

Gastrointestinal parasites in dogs and cats in line with the One Health' approach

[Parasitos gastrointestinais em cães e gatos em linha com a abordagem "Saúde Única"]

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ABSTRACT

Dogs and cats are frequently affected by gastrointestinal parasites of medical and veterinary concern. The correct diagnosis is pivotal to the treatment outcome, reducing the risk of environmental contamination and spreading of these pathogens. The aim of this study was to determine the prevalence of gastrointestinal parasites of domiciled dogs and cats in an urban area of Northeastern Brazil, as well as to discuss the findings from a "One Health" perspective. Fecal samples ($n = 231$) of dogs ($n = 126$) and cats ($n = 105$) were obtained directly from the environment after spontaneous defecation, and subsequently analyzed through the Mini-FLOTAC and Baermann techniques. Of all samples, 28.14% (65/231) presented immature forms of gastrointestinal parasites, with 31.75% (40/126) and 23.81% (25/105) of dogs and cats positive, respectively ($\chi^2 = 1.413$; $p = 0.2345$). Two genera of helminths (*Ancylostoma* and *Toxocara*) and two genera of protozoa (*Cystoisospora* and *Entamoeba*) were identified. Additionally, co-infections were observed in 15% (6/40) of positive dogs and 28% (7/25) of positive cats ($p = 0.2207$). None metastrongyloid larvae were detected. In conclusion, animals herein assessed presented a high prevalence of zoonotic gastrointestinal nematodes. Therefore, preventive measures against these neglected parasites should be stimulated.

Keywords: cat, dog, copromicroscopic diagnosis, Mini-FLOTAC, zoonosis

RESUMO

Cães e gatos são frequentemente afetados por parasitos gastrointestinais de interesse médico e veterinário. Em áreas urbanas, onde esses animais apresentam uma relação próxima com o homem, o diagnóstico correto é fundamental para o resultado do tratamento, reduzindo o risco de contaminação ambiental e de disseminação desses patógenos. O objetivo deste estudo foi determinar a prevalência de parasitos gastrointestinais infectando cães e gatos domiciliados em uma área urbana do Nordeste do Brasil, bem como discutir os achados sob a perspectiva de "Saúde Única". Amostras fecais ($n = 231$) de cães ($n = 126$) e gatos ($n = 105$) foram obtidas diretamente do meio ambiente após a defecação espontânea e, posteriormente, analisadas pelas técnicas Mini-FLOTAC e Baermann. Do total de amostras, 28,14% (65/231) apresentaram formas imaturas de parasitos gastrointestinais (ovos, cistos ou oocistos), com 31,75% (40/126) e 23,81% (25/105) de cães e gatos positivos, respectivamente ($\chi^2 = 1,413$; $P = 0,2345$). Dois gêneros de helmintos (*Ancylostoma* e *Toxocara*) e dois gêneros de protozoários (*Cystoisospora* e *Entamoeba*) foram identificados em cães e gatos. Além disso, coinfeções foram observadas em 15% (6/40) dos cães positivos e em 28% (7/25) dos gatos positivos ($P = 0,2207$). Nenhuma larva de metastrongilídeo foi detectada. Em conclusão, os animais aqui avaliados apresentaram alta prevalência de nematódeos gastrointestinais de importância zoonótica. Portanto, medidas preventivas contra esses parasitos negligenciados devem ser estimuladas.

Palavras-chave: gato, cão, diagnóstico copromicroscópico, Mini-FLOTAC, zoonose

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INTRODUCTION

Over the last years, the close relationship between domestic animals and humans has promoted significant benefits for all species involved, especially due to the social, emotional, and physical welfare (Robertson *et al.*, 2000). However, it is important to note that this proximity may have important implications for human health, especially when the sanitary conditions of animals are neglected.

