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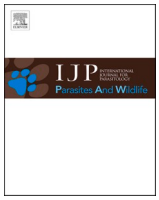

Successful treatment of *Trypanoxyuris* sp. infection in naturally infected southern brown-howlers (*Alouatta guariba clamitans*)

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ABSTRACT

Southern brown-howler monkeys (*Alouatta guariba clamitans*) may harbor *Trypanoxyuris* sp., a pinworm parasite with documented fatal consequences in this species. Despite this risk, effective treatment protocols remain unclear. Therefore, the present study aimed to evaluate the efficacy of two anthelmintic protocols against natural infections in two brown-howler monkeys received at the Wild Animal Care and Rehabilitation Sector (SARAS-CAV-UDESC). The protocols utilized pyrantel pamoate & praziquantel (600.0 mg, PO, single dose) and albendazole (20.0 mg, PO, daily for 5 days). Fecal egg counts were carried out daily at the Laboratory of Parasitology and Parasitic Diseases (LAPAR-CAV-UDESC) before and after drug administration. Both treatments successfully eliminated *Trypanoxyuris* sp. infections. The animal treated with pyrantel pamoate & praziquantel achieved egg clearance by day 6 (144 h), demonstrating effectiveness with a single administration. Albendazole cleared the infection within 2 days of treatment, indicating its potential as a fast-acting treatment. No adverse effect were observed in the treated monkeys. These findings contribute to the development of evidence-based treatment protocols for *Trypanoxyuris* sp. in primates, enhancing animal health and welfare of captive and wild populations.

1. Introduction

Oxyurids, nematodes from the Oxyuridae family, can infect a wide variety of primates, including humans (Hugot, 1999; Hugot et al., 1996). Phylogenetic analysis and co-evolutionary studies suggests that Old World Primates are typically infected by oxyurids from the genus *Enterobius*, while New World Primates are infected by *Trypanoxyuris* spp. (Hugot, 1985, 1999; Hugot et al., 1996). Similarly, humans are commonly infected by *Enterobius* spp. (Ohnishi et al., 2011; Patel et al., 2015).

The *Alouatta* genus is comprised of twelve species of Neotropical primates, distributed from the southern Mexico to southern of Brazil and Argentina (Cortés-Ortiz et al., 2015; Kowalewski and Raño, 2017). The southern brown-howler (*Alouatta guariba clamitans*) is one of those species, inhabiting the Atlantic Forests of Brazil (Cortés-Ortiz et al., 2015). This species demonstrates adaptability, occupying diverse habitats ranging from tropical to deciduous and semideciduous seasonal forests, as well as small and fragmented forest patches (Kowalewski and Raño, 2017).

Trypanoxyuris sp. have a direct life cycle, with transmission occurring through the ingestion of eggs containing infective larvae, shed by infected hosts. These eggs can contaminate the environment, and retroinfection (self-reinfection) is also possible (Felt and White, 2005). *Trypanoxyuris* sp. primarily infects the cecum and large intestine of primates (Amato et al., 2002; Souza et al., 2010). Clinical signs can include anal or perineal pruritus (itching), diarrhea, altered mood and behavior, anorexia, weight loss, and in severe cases, death (Koehler et al., 2014). Diagnosis is typically achieved through the detection of eggs or adult worms in feces, using coproparasitological exams, or the perianal tape test (Bentzel et al., 2007; Ikeda et al., 2016; Stuart et al., 1998).

