CHARACTERIZATION OF INTESTINAL ENDOPARASITES FAUNA WITH ZOONOTIC POTENTIAL OF ROAD KILLED WILD MAMMALS IN NORTHERN PARANÁ

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ABSTRACT: Roads and highways are of paramount importance for national trade and transportation. Linked to this, we observe various environmental impacts, including the invasion of wildlife habitat, the roadkill of wild animals, and consequent harm to the fauna in the vicinity of the highways. The aim of the study was to characterize the intestinal parasitic fauna of wild animals killed by roadkill in Northern Paraná. Wild animals killed by roadkill were collected in situ on the main highways in the northern region of Paraná and were sent to the State University of Londrina for autopsy and biological material collection. The parasitological fecal examinations performed included Willis, Faust, and Hoffmann methods. Our results revealed a large quantity of parasites, and even though the research was conducted with a small number of animals, the results may have broader implications. Many of the parasites found have zoonotic potential, such as parasites from the classes Trematoda and Cestoda, subclass Coccidia, family Trichostrongylidae, and genera *Ancylostoma* spp. and *Capillaria* spp. It can be concluded

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that the identification of the intestinal parasitic fauna of wild animals killed by roadkill is a valuable tool for investigating parasites with zoonotic potential. Further studies focusing on this characterization are necessary due to the increasing interaction between humans and wild animals. This is the first report of *Octosporella*-like oocysts in *Nasua nasua* in Brazil. Molecular studies are required for confirmation.
KEYWORDS: Free-living animals; Parasitic agents; Roadkill; Highways; Zoonoses.

CARACTERIZAÇÃO DE ENDOPARASITOS INTESTINAIS DE MAMÍFEROS SILVESTRES MORTOS POR ATROPELAMENTO NO NORTE DO PARANÁ

RESUMO: Estradas e rodovias são de suma importância para o comércio e transporte nacional. Atrelado a isso, observa-se diversos impactos ambientais, dentre eles, a invasão do ambiente silvestre, o atropelamento de animais silvestres e consequente prejuízo à fauna do entorno das rodovias. O objetivo do estudo foi a caracterização da fauna parasitária intestinal de animais silvestres mortos por atropelamento no Norte do Paraná. Os animais silvestres mortos por atropelamento foram coletados *in situ* nas principais rodovias da região norte do Paraná e encaminhados a Universidade Estadual de Londrina para a realização de autópsia e coleta de material biológico. Os exames parasitológicos de fezes realizados foram Willis, Faust e Hoffmann. Em nossos resultados encontra-se uma grande quantidade de parasitos, e mesmo que a pesquisa tenha sido realizada com poucos animais, os resultados podem refletir em grande escala. Muito dos parasitos encontrados tem potencial zoonóticos como parasitos das Classes Trematoda, Cestoda, subclasse Coccidia, da família Trichostrongylidae e gêneros *Ancylostoma* spp. e *Capillaria* spp. Pode-se concluir que a identificação da fauna intestinal parasitária de animais silvestres mortos por atropelamento é uma boa ferramenta de investigação de parasitos com potencial zoonótico. Novos estudos voltados para essa caracterização são necessários devido a crescente relação entre seres humanos e animais silvestres. Esse é o primeiro relato de oocisto semelhante à *Octosporella* em *Nasua nasua* no Brasil. Estudos moleculares são necessários para essa confirmação.
PALAVRAS-CHAVE: Animais de vida livre; Agentes parasitários; Atropelados; Rodovias; Zoonoses.

