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ORIGINAL ARTICLE

Frequency and risk factors of intestinal parasites in pet dogs from Mexicali, Mexico

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ABSTRACT. Parasitic intestinal infections in dogs represent a problem for human health, because a wide variety of these parasites have zoonotic potential. Therefore, proximity to pets puts us at risk. The objective of this study was to determine the frequency and risk factors (age, sex, size, breed, presence of ectoparasites and gastrointestinal disorders) of intestinal parasites in the feces of dogs attending a Veterinary Hospital in the City of Mexicali, Baja California, Mexico. A total of 148 fecal samples were collected from canine patients and analyzed for parasite identification and parasite load. A 12.2% (18/148) of the samples were positive to parasitic intestinal infections. The frequency of specific infections was an 8.1% of *Cryptosporidium* sp., followed by a 2.7% of *Cystoisospora* sp., and 1.4% of *Toxascaris leonina.* A statistical significance was identified between the presence of intestinal parasites and mongrel breed. The predominance of protozoa shows the importance of diagnosis prior to treatment with anthelminthic drugs, since preventive antiparasitic protocols are commonly used, although these particular parasites are out of the spectrum of those drugs. *Cryptosporidium* spp. have zoonotic potential, particularly in immunocompromised patients, and there are few or no treatment options.

Keywords: zoonosis, *Cryptosporidium*, intestinal parasites, dogs.

INTRODUCTION

Animal ownership, particularly in dogs, has brought many physical and psychological health benefits to humans (Wells *et al*., 2022), such as increased physical activity, and social and emotional support (Martín, 2020). However, these companion animals can also be carriers of zoonotic diseases (Medina-Pinto *et al*., 2018). Among the wide variety of diseases, intestinal parasitic infections stand out, in which dogs act as reservoirs by dispersing them through feces containing eggs, cysts, and oocysts (Morales *et al*., 2016).

Previous studies in northwestern Mexico have reported the presence of dog parasite forms in public spaces (Ramírez-Rubio *et al*., 2019) and in stray dogs from urban and agricultural areas (Trasviña-Muñoz *et al.,* 2017, 2020), highlighting a large proportion of dogs infected with zoonotic parasites such as *Toxocara canis, Toxascaris leonina, Ancylostoma caninum, Dipylidium caninum,* and *Taenia* sp. Nevertheless, the greatest risk of exposure to humans is represented by domestic dogs owing to regular contact between owners and pets (El-Tras *et al*., 2011). In addition, due to COVID-19, a reduced number of veterinary visits has been reported, which could lead to an increase in the number of parasites in owned dogs (Yee *et al*., 2021). Moreover, it is important to identify the diversity and abundance of parasites carried by pet dogs to generate information that will enable clinicians and health personnel to establish appropriate preventive and control measures (Aguillon-Gutierrez *et al*., 2021). Therefore, the objective of the present study was to determine the frequency and risk factors for intestinal parasites in stool samples of dogs from a Veterinary Hospital in Mexicali, Baja California, Mexico.

MATERIALS AND METHODS

STUDY DESIGN

The study was conducted on dogs received at the Small Species Veterinary Hospital (IICV-UABC) between August 2021 and December 2021 in the district of Mexicali, Baja California (32° 39’ 48’’ north latitude). For this study, samples were collected from 148 owned dogs with the following inclusion criteria: older than 1-month, different sexes, breed, and body size. Sampling was performed when all owners stated their consent to participate. As a requirement to participate, the owners had to provide a fresh stool sample from the dog in a closed container (minimum of 3 g). The personal data, such as the address and phone number provided by the owner, were handled in accordance with Federal Law for the Protection of Personal Data in Possession of Individuals (DOF, 2010). The stool samples were refrigerated at 4ºC, placed in a cooler, and transported to the Parasitology Laboratory at the Institute of Research in Veterinary Science (UABC) in Mexicali, Baja California, Mexico for processing. Originally, the study design had considered a total of 280 dogs, a sample size calculated (Thrusfield, 2005) based on the prevalence reported in previous studies in stray

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dogs (Trasviña-Muñoz *et al*., 2017), an error of 5%, a 95% confidence, and an expected proportion of 21.5%. However, due to COVID-19 restrictions, there was a low demand for hospital services; consequently, our sample size was reduced to 148.