There is a lack of literature regarding the effectiveness of treatment

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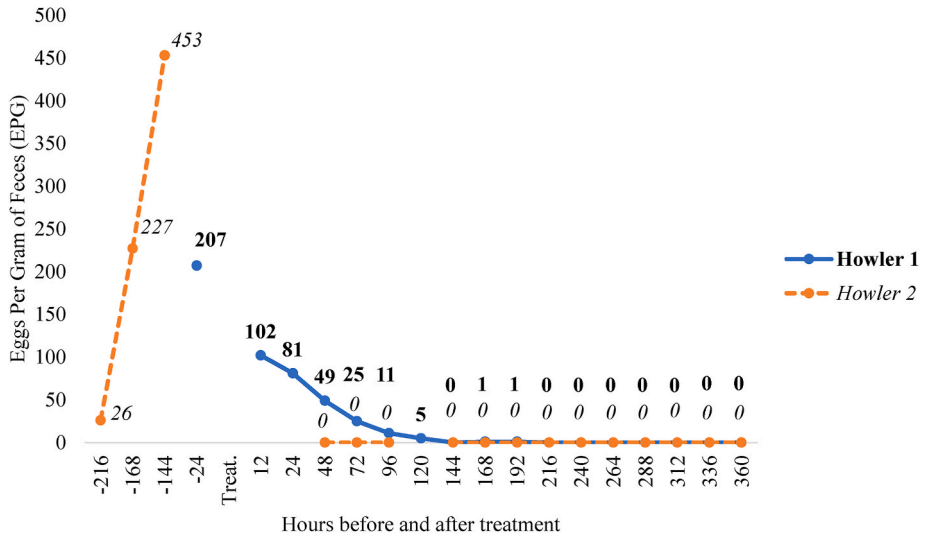

Fig. 1. *Trypanoxyuris* sp. fecal egg count before and after the administration of pyrantel pamoate & praziquantel (Howler 1) and Albendazole (Howler 2).

protocols for *Trypanoxyuris* sp. infection in non-human primates, particularly howlers. Thus, the objective of this study was to evaluate the effectiveness of the association of the anthelmintics pyrantel pamoate & praziquantel and albendazole against naturally occurring *Trypanoxyuris* sp. infections in wild southern brown-howlers undergoing medical care and rehabilitation for eventual release into their natural habitat, when deemed able to.

2. Material and methods

2.1. The southern Brown-howlers

Two female southern brown-howlers were attended by the Santa Catarina Environmental Police in Brazil and sent to the Wild Animal Care and Rehabilitation Sector (SARAS-CAV-UDESC), located in Lages, Santa Catarina State (SC).

The first howler (Howler 1) was rescued following a car accident in a highway close to the city of São Joaquim (SC). Over anamnesis and physical examination, the primate was diagnosed with a broken jaw, being stabilized, treated and receiving drugs for pain control. Howler 1 remained in captivity for one month for clinical and medical observation. Following a comprehensive health assessment, including successful parasite treatment, the primate was deemed fit for release back into the wild. Its broken jaw had healed properly, it maintained a healthy weight, and displayed normal primate behaviors, including appropriate aggression towards humans. The animal was then reintroduced to its original capture location, with the expectation of successful reintegration into the local howler population.

The second animal (Howler 2) was rescued from a rural zone near the city of Curitibanos (SC), following reports of a dog attack on a group of primates. During anamnesis and physical examination, the animal did not present clinical signs, except low weight. Howler 2 was rescued as an infant and remained in captivity for 16 months. Due to extensive and early contact with humans, the howler was deemed unfit for release, despite its good health and successful parasite treatment. Consequently, it was transferred to the Howler Project in Indaial, Santa Catarina, for continued care. It is noteworthy that the Howlers 1 and 2 never interacted, as they were cared for at SARAS-CAV-UDESC during different times.

2.2. Parasitological examination

Fecal samples were collected from the animal enclosure shortly after defecation, usually in the mornings. One sample was collected per animal, daily, for 15 days. Samples were placed in polypropylene flasks and transported in a styrofoam cooler to the Laboratory of Parasitology and Parasitic Diseases (LAPAR-CAV-UDESC), where they were promptly refrigerated at 4 °C. Examinations were performed on the same day of collection or the following morning. Fecal analysis was performed in duplicates using a modified Willis quantitative flotation technique with saturated NaCl solution (Willis, 1921).

Briefly, 3 g of feces were homogenized in 42 ml of common water, followed by the filtration of the material in a tea sieve. Subsequently, 15 ml of the material was transferred to two centrifuge conic tubes of the same volume. The tubes were then centrifuged for 4 min at 1500 rpm. After centrifugation, the supernatant was discarded and the resulting pellet resuspended in saturated NaCl solution, filling each tube to form a meniscus. A glass slide was placed over the meniscus of each tube. After 15 min the slide was removed, cover slipped and examined under optical microscope (Eclipse E200, Nikon). The eggs found were counted, and the simple mean calculated between the duplicates of each sample to determine the approximated eggs per gram of feces (EPG).