CARACTERIZACIÓN DE ENDOPARÁSITOS INTESTINALES DE MAMÍFEROS SILVESTRES MUERTOS POR ATROPELLO EN EL NORTE DE PARANÁ

RESUMEN: Las carreteras y autopistas son de suma importancia para el comercio y el transporte nacionales. Vinculado a esto, existen varios impactos ambientales, entre ellos la invasión del medio silvestre, el atropello de animales salvajes y los consiguientes daños a la fauna circundante a las carreteras. El objetivo del estudio fue caracterizar la fauna parasitaria intestinal de animales salvajes atropellados en el Norte de Paraná. Los animales salvajes atropellados fueron recolectados *in situ* en las principales carreteras de la región norte de Paraná y enviados a la Universidad Estadual de Londrina para autopsia y recolección de material biológico. Los exámenes parasitológicos fecales realizados fueron Willis, Faust y Hoffmann. En nuestros resultados encontramos una gran cantidad de parásitos, y aunque la investigación se realizó con pocos animales, los resultados pueden reflejarse a gran escala. Muchos de los parásitos encontrados tienen potencial

zoonótico, como los parásitos de las Clases Trematoda, Cestoda, subclase Coccidia, de la familia Trichostrongylidae y géneros *Ancylostoma spp*. y *Capillaria spp*. Se puede concluir que la identificación de la fauna intestinal parasitaria de animales salvajes atropellados es una buena herramienta para la investigación de parásitos con potencial zoonótico. Nuevos estudios encaminados a esta caracterización son necesarios debido a la creciente relación entre los seres humanos y los animales salvajes. Este es el primer reporte de un ooquiste similar a *Octosporella* en *Nasua nasua* en Brasil. Para esta confirmación son necesarios estudios moleculares.
PALABRAS CLAVE: Animales en libertad; Agentes parásitos; Atropellar; Carreteras; Zoonosis.

1. INTRODUCTION

Brazil is the fifth largest country in the world and occupies about half of South America, being globally renowned for its biodiversity, as it encompasses over 14% of the world's biota (Lewinsohn; Prado, 2002). More than eight thousand species of vertebrates inhabiting the Atlantic Forest, Pampas, Cerrado, Pantanal, Amazon Rainforest, and Caatinga have already been cataloged. Within these regions, the group of mammals is represented by more than 730 species, spread across 11 distinct orders (Instituto Chico Mendes de Conservação da Biodiversidade [ICMBIO], 2017).

Throughout the years, mankind, in its pursuit of progress, has repositioned the resources of nature, giving rise to a mixed geographical landscape composed of natural and processed elements with varying degrees of human impact on its territory (Kay; Hoekstra, 2008). Such types of changes have influenced alterations in climate, flooding, depletion of water sources, erosion, pollution, ozone layer depletion, and habitat destruction, thereby exposing wildlife to various pathogens and parasites, especially those with zoonotic potential (Taylor *et al*., 2010; Krauss *et al*., 2003).

According to the World Health Organization (2017), 71.8% of zoonotic diseases originate from wildlife. Fifty eight percent of all diseases affecting humans and animals being connected to ecosystem alterations directly influenced by globalization. This has led to the rapid proliferation of parasites with zoonotic potential (Taylor *et al*., 2010; Page *et al*., 2011). For instance, around 90% of the raccoon population (*Procyon lotor*) in the United States is parasitized by the nematode *Baylisascaris procyonis*, which can infect domestic animals and poses a threat to humans due to its zoonotic potential (Page *et al*., 2011). Similarly, the helminth *Gnathostoma spinigerum* has infected humans who commonly consume undercooked or raw freshwater fish, eels, frogs, birds, and reptiles from areas inhabited by populations of wild canids, felids, and suids, which play a role in

maintaining the biological cycle of this parasite (Centers for Disease Control and Prevention [CDC], 2017).

The relationship between parasites and wild animals is an area of research of utmost importance and interest in the biological and ecological sciences. Parasites, organisms that depend on other living beings for their survival and reproduction, play an essential role in the dynamics of natural ecosystems. By directly and indirectly affecting the health of both animal and human hosts (in cases of a zoonotic nature), as well as the behavior and ecology of their hosts, these microorganisms trigger a complex web of interactions that shape biodiversity and the structure of biological communities. Hence, the study of parasitic diseases and the interaction between parasite and host among captive and/or free-living wild animals is an indispensable tool to contribute to conservation and preservation programs, preventing negative impacts on biodiversity and public health (Catão; Dias, 2003; Zhu *et al.*, 2019).The significance of researching parasitic fauna is recognized and required in reintroduction protocols and in the clinical routine of wild animals (União Internacional para a Conservação da Natureza [UICN], 2014; Balansard *et al.*, 2019)

The objective of this current work was to characterize the helminth fauna of wild mammals killed by road accidents in northern Paraná, a southern state of Brazil. This was accomplished through reports on ethical aspects, introduction of materials and methodologies used, as well as the obtained results, while also providing the first report of *Octosporella*-like oocysts in the ring-tailed coati (*Nasua nasua*). Additionally, this work highlights the importance of conducting further studies on the relationship and interaction between humans and wild animals.