EPIDEMIOLOGICAL QUESTIONNAIRE

A questionnaire was administered to all owners who participated in the study by hospital veterinarians and interns during sample reception. Age (>12 months or <12 months) and sex (male or female) were recorded, and breed was determined based on the American Kennel Club classification. If the dog did not present characteristics that determined a single breed, it was classified as "mongrel". Size was recorded as small, medium, and large, determined by the weight of the animal (<5 kg, 5-15 kg, and >15 kg, respectively). Individual data were omitted by the owner, and other risk factors were also recorded, such as the presence of ectoparasites and signs associated with gastrointestinal disorders (vomiting, diarrhea, and constipation).

COPROLOGIC ANALYSIS

Parasitic forms of intestinal parasites present in the feces were identified using the Hendrix & Robinson method (2012). The samples were examined macroscopically for helminths or proglottids. Subsequently, the flotation concentration technique described by Zajac & Conboy (2012) was performed on 2 g of stool with a slight modification because a saturated saline (NaCl) solution (specific gravity 1.20) was substituted for zinc sulfate. The quantitative analysis was carried out with Mc Master's technique described by Serrano *et al*. (2010) by mixing 2 g of stool with saturated NaCl solution to a total volume of 60 ml, filtered, and then filled both compartments of the McMaster´s counting chamber to obtain the estimation of the parasite load in 1 g of stool.

To detect *Cryptosporidium* sp. oocysts, the modified Ziehl-Neelsen staining method described by Serrano *et al*. (2010) was applied.

STATISTICAL ANALYSIS

Descriptive statistics were used to calculate the overall and specific frequencies of intestinal parasitic infections. Inferential analyses were performed using the Statistix 9® software.The Chi-square (χ^2) test was used to establish associations between parasitic infections and risk factors. Risk factors with a p-value < 0.10 was further analyzed using a logistic-binomial regression model, which provides exact regression estimates, 95% confidence intervals, odds ratios, and p-values.

RESULTS

None of the 148 samples analyzed showed evidenced presence of adult parasites, and 18 (18/148) were positive for a single parasite. No co-infections were recorded. The genera and species found, in order of frequency, were as follows: *Cryptosporidium* sp., *Cystoisospora* sp., and *Toxascaris leonina* (Table 1). There was a notable predominance of protozoa, 88.8% (16/18) in the total positive cases compared to nematodes 11.1% (2/18).

Dogs younger than 12 months presented the highest frequency of positive cases of intestinal parasitic infections (14.2%, 3/21) compared to dogs older than 12 months (12.8%, 15/117). Males presented a higher frequency of cases of infection (14.4%, 11/76) than females. The most infected breed in this study was the Mongrel (21.5%, 11/51), compared to 7.9% (7/88) of purebred animals. The most frequently infected purebreds were Husky (25%, 1/4), American Staffordshire Terrier (9.5%, 2/21), and Poodle (15.3%, 2/13). Small dogs were more frequently infected (13.8%, 5/36), followed by medium (13.7%, 8/58), and large (10.6%, 5/47) size dogs. Dogs did not present ectoparasites associated with the biological cycle of gastrointestinal parasites and only ticks were found. It should be noted that most animals with intestinal parasitic infections did not show gastrointestinal disorders (88.8%, 16/18) (Table 2). The only statistically significant risk factor was breed (p = 0.026) (Table 3); and an association was identified between the overall frequency of cases and mongrel dogs (OR= 3.1821; 95% CI = 1.15-8.83). Regarding

Table 1. Frequency, genera, and parasite loads calculated using the McMaster technique for parasites detected in the feces of dogs at the Small Species Veterinary Hospital of the UABC in Mexicali, Baja California, Mexico. *Missing value: Because of the small size of Cryptosporidium sp. oocysts, the parasitic load was not determined. Low: 50-100 EPG, OPG; Middle: 101-500 EPG, OPG; high: 500 EPG, OPG (Rodriguez-Vivas *et al.*, 2011).

Genera/species	Frequency (%)	Positives/total samples	Parasitic load per gram of feces
Cystoisospora sp.	2.7	4/148	(50 -150)
Cryptosporidium sp.*	8.1	12/148	-
Toxascaris leonina	1.3	2/148	(100 -200)

Table 2. Characteristics of the population of owned dogs sampled at the UABC Small Species Veterinary Hospital in Mexicali, Baja California, Mexico.

