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Original Article

Endoparasites in domestic animals surrounding an Atlantic Forest remnant, in São Paulo State, Brazil

Endoparasitas em animais domésticos que vivem ao redor de uma reserva florestal, no Estado de São Paulo, Brasil

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Abstract

Morro do Diabo State Park (MDSP) is a significant remnant of the Atlantic Rain Forest in Brazil and is surrounded by rural properties. In that area, wild and domestic animals and humans are in close contact, which facilitates the two-way flow of infectious diseases among them. We assessed endoparasites in domestic livestock from all rural properties surrounding MDSP. There were sampled 197 cattle, 37 horses, 11 sheep, 25 swine, 21 dogs, one cat and 62 groups of chickens from 10 large private properties and 75 rural settlements. *Eimeria* spp. was present in almost all hosts, excepted in horses, dogs and cats. *Giardia* cysts were present only in cattle. Nematodes were found in swine, ruminants and horses in high prevalence. *Ancylostoma*, *Toxocara* and *Sarcocystis* were found in dogs. Chickens were found with coccidia, Ascaridida and *Capillaria* spp.. These parasites can cause malnutrition and reproductive disorders for their hosts. Strategies to prevent and control the spread of endoparasites can improve wildlife, animal and human health in this area.

Keywords: Helminths, protozoan, Morro do Diabo State Park, wildlife conservation, rural settlements, Brazil.

Resumo

O Parque Estadual Morro do diabo (PEMD) é um significante remanescente de Mata Atlântica no Brasil, e rodeado de propriedades rurais. Nesta área humanos, animais domésticos e silvestres vivem próximos, o que facilita o fluxo de agentes infecciosas entre eles. Nós avaliamos a presença de endoparasitas, por meio de exame coproparasitológico dos animais domésticos de todas as propriedades rurais do entorno do PEMD. Foram amostrados 197 bovinos, 37 equinos, 11 ovinos, 25 suínos, 62 grupos de galinhas, 22 cães e 1 gato, residentes em 10 grandes propriedades privadas e 75 assentamentos rurais. *Eimeria* spp. estava presente em quase todas as espécies hospedeiras, com excessão de equinos, cães e gatos. Cistos de *Giardia* estavam presentes somente em bovinos. Nematodes foram encontrados em suínos, ruminantes e equinos em alta prevalência. *Ancylostoma*, *Toxocara* e *Sarcocystis* foram encontrados em cães. Galinhas foram encontradas com coccidia, Ascaridida e *Capillaria* spp.. Os parasitas encontrados podem causar má nutrição e problemas reprodutivos para seus hospedeiros. Medidas de prevenção e controle da dispersão de endoparasitas podem melhorar a condição de saúde pública, animal e ambiental nesta área.

Palavras-chave: Helmintos, protozoários, Parque Estadual Morro do Diabo, conservação ambiental, assentamentos rurais, Brasil.

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Introduction

Morro do Diabo State Park (MDSP) is the last significant remnant of the Atlantic Rain Forest in western São Paulo State, Brazil, where only 1.8% of the original natural vegetation remains. The Atlantic Rain Forest is considered a biodiversity hotspot (GALETTI & SAZIMA, 2006). This forest is surrounded by rural properties, some of which are owned by low-income farmers (settlers) who survive on subsistence agriculture (SEVÁ et al., 2010). These properties make up a settlement that originated from the Landless Workers Movement. This scenario allows close contact between wild and domestic animals and humans, which facilitates the two-way flow of infectious diseases between these groups.

Some parasites can affect different host species. Endoparasites, such as worms and protozoans, have great impacts on public and animal health and wildlife conservation around the world, mainly in developing countries. Research has reported 11 previously unidentified endoparasite hosts in wild animals from different biomes in Brazil (RAMOS et al., 2016).

In areas with high biodiversity such as Latin America, domestic dogs pose serious risks to native species due to the ease with which they enter the forest, by acting as a reservoir for some infectious diseases and by preying on and competing with wild species (BUTLER & BINGHAM, 2000; BUTLER & DU TOIT, 2002; FIORELLO et al., 2006; GALETTI & SAZIMA, 2006; OLIVEIRA et al., 2008).