2. MATERIAL AND METHODS

2.1 Ethical aspects

This project was carried out with the approval of the Ethics Committee on Animal Use (CEUA) of the Universidade Estadual de Londrina (UEL) in October 2017 (No 30/2017) and was also authorized by the System of Authorization and Information on Biodiversity (SISBIO) in October 2016 under permit number 55384-1.

2.2 Study area and collecting road killed animals

The animals were collected post-mortem on urban and inter-municipal roads located in the north-central and pioneer north regions of Paraná. Transects (T) were designed to encompass the main counties in these regions (Figure 1): T1 (South transect) – Londrina, California, and Mauá da Serra; T2 (East transect) – Londrina, Cornélio Procópio, and Sertaneja; T3 (North transect) - Londrina, Rolândia, Prado Ferreira, Alvorada do Sul, Bela Vista do Paraíso; T4 (West transect) – Londrina, Mandaguari, Maringá, Astorga, and Jaguapitã. The starting and ending point for all transects was always the city of Londrina (-23.2927°, -51.1732°).

Once a week, each transect was traveled at an average speed of 40 to 60 km/h with two or three observers. To expand the coverage, the 2nd Environmental Police Company, 2nd Company of the Highway Police Battalion, and Paraná Health Regionals (15th, 16th, 17th, and 18th Regional) were requested to co-participate. The carcasses of wild animals were collected without evisceration and preferably in rigor mortis, and they were placed in specific bags for the transportation of biological material. Species identification was performed in accordance with Reis *et al.* (2011) using photographic documentation. Feces and adult parasites were directly collected from the intestines during the autopsy.

2.3 Laboratory diagnosis

The Willis method (1921), Hoffman method (Hoffman; Pons; Janer, 1934), and Faust method (Faust *et al*., 1939) were employed for analyzing fecal samples. The Willis method is a direct qualitative technique based on flotation in a saturated solution, and it is recommended for the detection of eggs, oocysts, and cysts in fecal samples. The Hoffman method is another direct qualitative approach that relies on spontaneous sedimentation, making it suitable for identifying heavier eggs in feces, as well as trematodes and cestodes. On the other hand, the Faust method utilizes centrifugation and flotation, offering a direct qualitative approach that is particularly effective for diagnosing lighter eggs and protozoa in fecal samples.

3. RESULTS

Samples from a total of 5 *Cerdocyon thous* (Crab-eating Fox), 2 *Hydrochoeris hydrochaeris* (Capybara), 1 *Galictis cuja* (Lesser Grison), 1 *Lepus europaeus* (European Hare), 4 *Sapajus apella* (Black-capped Capuchin), 1 *Procyon cancrivorus* (Crab-eating

Raccoon), 2 *Coendou spinosus* (Porcupine), 3 *Cavia aperea* (Brazilian Guinea Pig), 5 *Nasua nasua* (South American Coati), 4 *Tamandua tetradactyla* (Southern Tamandua), 1 *Mazama gouazoubira* (Gray Brocket), and 4 *Dasypus novemcinctus* (Nine-banded Armadillo) were included in the evaluation. Detailed 'the observed parasites, is provided in Table 1. It is important to note that all these animals are categorized as 'least concern' in terms of conservation status by the International Union for the Conservation of Nature and Natural Resources (IUCN, 2019).

Table 1. Results obtained through parasitological techniques following the protocols of Faust *et al*. (1939), Hoffman *et al*. (1934), and Willis (1921) using samples collected from road-killed wild mammals in the North of Paraná, Brazil, spanning the years 2016 to 2018.

