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RESEARCH REVIEW WITH CASE STUDIES

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Pterygodermatites nycticebi infections in golden lion tamarins (*Leontopithecus rosalia rosalia*) and aye-ayes (*Daubentonia madagascariensis*) from a German zoo

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Abstract

In a golden lion tamarin (*Leontopithecus rosalia rosalia*) colony kept indoors in a German zoo, two animals presented a sudden onset of reduced general condition, lethargy, and diarrhea. At animal capture for clinical examination, adult nematode stages were observed after stress-induced defecation. Despite treatment, two golden lion tamarins died in the following 2 days. At necropsy, spirurid stages were found in the lungs and intestine. Additionally, adult *Pterygodermatites* spp. were identified in histopathological samples of intestine and pancreas, confirming the previous diagnosis. Upon diagnosis, all animals were treated with ivermectin (0.2 mg/kg; SC). Thereafter, the general condition of the golden lion tamarins improved, whereby some of them excreted spirurid nematodes over 3 days. Four weeks after treatment, 20 fecal samples from the colony were examined and proved negative for parasitic stages. Given that common German cockroaches (*Blattella germanica*) are suitable intermediate hosts of *Pterygodermatites nycticebi*, 30 specimens were collected from seven different locations around the golden lion tamarins housing. Third-stage larvae of *Pterygodermatites* spp. were recovered from those cockroaches. Regular anthelmintic treatments, coprological screenings, and controls for intermediate hosts were recommended. More than 2 years later, *P. nycticebi* infection was diagnosed again histopathologically in an aye-aye (*Daubentonia madagascariensis*) which suddenly died. Coprological analysis confirmed the presence of spirurid eggs. Due to prosimian primates' cockroach-eating habits and given that total cockroach eradication proved impossible, continuous cockroach control strategies and regular treatments of primates are currently performed to prevent further *P. nycticebi* infections.

KEYWORDS

Blattella germanica, cockroach, intermediate host, prosimian primate, pterygodermatitosis

1 | INTRODUCTION

Pterygodermatites nycticebi (Quentin, 1969; syn. *Rictularia nycticebi* Mönnig, 1920) is a spirurid nematode parasite from the family Rictulariidae. The first description of this parasite in slow loris (*Nycticebus coucang*) originated from Java, Indonesia (Montali et al., 1983; Sato et al., 2003). Nowadays, *P. nycticebi* infections are occasional, and the cause of severe problems in zoo-living prosimian primates worldwide (Sato et al., 2003). Balsiger et al. (2018) presented a record of currently known monkey species susceptible to *P. nycticebi*, which also lists the golden lion tamarin (*Leontopithecus rosalia rosalia*).

Parasitic stages of *Pterygodermatites* spp. are usually found luminal or firmly attached to the mucosa of the stomach and the small intestine of American monkeys (Anderson, 1992; Balsiger et al., 2018). After mating, *P. nycticebi* females deposit oval eggs containing first-stage larvae (L1), that, after release with defecation, are ingested by suitable intermediate hosts, such as cockroaches (e.g., genera *Blatta*, *Blattella*, *Periplanata*) and dung beetles (e.g., genera *Scarabaeus*, *Madateuchus*; Anderson, 1992; Balsiger et al., 2018). Subsequently, hatched L1 invade the arthropod intestinal wall and undergo two molts into infective third-stage larva (L3), which is found enclosed in a fibrous capsule in the intestinal wall close to the Malpighian tubules (Balsiger et al., 2018). After ingestion of infected intermediate hosts by prosimian primates, two further molts occur as the L3 develop to the adult stage. Heavily *P. nycticebi*-infected animals show profound weakness, anemia, hypoproteinemia (Montali et al., 1983), and diarrhea, the latter of which can lead to death (Balsiger et al., 2018). Here, we describe *P. nycticebi* infections of golden lion tamarins (*Leontopithecus rosalia rosalia*) and aye-ayes (*Daubentonia madagascariensis*) kept indoors in a German zoo, and the control measures applied.

