Original paper

Prevalence of zoonotic intestinal parasites in domiciled dogs living in the urban area of Alfenas, State of Minas Gerais, Brazil

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ABSTRACT. Companion animals can carry pathogens that cause many infectious diseases in humans. In this sense, the present study aims to analyse the prevalence of zoonotic intestinal parasites in domiciled dogs living in the urban area of Alfenas, State of Minas Gerais, Brazil, from February 2017 to July 2018. To collect data, four regions of the city were considered as strata and their respective neighbourhoods as conglomerates, and one neighbourhood per stratum was selected. Stool samples were collected from 336 domiciled dogs and processed using the spontaneous sedimentation technique. The dog owners filled a questionnaire with information on the animals' living conditions. The parasites identified were *Ancylostoma* sp. and *Toxocara canis*, with higher prevalence of the former in male dogs, and of the latter in female dogs. Dogs of defined breed, small size, and age >1 year old exhibited the highest infection rates. To teach concepts of parasite transmission and prevention, the researchers developed educational actions with 6- to 10-year-old children who studied at schools from the selected neighbourhoods, as well as distributed informative folders to the dog owners. Laboratory tests confirmed the presence of potentially zoonotic intestinal parasites in 2.98% of the domiciled dogs living in Alfenas. Insufficient administration of deworming drugs (p=0.018) was the risk factor that significantly correlated with the occurrence of parasitic infection in the studied dog population. Educational actions favoured adoption of personal hygiene habits and good animal care practices to promote dog health and welfare and human health.

Keywords: zoonoses, dog diseases, ancylostomosis, toxocarosis

Introduction

The relationship between humans and animals is ever closer due to the mutual physical, emotional, and social benefits that it brings. However, such relationship poses risk to human health because domiciled animals can carry intestinal parasites with zoonotic potential. That is why the sanitary control of domiciled animals is essential to preserve their health and their owners' health [1-3].

Ancylostoma, Dipylidium, Giardia, Toxocara, and Trichuris are the most common zoonotic intestinal parasites in dogs, which may become infected through ingestion of eggs or cysts, skin penetration of larvae, and transplacental or transmammary pathways [4–7]. The clinical relevance of dog parasitosis lies not only in the organic disturbances that they cause to the animals, but also in the fact that they can be easily transmitted to humans due to their close living together. In this sense, analysis of the prevalence of intestinal parasites in dogs is important to delineate and adopt strategies for prevention of infection and promotion of sanitary education [8].

The present study aims to examine the prevalence of zoonotic intestinal parasites in domiciled dogs living in the urban area of Alfenas, State of Minas Gerais, Brazil, and to analyse the risk factors associated with dog infection by the intestinal parasites identified.

Materials and Methods

Study model

This is an observational epidemiological study of the transversal, exploratory, and descriptive type to examine the presence of zoonotic intestinal parasites in stool samples from domiciled dogs living in four neighbourhoods that represent the North, South, East, West, and Central regions of the city of Alfenas, State of Minas Gerais, Brazil. The samples were collected from February 2017 to July 2018.

Study area

Alfenas is located at the South region of the State of Minas Gerais – more specifically in the South and Southwest mesoregion of the state, in the Southeast Region of Brazil. The estimated Alfenas population in 2015 was 78,712 inhabitants, distributed within 51 neighbourhoods and 23,209 domiciles [9].

Collection, processing, and analysis of stool samples

This study analysed stool samples of male and female domiciled dogs of different breeds, including undefined breed, at all ages, whose owners signed the informed consent form. Each owner received stool sample bottles and a folder with instructions for collecting the dog stool samples. The next day, the bottles filled with stool samples were identified with the dogs' data furnished by their respective owners, cooled to 4–8°C, and sent within 24 h to the Veterinary Parasitology Section from Laboratório Hermes Pardini in Belo Horizonte, State of Minas Gerais, Brazil. The stool samples were processed using the Spontaneous Sedimentation technique [10–11].