The gastrointestinal parasitic fauna of dogs and cats have been well documented and comprises a wide plethora of helminthes (e.g., *Ancylostoma* spp., *Strongyloides* spp., *Trichuris* spp. and *Toxocara* spp.) and protozoa (e.g., *Cystoisospora* spp., *Entamoeba* spp., *Giardia* spp. and *Cryptosporidium* spp.) species (Lima *et al.*, 2017; López-Osorio *et al.*, 2020; Monteiro *et al.*, 2016; Oliveira-Arbex *et al.*, 2017). Overall, the main route of infection is through the ingestion of infective forms (e.g., eggs, larvae, cysts and oocysts), but for some species the percutaneous infection is also reported. This latter form of infection achieves great relevance, especially considering that feces of dogs/cats may contaminate soil of parks, sand boxes and backyards (Del Giudice *et al.*, 2019; Silva *et al.*, 2019) increasing the risk of contact with humans.

It is estimated that more than 3 billion people are affected every year by parasitic infections (Okuy *et al.*, 2004), and many of the parasites causing these diseases involve dogs and cats in their life cycle. For a long time, *Ancylostoma* spp. and *Toxocara* spp., are respectively accounted as main causes of cutaneous larva migrans (CLM) and visceral larva migrans (VLM), classical examples of zoonotic pathogens transmitted by pets (dogs and cats) (Criado *et al.*, 2012; Del Giudice *et al.*, 2019; Despommier *et al.*, 2003; Özbakış and Doğanay, 2020). Nonetheless, attention should also be paid to other pathogens such as *Strongyloides* spp. (Sanpool *et al.*, 2019) and

Echinococcus spp. (Kesteren *et al.*, 2013), important parasites of public health concern. It is important to note that some protozoan such as *Cryptosporidium* and *Giardia* are frequently reported in domestic dogs and cats (Ryan and Cacciò, 2013). For instance, *Giardia* species has been responsible for more than ~ 280 million human cases of diarrhea every year (total giardiasis acquired by all transmission routes) (Horlock-Roberts *et al.*, 2017).

Apart from the veterinary and medical interest, many of these pathogens are relevant from a “One Health” perspective since the environmental conditions are important for the establishment and survival of immature forms of these parasites. Recently, there is a global interest in canine and feline fecal pollution as an emerging problem of public health (Traversa *et al.*, 2014). Despite of the availability of scientific data, it is evident the poor knowledge of the population about the risk of transmission of these parasites through contaminated environment.

Despite of the wide knowledge about gastrointestinal fauna of parasites of dogs and cats worldwide (Gennari *et al.*, 2016; Oliveira-Arbex *et al.*, 2017), some One Health aspects need to be better addressed, especially in areas where scientific evidence of the presence of these zoonotic parasites are lacking. Therefore, the aim of this study was to determine the prevalence of gastrointestinal parasites of domiciled dogs and cats in an urban area of Northeastern Brazil and to discuss the importance of the findings from a One Health perspective.

MATERIAL AND METHODS

A cross-sectional study was carried out in different municipalities of the state of Pernambuco (35°56'38", 37°8'38" W and 8°15'36", 9°23'31" S), Northeastern Brazil (Figure 1). The map was constructed using the QGIS software (QGIS 3.10.14, A Coruña).

Gastrointestinal parasites...

