BMC Veterinary Research

RESEARCH

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Survey of parasitic fauna data from wild animals through coproparasitological diagnosis in Southern Brazil

Julia Somavilla Lignon[1,2*], Diego Moscarelli Pinto[2], Tamires Silva dos Santos[2], Giulia Ribeiro Meireles[2], Camila Gonçalves da Silveira[2], Bianca Conrad Bohm[1], Felipe Geraldo Pappen[2], Silvia Gonzalez Monteiro[3], Mauro Pereira Soares[4], Raqueli Teresinha França[5] and Fábio Raphael Pascoti Bruhn[1]

Abstract

Background The proximity between people and their domestic animals with wild animal populations can result in the spread of diseases with a significant impact on public health. Infection by parasites in wildlife is considered an important bioindicator of the current state of ecosystems, and studying the epidemiology of these infections is essential for a better understanding of natural foci. However, research on parasites in southern Brazil, especially in Rio Grande do Sul (RS), is considered incipient. Therefore, in this study, we aimed to identify the parasitic fauna of wild animals in the southern region of RS through fecal parasitological diagnosis. We processed 82 fecal samples from wild animals - including birds, mammals, and reptiles - from cities within the microregion of Pelotas, using the Zinc Sulfate Centrifugal Flotation, Spontaneous Sedimentation and Oocyst Sporulation techniques.

Results In 69.5% of the samples (93.1% of mammals, 47% of birds and 50% of reptiles), we found helminth eggs and/or protozoan cysts/oocysts, with strongylid-type eggs being the most frequent parasites (44.11%). Additionally, 64.9% of the positive samples were parasitized by at least one morphogroup with zoonotic agents (Taeniidae, *Capillaria*, *Strongyloides*, *Spirometra*, *Lagochilascaris*, *Sarcocystis*, *Trichuris*, *Giardia*, Ancilostomid, *Physaloptera*, *Toxocara*, *Fasciola*). We also recorded the first finding of *Monocystis* spp. in a Southern tamandua (*Tamandua tetradactyla*).

Conclusions Thus, it was observed that the majority of the animals were parasitized and, consequently, susceptible to a wide range of pathogens of medical and veterinary interest, highlighting the importance of these hosts in the spread of parasites, especially those with zoonotic potential. However, the ecology of transmission and the role of these hosts in the life cycles of parasites should be further explored in other studies.

Keywords Endoparasites, Parasitic infections, One health, Wildlife, Zoonosis

*Correspondence:
Julia Somavilla Lignon
julialignon@gmail.com
Full list of author information is available at the end of the article

Background

Increasing urbanization, agricultural expansion, excessive deforestation, and the illegal wildlife trade have led to greater contact between people and their domestic animals with wild animal populations [6, 14]. Currently, around 75% of infectious diseases emerging from humans have animal origins [22] and 71.8% originate from wild fauna [25]. Environmental changes and their consequences disrupt ecosystem balance, promoting the spread of infections between species — a phenomenon known as zoonotic spillover, which can have a significant impact on One Health [23, 31].

Among the diseases that affect wildlife, parasitic infection is considered an important bioindicator of the current state of ecosystems, used to evaluate the spread of pathogens and behavioral changes [13]. Wild animals, both in the wild and in captivity, can be reservoirs and carriers of various parasitic diseases (including zoonoses) with significant potential impact on public health, wildlife conservation, and economic aspects [7].

In this context, studying the epidemiology of these infections is essential for a better understanding of natural foci to verify the circulation of these agents among wild animals and the local, regional, or national importance of the diseases they cause. This knowledge supports the actions of veterinary and public health services [2].

Given that environmental changes have triggered alterations in the epidemiological transmission chain of some parasites, particularly those of zoonotic nature, involving wild, synanthropic, domestic animals, and even humans in their epidemiological cycles, and considering the scarcity of research in Southern Brazil, this study aimed to identify the parasitic fauna of wild animals in the Southern region of Rio Grande do Sul through fecal parasitological diagnosis.