In addition, wild animals may act as opportunistic and/or primary agents for a wide range of parasites (GODOY & CUBAS, 2011) and can transmit these parasites to domestic animals and humans inside or outside their natural habitats. According to a previous study (DASZAK et al., 2000), pathogenic parasites represent a hazard for wildlife conservation, recovery and management programs, especially for endangered species. In Brazil, the conditions of rural workers are precarious and can lead to diseases that represent health risks to the population (SCOPINHO, 2010); this scenario is common in some rural settlements in the country.

A lack of information about the prevalence of endoparasites in these rural areas poses a possible health threat to wildlife populations and, primarily, to the settlers as they are in close contact with domestic animals as a result of managing livestock and consuming products of animal origin, including meat. In the current paper, we assessed the prevalence of endoparasites in domestic animals from settlers and farms surrounding MDSP to identify the environmental risk to local wildlife and human populations.

Methodology

Fecal samples from domestic animals raised as pets or for subsistence were collected from all rural properties directly surrounding the MDSP border. These rural properties are divided into 10 large private properties (up to 20 ha in total area) and 75 small rural settlements (total area ranging from 10 to 20 ha) and are all located around MDSP in Teodoro Sampaio Municipality of São Paulo State, Brazil (Figure 1).

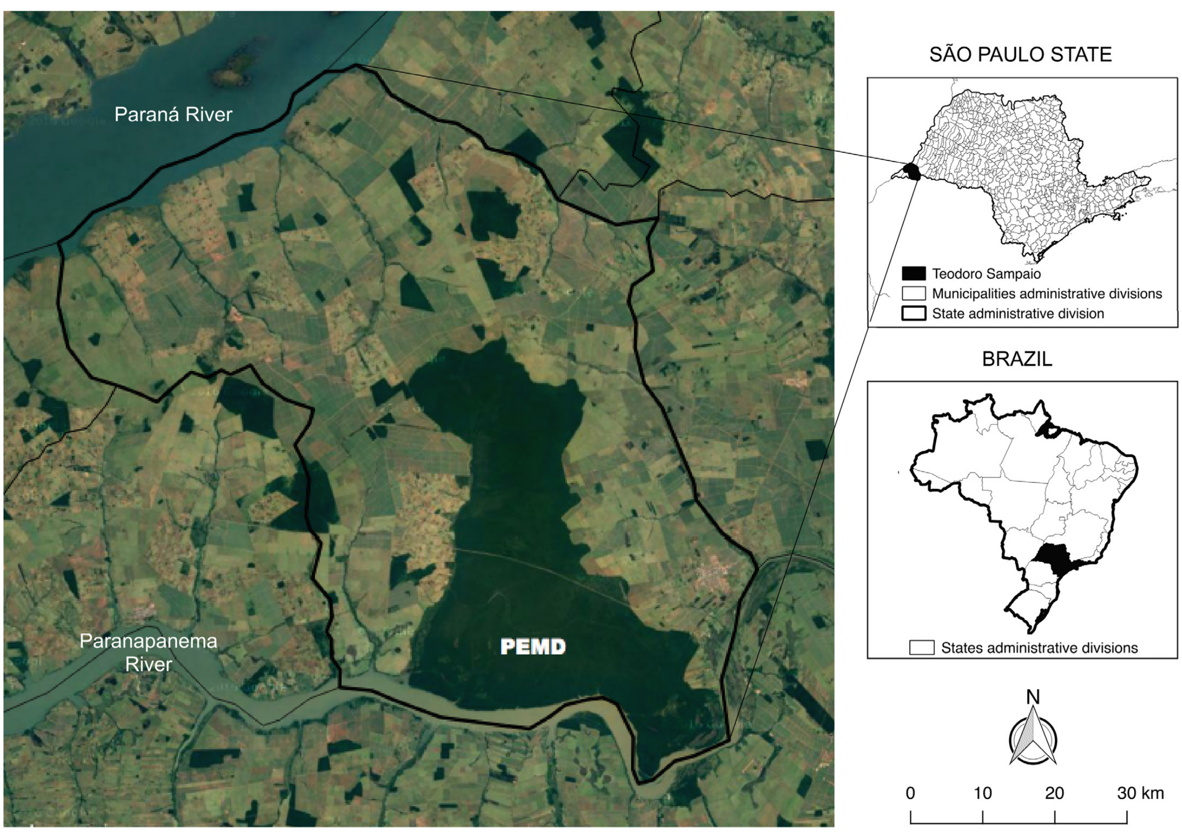

Figure 1. Morro do Diabo State Park (MDSP) in Teodoro Sampaio Municipality, State of São Paulo, Brazil.