Risk factors			Number of individuals	Positive	Negative
Age	<12 months		21	3	18
	>12 months		117	15	112
Sex	Female		65	7	58
	Male		76	11	65
Breed	Breeds withs at least 1 positive individual	Schnauzer	12	1	11
		American Staffordshire Terrier	21	2	19
		Poodle	13	2	11
		Chihuahua	15	1	14
		Husky	4	1	3
	Other breeds		22	0	22
	Mongrel		51	11	40
Size	Small		36	5	31
	Medium		58	8	50
	Big		47	5	42
Ectoparasites	Present		7	2	5
	Absent		133	16	117
Gastrointestinal disorders	Present		13	2	11
	Absent		126	16	110

Table 3. Association between the risk factors and intestinal parasites in feces of dogs owned by the Small Species Veterinary Hospital of the UABC in Mexicali, Baja California, Mexico. *p-value <0.05.

Risk factors		Positive/total samples	OR	95% IC	p-value
Age	<12 months	14.2% (3/21)	1.13	0.30 – 4.31	0.85
	>12 months	12.8% (15/117)			
Sex	Female	10.7% (7/65)	0.71	0.26 – 1.95	0.51
	Male	14.4% (11/76)			
Breed	Pure	7.9% (7/88)	3.18	1.15 – 8.83	0.02*
	Mongrel	21.5% (11/51)			
Size	Small	13.8% (5/36)	1.17	0.61 – 2.22	0.64
	Medium	13.7% (8/58)			
	Big	10.6% (5/47)			
Ectoparasites	Present	28.5% (2/7)	2.92	0.52 – 16.32	0.22
	Absent	12% (16/133)			
Gastrointestinal disorders	Present	15.3% (2/13)	1.26	0.26 – 6.22	0.77
	Absent	12.6% (16/126)			

the association between cases of parasitic infections for each detected parasite, no association was found between cases of *Cryptosporidium* sp., *Cystoisospora* sp., and *T. leonina* and the risk factors.

DISCUSSION

Companion animals, owing to their proximity to humans, are a potential source of more than 70 zoonotic diseases (Stull *et al*., 2015). Intestinal parasites are a clear example of this, as they are considered a global health problem (Zanzani *et al*., 2014). These infections sometimes present with a long prepatent period, and their clinical manifestations may not be evident (Torrecillas *et al*., 2021). The absence of clinical signs in the patients whose samples were analyzed in this study and had a positive result suggests that although the disease is not perceptible, subclinical parasitized dogs play an important role in allowing the parasites to develop their life cycle (Maggi & Krämer, 2019). This finding has been demonstrated in cryptosporidiosis; despite not presenting any signs, dogs can continue to excrete oocysts, which can contaminate the environment and facilitate subsequent transmission (OIE, 2018; Murnik *et al*., 2022).

The higher frequency of positive cases in the mongrel breed agrees with that reported by Plúas & Sánchez (2021) in Ecuador. This may be due to the lower level of veterinary care that mongrel dogs receive, which is related to their low economic value compared to that of purebred dogs (Ojo *et al*., 2019). The data from the present study disagree with those reported by Tortolero *et al.* (2008) in Venezuela and Vega *et al*. (2014) in Peru, where there was a statistically significant relationship between intestinal parasites and purebred dogs. The authors attributed this to genetic manipulation of these animals, which can increase their susceptibility to infection.

Although there is no zoonotic risk, *Cystoisospora* sp. are important for pet health. In young animals, strong signs of acute diarrhea, either catarrhal or hemorrhagic, are observed (Bowman, 2014; Villeneuve *et al*., 2015). In the present study, the animals infected with *Cystoisospora* sp. were older than 12 months, and the owners did not report the presence of previous gastrointestinal signs. However, no statistically significant relationship was observed between the presentation of this parasite and age. These results differ from those reported by Smith *et al*. (2014) in Canada, who reported that adult animals were considerably less infected than young animals.