Methods

A total of 82 fecal samples from wild animals, received during the years 2022 and 2023, were analyzed in the laboratory of the Grupo de Estudos em Enfermidades Parasitárias (GEEP) at the Universidade Federal de Pelotas (UFPel). The samples were sent by the Núcleo de Reabilitação da Fauna Silvestre (NURFS) through the Laboratório Regional de Diagnóstico (LRD), both affiliated with UFPel. All animals were free-living, although they were undergoing rehabilitation at NURFS. The rehabilitation time for each animal varied according to the type of pathology that affected it. In addition, each animal that arrives at NURFS/UFPel undergoes screening in a specific enclosure for it, before being relocated to larger enclosures with other animals of the same species, if possible or necessary.

During animal screening, mammalian and reptile feces were collected from each animal's enclosure immediately after defecation. Bird feces were collected in pools, also from each animal's enclosure, during one shift during the day. All samples were collected using disposable gloves, identified on the day of collection and transported in isothermal containers with ice to the laboratory for analysis. They were stored at refrigerated temperature (4 °C) for a maximum of 48 h in the laboratory, during which parasitological tests were performed. For diagnosis, the following techniques were used: Centrifugal Flotation with Zinc Sulfate, modified as described by Monteiro [16], Spontaneous Sedimentation described by Hoffmann et al. [11] and Oocyst Sporulation with 2% potassium dichromate described by Monteiro [16]. For identification purposes, all structures allowing the identification or differentiation of eggs/cysts/oocysts at the lowest possible taxonomic level were used, such as shell characteristics and ornaments, embryonic and larval formations, and the presence of opercula and spines. In some cases, such as strongylid-type eggs, ancilostomid eggs, anoplocephalid eggs, ascarid eggs, and some oocysts, identification remained at the morphogroup level due to the absence of diagnostic characters for species differentiation. Identification was performed by comparing the observed morphometry with that of species previously described in the literature for the host species [4, 16, 17, 19, 21, 29, 30, 32, 33], using an Olympus CX33 series optical microscope (Olympus Corporation, Tokyo, Japan) coupled with a digital camera, with variable magnification between 40x and 100x. Micrometric eyepieces were used for morphometric analyses.

The animals in the study came from cities in the Pelotas microregion, in Rio Grande do Sul, Southern Brazil. This region includes the municipalities of Pelotas, Capão do Leão, Pedro Osório, Cerrito, Canguçu, Morro Redondo, Turuçu, São Lourenço do Sul, Cristal, and Arroio do Padre (Fig. 1).

The collection of fecal samples from wild animals was authorized by the Biodiversity Authorization and Information System of the Ministry of the Environment under registration 82632-3 based on Normative Instruction number 03/2014.

Results and discussion

In total, fecal samples from 34 species of wild animals were processed. Of the 82 samples analyzed – 44 mammals, 34 birds, and 4 reptiles - helminth eggs and/or protozoan cysts/oocysts were found in 69.5% (57) (Table 1). Among mammals, 93.1% were infected, as well as 47% of birds and 50% of reptiles. Photographs of the parasitic forms found can be seen in Fig. 2. Our results reinforce that wild animals can be infected by