Fresh feces from 197 cattle, 37 horses, 25 porcine, 11 sheep, 22 dogs, 1 cat and 62 groups of chickens were collected directly from the ground and after defecation or from the anus in cases of bovine, equine, sheep and dogs. The 393 samples were stored refrigerated in 2.0% potassium dichromate at 4.0 °C for up to three weeks until analysis at the Laboratory of Parasitic Diseases, Department of Preventive Veterinary Medicine, School of Veterinary Medicine and Animal Health, University of São Paulo, São Paulo, Brazil. Samples were examined for endoparasite eggs, cysts and oocysts identification using a conventional sucrose flotation method, employing a sucrose of 1.203 specific gravity (SHEATHER, 1923). Therefore, stool of feces were diluted in sucrose solution and filtered using gauze. In the resultant mixture we add more sucrose solution until complete 12 mL to be centrifuged at 1,600*g* for 10 min in a 15 mL plastic tube. Floated material was transferred to a slide a under a coverslip, and the eggs were observed at 100x and 200x magnification and the cysts and oocysts at 400x magnification for identification by light microscopy.

Results

The percentages of the 85 properties that had each type of domestic animal were as follows: 93% cattle, 54% horses, 11% sheep, 24% swine, 25% dogs and 76% chickens. All 85 properties had rudimentary facilities for domestic animals, including facilities for sheep and swine where animals had direct contact with the soil and no concrete slabs. Additionally, livestock, horses, dogs, cats and poultry were raised with free access to the property, often sharing water from the state park with wild species and frequently entering the protected area.

The parasites found in the different animals' samples are described in Table 1. Although some properties had cats, their fecal samples were difficult to find; thus, only one sample was obtained, which was positive for *Ancylostoma* spp. eggs. Deer feces were found in the same area and were negative for endoparasites.

Discussion

Eimeria and *Isospora* spp. are ubiquitous coccidian parasites that are found in all orders of mammals. In the present study, *Eimeria* spp. were present with a high prevalence in almost all hosts (73% of sheep, 61% of cattle, 60% of swine and 90% of chickens). There are over 1000 species of *Eimeria*, and they are the majority parasite the intestinal epithelia of vertebrates. The species can infect horses, domestic dogs and cats and wildlife, along with economically significant species that infect rabbits, cattle, sheep, pigs, turkeys and chickens (WITCOMBE & SMITH, 2014). These protozoa produce changes in the intestinal mucosa that can lead to

Table 1. Prevalence rates of parasites in different hosts on the properties sampled.

Species	Positive (%)	Parasites	Number of animals (%)	
BOVINE	197 (78)	Strongylida	101	(51)
		Strongyloides papillosus	25	(13)
		Trichuris ovis	7	(4)
		Moniezia benedeni	5	(3)
		Eimeria spp.	121	(61)
		Giardia spp.	4	(2)
EQUINE	37 (59)	Strongylida	28	(44)
		Strongyloides westeri	7	(11)
SHEEP	11 (100)	Strongylida	9	(82)
		Eimeria spp.	8	(73)
PORCINE	25 (100)	Strongylida	14	(56)
		Strongyloides ransomi	5	(20)
		Ascaris suum	4	(16)
		Metastrongylus spp.	5	(20)
		Trichuris suis	2	(8)
		Coccidia*	8	(32)
		Eimeria spp.	15	(60)
DOG	22 (79)	*Strongyloides stercoralis*	1	(4)
		Toxocara canis	5	(18)
		Ancylostoma spp.	15	(54)
		Capillaria spp.	1	(4)
		Cystosisospora spp.	6	(21)
		Sarcocystis spp.	5	(18)
CHICKEN	62 (90)	Strongylida	3	(5)
		Ascaridida	15	(22)
		Capillaria spp.	15	(22)
		Cestoda	20	(29)
		Eimeria spp.	56	(90)

Legend: *Non sporulated oocysts (*Eimeria* spp. or *Cystoisospora suis)*.

bleeding for some species (PAIVA, 1996), reduced local nutrient absorption (PAIVA, 1996; URQUHART et al., 1998), and diarrhea, and can have a severe impacts on animal welfare, efficiency of feed conversion and weight gain (MARUGAN-HERNANDEZ et al., 2017).