Furthermore, the howlers shed some adult nematodes in their feces. Four females and two males with intact morphological features were recovered for analysis. The collected helminths were fixed in Railliet-Henry solution, mounted on microscope slides and clarified in lactophenol. Morphological examination was carried out by optical microscopy (Lab A1, Zeiss and Eclipse E200, Nikon) and an image software (ZEN 2.6, blue edition, Zeiss). Identification used morphological and biometric characteristics found in literature (Amato et al., 2002; Barbosa et al., 2017; Souza et al., 2010).

2.3. Treatment and follow-up

Howler 1 weighed 4,5 kg and was treated with a single 600 mg oral dose of pyrantel pamoate & praziquantel (Chemital Gatos®) (containing 348 mg of pyrantel pamoate and 30 mg of praziquantel). Howler 2 weighed 1.8 kg and received albendazole (Albel®) (40 mg/ml) orally, 0.5 ml (20 mg), daily, for five days. The treatment of Howler 2 followed a safe dose of 10 mg/kg, and it was repeated due to reports of treatment failure using a single dose (Amato Neto et al., 1985; Horton, 2000).

To evaluate treatment effectiveness, fecal samples were collected before and after treatment, as depicted in Fig. 1, for both monkeys. All collections followed the already described protocol. For Howler 1, a sample was collected 24 h before treatment, while for Howler 2216 h (9 days), 168 h (7 days) and 144 h (6 days) before treatment. Post-treatment examinations were performed for 360 h (15 days). Additional fecal examinations were carried out 40 days after treatment in

