

Necropsy findings in slender Lorises (*Loris lydekkerianus*)

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Key words:

Summary

Little has been published about necropsy findings in slender lorises. Necropsy findings of 28 adult slender lorises and 4 additional newborns are presented in this report. Since two species of lorises occurring in Vietnam, pygmy loris (*Nycticebus pygmaeus*) and northern slow loris (*Nycticebus bengalensis*) are closely related to slender lorises the given necropsy data might also be of interest for these two Vietnamese species.

A total of 32 slender lorises mainly from one colony at Bochum University were investigated post mortem. Five pathological changes in slender lorises which appear to be found more often and to be more important than others: Polycystic nephropathy, Tooth alterations, Gallstones, Cataracts and Trichobezoar. Other findings were a hint of diabetes mellitus, intensive iron storage in the liver of one loris, ovarian cysts, and some probably mostly age related alterations

Những phát hiện trong giải phẫu tử thi ở loài Culi slender (*Loris lydekkerianus*)

Tóm tắt

Hiện nay vẫn còn rất ít những tài liệu xuất bản về giải phẫu tử thi đối với loài Culi slender. Nghiên cứu này công bố những phát hiện về giải phẫu tử thi của 28 cá thể trưởng thành, 4 cá thể con non của loài Culi slender. Ở Việt Nam, hai loài Culi nhỏ (*Nycticebus pygmaeus*) và Culi lớn (*Nycticebus bengalensis*) có quan hệ gần gũi với loài Culi slender, do vậy những hiểu biết về giải phẫu tử thi của loài Culi slender sẽ rất có ý nghĩa. Tổng cộng có 32 cá thể từ một quần thể ở Đại học Bochum đã được nghiên cứu. Có 5 sự thay đổi bệnh lý quan trọng và thường xảy ra ở loài Culi slender đó là: viêm thận, thay răng, sỏi mật, mất nước, và búi lông dạ dày. Ngoài ra, những phát hiện khác như tiểu đường, nhiễm kim loại nặng trong gan, buồng trứng cũng được tìm thấy ở một số cá thể.

Introduction

Little has been published about necropsy findings in slender lorises. Most reports include few slender lorises in a large group of prosimians (Boraski, 1981; Griner, 1983; Benirschke et al., 1985). Therefore necropsy findings of 28 adult slender lorises and 4 additional newborns are presented in this report.

Post mortem results, diseases and treatment recorded at the colony at Bochum University and cooperating zoos as well as additional literature data are also published in the internet database for

conservation of lorises and pottos (<http://www..loris-conservation.org/database>).

Since two species of lorises occurring in Vietnam, pygmy loris (*Nycticebus pygmaeus*) and northern slow loris (*Nycticebus bengalensis*) are closely related to slender lorises the given necropsy data might also be of interest for these two Vietnamese species. Additional data on the health of *Nycticebus pygmaeus* and *Nycticebus bengalensis* are also available in the database.

Material and Methods

Animals

A total of 32 slender lorises mainly from one colony at Bochum University were investigated post mortem. The results of 4 newborn slender lorises babies (2 males, 2 females), one juvenile female of six month and 25 adults (9 males, 16 females) between 5 and 16 years of age of *Loris lydekkerianus nordicus* were reported. In addition, the results of a 9 years old female and a 16 years old male probably of *Loris. tardigradus* were also included.

Housing and clinical colony history

The animals were kept alone or in family groups of up to eight animals. The cages, made of wood and wire mesh, measured 2.5 to 40 m³ and were densely equipped with natural branches. Frequent cleaning of the branches was avoided. Instead, new cages were offered after several months. The breeding facility was rather quiet, and taming of the animals had reduced environmental stress. In order to decrease the susceptibility of the lorises to psychic stress, some unfamiliar stimuli were provided, for instance access to unfamiliar cages or passages, invitation of human visitors or a radio automatically switched on at intervals. The day length was kept constantly at 13 hours (neon light). Temperature was about 24°C with a relative humidity of 60-70%.

Nutrition included milk formula with some milk-protein and egg yolk, calcium and vitamins added, live insects (locusts, crickets, mealworms), marmoset pellets and pieces of fruits and vegetable. Fennel tea or water was regularly offered, but drinking is regarded as unusual in this species. The daily quantity of food was usually kept constant with meagre food supply one day per week.

Necropsy and histopathology

At necropsy, the animals were fresh, frozen or fixed in 4% formaldehyde solution. The degree of post-mortem conservation was in a large range between fresh and putrefied/autolytic. In one case the body of an animal was available without head and neck. Photographs of the organs of interest were taken. Afterwards the organs were fixed in a 4% formaldehyde solution for at least 24 hours. Tissue samples were paraffin embedded, 8 µm slides were prepared and a haematoxylin-eosin-stain was performed according to standard methods. In one euthanized animal an immunohistology was performed for insulin and glucagon using antibodies against the human hormones.