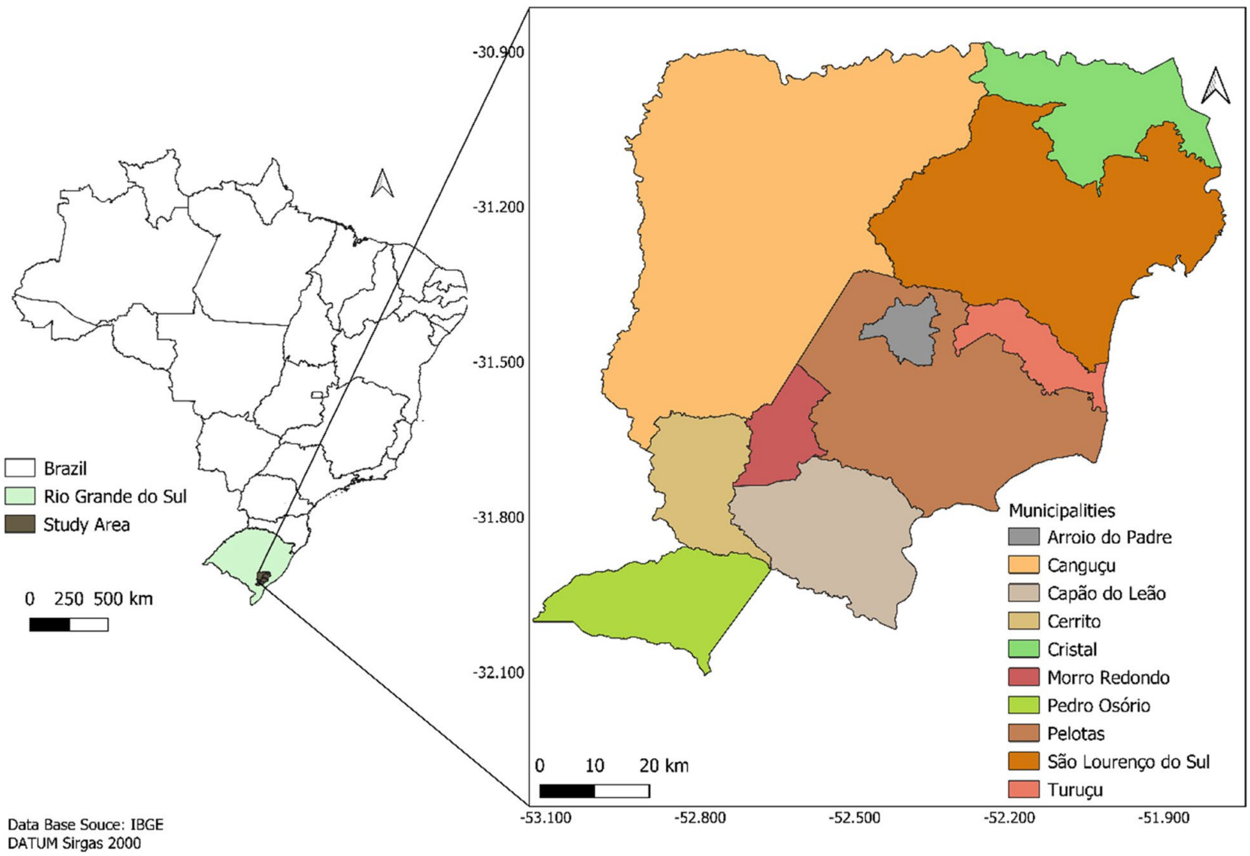

Fig. 1 Study area including the cities belonging to the Microregion of Pelotas, Rio Grande do Sul, Brazil

a wide variety of endoparasites, which typically result in subclinical infections in healthy free-living hosts but are among the main sanitary problems in captive animals [3, 29]. High population density, stress, adaptation to a new environment, or prolonged periods in a confined space can exacerbate these situations [19], highlighting the importance of complementary examinations such as coproparasitological diagnosis, given that many of the animals in this study were undergoing rehabilitation.

Overall, strongylid-type eggs were the most frequent parasites (44.11%), followed by *Capillaria* spp. eggs (26.47%), demonstrating the diversity of parasitic species and hosts these taxa can infect. In this context, helminth infections were more common (67.64%) than protozoan infections, which were observed in 35.29% of animal species, as described by previous studies [9, 18]. However, the finding of *Giardia* spp. infections in *Cerdocyon thous* deserves attention because, besides being important causes of diarrhea in animals, they have zoonotic potential [8]. Furthermore, a study on the genotypes of these protozoa demonstrated that humans are likely the source of infection for these animals [28].

Among helminths, nematode infections were more prevalent compared to other classes, as reported in previous studies [24, 27]. This may have occurred due to the direct life cycle (at least in most species), without involvement of intermediate hosts and can be transmitted through contaminated food, water, and soil [15]. On the other hand, trematodes and most cestodes require at least one intermediate host to complete their life cycle for transmission to occur. This may be the reason for the lower occurrence of infections by these helminths in this study [1, 15].

Although species-level identification is challenging through coproparasitological diagnosis, being a limiting factor in studies like this, many of the identified morphogroups contain species with zoonotic potential and, therefore, can infect humans. In this study, more than half of the animal species (64.9%) were parasitized by at least one morphogroup with zoonotic agents. Many of the animals evaluated here, such as capybaras, opossums, and crab-eating foxes, are known reservoirs of various pathogens, and their proximity to other animals, including domestic (livestock and pets) and humans, in urban, peri-urban, and rural environments, can have significant

Table 1 Data on the parasitic fauna of wild animals, through coproparasitological diagnosis, in Southern Brazil