The Ethics Committee for Human Research (protocol n° 2.144.344) and The Ethics Committee

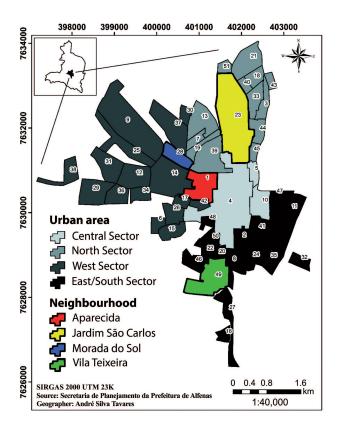


Figure 1. City map of Alfenas, State of Minas Gerais, Brazil representing the conglomerates of neighbourhoods studied from 2017 to 2018

for Laboratory Animal Use (protocol n° 18 A/2017) approved the study protocol.

Analysis of the risk parameters associated with parasite transmission

Data from dogs and their respective owners and domiciles were collected using a semistructured questionnaire with three sections: (1) personal data and socioeconomic and cultural profile of the owners; (2) physical characteristics of the domiciles that could be associated with parasite transmission; (3) the animal profile, the implemented care measures, and clinical signs of intestinal parasitosis. The dogs were classified according to their age as younger or older than 1 year [12], and to their size as small (up to 10 kg), medium (10 to 20 kg), or large (more than 20 kg).

Sampling design and statistical data analysis

The present study used a three-stage sampling design. The city of Alfenas-MG was divided into four regions (Fig. 1), where each region was composed of neighbourhoods. The conglomerate sampling technique was used in the first stage by raffling one neighbourhood (conglomerate) per region. The second stage used a stratified sampling technique because the neighbourhoods selected in the first stage differed with respect to the number of domiciles; hence, the number of samples was pondered by the number of domiciles. The third and last stage used the systematic sampling technique based on the domiciles at the streets from each neighbourhood.

Four neighbourhoods were raffled (one per region) using the software R [14], followed by a systematic sampling that was proportional to the number of domiciles from each raffled neighbourhood. The selected neighbourhoods were Aparecida (Central region), Vila Teixeira (South and East regions), Jardim São Carlos (North region), and Morada do Sol (West region), which had 1,383, 960, 1,864, and 779 domiciles, respectively, according to the Demographic Census data from 2010. A total of 94, 63, 130, and 49 domiciles were visited in such neighbourhoods, respectively, and the total sample size was 336 animals.

The sample size of dogs domiciled in Alfenas was calculated from the prevalence of protozoans and helminths in dog stool samples reported in the literature [13]. The number of dogs was estimated from the anti-rabies vaccination goal reported in the Information System of the National Immunization Program website in 2016.

The responses of the questionnaires and stool examination results were analysed using descriptive statistics and the Fisher's exact test among qualitative variables, at the significance level of 5%.

Results

The present study reports data from parasitological stool examination from dogs domiciled in Alfenas-MG, and data from interviews held with the respective dog owners. Analysis of the sociodemographic profile of the owners revealed that most of them were women (65.48%), at ages from 21 to 60 years (73.21%), who lived in homes with 1-3 inhabitants per domicile (56.25%), had monthly income of up to three minimum salaries (60.12%), and had education level at high school or college (57.74%). The mean age of dog owners was 44.7 years, and the mean number of inhabitants per domicile was 3.3. About one-third of the domiciles had children <10 years old (24.40%) and adults >60 years old (33.04%). The mean number of children <10 years old and adults >60 years old was 0.32 and 0.45, respectively.

All the domiciles had public drinking water supply, sewage network, and municipal waste collection, and most of them had cemented yard (62.80%) and belonged to the dog owners (61.90%). About half of the domiciles (51.49%) were inhabited by two or more dogs, which lived together with other animal species such as cat, bird, turtle, and fish in 25.60% of the domiciles.

