Zool. Garten N.F. 88 (2020) 79-90

DER ZOOLOGISCHE GARTEN

Lethal bloat syndrome in two lemur species associated with *Clostridium perfringens* infection at Tierpark Berlin

Tödlich verlaufendes Aufblähungs-Syndrom in Zusammenhang mit *Clostridium perfringens* -Infektionen bei zwei Lemuren-Arten im Tierpark Berlin

Andreas Pauly[1*], Claudia A. Szentiks[2], Kristin Mühldorfer[2], Alexander Haake[3], Achim D. Gruber[3], Antina Lübke-Becker[4], Astrid Bethe[4], Martina Bleyer[5] & Andreas Knieriem[1]

[1)] Tierpark Berlin-Friedrichsfelde GmbH, Am Tierpark 125, 10319 Berlin, Germany
[2)] Abteilung für Wildtierkrankheiten, Leibniz-Institut für Zoo- und Wildtierforschung, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany
[3)] Institut für Tierpathologie, Freie Universität Berlin, Robert-von-Ostertag-Str. 15, 14163 Berlin, Germany
[4)] Institut für Mikrobiologie und Tierseuchen, Freie Universität Berlin, Robert-von-Ostertag-Str. 7-13, 14163 Berlin, Germany
[5)] Serviceeinheit Pathologie, Deutsches Primatenzentrum GmbH (DPZ), Leibniz-Institut für Primatenforschung, Kellnerweg 4, 37077 Göttingen, Germany

Summary

In 2019 and 2020 five cases of a bloat syndrome occurred in the lemur stock of Tierpark Berlin. It is very likely, that this syndrome was caused by a *Clostridum perfringens* infection. The authors describe each of these five cases in detail and give recommendations for the therapy and prophylaxis of this potentially lethal condition.

Keywords: Lemurs, bloat syndrome, symptoms, pathology, therapy, *Clostridium perfringens*, *Eulemur*

*Corresp. author:
E-Mail: a.pauly@tierpark-berlin.de (Andreas Pauly)

Introduction

Tierpark Berlin (Germany) has kept a large collection of lemur species for many years. Since 2012 the endangered red-collared brown lemur (*Eulemur collaris*) has been held there. The species was first reared successfully in Germany in 2019 at Tierpark Berlin. In the same year, Tierpark Berlin received, for the first time in its history, the critically endangered blue-eyed black lemur (*Eulemur flavifrons*) on recommendation of the EAZA Ex situ Programme (EEP) coordinator. This lemur is the flagship species of the conservation organisation Association Européenne pour l'Étude et la Conservation des Lémuriens (AEECL), which has been financially supported by Tierpark Berlin for many years. The diet of the lemurs at Tierpark Berlin consists of vegetables and apple. From time to time, they receive browse. Bark chips are used as substrate to cover the floor in the inside exhibit. The ground of the outside enclosure is covered with topsoil mainly overgrown with grass.

Pond et al. (1982) described an acute gastric dilation in 21 Old World monkeys (OWM), which were wild-caught and kept for experimental studies. The syndrome occurred after food restriction followed by ad libitum feeding (biscuit-type diet with a high amount of carbohydrates) or associated with anaesthesia. In four of these cases, *Clostridium perfringens* was isolated from the intestine. Pathologic examination revealed an atonic stomach, greatly distended with gaseous fermented ingesta and in some cases rupture of the stomach. Terio et al. (2018) mentioned an acute gastric dilation or "bloat syndrome" caused by a *Clostridium perfringens* infection in OWM and New World monkeys (NWM) after overeating and drinking, following anaesthesia or after disturbances of the gastric flora induced by antimicrobial treatment. This syndrome is a medical emergency and, if left untreated, leads to acute shock and death. Acute gastric dilation was also described by Abee et al. (2012) and Yasuda et al. (2015) in OWM and NWM. Williams (2002) published eight cases of *Clostridium difficile* infections in lemurs associated with diarrhoea and partial bloating of the abdomen at the Duke Lemur Center in North Carolina (USA). However, the authors failed to find any reports of a bloat syndrome in lemurs caused by *Clostridium perfringens*.

The following report describes five cases of a bloat syndrome in three blue-eyed black lemurs and two red-collared brown lemurs at Tierpark Berlin.