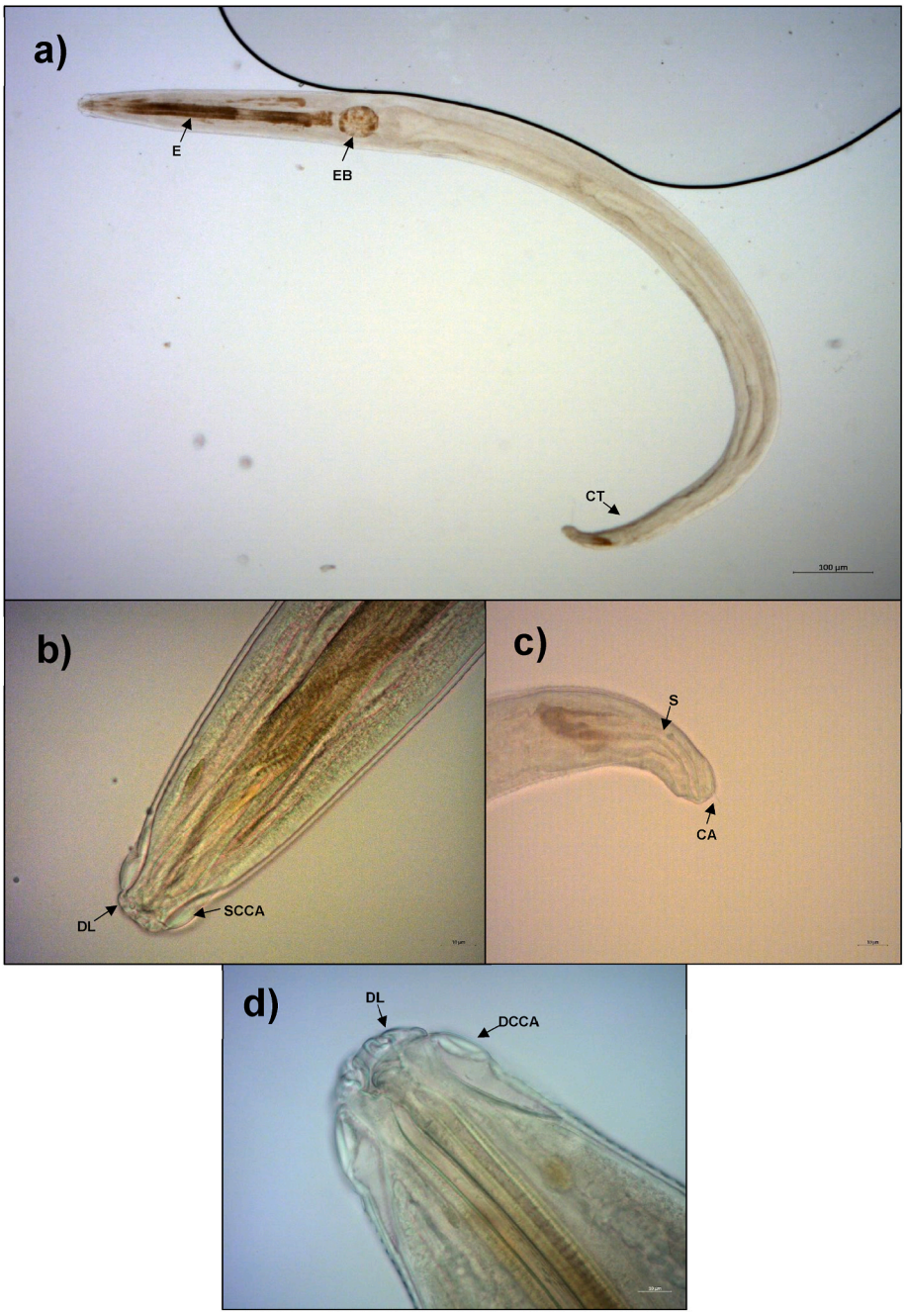

Fig. 2. *Trypanoxyuris* sp. morphology. a) Male specimen; b) Male anterior region; c) Male posterior region, d) Female anterior region. (E = esophagus; EB = esophageal bulb; CT = curved tail; DL = dorsal lips; SCCA = single-crested cephalic alae; S = spicule; CA = caudal appendix; DCCA = double-crested cephalic alae).

Howler 1, and 33 days after treatment in Howler 2. To avoid daily physical restraint, the perianal tape test was not utilized in this study, adhering to local animal welfare protocols.

3. Results and discussion

During the initial parasitological examination of the rescued southern brown howler monkeys, prior to the initiation of any treatment, eggs consistent with the Oxyuridae family were detected in their fecal samples (Ikeda et al., 2016). The mean EPG detected in these animals, before and after anthelminthic treatment, is presented in Fig. 1. The treatment was considered successful if the howler did not shed any eggs on their feces after the 15-day analysis period. Therefore, both anthelmintics effectively eliminated the parasitic load of both treated monkeys. The investigation of reinfection in both animals resulted negative, as no eggs were detected 40 days after treatment in Howler 1, and 33 days after treatment in Howler 2.

The number of *Trypanoxyuris* sp. eggs found in the fecal samples of Howler 1, treated with pyrantel pamoate & praziquantel, steadily decreased after the drug administration, reaching egg clearance by the sixth day (144 h) after treatment. However, positive results (1 EPG) were found in samples collected at the seventh and eighth days (168 and 192 h) post-treatment. All subsequent samples were negative for eggs.

No fecal samples of Howler 2 were collected during the first (12 and 24h) and fifth day (120h) after treatment with albendazole, nor the day before treatment (−24h), as the animals did not defecate or the feces were outside the cage and disposed. All samples from this howler showed negative results for *Trypanoxyuris* sp. eggs over the 15 days

Table 1
Biometric measurements of female and male specimens of *Trypanoxyuris* sp. shed by *A. guariba clamitans* in this study, compared to specimens of *Trypanoxyuris minutus* collected in postmortem analysis carried out in previous studies.