Scientific name	Common name	Samples	Positive samples	Endoparasites
Alouatta guariba	Howler monkey	2	1	Taeniidae eggs
Aramides saracura	Slaty-breasted wood-rail	3	2	*Capillaria* spp., *Heterakis* spp.
Aramus guarauna	Limpkin	2	0	-
Athene cunicularia	Burrowing owl	1	1	*Capillaria* spp.
Bubo virginianus	Great Horned owl	2	1	*Capillaria* spp.
Caiman latirostris	Broad-snouted caiman	1	1	*Strongyloides* spp., *Capillaria* spp., Strongylida eggs
Cavia aperea	Brazilian guinea pig	2	2	Strongylida eggs, Anoplocephalid eggs
Cerdocyon thous	Crab-eating fox	6	6	*Alaria* spp., *Capillaria* spp., *Spirometra* spp., *Cystoisospora* spp., *Lagochilascaris* spp., *Sarcocystis* spp., *Trichuris* spp., *Giardia* spp., Ancilostomid eggs, Anoplocephalid eggs
Colaptes campestris	Woodpecker	1	1	Anoplocephalid eggs
Conepatus chinga	Molina's Hog-nosed skunk	2	2	*Physaloptera* spp., *Spirometra* spp., Ancilostomid eggs, Anoplocephalid eggs
Dasypus novemcinctus	Nine-banded armadillo	1	1	Strongylida eggs
Didelphis albiventris	White-eared opossum	17	16	*Aspidodera* spp., *Cruzia* spp., *Physaloptera* spp., *Capillaria* spp., *Trichuris* spp., Strongylida eggs, *Alaria* spp., *Sarcocystis* spp., *Monocystis* spp., *Toxocara* spp., *Eimeria* spp., Anoplocephalid eggs
Euphractus sexcinctus	Six-banded armadillo	1	1	Strongylida eggs
Furnarius rufus	Rufous hornero	1	0	-
Hydrochoerus hydrochaeris	Capybara	2	2	*Protozoophaga obesa, Hippocrepis hippocrepis, Eimeria* spp., *Strongyloides* spp., Strongylida eggs, *Monoecocestus* spp., *Fasciola* spp., Ascarid eggs, Oocysts
Leopardus geoffroyi	Geoffroy's cat	3	1	Ancilostomid eggs, *Toxocara cati*, Taeniidae eggs
Lycalopex gymnocercus	Pampas fox	1	1	*Capillaria* spp., *Spirometra* spp., *Sarcocystis* spp., *Trichuris* spp., *Toxocara* spp., Ancilostomid eggs
Molothrus bonariensis	Shiny cowbird	1	0	-
Myocastor coypus	Coypu	2	2	*Fasciola* spp., *Paramphistomum* spp., Strongylida eggs
Myiopsitta monachus	Monk parakeet	7	3	*Isospora* spp.
Nasua nasua	Coati	1	1	*Sarcocystis* spp., *Cruzia* spp., *Monocystis* spp., Strongylida eggs, Oocysts
Ozotoceros bezoarticus	Pampas deer	5	3	*Eimeria* spp., Strongylida eggs
Paroaria coronata	Red-crested Cardinal	1	1	*Isospora* spp.
Passer domesticus	House sparrow	1	0	-
Pitangus sulphuratus	Great kiskadee	7	3	*Capillaria* spp., *Isospora* spp.
Procyon cancrivorus	Crab-eating raccoon	1	0	-
Ramphastos dicolorus	Green-billed toucan	2	1	*Capillaria* spp.
Saltator similis	Green-winged saltator	1	1	*Isospora* spp.
Salvator merianae	White-and-black tegu lizard	2	1	*Physaloptera* spp., Strongylida eggs, Oyxurid eggs
Spatula querquedula	Garganey	1	0	-
Stephanophorus diadematus	Diademed tanager	1	1	*Isospora* spp., *Ascaridia* spp.
Tamandua tetradactyla	Southern tamandua	1	1	*Eimeria* spp., *Monocystis* spp., Strongylida eggs, Oocysts
Trachemys dorbigni	D'Orbigny's slider	1	0	-
Vanellus chilensis	Southern lapwing	2	1	*Heterakis* spp.
Total		82	57	

public health implications. Coincidentally, these same animals (capybaras, opossums, and crab-eating foxes) were those with the highest diversity of endoparasites in this study.