Case reports

1st case (January 2019): 1.0 blue-eyed black lemur (*Eulemur flavifrons*) "Olivier", 20 years old

Tierpark Berlin received this animal on 31/08/2018 from Cologne Zoo (Germany). Until 06/01/2019, the lemur showed normal behaviour and appetite. On this day, "Olivier" ate only half of the daily ration and had bloody diarrhoea in the afternoon. The lemur received injections of meloxicam (anti-inflammatory drug, 2.0 mg/kg BW Metacam® 2% s.c.) and amoxicillin (antibiotic, 7.5 mg/kg BW Amoxicillin WDT® 15% s.c.). The following day, "Olivier" exhibited strange behaviour: He chewed food items without swallowing them, followed by spitting out little pieces of food. Furthermore, the lemur seemed to be bloated. Parasitological analysis of faecal samples showed only few oxyurid eggs in fresh stool. Quick tests for *Giardia, Cryptosporidium* and *Entamoeba histolytica* were negative. The lemur received injections of enrofloxacin (antibiotic, 5.0 mg/kg BW Enrofloxacin® 2.5% s.c.) and vitamins (0.5 ml Ursovit® AD3EC s.c.). Additionally, he received an infusion consisting of glucose and sodium chloride

and a spot-on with selamectin (anti-parasitic, 7.5 mg/kg BW Stronghold® Katze). On the morning of 08/01/2019, the animal was found dead. Pathological findings included petechial and regional haemorrhages into the wall of the small and large intestine (Fig. 1). The mucosa of the intestine was not affected. Histology revealed an erosive, partly ulcerative enteritis associated with a dysbacteriosis. Endoparasites were not found. The liver had hepatocellular necrosis. In a subsequent bacteriological investigation, *Escherichia coli* (different phenotypes) and alpha-haemolytic streptococci were isolated in severe growth from the small and large intestine. *Salmonella* or *Yersinia* (both from enrichments) as well as *Clostridia* (in anaerobic cultures, including *Clostridium perfringens*) were not detected.

Fig. 1: Digestive tract of 1.0 blue-eyed black lemur "Olivier" at necropsy. Photo: A. Pauly.

2nd case (March 2019): 1.0 blue-eyed black lemur (*Eulemur flavifrons*) "Cesar", 11 years old, son of "Olivier"

This lemur was obtained on 21/02/2019 from Mulhouse Zoo (France) as a new partner for the remaining female "Zazou" after the death of "Olivier". On 04/03/2019, the animal suffered from anorexia, vomitus and watery diarrhoea and showed the strange behaviour of spitting out small pieces of food (as mentioned above). After immobilisation with medetomidine (0.1 mg/kg BW Sedator® i.m.) and ketamine (10.0 mg/kg BW Ketamin 10% WDT® i.m.), blood was drawn and radiographs were taken. The stomach was filled with food and the intestine was bloated. Blood values (haematology and chemistry) were in normal range, except values for sodium, chloride, albumin and total protein, which were below normal values. Therapy was initiated immediately with daily infusions of sodium chloride and glucose, antibiotics (enrofloxacin (5.0 mg/kg BW Enrofloxacin® 2.5% s.c.) and cefovecin (8.0 mg/kg BW Convenia® s.c.)) and metoclopramide (0.5 mg/kg BW BID MCP-ratiopharm® p.o.) to accelerate the gastrointestinal passage. A slight improvement of the symptoms was observed in the following days. The lemur ate small amounts of food and no longer had diarrhoea. Unexpectedly, "Cesar" was found dead in the morning on 09/03/2019. During necropsy, a rupture of the small intestine

without detectable marginal bleeding and a fibrinous serositis with a large amount of brownish fluid in the abdominal cavity were found (Figs. 2 and 3). There were no foreign objects in the intestine. Histologically, a severe lympho-histiocytic infiltration in the small intestine and hepatocellular necrosis was recorded. In a subsequent bacteriological investigation *Escherichia coli* and *Enterococcus* species were isolated in severe growth from liver, spleen and urine and from the small and large intestines. *Salmonella* or *Yersinia* (both from enrichments) were not detected, but *Clostridium perfringens* was cultured in severe growth from small and large intestines together with other concomitant anaerobic bacteria (i.e., *Clostridium, Fusobacterium* and *Bacteroides* species). *Clostridium*-like bacteria were also detected histologically in the blood vessels from different organs.