FEMALE SPECIMENS				
MEASUREMENTS	**This study (n = 4)**	**Barbosa et al. (2017) (n = 20)**	**Souza et al. (2010) (n = 17)**	**Amato-Neto et al. (2002) (n = 11)**
TOTAL BODY LENGTH	8.12–8.77 mm 8.34 ± 0.26	3.39–6.7 mm 4.47 ± 0.96	5.52–7.86 mm 6.65 ± 0.69	7.10–8.50 mm 7.70 ± 45
MAXIMUM BODY WIDTH	600–700 µm 637.5 ± 38.97	–	–	–
LENGTH OF THE ESOPHAGUS	1.76–1.80 mm (Including bulb) 1.78 ± 0.02	1.04–1.65 mm 1.26 ± 0.16	1.45–1.67 mm 1.6 ± 0.05	1.62–1.80 mm (Including bulb) 1.72 ± 56
LENGTH OF THE ESOPHAGEAL BULB	140–170 µm 155 ± 11.18	–	–	97–153 µm 141 ± 20
WIDTH OF THE ESOPHAGEAL BULB	140–160 µm 147.5 ± 8.29	–	–	129–161 µm 151 ± 20
WIDTH OF THE INTESTINAL-ESOPHAGEAL JUNCTION REGION[a]	200–270 µm 235 ± 35	175–382 µm 231 ± 57.28	260–385 µm 307.08 ± 44.79	–
EGGS LENGTH	45–47.5 µm 46.3 ± 1.25	45–50 µm 47.5 ± 1.66	41.45–50.12 µm 47.42 ± 2.8	44–46 µm 45 ± 1
EGGS WIDTH	20–27.5 µm 24.4 ± 2.72	20–25 µm 23.2 ± 1.68	21.87–24.5 µm 23.45 ± 0.85	22–26 µm 24 ± 2
MALE SPECIMENS				
MEASUREMENTS	**This study (n = 2)**	**Barbosa et al. (2017) (n = 10)**	**Souza et al. (2010) (n = 17)**	**Amato-Neto et al. (2002) (n = 38)**
TOTAL BODY LENGTH	2.35–2.89 mm 2.62 ± 0.27	2.29–2.8 mm 2.49 ± 0.12	2.15–2.69 mm 2.46 ± 0.17	2.40–3.02 mm 2.72 ± 1.8
MAXIMUM BODY WIDTH	120–150 µm 135 ± 15	–	–	129–153 µm 136 ± 12
LENGTH OF THE ESOPHAGUS	700 µm (Including bulb) 700 ± 0	612–791 µm 705.41 ± 41.42	556.25–775 µm 681.1 ± 49.97	677–838 µm (Including bulb) 761 ± 59
LENGTH OF THE ESOPHAGEAL BULB	90–92 µm 91 ± 1	–	–	80–89 µm 87 ± 3
WIDTH OF THE ESOPHAGEAL BULB	80–82 µm 81 ± 1	–	–	81–88 µm 81 ± 4
WIDTH OF THE INTESTINAL-ESOPHAGEAL JUNCTION REGION	95–110 µm 102.5 ± 7.5	102–153 µm 134.04 ± 13.97	105–125 µm 111.94 ± 5.05	–
SPICULE LENGTH	50–62.15 µm 56.08 ± 6.07	40–60 µm 46.21 ± 5.45	43.75–56.25 µm 52.05 ± 2.79	40–44 µm 45 ± 2
CAUDAL APPENDIX LENGTH	10 µm 10 ± 0	11.1–18.5 µm 11.9 ± 3.50	15–22.50 µm 18.93 ± 2.44	8–10 µm 9.5 ± 1.3

Minimum-Maximum value/Simple mean ± Standard deviation.
[a] The measurements were taken on only two viable female specimens.

(treatment to 360h) of analysis. Howler 2 eliminated adult nematode specimens 48 and 96 h after treatment, due to the treatment, but no egg was detected in the samples.

Adult nematodes shed in the feces and recovered were morphologically identified as *Trypanoxyuris* sp. (Fig. 2) using microscopy at 40, 100× and 400× magnification. The female nematodes were larger than the males. Males had a curved posterior third, while females a straight one. Both sexes had a bilobate dorsal lip, while females had a double-crested cephalic alae and males a single-crested cephalic alae (Amato et al., 2002; Barbosa et al., 2017; Hugot, 1985; Souza et al., 2010). Morphological biometric criteria used for identification and its results are presented in Table 1 (minimum and maximum value; simple mean ± standard deviation).

Howler monkeys can live close to environments that have been altered by human activity such as agriculture and livestock, but they prefer living in untouched forests (Estrada, 2015). The increasing land usage, deforestation, climatic change and fragmentation of habitats have increasingly confined these animals to the edges or smaller patches of forests (Behie et al., 2014; Chapman et al., 2005; Chapman and Peres, 2001; Cruz et al., 2000; Gillespie et al., 2005; Trejo-Macías et al., 2007). These factors may increase the chances of contact between human and non-human primates and the cross infection by zoonotic pathogens (Chapman et al., 2006, 2005; Solórzano-García and Pérez-Ponce de León, 2017).