Furthermore, since the diagnosis was based on fecal examination and many animals are predators, there is a possibility that some eggs, cysts, and oocysts found in the examinations belong to the preyed animal rather than the predator (spurious infection or pseudoparasitism). Thus, they act as dispersers of pathogens in the environment, representing a risk for other susceptible animals, as well as for caretakers and handlers of

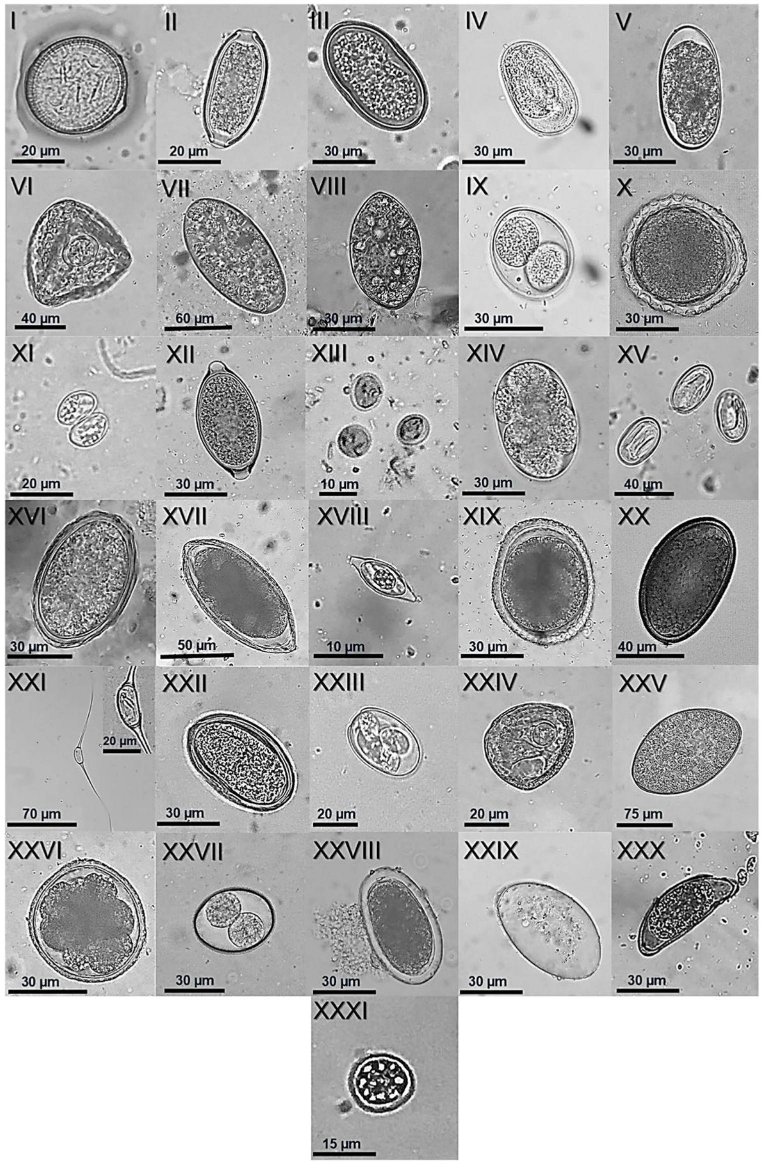

Fig. 2 Illustrations of parasitic forms identified in fecal samples of wild animals evaluated in southern Brazil. I – Taeniidae egg; II – *Capillaria* spp. egg; III – *Heterakis* spp. egg; IV – *Strongyloides* spp. egg; V - Strongylida egg; VI – Anoplocephalid egg; VII – *Alaria* spp. egg; VIII – *Spirometra* spp. egg; IX – *Cystoisospora* spp. oocyst; X – *Lagochilascaris* spp. egg; XI – *Sarcocystis* spp. oocyst; XII – Trichuris spp. egg; XIII - *Giardia* spp. cysts; XIV – Ancilostomatid egg; XV – Physaloptera spp. eggs; XVI – *Aspidodera* spp. egg; XVII – *Cruzia* spp. egg; XVIII – *Monocystis* spp. sporocystis; XIX – *Toxocara* spp. egg; XX - Ascarid eggs; XXI - *Hippocrepis hippocrepis* egg (in detail is the miracidium capsule); XXII - *Protozoophaga obesa* egg; XXIII – *Eimeria* spp. oocyst; XXIV - *Monoecocestus* spp. egg; XXV – *Fasciola* spp. egg; XXVI – *Toxocara cati* egg; XXVII – *Isospora* spp. oocyst; XXVIII – *Ascaridia* spp. egg; XXIX – *Paramphistomum* spp. egg; XXX – Oxyurid egg; XXXI – Unidentified oocyst

