73) with two eggs shining through the abdominal wall.](image)

**Fig. 1:** Adult female of *Pedicinus obtusus* (x 73) with two eggs shining through the abdominal wall.

**Discussion**

Usually, a lot of information is lacking about the past life of a primate, when custom authorities impound it. This lack of information mostly includes knowledge about the microbiological and
parasitological status of the animal. Therefore, a quarantine period after the import of monkeys is laid down by law in Germany. During this period it is essential to gain as much information as possible about the past life of the animal, its microbiological and its parasitological status. This is necessary not only for zoonotic reasons but also in order to protect the state of hygiene of the primate facility. Strict isolation is one element in these quarantine procedures, even if it is depressing for the individual as much as it could be seen in this particular case. One important element in the quarantine period is informing of the animal caretakers – not only about the risks and the necessary procedures – but also about possible "surprises" concerning the state of hygiene of the arrival. If an animal caretaker knows that sucking lice are usually "host specific", the reaction after the detection of e. g. *Pedicinus obtusus* will be different from that without this knowledge.

The consequences for an infested animal are not severe, unless the exposure is repeated: the host can develop an inflammatory hypersensitivity reaction, pruritus, dermatitis and possible secondary infections (4). However, this is the reason why the consequences for the affected colony can be severe, if the parasite is spread in the facility. In order to successfully eliminate the ectoparasites, it is recommended not only to treat the affected animals but also to clean the particular cage and the room carefully with steam (4,6).

**References**