Data regarding the profile of infected and noninfected dogs, as well as the sanitary care and clinical symptoms of possible intestinal parasitosis are summarized in Table 1. The dog population had equivalent gender distribution, with predominance of those with defined breed (54.17%), small size (61.61%), and age >1 year old (83.04%). Regarding the animal care, most of them took deworming drugs in the last 12 months (75%), received antirabies vaccine (90.31%), and were exclusively fed with commercial chow (85.12%). The rates of animals that lived in the yard during the day (65.18%) and at night (66.67%) were similar, and they spent the remaining time in the interior of the home. Half of the animals (50.89%) had no access to the street or other public places.

The clinical symptoms suggestive of intestinal parasitosis reported by the dog owners were diarrhoea (8.63%), weight loss (10.71%), and anal itch (15.18%). About one-fifth of the animals (21.73%) were infested with fleas, probably due to infection with *Dipylidium caninum*. Seven out of the 279 dogs >1 year old (2.50%) and three out of the 32 dogs <1 year old (9.40%) – i.e. 10 stool samples (2.98%; IC_{95%}: 1.44–5.40) – were infected with zoonotic intestinal parasites. *Ancylostoma* sp. (0.89%; IC_{95%}: 0.18–2.59) and *Toxocara canis* (2.08%; IC_{95%}: 0.84–4.24) were the parasite species identified.

Analysis of the distribution of infected dogs per neighbourhood in Alfenas (Fig. 2) revealed that four (57.14%) and three (42.86%) out of the seven (70.00%) dogs infected with *T. canis* were domiciled in Vila Teixeira and Morada do Sol, respectively; most of them were female >1 year old, with defined breed and small size. Two (66.66%) and one (33.34%) out of the three (30.00%) dogs infected with *Ancylostoma* sp. were domiciled in Vila Teixeira and Aparecida, respectively; most of them were male >1 year old, with no defined breed and small size. The dog age (p=0.072), gender (p=1.000), breed (p=0.760), and size (p=0.782) did not significantly correlate with parasite infection. Five out of the 10 infected dogs (50%) lived

Variables	Characteristics	Infected	Non-infected (%)		\mathbf{p}^{1}
Gender	Male	5	165	(49.11)	1.000
	Female	5	171	(50.89)	
Breed	Defined breed	6	182	(54.17)	0.760
	No defined breed	4	152	(45.24)	
	Not informed	-	2	(0.60)	
Age	<1 year old	3	32	(9.52)	0.072
	>1 year old	7	279	(83.04)	
	Not informed	-	25	(7.44)	
Size	Small	7	207	(61.61)	0.782
	Medium	2	44	(13.10)	
	Large	1	52	(15.48)	
	Not informed	-	33	(9.82)	
Vaccines	Yes	7	304	(90.48)	0.036*
	Not	3	26	(7.74)	
	Not informed	_	6	(1.79)	
Deworming drugs	Yes	4	252	(75.00)	0.018*
	Not	6	84	(25.00)	
Commercial chow	Yes	9	286	(85.12)	0.268
	Not	1	9	(2.68)	
	Chow + food	_	41	(12.20)	
Stay during the day	Inside home	5	117	(34.82)	0.326
	Yard	5	219	(65.18)	
Stay at night	Inside home	5	112	(33.33)	0.311
	Yard	5	224	(66.67)	
Access to street / public places	Yes	5	162	(48.21)	1.000
	Not	5	171	(50.89)	
	Not informed	_	3	(0.89)	
Fleas	Yes	2	73	(21.73)	1.000
	Not	8	262	(77.98)	
	Not informed	_	1	(0.30)	
Diarrhea	Yes	0	29	(8.63)	1.000
	Not	10	304	(90.48)	
	Not informed	_	3	(0.89)	
Anal itch	Yes	1	51	(15.18)	1.000
	Not	9	282	(83.93)	
	Not informed	_	3	(0.89)	
Weight loss	Yes	3	36	(10.71)	0.082
	Not	7	296	(88.10)	
	Not informed	-	4	(1.19)	

Table 1. Association of dog profile, implemented care measures, and clinical signs of intestinal parasitosis, with the number of domiciled dogs infected with intestinal parasites in the urban area of Alfenas, State of Minas Gerais, Brazil, from 2017 to 2018 (n = 336)

¹ Fisher's exact test performed with the number of infected dogs according to the variables

* Significant correlation with the occurrence of parasitic infection

together with children and older adults, as reported by their owners.