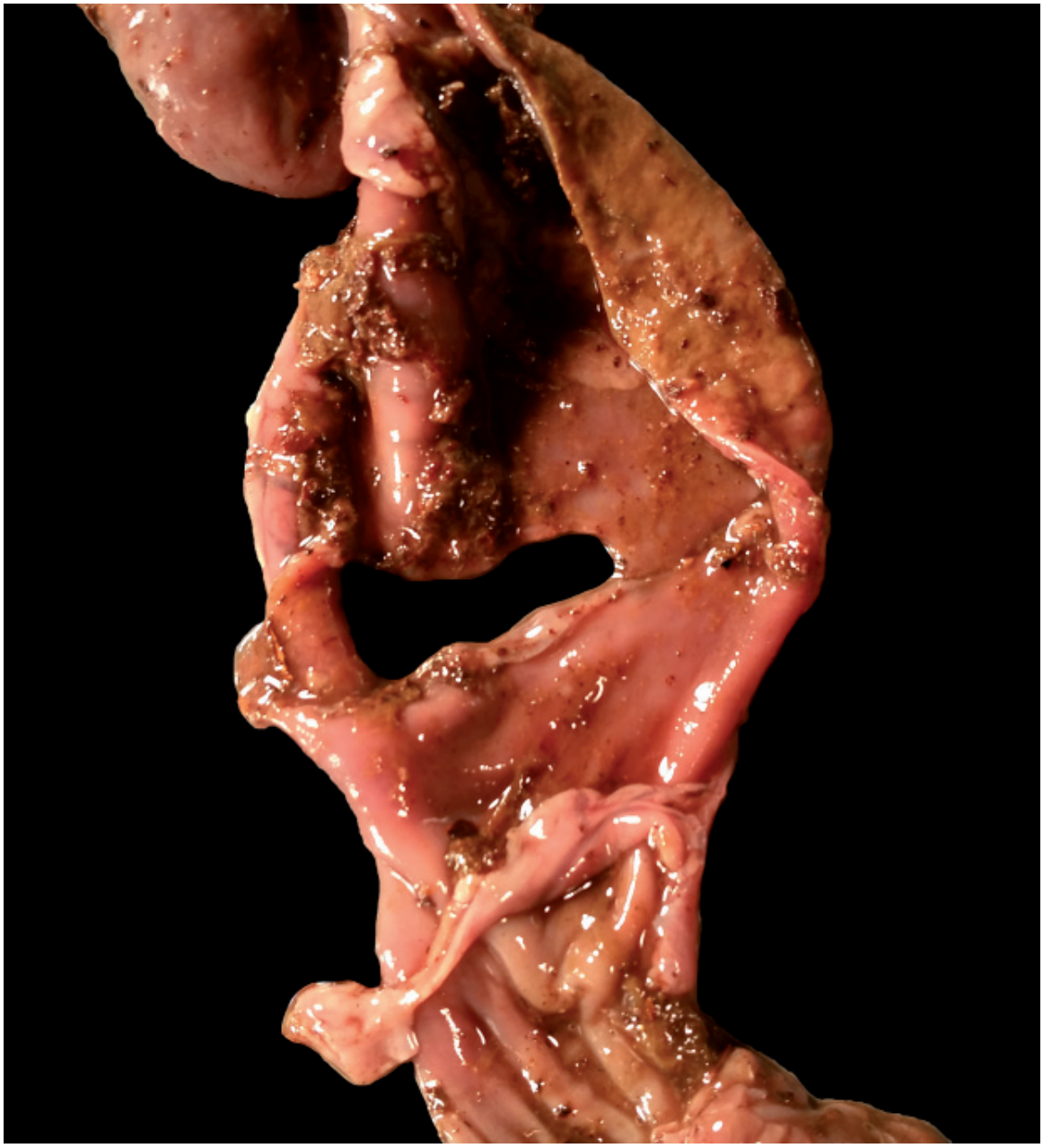

Fig. 2: Rupture of the small intestine of 1.0 blue-eyed black lemur “Cesar”. Photo: A. Pauly.

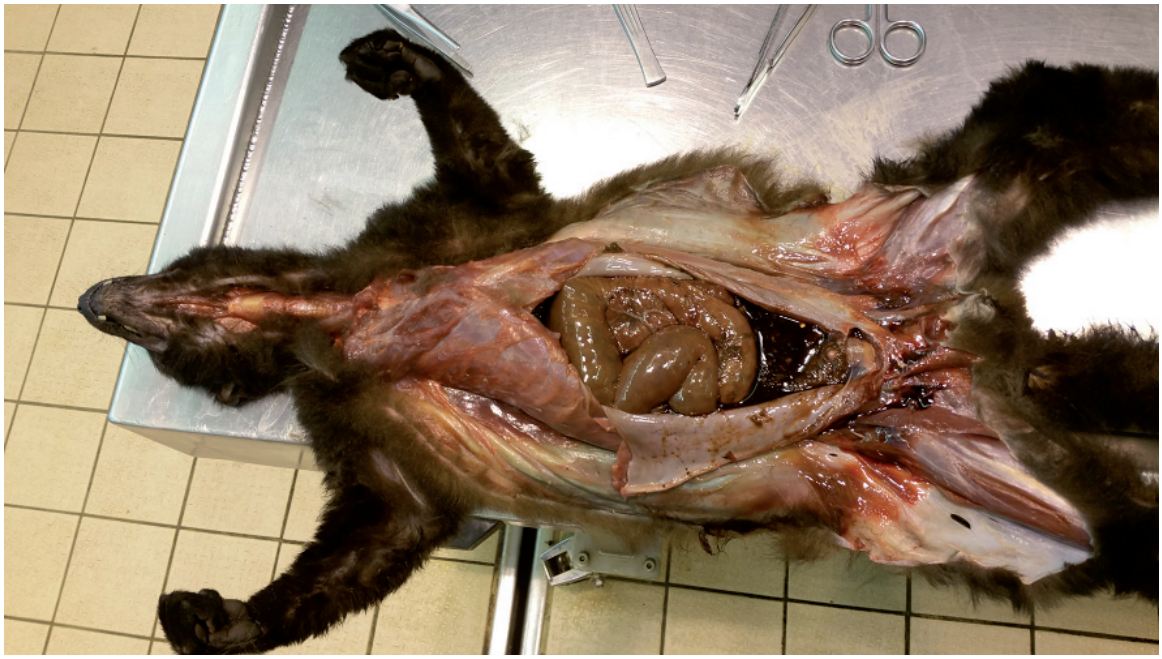

Fig. 3: Situs of 1.0 blue-eyed black lemur "Cesar" at necropsy. Note the distended intestine and the brownish fluid in the abdominal cavity. Photo: A. Pauly.