Animal	CN	Species	Municipality	Season	Gen	Age	Faust	Hoffman	Willis
1	CF	*C. thous*	Londrina	Fall	F	A	COC	TRE	TRE, COC
2	CF	*C. thous*	Santa Mariana	Fall	M	J	TRE	TRE	TRE, ASC
3	CF	*C. thous*	Prado Ferreira	Winter	M	A	NI	NI	COC
4	CF	*C. thous*	Marilândia do Sul	Winter	M	J	NEG	NEG	NEG
5	CF	*C. thous*	Mauá da Serra	Summer	F	A	NEG	NEG	ANC
6	CP	*H. hydrochaeris*	Cornélio Procópio	Spring	F	A	NEG	NEG	NEG
7	CP	*H. hydrochaeris*	Londrina	Summer	F	J	ANC	TRE	ANC
8	LG	*G. cuja*	Apucarana	Fall	M	A	NI	ANC	NEG
9	EH	*L. europaeus*	Londrina	Fall	M	A	COC	ASC	COC
10	BC	*S. apella*	Londrina	Spring	M	J	NEG	NEG	NEG
11	BC	*S. apella*	Londrina	Summer	NI	NI	NEG	NEG	NEG
12	BC	*S. apella*	São Pedro do Ivaí	Winter	M	A	ANC	NI	NI
13	BC	*S. apella*	Jandaia do Sul	Winter	NI	NI	NEG	NEG	NEG
14	CR	*P. cancrivorus*	Mauá da Serra	Fall	M	A	OXY	OXY	OXY
15	Por	*C. spinosus*	Londrina	Spring	F	A	NEG	NEG	NEG
16	Por	*C. spinosus*	São Jerônimo da Serra	Winter	NI	NI	NI	NI	MET
17	BP	*C. aperea*	Londrina	Summer	F	A	NEG	NEG	NEG
18	BP	*C. aperea*	Bela Vista do Paraíso	Fall	F	A	NEG	NEG	NEG
19	BP	*C. aperea*	Londrina	Winter	M	A	ANC	NI	NI
20	SC	*N. nasua*	Rolândia	Fall	F	A	CES, TRE	CES	TRE
21	SC	*N. nasua*	Mauá da Serra	Spring	M	A	NEG	NEG	NEG
22	SC	*N. nasua*	Londrina	Spring	M	J	CES	NI	ANC, TRE, CES, *Trichuris sp*
23	SC	*N. nasua*	Borrazópolis	Winter	M	A	ANC, OCT	TRE	NI
24	SC	*N. nasua*	São Pedro do Ivaí	Winter	M	J	NI	ASC	TRI
25	ST	*T. tetradactyla*	Mauá da Serra	Spring	F	A	NEG	NEG	NEG
26	ST	*T. tetradactyla*	Cambé	Fall	M	A	COC	TRE, Ct	ANC
27	ST	*T. tetradactyla*	Sertaneja	Spring	M	A	TRI	NI	TRI, SrL
28	ST	*T. tetradactyla*	Borrazópolis	Spring	F	A	NI	NI	CES
29	NA	*D. novemcinctus*	Londrina	Spring	F	A	TRI, CES, TRE	TRE, CES	NI
30	NA	*D. novemcinctus*	Arapongas	Summer	F	A	TRI, CES	TRE	TRI
31	NA	*D. novemcinctus*	Mauá da Serra	Fall	M	J	*Capillaria sp.*	CES	CES, ASC, TRI
32	NA	*D. novemcinctus*	Londrina	Spring	M	A	NEG	NEG	NEG
33	GB	*M. gouazoubira*	Cornélio Procópio	Spring	F	A	NEG	NEG	NEG

CN: Common Name, CF: Crab-eating Fox, CP: Capybara, LG: Lesser Grison, EH: European Hare, BC: Black-capped Capuchin, CR: Crab-eating Raccoon, Por: Porcupine, BP: Brazilian Guinea Pig, SC: South American Coati, ST: Southern Tamandua, NA: Nine-banded Armadillo, GB: Gray Brocket, COC: Coccidiida Oocysts, TRE: Trematode Eggs, Gen: Gender, F: Female, M: Male, A: Adult, J: Juvenile, NI: No information, NEG: Negative Sample, ANC: *Ancylostoma sp*., OXY: Oxyuroidea Eggs, CES: Cestoda Eggs, OCT: *Octosporella sp*.-like oocyst, TRI: Trichostrongylidae Eggs, ASC: Ascarididae Eggs, Ct: *Cruzia tentaculata*, MET: *Metastrongylus sp*., SrL: Schneidernema retusa Larvae.