Analysis of the care provided to the 10 infected dogs (Table 1) revealed that nine dogs (90%) were exclusively fed with commercial chow, indicating that they received a balanced diet; seven dogs (70%)received anti-rabies vaccine; and four dogs (40%) regularly took deworming drugs. The insufficient treatment with deworming drugs in six dogs (60%) could be a determining factor for parasite infection in these animals. This hypothesis is supported by the statistical data analysis reported in Table 1, where analysis of the risk factors for intestinal parasite infection indicated the prevalence of parasites in dogs that did not receive deworming drugs (p=0.018). In addition, eight out of the 10 infected animals (80%) lived together with other dogs, and five of them (50%) had access to the street or other public places; however, these parameters did not correlate with intestinal parasite infection (p=1.000).

Reports from teachers and school workers evidenced that the educative actions developed by the researchers at schools aroused the interest of children, who interacted with the theatre characters and started following the instructions proposed, such as washing hands before all the meals and after playing and using the restroom.

At the moment the dog owners received the laboratory test result and the educative folder, they demonstrated interest and curiosity to learn about the biology and mechanisms of transmission of zoonotic intestinal parasites, as well as to know what preventive actions they could take and what habits they should change to provide better care to their dogs.

Discussion

The fact that most of the dog caregivers were women (65.48%) with mean age of 44.7 years old indicated that they had maturity and responsibility to provide welfare and healthcare to the animals. The findings of the present study corroborate literature reports that women are the main caregivers of domiciled dogs, at a similar rate (64.6%) [15] or at rates as high as 84.5% [16] and 92.5% [17]. The prevalence of women at rates and age ranges similar to those reported herein is in line with those found in Botucatu-SP - 61.86% of female owners and 71.7% of the owners at 21-60 years old [18] - and in Canoinhas-SC - 73% of female owners at mean age of 45 years [19]. These findings demonstrate that women were more present than men at home during the day, when the researchers visited the domiciles [15].

The mean number of inhabitants per domicile (3.3) found in this study was slightly greater than the estimated mean in Brazil and in the Southeast Region of Brazil (3.1 and 2.9, respectively) [20]. The prevalence of dog owners with monthly income ≤ 3 minimum salaries (60.12%) and education level

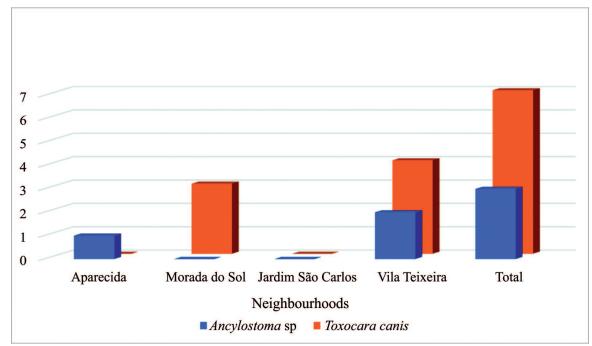


Figure 2. Distribution of domiciled dogs infected with intestinal parasites across the neighbourhoods in the urban area of Alfenas, state of Minas Gerais, Brazil, from 2017 to 2018

at high school/college (57.74%) differed from the socioeconomic profile reported in Franca-SP [21] and Patos de Minas-MG [15]: in the former, 32% of the owners had higher education and 39% had monthly family income of 5–15 minimum salaries; in the latter, 52.33% of the owners had studied at high school and 43.66% had monthly family income of 1–3 minimum salaries.

The socioeconomic profile and education level of the owners can be risk factors for intestinal parasitosis in dogs; such diseases are more prevalent in dogs belonging to people from less favoured social classes, indicating that people with higher education level have sufficient knowledge on the good care practices to preserve health of their pets [2,22]. The increased number of positive results in parasitological stool examination from dogs belonging to people who had only elementary school degree and lived in domiciles located on the outskirts of the city of Lages-SC strengthens the hypothesis that socioeconomic and cultural factors correlate with the parasite infection rate in dogs [23].