3rd case (April 2019): 0.1 blue-eyed black lemur (*Eulemur flavifrons*) "Zazou", 20 years old

Tierpark Berlin received this female on the same day as the male "Olivier". She came from La Palmyre Zoo (France). After "Olivier's" death, she lived together with the new male "Cesar". "Zazou" harmonised very well with both males. On 14/01/2019, six days after "Olivier" passed away, a fresh stool sample of "Zazou" was taken and no pathogenic bacteria were detected (result of the bacteriological examination: physiological intestinal flora). The female showed normal behaviour and appetite until 20/04/2019. Over a period of five days, "Zazou" was inconspicuous in her behaviour in the morning, than she refused to eat the whole amount of her diet in the afternoon and was further observed to be very calm. Additionally, she was observed to apparently drink more water than before. Her vulva was slightly opened and she appeared pregnant. On 23/04/2019, another stool sample was checked for pathogenic bacteria and parasites. Only a few oxyurid eggs were detected. The lemur was treated with fenbendazole (anti-parasitic, 25.0 mg/kg BW Panacur® PetPaste p.o.) over a period of three days. Gestation diabetes was suspected after a urine stick was positive for glucose on 27/04/2019. In the afternoon of the same day, "Zazou" choked and vomited severely. She was anaesthetised with midazolam (0.2 mg/kg BW Midazolam-ratiopharm® i.m.) and ketamine (10.0 mg/kg BW Ketamin 10% WDT® i.m.) for x-ray examination. Her stomach was fully filled with food and the intestine contained large amounts of gas (Fig. 4). Anaesthesia was prolonged with isoflurane (1.5 % Isofluran CP®, 1 l O2/min) for laparotomy. The blood vessels of the stomach were highly injected and no peristaltic movement in the whole digestive tract was detectable (Fig. 5), not even after stimulating the intestine with sodium chloride. A gastro- and enterotomy to remove food and gas were performed. Additionally, the lemur received infusions with sodium chloride and glucose, cefovecin (antibiotic, 8.0 mg/kg BW Convenia® s.c.) and prednisolone (cortisone, 1.0 mg/kg Prednisolonacetat® i.m.). Despite the successful surgery, the lemur died three hours

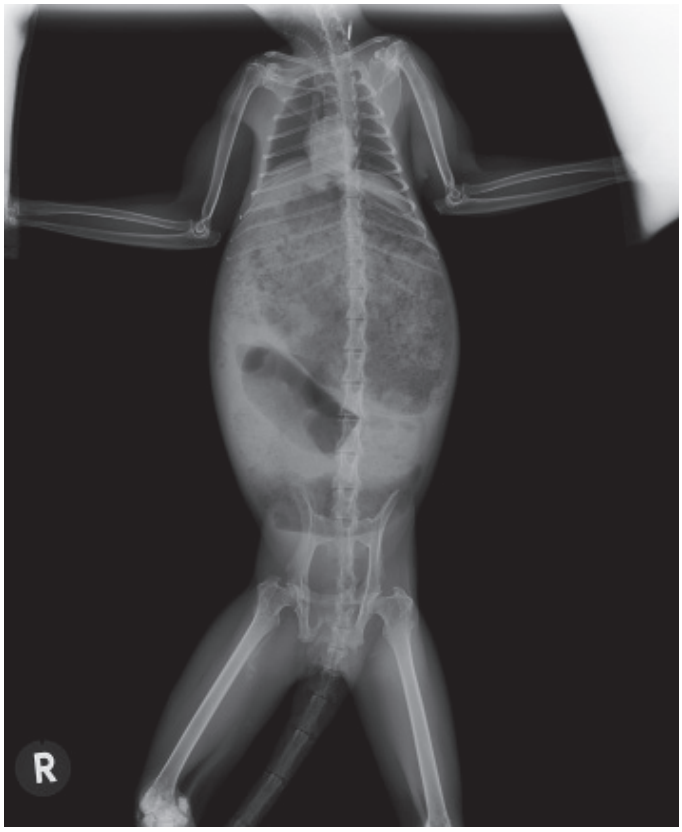

Fig. 4: Ventrodorsal radiograph of 0.1 blue-eyed black lemur “Zazou”. Note the distended stomach filled with food and gas and the gas-filled intestine. Photo: A. Pauly.