The transmission of *Trypanoxyuris* spp. occurs by direct contact between primates, after infective larvae develops within eggs present in their perineal region, or indirectly by environmental contamination. Therefore, the reduction in their natural habitat, by anthropic influence, may increase the transmission of parasites, due to closer contact between individuals.

There are several reports of infection of wild howler monkeys by *Trypanoxyuris* spp., with the helminths being detected by fecal examination, adult worm collection, or perianal tape test (Amato et al., 2002; Ikeda et al., 2016; Martins et al., 2008; Solórzano-García et al., 2020, 2016; Solórzano-García and Pérez-Ponce de León, 2017; Souza et al., 2010), with a report of hyper infection of *A. guariba clamitans* by *Trypanoxyuris minutus* associated to its death (Amato et al., 2002).

In the present study, the pyrantel pamoate & praziquantel treatment was 100% efficient in eliminating the infection by *Trypanoxyuris* sp. This is the first report of the treatment with these compounds in southern brown-howlers. These findings corroborate with a previous report of 100% of success using pyrantel pamoate, for treating owl monkeys (*Aotus nancymae*) infected by *Trypanoxyuris microon* (Bentzel and Bacon, 2007). The authors also reported 100% effectiveness with ivermectin, which was considered a feasible treatment alternative, with both drugs having better results than thiabendazole (60% effectiveness).

This report also provides the first description of the treatment of *A. guariba clamitans*, naturally infected by *Trypanoxyuris* sp., with albendazole. This treatment reached 100% of efficiency, even though it is a compound from the same pharmacological class of thiabendazole (Bentzel and Bacon, 2007).

The only other report found in literature regarding southern-brown howlers treated for *Trypanoxyuris* sp. infection described unsuccessful treatment protocols using ivermectin and pyrantel pamoate (Ikeda et al., 2016). In that report, the therapeutical aim was only achieved by the administration of ivermectin associated with fenbendazole.

Furthermore, combining treatment with proper disinfection of the primate living environment is crucial for therapeutical success (Ikeda et al., 2016), as is the potential need for a second round of treatment, based on individual animal assessment, to reduce the chances of reinfection (Ikeda et al., 2016; Koehler et al., 2014). In this study, the animals were examined approximately five weeks after treatment (40 and 33 days after treatment for Howler 1 and 2, respectively), and no reinfection was detected. This is probably due to the SARAS adequate sanitary management of the animals under treatment.

The importance of establishing effective therapeutical protocols for the treatment of infection by *Trypanoxyuris* sp. in *A. guariba clamitans* is of paramount importance due to their classification as a threatened species in Brazil, and the potential for a fatal outcome of such infection (Amato et al., 2002; Ministério do Meio Ambiente, 2022). The concern extends to captive primates given the high number of viable oxyurid eggs shed by the animals. Therefore, adequate disinfection of enclosures and living spaces, together with efficient anthelminthic treatment protocols, are crucial for the guarantee of animal wellbeing and health when maintained *ex situ* (Koehler et al., 2014).

4. Conclusion

This report details the antemortem diagnosis of tripanoxiurosis and the successful treatment of *Trypanoxyuris* sp. infection in two female *A. guariba clamitans* using either a single dose of pyrantel pamoate & praziquantel (600 mg) or five daily oral doses of albendazole (20 mg). These findings contribute to the development of evidence-based treatment protocols for *Trypanoxyuris* sp. in primates, potentially improving captive and wild animal welfare.

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Data statement

All data will be made available based on request by interested parties.

CRediT authorship contribution statement

Felipe Rieth de Lima: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Luísa Barreto Rippel:** Writing – review & editing, Investigation, Formal analysis. **Sandy Gabrielly Radünz Machado:** Writing – review & editing, Investigation, Formal analysis. **Aury Nunes de Moraes:** Writing – review & editing, Supervision. **Bárbara Corbellini Rovaris:** Writing – review & editing, Investigation, Formal analysis. **Anderson Barbosa de Moura:** Writing – review & editing, Supervision. **Andreas Lazaros Chryssafidis:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declarations of competing interest

The authors declare that there is no conflict of interest in the production of this manuscript.

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