2 | CASE PRESENTATION

At the beginning of June 2017, two golden lion tamarins (*L. rosalia rosalia*) from a group of nine animals, were reported with sudden onset of reduced general condition, lethargy, apathy, and diarrhea. During animal capture for individual health assessment, some animals presented fever, and one of the females shed nematode parasites after stress-induced defecation. At the same time, a recurrent lymphocytic choriomeningitis virus outbreak was suspected and, consequently, parasitic infections were not considered responsible for such severe clinical signs. All animals were treated with enrofloxacin (5 mg/kg bodyweight [bw]; SC; Baytril® 2.5%), metamizole (20 mg/kg bw; SC; Vetalgin®), inactivated *Parapoxvirus ovis* vaccine (1 ml; SC; Zylexis®), and fluid therapy (20 ml/kg bw; SC; Ringer's lactate and glucose 5%) for seven consecutive days. In the following 2 days, two other golden lion tamarins died (one male and one female) and the group was treated with ivermectin [0.2 mg/kg bw; SC; Ivomec-R® diluted in propylene glycol (1:10)]. On the 4thday of treatment (1 day after the ivermectin application) numerous adult nematode parasites were observed in the feces (Figure 1), but all animals presented good appetence. On the 5th day, no animal presented diarrhea or parasite shedding, but on the 6th day of treatment, adult parasites were again observed in feces. After repeated administration of ivermectin (0.2 mg/kg bw; SC; Ivomec-R diluted in propylene glycol) on the last day of treatment, adult nematodes were again shed.

FIGURE 1 Adult stages of nematode parasites in stressed-induced defecation and after treatment with ivermectin [Color figure can be viewed at wileyonlinelibrary.com]

At necropsy, the tamarins presented incipient cachexia. Multiple nematodes (5 mm long × 0.5 mm wide) were found in the pleural cavity and the esophagus. The lungs presented a mild multifocal lymphohistiocytic interstitial inflammation. Additionally, adult female parasites were identified in histopathological samples of intestine and pancreas (Figure 2). Low-grade multifocal lymphohistiocytic inflammation was observed in the intestine. Severe focal-extensive necrotizing inflammation was detected in the pancreas and numerous intralesional nematodes were found via periodic acid–Schiff staining. Other findings included mild to moderate hemosiderosis in spleen, moderate hepatitis showing multifocal periportal lymphohistiocytic inflammation with low-grade hemosiderosis, and low-grade interstitial nephritis showing multifocal lymphohistiocytic inflammation. At this moment, no parasitic stages were diagnosed by coprology. The cause of death of the animals was cardiac failure due to cachexia caused by a severe helminthosis. The nematodes were identified as *P. nycticebi* (Rictulariidae, Spirurida) based on their morphological features: two subventral rows of combs (88 pairs) from behind the buccal cavity and terminating at the level of the anus, being 40–42 pairs of prevulvar spines, dorsally inclined buccal opening, never totally dorsal and transversal and surrounded by denticles, oral cavity with three well-developed esophageal teeth (Ikeda et al., 2003; Quentin, 1969; Simões, 2016).

At the end of June 2017, another female golden lion tamarin from the same group suddenly presented bad body condition, hypothermia, and seizures. Despite intensive therapy (diazepam [0.5 mg/kg bw; Diazepam-Ratiopharm® 5 mg], enrofloxacin [5 mg/kg bw; SC; Baytril® 2.5%], fluids [20 ml/kg bw; SC; Ringer's lactate and glucose 5%], meloxicam [0.2 mg/kg bw; SC; Metacam® 2 mg/ml], and