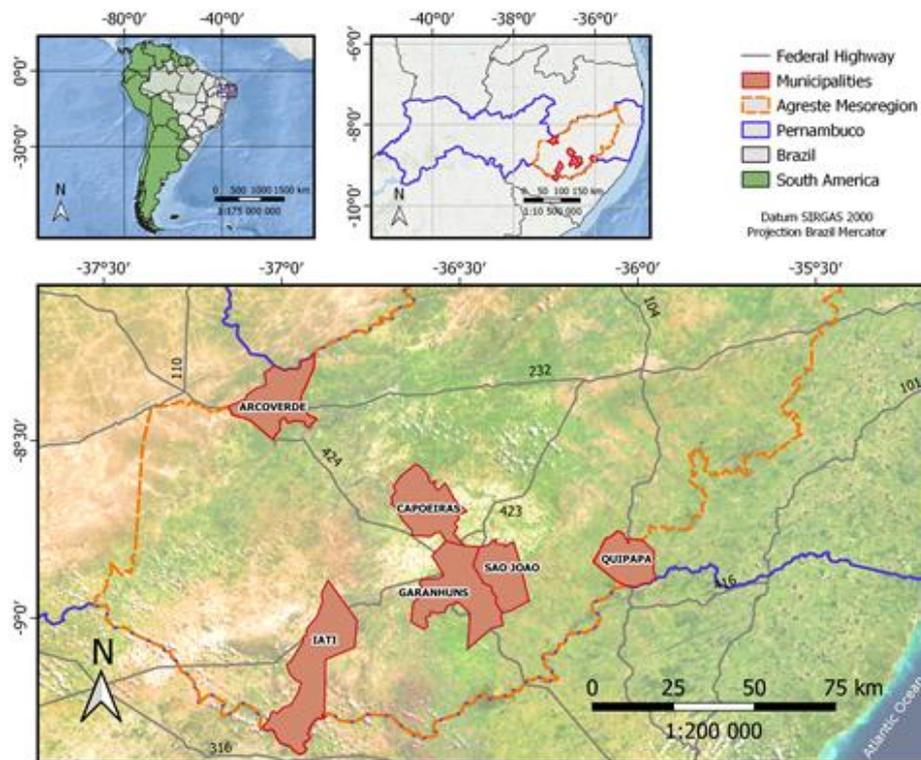


Figure 1. Distribution of the samples according to the municipalities of the state of Pernambuco, Northeastern Brazil.

This area is characterized by a tropical climate with distinct wet (rainy) and dry seasons. The rainy period occurs in autumn and winter (from March to August), and the mean annual temperature is above 18°C. With altitude ranging from the sea level up to 1,260 meters, Pernambuco has three climate classifications: hot and humid tropical climate (*As*), hot semi-arid climate (*Bsh*) and tropical monsoon climate (*Am*) (Medeiros *et al.*, 2018). All area of collection was in hot and humid tropical climate (*As*).

The minimum sample size ($n = 231$) was calculated considering the dog ($n = 14,057$) and cat ($n = 7,272$) populations of the study area following the proportion ratio presented in a previous study (Canatto *et al.*, 2012). An estimated prevalence of 50%, confidence level of 95% and statistical error of 5% were considered (Thrusfield, 2004).

From August 2018 to July 2019, fecal samples were obtained from domiciled dogs ($n = 126$) and cats ($n = 105$) after spontaneous defecation. The distribution of samples across different

municipalities of the study area is as follows: Garanhuns (75 dogs and 56 cats), São João (12 dogs and 13 cats), Capoeiras (11 dogs and 12 cats), Quipapá (10 dogs and 12 cats), Iati (10 dogs and 8 cats) and Arcoverde (8 dogs and 4 cats).

Each pet owner performed the sampling. The fecal part that was in contact with the soil was discarded, using only the upper portion. Samples were stored at 8°C until laboratory procedures. The selection of animals occurred by convenience, irrespective of their sex, age, or breed. No animal had been dewormed on the last three months before sample collection. According to the owners, all animals were maintained in an indoor environment, but with access to the backyard. They were fed with commercial dry food and water was provided *ad libitum*.

All samples were analyzed through the Mini-FLOTAC technique following all manufacturers' recommendations. Sodium chloride ($d = 1.20\text{g/cm}^3$) was used as flotation solution (Lima *et al.*, 2015). In addition, they were also subjected to

the Baermann technique (Euzéby, 1981) to search lungworms parasites.

Results were statistically analyzed for absolute and relative frequencies. In addition, the Lilliefors test was used to verify the normality of the data. The Qui-square with Yates correction (χ^2) was used to compare the frequency of gastrointestinal parasites infecting dogs and cats, as well as occurrence of the parasites in males and females and in different ages. The Fisher's Exact Test was used to compare the co-infections and single infections.

The significance level was set at 5%. All analyzes were performed using the statistical software BioEstat version 5.0 (Ayres et al., 2007).