Results

Kidneys

At necropsy, in one new-born and 21 adults different degrees of juvenile/adult polycystic nephropathy were observed (see also Plesker & Schulze, 2006). Whereas in the affected new-born

only histological changes were detected in the form of some slightly dilatated distal tubuli, in 21 adults already macroscopically a wavy, nodular surface of the kidneys was observed. Mostly miliary, in some cases up to 4 mm large, unilocular fluid filled cysts were detected on the surface as well as on the cortex part of the sections (Fig. 1). Histologically extensive tubular dilatation varying from very mild to extreme was seen. Significant sclerosis and fibrosis in combination with moderately severe infiltrates of lymphocytes and histiocytes were seen in the tissue between the cysts with the consequence that only few areas of functional active parenchyma remained. The remaining glomeruli revealed - in part - a spectrum from mild proliferation of the mesangial matrix/cells and local proliferation of the parietal endothelial cells to totally obliterating fibrosis or homogenic sclerosis of the glomerulum.



Fig.1. Multiple cysts in the cortex of the kidney of a slender loris (*Loris lydekkerianus*) affected by polycystic nephropathy (section of the kidney). Photo: Roland Plesker.

In eight of these lorises emaciation was confirmed, in 7 of these individuals a clear uremia was noted at necropsy. One individual displayed clear anemia.

Spleen

In histology, in 11 adult individuals only very few follicles were detectable in the spleen. This was interpreted as a sign of immunosuppression. In addition, in one case of a 9 years old female moderate extramedullary hemopoiesis was observed in the red pulpe of the spleen.

Teeth

In seven individuals, alterations affecting the teeth were seen such as dental calculus or

loosening/loss of single teeth (Fig. 2). In one of these cases, a severe periodontal disease was seen without abscess formation. In four additional of these cases, teeth alterations were combined with purulent/gangrenous inflammation in the surrounding maxillar or mandibular tissues (Plesker & Schulze, 2013).

Gallbladder and liver

In 5 adults (4 females, 1 male) the gallbladder was completely filled with either one or multiple gallstones. All of them were made of 100% cholesterol as determined by infrared spectroscopy (see also Plesker et al., 2012). In two of these lorises a slight icterus was diagnosed macroscopically. In a more than 15 years old male two moderately large areas with adenoid proliferation of the gall vessels in combination with moderate lymphatic infiltration of the liver were detected. In a 9 year old female multiple regenerative nodes were seen in the liver, in part with fibrosis. Histologically both local fibrosis and proliferation of the bile ducts was present.

In one case of a 13 year old female, histologically intensive iron storage in the liver (hemochromatosis) was proven by using a Berlin-blue-staining.

Eyes

In three adult individuals, cataracts were detected (Fig. 3).

Ovaries

Large unilocular fluid filled cysts of both ovaries, up to 7 mm in diameter, were noticed in a 9 years old female. This animal was reported to show male behaviour in the cage.

Gut (Bezoar)

One animal died of an occluding trichobezoar in the beginning of the jejunum.

Additional pathological alterations include

Myocardial infarcts (female, 12 years), arteriosclerosis (male, 15 years), demineralisation of bones (female, 6 month) as well as serial broken ribs of one thorax side (female, 11 years). seminoma (male, 15 years) and testical atrophy (male, 16 years).



Fig.2. Hole after surgical treatment of a teeth-related abscess in the throat of a slender loris (*Loris lydekkerianus*) (arrow). Yellow colour due to disinfection. Photo: Roland Plesker.



Fig.3. Bilateral cataract in a young-adult slender loris (*Loris lydekkerianus*). Photo: Roland Plesker.

Discussion

First of all, we would like to stress five pathological changes in slender lorises which appear to be found more often and to be more important than others:

Polycystic nephropathy

The occurrence of such a large number of individuals with polycystic nephropathy in one colony is unusual, since usually only single cases of polycystic nephropathy in primates are reported in literature. This implies, that a systemic factor in the colony, e.g. the specific nutrition, might be responsible/contributes to the occurrence of the disease.

In humans, at least some forms of polycystic nephropathy are known to be genetically fixed and there are strains of rats and mice available with a genetic fixation of the disease.

In addition, the alterations are inducible with insecticides or corticosteroids in rats. Third, the occurrence of “chronic nephrosis with nephritis” associated with extensive tubular dilatations in *Microcebus murinus* was correlated to stress-induced increased corticoadrenal activity (PERRET, 1982). The permanent stress was thought to be induced by housing conditions in captivity since the degree of kidney alterations in *Microcebus murinus* could be correlated to the period of time the animals were kept in captivity. In our cases in *Lorises*, there was no behavioural evidence that the animals that had been affected had suffered from more severe stress than others. However, the loss of follicles (immunosuppression) in the spleen as seen histologically in 11 individuals might be an indication for chronic stress at least in these individuals.