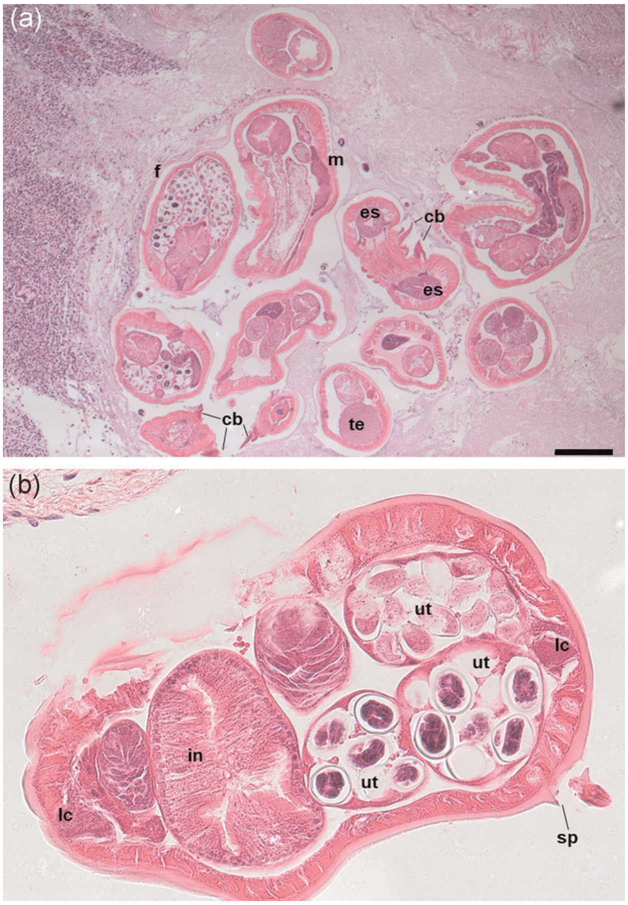

FIGURE 2 Histopathological samples of (a) pancreas (multiple parasites: f, female and m, male), and (b) intestine with gravide female *Pterygodermatites nycticebi*. Scale bar = 200 µm (a) and 50 µm (b). cb, comb; es, esophagus; in, intestine; lc, lateral cord; sp, spine; te, testis; ut, uterus with eggs [Color figure can be viewed at wileyonlinelibrary.com]

virostatic agents [2 × 10 mg/kg bw; orally; Aciclovir-Ratiopharm®]), the animal condition deteriorated and it was found dead 2 days later. Curiously, this female presented fever in previous medical controls, but in the last 2 days before its death presented hypothermia. One week later, a subadult male golden lion tamarin abruptly showed reduced general condition, pale mucous membranes, and dehydration. It was treated with intensive therapy as previously described and despite general condition improvement, it was found dead 2 days thereafter. *Postmortem* examinations were carried out on both animals. The female presented the same nematodes described previously and concomitant lymphocytic choriomeningitis virus infection, while the male was diagnosed with lymphocytic choriomeningitis virus infection but no parasitic infection.

At the end of August 2017, 4 weeks after the ivermectin treatments of all animals from the same housing, 20 fecal samples of different monkey species were analyzed using sodium acetate acetic acid formalin (SAF) method (Yang & Scholten, 1977; Young et al., 1979). A nematode specimen excreted by a golden lion tamarin was morphologically identified as *P. nycticebi* (as previously described; Figure 3a–c); no other parasite stages were found coproscopically. This could be related with the sporadic egg shedding and the fact that they are rather rare to be found, or with maybe the analyses were performed during the prepatent period. Moreover, given that common German cockroaches (*Blattella germanica*) are suitable intermediate hosts of *Pterygodermatites* spp., alive cockroaches (*n* = 30; *B. germanica*) were collected from seven different locations (*n* = 4–5 per location) around tamarins housing for parasitological diagnosis. Cockroaches were frozen at −20°C for 2 h before homogenization with a mortar and pestle. Third-stage larvae of *Pterygodermatites* spp. (Figure 3d) presented well-developed buccal capsules with denticles, prominent cross-striated lateral alae which extended until near the anus, and a sharply pointed tail (Simões, 2016). Furthermore, L3 larvae were exclusively recovered from those cockroaches originating from the same housing where two animals had died previously, confirming the presence of this spirurid parasite not only in definitive hosts but also in suitable invertebrate intermediate hosts.

At the end of 2019, regardless of regular coprological analysis (every 2–3 months) and consequent anthelmintic treatments (ivermectin; 0.2 mg/kg bw; orally) following positive results, an aye-aye (*D. madagascariensis*) succumbed to sudden death and a *P. nycticebi* infection was confirmed (as previously described) at *postmortem* examinations. Consequently, 44 scat samples from different primate animals kept at the zoo were analyzed via SAF methodology. Spirurid eggs were identified exclusively in Goeldi monkeys (*Callimico goeldii*) fecal samples (6.8%; *n* = 3). Oval thick-shelled embryonated eggs (Figure 3a) measured between 30–39 × 22–30 µm. Moreover, several American primate samples, including golden lion tamarins (*L. rosalia rosalia*) and pygmy marmoset (*C. pygmaea*), proved positive for *Giardia* antigen (31.8%, *n* = 14, coproantigen ELISA). Furthermore, *Entamoeba coli* cysts (13.6%, *n* = 6) and *Neobalantidium coli* cysts (2.3%, *n* = 1) were observed in other primate samples.