Analysis of the family configuration evidenced the presence of children <10 years old and adults >60 years old in 24.40% and 33.04% of the domiciles, respectively; these percentages were respectively smaller and greater than those reported 32.70% and 23.60% in Recife-PE: [22]. Cohabitation of dogs with children and older adults is relevant from the zoonotic perspective because people at these ages are more susceptible to intestinal parasite infection. Thus, it is necessary to aware the dog owners to provide appropriate veterinary care to their pets, give deworming drugs regularly, and immediately collect their stool [24-28]. Parasite infection in dogs correlated with parasite infection in children in Lages-SC [29], but not with parasite infection in older adults in Porto Alegre-RS [24].

The appropriate sanitary conditions of the domiciles visited in this study and the reasonable income and education level of the dog owners corroborate the World Health Organization reports that these parameters are indicators of health promotion [30,31]. The lack of land and sand in 63% of the yards of the domiciles visited was relevant to inhibit parasite egg embryonic development, evolution of helminth larvae, and survival of protozoan cysts that were eventually present in dog stool, in agreement with literature reports [32,33]. It is worth to note that 65% of the

dog owners considered the yard where the pets lived as extension of their homes and maintained it in ideal hygiene conditions.

Most of the dogs studied herein were small size adults (Table 1), corroborating the percentage (51.58%) reported in a distant geographic location – Gurupi-TO [34]. The small size and breed are the prevalent criteria to select a pet for companionship [35]. Nowadays, few pets are considered as security guards and fed with human food leftovers; in fact, most of them are considered as family members and their expenses compose the family budget and consume a significant part of the monthly income [36].

The clinical signs of intestinal parasite infection in the dogs studied are in line with those reported by a study performed with domiciled dogs assisted in an animal healthcare centre in Rio de Janeiro-RJ [37]: there was no statistical correlation between the clinical signs and the presence of intestinal parasites in dogs, which emphasizes the importance of making regular parasitological stool examination, independently of the clinical manifestations. It is also vital to aware the population about the risks to which they are exposed when cohabiting with animals that are asymptomatic but eliminate infectious forms of parasites in their faeces [21].

Even though the studied dogs lived in domiciles with appropriate feeding and sanitary conditions, 2.98% of them were infected with zoonotic intestinal parasites, as detected in the parasitological stool examination. Our findings diverged from the presence of zoonotic intestinal parasites in 9.7% of the stool samples from domiciled dogs living in the Central and Northeastern Italy [27] and in 39.99%, 41.17%, and 60.10% of the stool samples from dogs living in the Brazilian municipalities of São Paulo-SP [1], Rio de Janeiro-RJ [37], and Dois Vizinhos-PR [38], respectively. The infection rates may vary according to geographic location, climate, socioeconomic conditions, and sampling criteria used [39].

In addition to causing organic damage to the animals, intestinal parasites can be directly transmitted to other animals and zoonotically transmitted to people who are healthy, immuno-compromised, or have immature immune system [40]. The prevalence of *T. canis* found in this study is similar to that reported in the literature (2.34–4.30%) for domiciled dogs [27,37,38,41–43]. On the other hand, the prevalence of *Ancylostoma* sp identified herein was lower than that reported by

other authors in Brazil, which ranged from 2.22% to 18.50% [1,8,41,42], and higher than the prevalence of 0.6% reported in Italy [27].

The dogs infected with *T. canis* and *Ancylostoma* sp. lived in geographically distant neighbourhoods (Fig. 2). Vila Teixeira is characterized by the presence of college students who usually adopt abandoned animals, which may explain, at least in part, the prevalence of parasites in this location. We did not identify a particular feature of Jardim Săo Carlos that distinguished it from the other neighbourhoods and could explain the absence of infected dogs in this location.