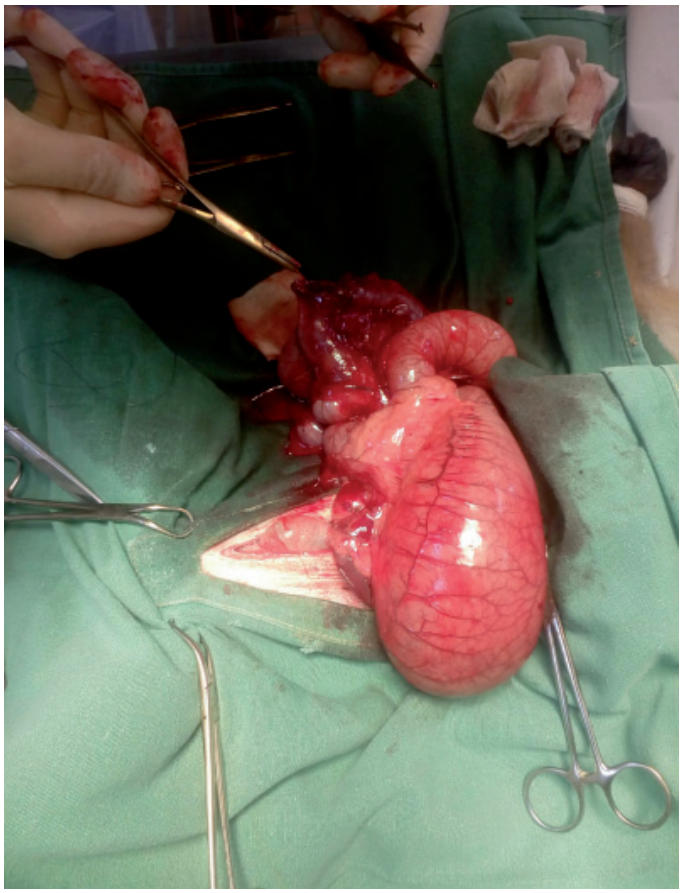

Fig. 5: Gastric dilation of 0.1 blue-eyed lemur “Zazou” during laparotomy. Note the injected blood vessels. Photo: A. Pauly.

later with shock symptoms. Pathohistology revealed diffuse oedema within the mucosa of the small intestine and a diffuse lymphocytic to histiocytic enteritis in the large intestine. In a subsequent bacteriological investigation, *Escherichia coli* (two phenotypes), *Streptoccocus* species and Gram-negative anaerobic species were isolated in severe growth. *Salmonella* or *Yersinia* (both from enrichments) were not detected, as well as *Clostridia* (in anaerobic cultures, including *Clostridium perfringens*).

4th case (May 2019): 1.0 red-collared brown lemur (*Eulemur collaris*) "Tonik", 9 years old

The animal was obtained from Plzeň Zoo (Czech Republic) in 2012. On 29/05/2019, the lemur showed the strange behaviour of chewing and spitting out food items as mentioned in the reported blue-eyed black lemur cases. He was treated with doxycycline (antibiotic, 10.0 mg/kg Doxycyclin-ratiopharm®SF i.m.), prednisolone (cortisone, 1.0 mg/kg Prednisolon-acetat® i.m.) and glucose infusion the following day. In the morning of 31/05/2019, "Tonik" was found dead in the indoor exhibit. This male red-collared brown lemur lived together with