animals in captivity. Pseudoparasitism by *Monocystis* spp., for example, has been reported in coatis (*Nasua nasua*) [17], nine-banded armadillos (*Dasypus novemcinctus*) [21], and more recently in opossums (*Didelphis albiventris*) [12]. Its presence is closely related to the omnivorous feeding habits of these animals since this protozoan has annelids as hosts [34]. Here, we report the finding, for the first time, in southern tamanduas (*Tamandua tetradactyla*). Although its apathogenic effect is not fully understood in vertebrates, the identification of these protozoa in the feces of individuals can lead to a misconception about the need for treatment of these animals [21], considering that the sporocyst has a similar appearance to *Trichuris* spp. eggs, albeit smaller, with approximately 10 µm in length, while those of the nematode are around 55 µm [16].

In aadition, the discovery of *Toxocara* spp. eggs in opossum feces and *Capillaria* spp. in *C. thous* and *Lycalopex gymnocercus* feces does not rule out the possibility of pseudoparasitism. Although the parasitic species were not identified in our study, other authors have reported the identification of spurious infection by *Toxocara cati* eggs in *D. albiventris* [20] and the participation of wild canids in the dispersal of *Capillaria hepatica* eggs [26]. In opossums, the possibility of interspecific coprophagy has already been suggested [10] and their omnivorous diet al.so allows the ingestion of items related to their usual diet (e.g., arthropods and vegetables) contaminated by feces of other animals containing *Toxocara* spp. eggs [5]. On the other hand, *C. thous* and *L. gymnocercus* can prey on hosts infected by *C. hepatica*, with the harmless passage of non-embryonated eggs through the gastrointestinal tract of these animals, eliminating them in their feces [26]. Thus, new findings in wild animals should be described, allowing the avoidance of false-positive diagnoses.

The present study represents the first survey of gastrointestinal parasite diversity, through coproparasitological diagnosis, in wild animals in Rio Grande do Sul, southern Brazil. The difficulty in species identification through fecal parasitological diagnosis, as well as the possibility of spurious infection, highlights the importance of further research including adult helminth identification, diagnostics through molecular methods, and experimental infections, which can aid in specific taxonomic identification and the epidemiology of the agents' life cycle to prove the real impact of these parasites on human and animal medicine. Furthermore, prospective epidemiological studies are suggested to be conducted over longer periods, maintaining active surveillance in the local wildlife, aiming to prevent potential future epidemics of parasitic zoonoses. Nevertheless, the findings of the present study can contribute to future diagnoses of diseases affecting these animals, as regular monitoring, coupled with appropriate therapeutic measures, can help reduce the serious consequences of gastrointestinal parasitic infections in captive wild animals, making preventive planning and early control more effective.

Conclusions

It was observed that the majority of the animals were parasitized, making them susceptible to a wide range of pathogens of medical and veterinary interest. The importance of these hosts in the dispersal of parasites, especially those with zoonotic potential, is emphasized. However, the transmission ecology and the role of these hosts in the life cycles of parasites should be further explored.

Acknowledgements
We would like to thank the Núcleo de Reabilitação da Fauna Silvestre (NURFS) and the Laboratório Regional de Diagnóstico (LRD) at UFPel for sending the samples to carry out the study.

Authors' contributions
JSL, DMP, TSS, GRM, CGS, BCB, FGP, SGM, MPS, RTF and FRPB analyzed and interpreted the results. JSL was one of the main sources in writing the manuscript. All authors read and approved the final manuscript.

Funding
This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This work was approved by the Ethics Committee on the Use of Animals at UFPel (process number 23110.046990/2022-02).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Laboratório de Epidemiologia Veterinária, Departamento de Veterinária Preventiva, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brasil. [2]Laboratório do Grupo de Estudos em Enfermidades Parasitárias, Departamento de Veterinária Preventiva, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brasil. [3]Laboratório de Parasitologia Veterinária, Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brasil. [4]Laboratório Regional de Diagnóstico, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brasil. [5]Núcleo de Reabilitação da Fauna Silvestre, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brasil.

Received: 12 June 2024 Accepted: 19 December 2024
Published online: 07 January 2025

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