Among the coccidia in swine, 13 species of *Eimeria* and one of *Cystoisospora suis* were described; however, only *C. suis* has been found to cause serious diarrhea in piglets (MATSUBAYASHI et al., 2016). In our study, we did not differentiate between these two species of coccidian due to the fact that the oocysts were not sporulated. However, coccidiosis is of great economic importance because of the losses it causes due to clinical infections; in particular, it causes reduced weight gain (CHARTIER & PARAUD, 2012).

Sheep act as hosts of some species of *Eimeria* (TAYLOR et al., 2011), two of the most frequent of which (*E. crandallis* and *E. ovinoidalis*) are highly pathogenic, resulting in high mortality rates in young animals (DENIZ, 2008). These parasites are common on farms with high-density populations of sheep, which leads to poor hygiene and stress (LAGARES, 2008). In practice, it is impossible to eradicate *Eimeria* in this host species; and farmers can only attempt to reduce it to tolerable limits.

Avian coccidiosis is an enteric disease caused by several species of *Eimeria.* It is considered one of the most important diseases in birds around the world because of its importance in the poultry industry, where it can cause losses in production, increased mortality, reduced bird welfare and the risk of contamination of products for human consumption (CARDOZO & YAMAMURA, 2004). Thus, the presence of *Eimeria* in 90% of the chicken in our study may affect the people who raise these animals for subsistence. This parasite prevalence may also indicate high soil contamination, representing a good source for infecting other avian species. Although *Eimeria* species are host-specific, they can infect a single host order or group of closely related hosts (ATKINSON et al., 2008). In the properties sampled and in the interior of the Atlantic Rain Forest - MDSP, there were observed other species of the order Galliformes were observed, including poultry and the black-fronted piping-guan (*Aburria jacutinga*) (personal observation).

Hygienic practices in farm facilities plays a fundamental role in the control of coccidiosis, even when using prophylactic drug therapy (KREINER et al., 2011). On the basis of this study's results and our field observations, preventive measures to reduce domestic stock parasite loads have not been adopted in either large or small properties surrounding MDSP.

We found *Giardia* cysts only in cattle and with a low prevalence. This protozoan is intermittently eliminated in feces; in addition, we used only the sucrose flotation methodology for the analyses, which we recognize has low sensitivity for protozoan diagnostics. Therefore, we cannot consider our prevalence data for these parasites as reliable because it likely represents an underestimation. *Giardia* parasitizes a variety of crops and animal species (PATZ et al., 2000).

Most wild mammals are found to be infected with coccidia one or more times during their life (SAMUEL et al., 2001). The presence of this protozoa in domestic animals may implicate the wild animals that frequent the properties studied, along with the white-lipped peccary (*Tayassu pecari*), which was found parasitized by protozoans of the genera *Eimeria* and *Giardia* in MDSP (NAVA, 2008).

Some protozoans, including *Eimeria*, *Isospora* and *Giardia*, are also transmitted by drinking contaminated water (GODOY et al., 2004). The spread of waterborne zoonotic parasitic diseases is associated with domestic livestock, and some of these diseases are readily transmitted to humans (PATZ et al., 2000) and wild animals (SEVÁ et al., 2010). In areas with poor sanitary conditions, as in the area sampled in the present study, the control of this parasite is complicated. This scenario of a high prevalence of protozoa is likely intensified during periods of rainfall, when intense runoff leads to heavily contaminated water sources. In Teodoro Sampaio Municipality, the annual average rainfall is high, reaching up to 1359 mm (CEPAGRI, 2007). In addition, some rivers in MDSP that are shared with rural properties.