RESULTS

Of all fecal samples analyzed (n = 231) immature forms of gastrointestinal parasites (eggs, cysts, or oocysts) were detected in 28.14% (65/231) through the Mini-FLOTAC technique. In particular 31.75% (40/126) and 23.81% (25/105) of canine and feline samples scored positive, respectively ($\chi^2 = 1.413$; p = 0.2345), but without statistical difference. Additionally, co-infections were observed in 15% (6/40) of positive dogs and 28% (7/25) of positive cats (p = 0.2207).

The results of single and co-infections for dogs and cats are reported on Table 1.

Table 1. Single and co-infections by gastrointestinal parasites detected in fecal samples of domiciled dogs and cats from Northeastern Brazil

Parasites	Prevalence % (no. positive/total positive)	
	Cats	Dogs
<i>Ancylostoma</i> spp.	36 (9/25)	65 (26/40)
<i>Cystoisospora</i> spp.	16 (4/25)	5 (2/40)
<i>Toxocara</i> spp.	8 (2/25)	15 (6/40)
<i>Entamoeba</i> spp.	12 (3/25)	-
<i>Toxocara</i> spp. + <i>Ancylostoma</i> spp.	12 (3/25)	7.5 (3/40)
<i>Toxocara</i> spp.+ <i>Entamoeba</i> spp.	4 (1/25)	-
<i>Toxocara</i> spp. + <i>Cystoisospora</i> spp.	4 (1/25)	-
<i>Ancylostoma</i> spp. + <i>Cystoisospora</i> spp.	8 (2/25)	7.5 (3/40)

Overall, females were more positive than males in both dogs ($\chi^2 = 0.883$; p = 0.3473) and cats ($\chi^2 = 0.064$; p = 0.8003) species, but without statistical difference. Dogs with age ranging from 1 to 3 years old were more parasitized ($\chi^2 = 8.996$;

p = 0.0111), whereas cats with less than one year old were more infected ($\chi^2 = 15.522$; p = 0.0004) (Table 2). In both cases statistical significance has been detected.

Table 2. Infections by gastrointestinal parasites detected in fecal samples of domiciled dogs and cats according to the sex and age

	Dogs			
	No. examined	No. positive	Prevalence (%)	p-value
Male	66	18	27	
Female	60	22	37	0.3473
< 1 year old	27	15	55.5	0.0123
1 to 3 years old	35	9	26	0.0984
>3 years old	64	16	25	0.0111
	Cats			
	No. examined	No. positive	Prevalence (%)	p-value
Male	46	12	26	
Female	59	13	22	0.8003
< 1 year old	29	14	48	0.0108
1 to 3 years old	18	5	28	0.0003
>3 years old	58	6	10.5	0.004

The majority of dogs positive for *Toxocara* sp. (77.7%; 7/9) had less than one year old. None metastrongyloid larvae were detected at the Baermann and Mini-FLOTAC techniques.

DISCUSSION

This study reveals a high prevalence (28.1%; 65/231) of gastrointestinal parasites in dogs and cats from an urban area of Northeastern Brazil. Although high, the positivity of each animal species (31.75% for dogs and 23.81% for cats) presented a reduction when compared with other studies that reported frequencies of 70.94% (327/640) for samples of dogs collected from the environment (Macedo *et al.*, 2019) and 65.31% (113/173) for domiciled cats (Monteiro *et al.*, 2016), both in urban areas of Northeastern Brazil. These differences may be related to several factors such as parasite load, age of the animals, conservation of the sample and type of diagnostic test employed (Funada *et al.*, 2007). Additionally, in the present study only domiciled animals were sampled, which may also explain this reduced number of positive animals when compared with previous studies.