The relevance of the intestinal parasites identified relies on the fact that they interfere with dogs' health, are a source of infection to other pets that live in the same domicile, and can transmit zoonotic parasites to humans, such as visceral and cutaneous larva migrans [21]. Positive cases of larva migrans syndrome have been reported in different regions of Brazil [44,45], and serological data have confirmed the high level of human exposure to T. canis [46-51]. The seroprevalence of toxocarosis in Alfenas-MG was significant (53.8%) [7], but it can vary due to the age range, personal hygiene habits, and social, economic and sanitary conditions of the studied population [49]. The relative prevalence of intestinal parasites in dogs <1 year old (9.40%) and dogs >1 year old (2.50%) was similar to those reported in the Central and Northeastern Italy [27] but differed from other literature reports [52-54]. The presence of Ancylostoma sp. and T. canis in three- to six-month-old dogs may be explained by the possibility of transplacental and transmammary transmission of these parasite species [6].

The present study did not find significant association of dog gender, breed, and size with the presence of intestinal parasites (p>0.05). This finding corroborates the reports that both male and female dogs are equally susceptible to infection with intestinal parasites, and the infection rate is related to environmental and sanitary factors [55,56]. The prevalence of intestinal parasite infection in small dogs is in line with data reported by the Zoonosis Control Centre of Gurupi-TO [34], but contrasts with the higher prevalence of intestinal parasite infection in large dogs in São Paulo-SP [40]. The increased incidence of parasitic diseases in dogs with defined breed was also reported in the animals assisted in the Veterinary Hospital from the State University of Londrina-PR [57]. In Viçosa-MG and Rio Branco-AC, parasitic diseases were more prevalent in animals with no defined breed [39,58].

The care practices provided by the owners, including feeding with commercial chow and administration of deworming drugs and vaccines, were clearly appropriated to preserve dogs' health, in agreement with data from a study with domiciled dogs conducted in Franca-SP [59]. The presence of intestinal parasites in domiciled dogs correlates with the sanitary conditions of their homes and the care provided to them, stressing that the owners should understand the importance of sanitary care and put it into practice [2].

Considering that the increased rate of intestinal parasite infection correlated with the insufficient administration of deworming drugs this parameter was a protective factor that lowered the number of infected dogs. A previous study with stool samples collected from 100 stray dogs living in Campus I from Federal University of Alfenas (UNIFAL-MG) and its neighbourhoods, in Alfenas-MG, detected the presence of parasites in 36% of the samples (unpublished data), demonstrating the significant risk of infection with potentially zoonotic intestinal parasites. In line with these findings, the Laboratory of Clinical Analyses at UNIFAL-MG detected a high frequency (53.8%) of positivity for anti-T. canis IgG antibodies in serum samples from patients who lived in Alfenas-MG [60].

It is worth noting that the low prevalence of intestinal parasite infection in the studied dog population can be associated with the environmental conditions, the sanitary care provided to the animals, and the reasonable socioeconomic and cultural conditions of their owners; together, these parameters positively impacted on the animal's quality of life. The relevance of providing sanitary care and making regular parasitological stool examination in domiciled dogs and serological tests to detect anti-T. canis antibodies [60], associated with the high incidence of parasitic diseases in stray dogs (unpublished data), emphasize the need for implementing and maintaining sanitary programs to control parasitic diseases and developing educative actions to aware the population about the risks of transmission of zoonoses to humans [19,49,61,62]. In this sense, playful teaching strategies have favoured adoption of personal hygiene habits and actions that mitigate transmission of both zoonotic and non-zoonotic intestinal parasites [61].

In summary, the present study demonstrated the presence of potentially zoonotic intestinal parasites

in domiciled dogs living in the urban area of Alfenas - MG. The insufficient administration of deworming drugs was the determining factor for these infections. The non-adherence to the annual antirabies vaccination campaigns, which are free of charge, indicated the pet owner's negligence in taking care and promoting health and welfare of the dog. The lack of administration of deworming drugs to the dogs was a possible act of negligence that favoured parasite infection. To make the owners aware of the proper treatment to their pets, the researchers taught educational strategies that led them to adopt good care practices to promote the animal health and welfare, which consequently improved the human health.

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