In the present study, nematodes were found with high prevalence rates in ruminants and horses (Figure 2), with nematodes of the Strongylida suborder the most frequent. These nematodes can cause intestinal damage, anemia, anorexia, depression, weight loss and dehydration in animal hosts (LAGARES, 2008). Some animals had concomitant infections with Strongylida and *Eimeria*, including 32% of swine, 37% of cattle and 64% of sheep. This result suggests that these parasites in combination could cause more intense damage to hosts.

Some nematodes were found in domestic swine in the present study. The most important symptoms of *Ascaris suum, Trichuris suis* and *Hyostrongylus rubidus* are malnutrition and growth retardation (STEPHENSON et al., 1980; HALE & STEWART, 1985; JESUS & MÜLLER, 2000). *Metastrongylus* species that affect domestic swine can cause symptoms related to respiratory disorders and, hence, retard growth (OPRIESSNIG et al., 2011). The prevalence of these parasites in swine is related directly to overcrowding, excessive heat and humidity, the sex and age of animals, dirty or contaminated soil beds, mixed grazing with sheep and cattle and the presence of Coleoptera and too much organic matter, which typify the conditions found in the areas sampled (URQUHART et al., 1998).

The overlap of areas containing wildlife and domestic animals in this study points to the risk of parasite exchange among species. In fact, endoparasites such as Strongyloidea-like, *Strongyloides*-like, Ascaridae-like and *Ascaris suum*-like parasites were found in collared

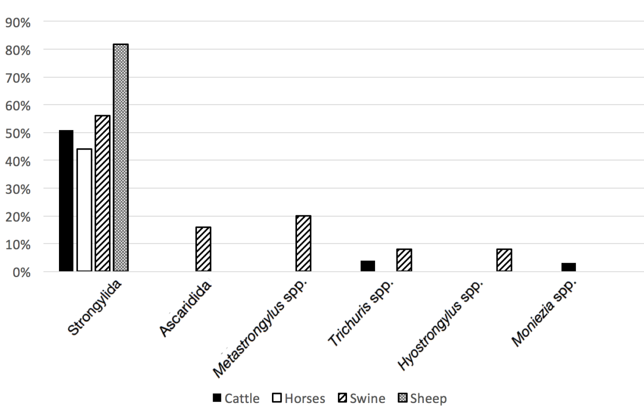

Figure 2. Prevalence rates of nematodes in large domestic animals.

peccaries (*Pecaritajacu*) and white-lipped peccaries (NAVA, 2008) captured in areas bordering the settlements of the study area.

Herein, domestic dogs were present on 21 (24.7%) properties, and *Ancylostoma* and *Toxocara* were found in 54% and 18% of them, respectively. For humans these parasites cause Cutaneous and Visceral Larva Migrans, respectively, which cause disorders in some organs (QUEIROZ & CHIEFFI, 2006). For wild animals infected with these parasites, movement patterns in the environment may also differ, which may have consequences for their survival or reproduction (HAY et al., 1985). In addition, toxocariasis can reduce population sizes once it becomes frequent in young animals and causes mortality (SANTARÉM et al., 2009).

Domestic dogs (*Canis lupus familiaris*) are part of the process of landscape occupation by humans. They have become the most abundant carnivore in rural areas, and the traditional management practice in Brazilian rural areas is to let them move about unrestricted, leaving them free to enter forest patches. In a study of management practices, Kitala et al. (2001) found that many dogs spend considerable time roaming freely since 69% of the dogs were never restricted (TORRES & PRADO, 2010). It is important to consider that dogs on rural properties surrounding MDSP commonly enter forested areas to hunt on their own, establish a territory or hunt with their owner. Furthermore, there is a lack of proper management of domestic dogs, including the absence of vaccinations, deworming and population control (NAVA, 2008). Domestic dog hosts have been frequently implicated as a source of infection for wild canids (LAURANCE et al., 1998; CLEAVELAND et al., 2000; RANDALL et al., 2004; WOODROFFE et al., 2004; MEGID et al., 2010) and wild felids (NAVA, 2008). Disease transmission from domestic dogs may constitute an important anthropogenic 'edge effect' (WOODROFFE et al., 2004). In addition, the contrary effect, related to the transfer of parasitic infections from wild animals to domestic dogs, may occur, and in this case, the prevalence of dog parasites represent a bio-indicator of environmental contamination. Wild canids and felids, such as crab-eating fox (*Cerdocyon thous*), pumas (*Puma concolor*) and jaguars (*Panthera onca*), are present inside and surround the MDSP and are also hosts of these parasites founded in domestic dogs, such as *Toxocara* spp., *Capillaria* spp., *Trichuris* spp., *Cystoisospora* and *Giardia* spp., (FARRET et al., 2008; HOLSBACK et al., 2013; LIMA, 2009; SANTOS et al., 2015).