For control reasons, in January 2020, 35 primate fecal samples were analyzed. In none of these samples, spirurid eggs were observed. Overall, the parasitological examination revealed 8.6% (*n* = 3) galago samples positive for *Giardia* cysts via SAF method, 8.6% (*n* = 3) samples positive for *N. coli* cysts, and 20% of all samples (*n* = 7) positive for *Giardia* spp. coproantigen (ELISA).

3 | DISCUSSION AND CONCLUSION

Zoo animals are considered as a valuable resource of endangered species, such as golden lion tamarins (*L. rosalia rosalia*) endemic in the Atlantic forest in Rio de Janeiro State, Brazil (Dietz et al., 2019; Ruiz-Miranda et al., 2019). *P. nycticebi* infections have been associated with severe or even fatal outcome in prosimian primates (Ikeda et al., 2003; Montali et al., 1983). Especially in golden lion tamarins, pterygodermatitosis was reported to be associated with high morbidity and mortality (Ikeda et al., 2003; Montali et al., 1983), but other prosimians are also known to be susceptible for *P. nycticebi* (Balsiger et al., 2018). Here, we present a case report of a *P. nycticebi* outbreak in a German zoo, which caused the death of four golden lion tamarins and one aye-aye. *P. nycticebi* infections of *L. rosalia*

FIGURE 3 Adult *Pterygodermatites nycticebi* (a) presents subventral combs from behind the buccal cavity (b, anterior part) till the level of the anus (c, posterior part). Third-stage larva of *P. nycticebi* isolated from *B. germanica*. L3 with prominent cross-striated lateral alae (arrow) which extend until near the anus, and a sharply pointed tail. (a) Oval thick-shelled embryonated eggs (30–39 × 22–30 μm). Scale bar = 10 μm (a); 200 μm (a, b); 100 μm (c); 50 μm (d) [Color figure can be viewed at wileyonlinelibrary.com]

rosalia had been previously reported in the same zoo back to the year 1985 (Matern & Fiege, 1989).

At necropsy, adult *P. nycticebi* parasites are typically found in the small intestine and colon of final hosts; as additional locations, stomach, cecum, and rectum were described (Anderson, 1992; Ikeda et al., 2003; Montali et al., 1983). In the current case, the first histological examinations revealed the presence of adult gravid females in the intestine but also in the pancreas. So far, only one previous study recorded the occurrence of *P. nycticebi* stages in pancreatic ducts (Montali et al., 1983). However, other spirurid species parasitizing exclusively this organ, namely *Trichospirura leptostoma* (Hawkins et al., 1997), cannot entirely be ruled out since mixed infections could have been present in this animal. In line to other findings (Balsiger et al., 2018; Matern & Fiege, 1989), intestinal multifocal lymphohistiocytic inflammation was here observed, but in contrast, *P. nycticebi* L3 were neither detected encapsulated in the intestinal wall nor in the mesenteric lymph nodes. *P. nycticebi* L3 were recovered from intermediate hosts German cockroaches. Given that *P. nycticebi* L3 are generally encapsulated in the intestinal cells of *B. germanica* (Anderson, 1992; Ikeda et al., 2003), cockroaches were homogenized to facilitate the isolation of these larvae. This analysis was crucial for a better understanding of the transmission mode within captivity. As stated before, the life cycle of this obligate heteroxenous spirurid nematode includes diverse coprophagous insects (e.g., dung beetles, cockroaches) as intermediate hosts (Ikeda et al., 2003) perpetuating pterygodermatitosis in zoos worldwide (Sato et al., 2003). Zoos that house callitrichid primates provide a good environment for the maintenance of stable cockroach populations, such as *B. germanica* (Montali et al., 1983; Yue & Jordan, 1986). Nonetheless, a recent report from Switzerland suggested that other insects, such as European earwigs (*Forficula auricularia*), also act as permissive intermediate hosts for *P. nycticebi* (Balsiger et al., 2018). Total eradication of these ubiquitous insect species seems impossible to achieve, but continuous insect control should reduce prosimians exposure to these spirurids. However, Balsiger et al. (2018) recorded that even after total decontamination of primates housing premises, infected insects were still present and monkeys shed spirurid eggs soon after reintroduction. So far, the prepatency of *P. nycticebi* is still unknown in American monkeys, but it is assumed to last 4 to 8 weeks, as reported for other spirurid species (Balsiger et al., 2018; Yue & Jordan, 1986). Therefore, in the current case, anthelmintic treatments were recommended to be applied every 4 to 6 weeks. As described by Montali et al. (1983), *P. nycticebi* control was achieved by a combination of regular fecal screening of all primates in the zoo, biannual prophylaxis with mebendazole (40 mg/kg bw), and a rigorous cockroach extermination program. In other reports, treatments included mebendazole, levamisole, and ivermectin (Balsiger et al., 2018; Matern & Fiege, 1989; Montali et al., 1983). However, anthelmintics might not be effective against all stages of the parasite, especially encapsulated stages. In line, levamisole treatments (7.5 mg/kg bw, repeated after 2 weeks) failed to effectively eliminate nematodes in another monkey population (Balsiger et al., 2018).