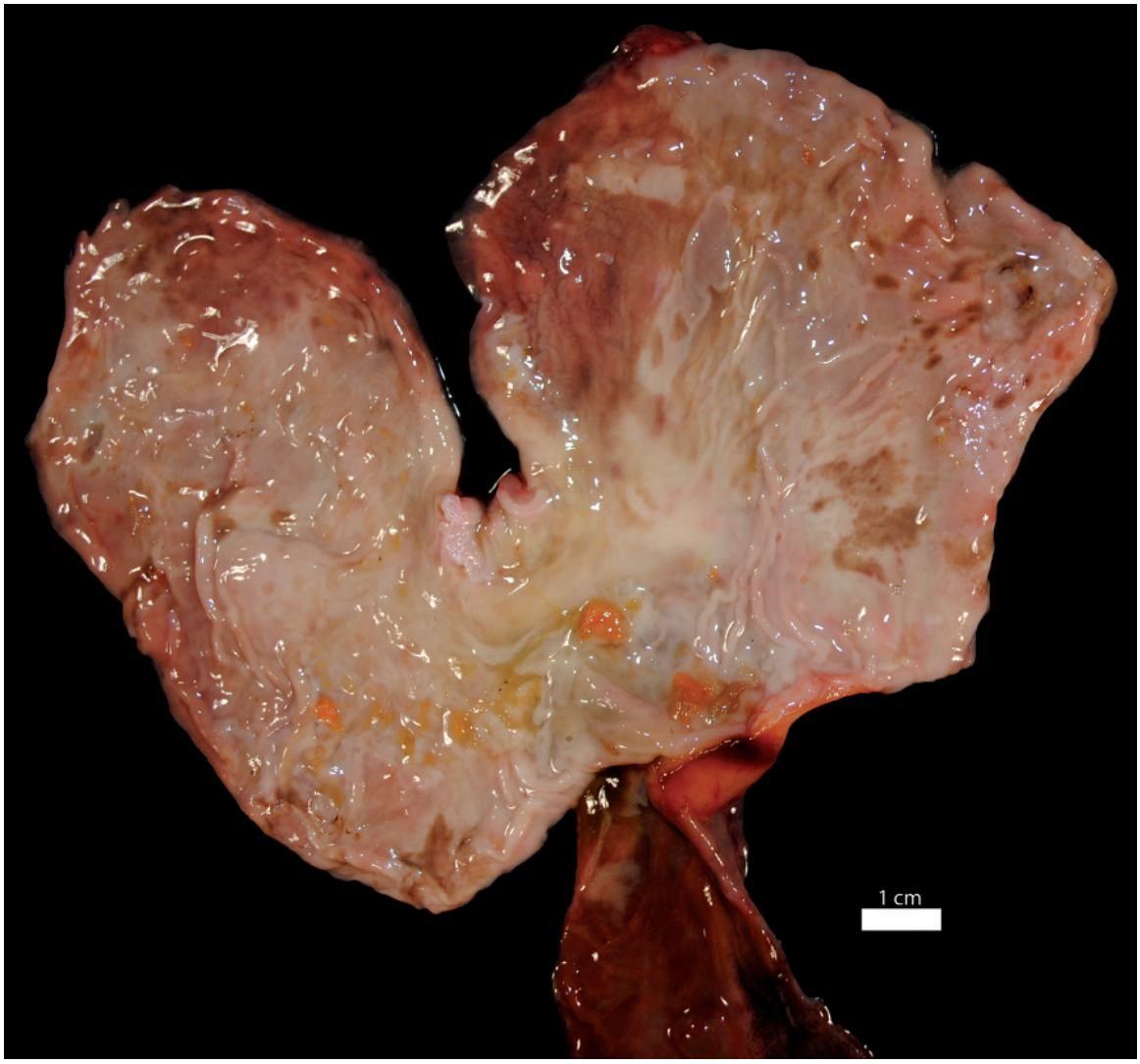

Fig. 6: Ulcerative and fibrinous gastritis of 1.0 red-collared brown lemur "Tonik". Photo: A. Haake.

two females of the same species in the former enclosure of the blue-eyed black lemurs for around three weeks. The inside exhibit was thoroughly cleaned and disinfected before the transfer of the red-collared brown lemur group with p-chlor-m-cresol (Neopredisan®, 4 % for 1 h). In necropsy, the stomach and intestine were massively distended with gas. In addition, a severe ulcerative and fibrinous gastritis (Fig. 6) and a severe ulcerative and diphtheroid enteritis of the small and large intestine (Fig. 7) were observed. In a subsequent bacteriological investigation, *Escherichia coli* and *Enterococcus hirae* were isolated in severe growth. *Salmonella* or *Yersinia* were not detected, but *Clostridum perfringens* (toxovar A) was cultured anaerobically in severe growth from the intestine.

Fig. 7: Ulcerative and diptheroid enteritis of jejunum, colon and rectum of 1.0 red-collared brown lemur "Tonik". Photo: A. Haake.

5th case (February 2020): 1.0 red-collared brown lemur (*Eulemur collaris*) "Thierry", 2 years old

Tierpark Berlin received this lemur on 14/08/2019 from Plzeň Zoo (Czech Republic) as a new breeding male after "Tonik" passed away. Until 09/02/2020, "Thierry" showed no clinical abnormalities. On this day, the lemur was apathetic and anorexic. He was observed chewing food items without swallowing. A bloating of the abdomen was not seen. Due to previous experience with the other lemurs (cases 1-4), an infection caused by *Clostridium perfringens* was suspected. "Thierry" was immediately treated with metronidazole (antibiotic, 50 mg/kg Eradia® p.o.) and received infusions with sodium chloride and glucose to accelerate the elimination of potentially, already absorbed *Clostridium*-toxins. The second day after the start of therapy the lemur recovered partially and was observed to forage slowly, consuming the whole amount of

the daily food ration. On 11/02/20, he additionally received a mixture of filtered faeces from the healthy female mixed with water to build up a physiological microbiome in his intestine. After ten days of treatment with metronidazole (50 mg/kg Eradia® p.o.), the lemur was considered to have totally recovered. On 18/02/20, he received a second faecal transplantation to boost his physiological microbiome.