4. DISCUSSION

The study of the intestinal helminth fauna of wild animals allows for a better understanding of the dynamics of parasites in ecological systems, being of great importance for monitoring parasites with zoonotic potential (Jenkins *et al*., 2015; Thompson, 2013).

The growth of urban areas and the expansion of agriculture lead to habitat fragmentation and the consequent increase in direct or indirect interactions between humans, domestic animals, and wildlife, predisposing to a higher risk of disease transmission among them (Winck *et al*., 2022). Considering the importance of identifying zoonotic parasites in this proximity, only parasites with such potential will be discussed.

In the family Ascarididae, found in this study in *C. thous, L. auropaeus, N. nasua*, and *D. novemcinctus*, there are zoonotic species such as *Toxocara canis* and *Toxocara cati*, which cause Visceral Larva Migrans and Ocular Larva Migrans in humans. *T. canis* has been reported in *C. thous and N. nasua* in other studies (Holsback *et al*., 2013; Santos *et al*., 2015; Vieira, Luque, Muniz-Pereira, 2008; Magnaval *et al*., 2001; Strube; Heuer; Janecek, 2013). The identification of these parasite species can indicate close contact with dogs. In *D. novemcinctus*, representatives of the genus *Capillaria* were found, one of which is a zoonotic species, *Capillaria hepatica*. It is common in rodents but has been identified in a range of different hosts and rarely in humans, with approximately 163 cases reported worldwide and 5 cases in Brazil until 2011. However, studies in some regions show the presence of positive coproparasitology for *C. hepatica* eggs and antibody prevalence in the population, indicating contact with eggs and even serology with high titers, from which true infection can be inferred (Neves *et al*., 2016; Rocha *et al*., 2015; Klisiowicz *et al*., 2014). Capillariasis is a disease that is difficult to diagnose, primarily depending on liver biopsy, and can lead to liver cirrhosis and death, both in humans and animals (Martins, 2019). Klisiowicz *et al.* (2014) found a high prevalence in humans in the city of Guaraqueçaba in Paraná, but the studied population had the habit of eating wild animals. Knowing its zoonotic potential, the identification of a sylvatic cycle allows us to increase surveillance of possible human cases and their circulation in domestic animals, including rodents that live in cities. Rocha *et al.* (2015) suggest that *C. hepatica* should be considered a differential diagnosis for Hepatitis A, B, C, and leptospirosis, common in the region they studied.

Parasites of the genus *Ancylostoma* were identified in more than half of the analyzed species in this study (*Cerdocyon thous, Hidrochoeris hidrochaerys, Galactis cuja, Sapajus apella*, *Cavia aperea*, *Nasua nasua*, and *Tamandua tetradactyla*) and have been reported in the literature in wild animals in Brazil by various studies, including recent ones by Santos *e al.* (2015) in *C. thous*, Figueiredo, Manrique, and Nogueira (2018) in *C. thous, N. nasua, S. apella*, and *T. tetradactyla*, and by Figueiredo, Manrique, Nogueira, and Chaves (2020) in *Sapajus apella*. This genus contains two species of great importance to public health, *Ancylostoma caninum* and *A. braziliense*, zoonotic parasites causing Cutaneous Larva Migrans, popularly known as "geographic itch." *A. braziliense* and *A. caninum* have already been described parasitizing *N. nasua* and *C. thous*, respectively (Moraes *et al*., 2019; Vieira, Luque, Muniz-Pereira, 2008). A. buckleyi has been reported in *C. thous*, and although there are no reports of this species parasitizing humans, we cannot ignore its proximity to other zoonotic species, suggesting that it also has the potential to cause diseases in humans. The occurrence of *Ancylostoma sp*. in *Galictis cuja* is possibly the first report in the literature.