Tooth alterations

are fairly common in the Bochum colony of *Lorises* (Plesker & Schulze, 2013). We have seen dental calculus formation as well as loose teeth or loss of teeth. As a consequence, in some cases, abscess formation/gangrenous inflammation was correlated to these former teeth alterations (see also Eisenberg et al., 2012). We believe that feeding *Lorises* with locusts may contribute significantly to teeth problems since sharp spikes on the hind legs of migratory and other locusts (used as food insects) may cause regular injuries in periodontal tissues. These wounds may serve as “point of tissue invasion” for pathogenic bacteria. Species of bacteria isolated from the oral cavity or tooth roots of lorises with symptoms of gingival infection or tooth root abscess included *Prevotella melaninogenica*, *Eikenella corrodens*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Proteus mirabilis*. All of them are ubiquitous, but may cause problems in humans or farm animals, however, the significance of these bacteria for lorises has to be further investigated. The same is the case for *Trueperella pyogenes* (Eisenberg et al., 2012).

Gallstones

are known to occur rarely in baboons (*Papio* sp.), orangutan (*Pongo*), *Callithrix kuhlii*, *Leontopithecus* sp. and in owl monkeys (*Aotes trivirgatus*) (Chalifoux & Anver, 1993). In owl monkeys, the gallstones are of cholesterol origin. The reason for this is unclear. In slender lorises no preference for one sex could be confirmed. However, the occurrence of these stones are known to be quite painful in humans and this is an excellent explanation for a behaviour seen in some individuals of slender lorises that is described as “having abdominal pain”. Another possible consequence of gallstones - icterus - was seen in two of our four cases.

Cataracts

are not only limited to slender lorises but have also been observed in two pygmy lorises at the EPRC in Cuc Phuong National Park, Vietnam (Fig. 4).

Trichobezoar

Although in our cases there is only one individual that died from a trichobezoar, in the history of this colony as well as in other necropsy reports from *slender lorises* sometimes the occurrence of trichobezoars is described. The occurrence is due to a special behaviour in prosimians: these animals clean their hairs with their lower incisors and canines. These teeth are arranged in the form of a comb that is cleaned with an additional sublingual tongue made of fibrous material. The removed loose hairs are swallowed, which is the reason for the normal occurrence of masses of hairs in the faeces.



Fig.4. Cataract in a pygmy loris (*Nycticebus pygmaeus*) at the Endangered Primate Rescue Center, Vietnam. Photo: Tilo Nadler.

Other findings

There were some hints for diabetes mellitus both in the colony history and at necropsy (like potentially necrotic ends of fingers and toes (2 individuals) in combination with slight cataract (one 11 years old female). Normally, diabetes mellitus can be identified clinically by glucose determination in the urine; only on one occasion blood glucose level has also been tested in the colony of Bochum University (using blood received via blood-sucking tropical bug, *Gromphadorhina portentosa*). However, the results of urine testing and of the blood testing must be interpreted very carefully (if the urine is not obtained under very clean conditions the testing mostly is false positive and blood results might have been changed by the passage within the bugs). The degree of post mortem conservation in most individuals made it impossible to make a diagnosis of the pancreas isles in a hematoxylin-eosin stained slide. In two cases in which the pancreas was fresh, slight oedematous disintegration and enlargement of the isles was noticed in routine histology. In immunohistology - using antibodies against human insulin and glucagon - the detected amount of insulin was very high and the amount of glucagon was reduced in comparison to the human reference. Since no slender loris reference was available for comparison the significance of this finding remains unclear. However this illness must be taken into consideration at least when there is an enlarged volume of urine observed in single individuals. Changes of the skin and loss of hair on the limbs, occasionally observed in lorises with severe wasting disease and also described as a usual symptom before death in captive slender lorises by Osman Hill (1937), might indicate diabetes or be a sign of malnutrition (possibly fatty acid deficiency).

We demonstrated intensive iron storage in the liver of one loris. However, this was the only individual that was tested. We are convinced that more affected individuals would have been

detected if they would have been tested. Intensive iron storage in the liver had been described in several prosimian species.

The occurrence of ovarian cysts is more frequent in certain mammal species than in others. In our material for example we see ovarian cysts mostly in guinea-pigs. A conclusion from the one case we observed in slender lorises is - of course - not possible. However for us it was interesting to find the ovarian cysts in an individual that revealed male behaviour when observed clinically. Biochemically this is due to testosterone's that produced normally in the ovary as an intermediate product and that is released in large amounts in the case of ovarian cysts.

Artherosclerosis and myocardial infarcts, osteoporosis, loss of teeth and dental stones, regenerative nodes of the liver and fibrosis of the gall vessels, reduction of follicles in the white pulpe of the spleen, residues of former traumata and even seminomas, all together are alterations that are more often seen in elder individuals and are well known in a variety of species. Therefore these alterations are regarded as more age related than slender lorises specific.

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