Results

All affected lemurs (n=5) showed the same unknown behaviour at the beginning of the disease (chewing food without swallowing, followed by spitting out little pieces of the food items or vomiting). In four cases, a bloating of the abdomen was observed. In each of the four dead lemurs, Escherichia coli was detected in the intestinal tract. However, an additional whole genome-based characterisation of three different Escherichia coli isolates from two lemurs (0.1 blue-eyed black lemur "Zazou" and 1.0 red-collared brown lemur "Tonik") revealed the presence of genetically distinct strains (phylogenetic group: A, B1 and B2) and virulence gene profiles that do not point to enteropathogenic *Escherichia coli* or other known pathovars. *Escherichia coli* as well as *Streptococcus* spp. and *Enterococcus* spp. are probably part of the indigenous microbiota. In two of the described cases, *Clostridium perfringens* was isolated. All dead lemurs exhibited signs of the bloat syndrome as described in the literature for OWM and NWM. An overview of clinical symptoms, the clinical course and the results of the bacteriological and pathological examinations of the described cases at Tierpark Berlin can be found (see Table 1).

Discussion

Clostridium perfringens is an anaerobic Gram-positive bacterium that can cause enterotoxaemia with acute gastric dilation in humans and nonhuman primates (Brack, 1987). It is able to form permanent stages; these spores are resistant to heat and can survive for a long time in the environment. Direct transmission from animal to animal was not observed. The minimal time period between two affected lemurs was around one month. This does not coincide with this acute clinical picture.

Clostridium perfringens was isolated from the intestine of only two out of five lemurs with symptoms of a bloat syndrome. However, it is very likely, that the presence of *Clostridium perfringens* or toxins produced by the bacterium were associated with this syndrome in the lemurs. This hypothesis is based on the following observations:

1. The clinical appearance and pathological findings in all five cases are identical to those described in the literature (Pond et al., 1982, Terio et al., 2018, Abee et al., 2012, Yasuda et al., 2015).
2. No food restriction or change in diet during the entire time occurred and not one of the lemurs was anaesthetised before developing the bloat syndrome. These reasons are listed as causal for developing a bloat syndrome in the literature (Terio et al., 2018).
3. The long time period between two infections provides a strong indication that the lemurs picked up spores from the soil or environment of the enclosure. Direct transmission from animal to animal is not assumed. The inside exhibits were cleaned and disinfected after the death of the last blue-eyed black lemur twice with p-chlor-m-cresol (Neopredisan®, 4% for 1 h), which is effective against the spores of *Clostridium perfringens*.
4. The successful treatment of the second affected red-collared brown lemur with metronidazole, an antibiotic highly effective against *Clostridium*, and his recovery after a short period.

Tab. 1: Clinical symptoms, clinical course, results of the bacteriological and pathological examination of the affected lemurs.

Case	Clinical symptoms	Clinical course	Bacteriology	Pathology
1.0 "Olivier"	chewing food without swallowing, followed by spitting out little pieces of the food items; bloody diarrhoea abdominal bloating	died	+++ *Escherichia coli* (different phenotypes) +++ alpha-haemolytic *Streptococcus* sp. (small and large intestine)	petechial and regional haemorrhages (wall of small and large intestine, mucosa not affected), erosive and ulcerative enteritis, hepatocellular necrosis
1.0 "Cesar"	chewing food without swallowing, followed by spitting out little pieces of the food items; vomitus; watery diarrhoea; abdominal bloating	died	+++ *Escherichia coli* +++ *Enterococcus* sp. (liver, spleen, urine, small and large intestine) +++ *Clostridium perfringens*, *Clostridium* sp., *Fusobacterium* sp., *Bacteroides* sp. (small and large intestine)	intestinal gas distention, rupture of small intestine, fibrinous serositis, lympho-histiocytic enteritis of small intestine, hepatocellular necrosis
0.1 "Zazou"	vomitus; abdominal bloating; no peristaltic movement in the whole digestive tract	died	+++ *Escherichia coli* (two phenotypes), +++ *Streptococcus* sp., +++ Gram-negative anaerobic species (small and large intestine)	oedema in the mucosa of small intestine, lympho-histiocytic enteritis of large intestine
1.0 "Tonik"	chewing food without swallowing, followed by spitting out little pieces of the food items; abdominal bloating	died	+++ *Escherichia coli,* +++ *Enterococcus hirae,* +++ *Clostridium perfringens* (toxovar A) (small and large intestine)	Gastrointestinal gas distention, ulcerative and fibrinous gastritis, ulcerative and diphtheroid enteritis of small and large intestine
1.0 "Thierry"	chewing food without swallowing, followed by spitting out little pieces of the food items	survived	-	-

+ 1 to 30 colonies of bacteria, ++ 30 to 100 colonies of bacteria, +++ > 100 colonies of bacteria

5. Similar cases occurred at the Duke Lemur Center (NC, USA) due to an infection with *Clostridium difficile*. Four of the eight described cases were blue-eyed black lemurs (*Eulemur flavifrons*) (Williams, 2002).