Sarcocystis was found in 18% of the dogs sampled in this study. Domestic dogs are definitive hosts of the *Sarcocystis* spp. listed below (the species in parenthesis are intermediary hosts): *S. ovicanis* (sheep), *S. tenella* (sheep); *S. miescheriana* (pig), *S. bertrami* (horse), *S. capracanis* (goat) and *S. canis* (cattle) (DUBEY, 1976; WADAJKAR et al., 1993; WEE & SHIN, 2001). *Sarcocystis canis* has been reported to cause acute canine sarcocystosis in very young animals (COOLEY et al., 2007). The prevalence of *Sarcocystis* spp. in a canine population is related to the eating habits of the animals, particularly the consumption of raw meat and viscera, undercooked meat, or through hunting small mammals and birds (LABRUNA et al., 2006). Therefore, it is possible that this parasite is present in other domestic and wild animals in the studied area and may act as sources of *Sarcocystis* in the canine population.

Cats were present in some rural properties; however, their fecal samples were difficult to find. The one sample collected and analyzed contained *Ancylostoma* eggs, which also parasitize human and wild felids, as mentioned above.

Chickens were present in 62 (72.9%) properties; 48% of them had coccidia, and 22% had Ascaridida and *Capillaria* spp. Symptoms such as malnutrition, growth reduction and respiratory disorders are caused by Ascaridida (SCOTT, 1988).

Some parasites found in all domestic animals have an indirect effect on reproduction by delaying growth, which leads to delayed sexual maturity and a decreased reproductive lifespan (STRINGER & LINKLATER, 2014).

As observed in the small and large properties in the region studied, domestic animals are infected by some endoparasites. The main factor for this finding is the poor herd management observed for all domestic species sampled in this study, such as the lack of preventive measures to reduce parasite loads and limit infections. Other factors include: 1) the rudimentary characteristics of sheep and swine facilities, which contributes to maintaining parasite populations on the property; 2) the high densities of sheep and swine held within enclosures; and 3) the fact that dogs are raised with free access to the forest and are being frequently in contact with wild animals, humans and other domestic species.

The close proximity of the state park and the rural settlement along with the presence of wildlife, such as peccaries, deer and birds in the crop fields and livestock pastures, along with the free access of dogs, cats, horses and livestock to the river that shares a boundary with the state park suggests a high probability of parasite exchange among these populations through their collection in soil and water.

Conclusion

Some strategies for preventing and controling parasites to avoid infections in domestic animals include the removal of carcasses, vaccination and deworming programs, creating a buffer zone between areas with wildlife and livestock and health education programs (STRINGER & LINKLATER, 2014). In conclusion, we suggest improvements in property management through a preventive strategy program to disrupt endoparasite life cycles. To obtain better results, the strategies should be enacted jointly with public authorities and health experts.

This scenario of rural properties raising domestic animals in poor conditions and with endoparasites presents an environmental risk to some local populations. Additionally, local water sources are likely contaminated with the parasites detected in this study. The high prevalence rates of coccidiosis in poultry and domestic livestock represent the low production yields of animals raised for subsistence. Dogs and cats also carry endoparasite loads with zoonotic potential, such as *Ancylostoma* and *Toxocara*. Therefore, the strategies proposed for the prevention and control of endoparasites found in the current study can benefit not only domestic animals but also wild animals and public health in the MDSP region, which represents the last significant remnant of the Atlantic Rain Forest in western São Paulo State, Brazil. These measures can also be applied in other areas with similar features and that require similar care.

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