The class Cestoda belongs to the *Phylum Platyhelminthes* and is quite diverse, with a variety of parasites that can be zoonotic. Representatives of this class were found in *Nasua nasua, Tamandua tetradactyla*, and *Dasypus novemcinctus* in this study. Cestodes with zoonotic potential have been reported in *S. apella* and *C. thous*. The genus *Dipylidium* contains a species of public health importance, *Dipylidium caninum*, which is an accidental parasite in humans and often overlooked. It can cause abdominal pain, flatulence, and occasional diarrhea (Rodríguez *et al*., 2012). Vieira *et al*. (2012) reported *D. caninum* in *C. thous*. This parasite lodges in the small intestine, mainly in dogs and cats, and occasionally in carnivorous mammals and humans. They relate this finding to anthropogenic proximity to the habitat of these animals.

In this study, parasites of the class Trematoda, belonging to the *Phylum Platyhelminthes*, were also found in *C. thous, H. hidrochaeris, N. nasua, T. tetradactyla*, and *D. novemcinctus*. *Alaria alata* is a zoonotic trematode that causes problems in Europe and can infect humans through the consumption of meat from paratenic hosts (mammals, birds, and reptiles), often occurring through hunting and consumption of organic meats, leading to alariosis. There are no published studies on this species parasitizing humans in Brazil (Korpysa-Dzirba *et al.*, 2021), but it has been reported in *C. thous* and *P. cancrivorus* (Fernandes *et al.*, 2015; Lima *et al*., 2013). Knowing that this species has

been described in Brazil, it is important to continue studying wild animals to identify regions where infection may occur in production animals intended for consumption, which could, in turn, lead to infection in humans. In the same class of parasites, we have the genus Fasciola, which includes the species *Fasciola hepatica*, a zoonotic parasite that leads to the destruction of liver parenchyma in humans (Martins, 2019). It has been reported in *H. hidrochaeris* in Brazil (Fernandes *et al*., 2015), including in the state of Paraná (Esprenger *et al*., 2018). The transmission of this zoonosis occurs through the consumption of water and vegetables contaminated with metacercariae (Neves *et al*., 2016). When considering the increasing proximity to the wild habitat, it is also necessary to consider the circulation of these infected animals near pastures and agricultural areas where the intermediate host is present, perpetuating the cycle of this parasite for both domestic animals that graze on the pasture and humans through crop consumption.

In the Coccidiida subclass, observed in this study in *C. thous*, *L. aeropaeus*, and *T. tetradactyla*, there are several parasites of public health importance. In this class, *Octosporella-like* oocysts were found in *N. nasua*, a poorly studied parasite with almost no data available. According to the Global Biodiversity Information Facility's digital platform database (GBIF, 2021), several species of this parasite have been found in Switzerland, with reports also in Sweden, Norway, Spain, the United Kingdom, France, the Czech Republic, Thailand, Denmark, Italy, the United States, Germany, Canada, Greece, and Venezuela. However, no species had been reported in Brazil. To our knowledge, this might be the first description of *Otosporella* in *Nasua nasua* in Brazil; however, since the results were of *Octosporella-like* oocysts, molecular tests are necessary for confirmation. The genus *Neospora* also belongs to the Coccidiida subclass, and although there are no reports in humans, due to its similarity to *Toxoplasma gondii*, the possibility of infection cannot be ruled out (Robayo-Sánchez, Gómez-Marín, Cortés-Vecino, 2017).

5. CONCLUSION

Based on the information generated by this study, it can be concluded that characterizing the intestinal helminth fauna of road-killed wild animals is a valuable tool for investigating parasites with zoonotic potential. To the best of our knowledge, this marks the first report of *Ancylostoma sp*. in *G. cuja* and the presence of *Octosporella-like* oocysts in *N. nasua* in Brazil.

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