In the current report, until the end of 2019, repeated coprology analysis and consequent treatments with ivermectin seemed effective, since no further *P. nycticebi* infections were apparent. Nevertheless, in December 2019, *P. nycticebi* infections reoccurred in two other primate species of the zoo, that is aye-aye (*D. madagascariensis*) and Goeldi monkeys (*Callimico goeldii*), and were diagnosed by both, necropsy and coprological examinations. It is worth noting that all newly introduced primates at the zoo went through a 4-week quarantine in a separate building and a coprological screening was performed before integration to prevent introduction of new pathogens into the colony. Also, coprological analysis is performed when animals will change the enclosures, or in case of any signs of illness. Following deworming depends on the results of the analysis.

In vivo diagnosis of *P. nycticebi* infections is easily based on the identification of typical spirurid thick-walled, embryonated eggs by standard SAF or zinc chloride flotation-based coprological analysis (Balsiger et al., 2018), or via molecular analyses that allow a species confirmation. The SAF method has a high sensitivity for protozoan parasitic stages (e.g., trophozoites, cysts, oocysts) and acceptable sensitivity for metazoan parasitic stages, (e.g., nematode eggs). In addition, it is inexpensive, easy to perform, and, due to the absence of mercury, it has low toxicity (Marti & Escher, 1990) when compared to other diagnostic methods. Furthermore, the SAF method reduces the infectivity of the fecal samples due to its fixation component. Consistently, this method also permitted the identification of concomitant protozoan infections in primates, such as giardiosis, amebosis, and neobalantidiosis. Even though animals were in good condition, early diagnosis of such infections allowed prompt treatment. This seems important, since any additional stress factor, such as concomitant infections, relocation of animals, weaning, or pregnancy, may support a considerable spread of *Pterygodermatites* spp. infections (Balsiger et al., 2018).

Improved hygiene measures and continuous control programs of arthropods support reduction of parasite exposure to these primates (Montali et al., 1983). In this case, some measures were reinforced, for example, positively tested animals were separated from the others, designated cleaning tools for this housing, personal protection equipment for keepers, and foot bath disinfection at the entrance of the housing, and, most importantly, increased pest control. It is of extreme importance that the life cycle of the parasite is abrogated, especially when considering that cockroaches are extremely successful in establishing new colonies in suitable environments. In the current case, animals remained infected and shed eggs even under the reported measures, showing that total eradication of *P. nycticebi* from zoos can hardly be achieved. Moreover, if rodents are involved in *P. nycticebi* life cycle as definitive hosts as previously demonstrated (Balsiger et al., 2018), their control in zoos is highly challenging.

In conclusion, several factors can be implicated in the failure of pterygodermatitosis control. First of all, the unknown prepatency period, which poses a problem when deciding when would be the best time to treat; second, the fact that some stages can remain encysted in the gut wall as a dormant stage, being able to resume development later on, and probably being not affected by the treatment; and third, the unfeasibility of total elimination of intermediate hosts, allowing for constant reinfection. Therefore, it is not possible to claim that the anthelmintic treated failed, but rather assume that an integrated and multidisciplinary control approach is necessary. Thus, continuous and regular anthelmintic treatments of primates with different compounds in combination with regular coprological screenings are highly recommended. Improved hygiene measures together with long-term monitoring programs to reduce intermediate host populations seem superior and absolutely necessary to prevent lethal *P. nycticebi* infections in zoo husbandries.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. All data are already included in the manuscript.

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