Considering the cases at the Duke Lemur Center and Tierpark Berlin, it is striking that most of the lemurs affected by a *Clostridium* enterotoxaemia belonged to two species, *Eulemur flavifrons* and *Eulemur collaris*. Tierpark Berlin has kept seven lemur species since 1984 and never had cases of this disease until introducing the blue-eyed black lemur into its collection. There were no changes in the feeding and hygiene regime either, which seems to indicate that the blue-eyed black lemur and the red-collared brown lemur are probably highly susceptible for this disease. Spores of *Clostridia* can survive in the soil for a very long time. Despite the thorough disinfection of the inside exhibit with Neopredisan®, two more lemurs became ill, indicating that the lemurs might have picked up spores from the topsoil of the outside enclosure.

Conclusions

It is strongly recommended by the veterinary advisor of the EAZA Prosimian TAG (Dr. Andreas Pauly, Tierpark Berlin, Germany) to immediately treat a lemur, showing symptoms of the described disease (chewing food without swallowing, followed by spitting out little pieces of the food items or vomiting), with metronidazole and to give the animal infusions to accelerate the elimination of the potentially absorbed toxins. If therapy is initiated too late, e.g. later than half a day after the first typical symptoms, the lemur will probably succumb to the disease within a few days. A faecal transplantation from a healthy lemur is very helpful to build up a physiological microbiome in the harmed intestine. This can support the recovery. To further prevent *Clostridum perfringens* infections in zoological collections, it is recommended to use a vaccine, if available. The production of an inactivated vaccine from one of the isolated bacterial strains is a viable option. After the successful treatment and recovery of the last infected red-collared brown lemur, all lemurs at Tierpark Berlin were vaccinated twice with Covexin 10® (0.05 ml per animal) at an interval of four weeks. Covexin 10® includes ten different toxoids of *Clostridium* species, among others toxoid A. No side effects from this vaccination were observed to date (June 2020).

Acknowledgements

The authors would like to thank M. Biering, N. Jahn, Z. Mezö and M. Scholz for their technical assistance with pathological and bacteriological investigations. Furthermore, the authors thank B. Walther, T. Semmler and V. Johanns (Robert-Koch-Institut, Berlin, Germany) for the genome-based virulence typing of *Escherichia coli* isolates.

Zusammenfassung

In den Jahren 2019 und 2020 traten insgesamt fünf Fälle eines Aufblähungs-Syndroms bei zwei Lemuren-Arten, Blauaugenmaki (*Eulemur flavifrons*) und Halsbandmaki (*Eulemur collaris*), im Tierpark Berlin auf. Als Leitsymptome zeigten die Tiere reduzierten Appetit und eine Aufblähung des Abdomens. Die Lemuren bissen vom Futter kleine Stücke ab und schluckten diese nicht ab, sondern spuckten sie zerraspelt wieder aus. Vier der fünf Tiere starben trotz in-

tensiver Therapie innerhalb weniger Tage. Bei zwei der verstorbenen Tiere konnte post mortem ein starker Befall mit *Clostridium perfringens* nachgewiesen werden. Bei einem Tier konnte das Toxovar A isoliert werden. Dieses Bakterium kann durch Bildung von Toxinen zu schwerwiegenden Entzündungen und Lähmungen im Verdauungstrakt führen. Untermauert wurde der Verdacht auf eine *Clostridium perfringens*-Enterotoxämie der verstorbenen Tiere durch einen im Februar 2020 erkrankten Halsbandmaki, der ähnliche Symptome wie die verstorbenen Lemuren zeigte. Dieses Tier konnte erfolgreich mit Metronidazol, einer Infusions-Therapie und durch Gabe von Kottransfusionen eines gesunden Tieres gerettet werden. Aufgrund der vorliegenden Fälle erhielten alle Lemuren des Tierpark Berlin zweimalig eine Clostridien-Impfung. Es wurden bis dato (Juni 2020) keine Nebenwirkungen der Impfung beobachtet.

References

Abbe, C.R., Mansfield, K., Tradif, S.D. & Morris, T. (eds) (2012). Nonhuman Primates in Biomedical Research: Diseases. Academic Press.

Brack, M. (1987). Agents transmissible from simians to man. Springer-Verlag.

Pond, C.L., Newcomer, C.E. & Anver, M.R. (1982). Veterinary Pathology 19 (7_supplement), 126–133.

Terio, K.A., McAloose, D. & St. Leger, J. (2018). Pathology of Wildlife and Zoo Animals. Academic Press.

Williams, C.V. (2002). Spontaneous Clostridium difficile-associated diarrhea in lemurs. Proceedings American Association of Zoo Veterinarians, Milwaukee, Wisconsin, 359–364.

Yasuda, M., Inone, T., Ueno, M., Morita, H., Hayashimoto, N., Kawai, K. & Itoh, T. (2015.) A case of nontraumatic gas gangrene in a common marmoset (Callithrix jacchus). Journal of Veterinary Medical Science 77 (12), 1673–1676.