Co-infections were also observed in few samples of dogs and cats. Albeit less predominant, this mixed infection may enhance the pathogenic role of some species involved causing various damages to the vertebrate host. Overall, female dogs ($\chi^2 = 0.883$; $p = 0.3473$) and cats ($\chi^2 = 0.064$; $p = 0.8003$) were more parasitized than males, but without statistical difference. Unfortunately, no information about the presence of pregnant females is available since this condition may influence the immunological status of animals and consequently the endoparasite eggs, oocysts and larvae shedding. Dogs from one to three years old ($\chi^2 = 8.996$; $p = 0.0111$) and cats under one year old ($\chi^2 = 15.522$; $p = 0.0004$) were more parasitized than other animals, in both cases significant differences were observed. It has been well documented that the occurrence of some gastrointestinal parasites (e.g., *Toxocara* and *Cystoisospora*) is more common in young animals since the immunological immaturity favors the multiplication of parasites and, consequently, an intense elimination of immature forms (Ferreira *et al.*, 2013; Pivoto *et al.*, 2013). Conversely, the parasitism by *Ancylostoma* does not depend on the immunity acquired during the animal's life (Boag *et al.*, 2003).

Ancylostoma spp. was the parasite most frequently detected in this study followed by *Toxocara* spp. This finding confirms previous observations that *Ancylostoma* spp. is the most prevalent nematode in Brazilian pet animals (Labruna *et al.*, 2006; Ferreira *et al.*, 2016). Undoubtedly, both genera of nematodes are considered important agents affecting dogs and cats worldwide (Dantas-Torres and Otranto, 2014), and being responsible for severe clinical manifestations, especially in young animals. Although less frequent, infections by *Cystoisospora* and *Entamoeba*, which are neglected protozoa, were also reported. In general, these parasites have been less detected than nematodes. For instance, a positivity of 13.27% (15/113) for *Cystoisospora* was observed for cats in a previous study conducted in Northeastern Brazil (Monteiro *et al.*, 2016). Although in the present study *Entamoeba* spp. had been retrieved in cats ($n = 4$), information on the prevalence of this parasite in dogs and cats has been poorly documented (Nguí *et al.*, 2014). It is important to note that all cats infected by *Entamoeba* spp. lived in the same area, suggesting the role of environmental contamination, and sharing of pathogens among these animals.

Metastrongyloid larvae was not detected in this study, following the same trend observed in previous studies in Brazil where lungworms have been scantily diagnosed in these animals. Recently, great attention has been paid to these nematodes due to the spreading beyond the frontiers of Europe and North America (Penagos-Tabares *et al.*, 2018). These parasites have been detected worldwide, including in Brazil (Ferreira *et al.*, 2007). However, the retrieval of these nematodes here is still limited, occurring especially in southern areas (Ehlers *et al.*, 2013).

The detection of zoonotic pathogens infecting dogs and cats is important from a One Health perspective. This study revealed the predominance of infections by *Ancylostoma* spp. and *Toxocara* spp., nematodes responsible for the occurrence of CLM and VLM, respectively (Del Giudice *et al.*, 2019; Özbakış and Doğanay, 2020). Although preventive measures against these syndromes are widely known, their persistence worldwide indicates the negligence of the population in face of this health threat. In fact, data herein obtained reflect a common scenario observed throughout Brazil, where high

prevalence by zoonotic gastrointestinal pathogens is frequently detected in dogs and cats (Seva *et al.*, 2018). The role of sanitary conditions and cultural behavior as determinant factors for the occurrence of these parasites is indisputable. It has already been proven that animals from owners with low purchasing power living in areas without basic sanitation are more parasitized (Nguí *et al.*, 2014), and consequently represent a high risk of environmental contamination by canine and feline feces.

CONCLUSIONS

Dogs and cats herein analyzed presented high prevalence of gastrointestinal nematodes of medical and veterinary concern. The positivity occurs in both species (e.g., dogs and cats) and male and females. Nonetheless, it is predominant in dogs with age ranging from 1 to 3 years old and in cats with less than one year old. Finally, preventive measures against these neglected parasites should be addressed to maintain animal health, to prevent the human infection and to reduce the environmental contamination by dogs and cats' feces that have been considered an emerging threat from a One Health perspective.

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