There are over 30 different species of *Cryptosporidium* sp. recognized; some are host-specific, and others infect a wider host range (Robertson *et al*., 2020). The most prevalent species reported in humans include *C. hominis, C. parvum,* and *C. canis* (Ryan *et al*., 2021). In the present study, a frequency of 8.10% was observed for *Cryptosporidium* spp. Thus, this study is the first to report cases of infection in dogs in Mexicali, Baja California, Mexico. Regarding cryptosporidiosis in dogs, there are discrepancies regarding its importance as a zoonosis, as some studies indicate that the risk is minimal (Lucio-Forster *et al.*, 2010). However, Mexicali borders the state of California in the United States, where historically, it has been indicated as the main risk of infection through contact with dogs at home (Acute Communicable Disease Control, 2014). In Mexico, there have been few studies on this protozoan in domestic dogs. In Mexico City, Martinez-Barbosa *et al*. (2015) reported a 11.5% prevalence of *Cryptosporidium* spp. in asymptomatic domestic dogs, which coincides with our findings, and the age of the dogs (younger than 12 months) was determined as a risk factor, which differs from our study. In Aguascalientes, Vitela-Mendoza *et al*. (2019) determined the frequency of *Cryptosporidium* sp. in dogs from dairy stables and an urban area captured by the Center for Control, Attention, and Animal Welfare (CCABA) and found that 20.5% of the canines were infected. The highest number of cases were observed in animals from dairy farms (30%), whereas the urban area showed a low level (9%). In Durango, Aguillón-Gutiérrez *et al*. (2021) reported a prevalence of 6% in pet dogs, which is the most frequent parasite in these canines. In Toluca, Lara-Reyes *et al*. (2019) reported that 4.7% of dogs submitted to different clinics in the area to be evaluated were positive for this parasite.

In humans, cryptosporidiosis can present with minimal to severe symptoms, such as watery and bulky diarrhea, abdominal pain, nausea, vomiting, anorexia, fatigue, and fever. These can last up to one month, with a risk of reinfection; in severe cases, it can compromise the human patient's life (Chalmers & Davies, 2010). Unfortunately, no drugs or vaccines are available to prevent this infection, and the treatment options for both animals and humans are extremely limited and may not be fully effective in immunocompromised patients (Innes *et al*., 2020). According to the CDC (2021), no single cleaning measure is effective in preventing cryptosporidiosis and has even shown resistance to chlorine in water, but it also strongly suggests washing hands often with soap and water.

Toxascaris leonina has zoonotic potential, can occasionally infect humans as accidental hosts, and is commonly present in asymptomatic dogs (Rostami *et al.*, 2020). Canines usually cause mild gastrointestinal disturbances, and in most cases in humans, they do not cause any signs or symptoms (Traversa, 2012). The frequency of *T. leonina* (1.35%) in our study differed from the results reported globally (2.9%) (Rostami *et al*., 2020) as well as from other states of Mexico, such as Querétaro (11.91%), Mexico City (4.16%), Oaxaca (7.22%) (Fernández & Canto, 2002; Eguía-Aguilar *et al*., 2005; Vélez-Hernández *et al*., 2014), and Mexicali, Baja California (5.5%) (Trasviña-Muñoz *et al*., 2017). The low frequency found in this study could be explained by the fact that the dogs in this study were domestic, whereas the previously mentioned studies investigated stray dogs, which are generally exposed to various risk factors that facilitate their transmission, such as the presence of rodents, which are paratenic hosts for this parasite, and the absence of health care for dogs (Trasviña-Muñoz *et al*., 2017). In addition, climate factors, as this nematode has a lower development in extreme climates (Rostami *et al*., 2020), such as those we have in this region during summer and winter.

It is important to note that there was a higher proportion of protozoa in this study, since in some countries "preventive" antiparasitic protocols are used, applying anthelmintic drugs, such as praziquantel and pyrantel (Stull *et al*., 2007; Matos *et al*., 2015) with no effects on the protozoa. The spectrum of these drugs could explain the absence of other helminths, such as *Toxocara canis* and *Ancylostoma caninum,* or cestodes such as *Dipylidium caninum,* which had been previously recorded in this region (Trasviña-Muñoz *et al*., 2017; Ramírez-Rubio *et al*., 2019). This highlights the importance of diagnosis prior to the initiation of any treatment as well as post-treatment diagnosis to evaluate its efficacy based on differences in parasite loads (D´Ambroso *et al*., 2022). If owners lack information regarding the possibility of transmission and how improper disposal of feces can affect the environment, there is an increased risk of infection; therefore, the role played by these animals as reservoirs must be considered (Raičević *et al*., 2021). In addition, diagnostic work together with the development of scientific communication strategies focused on the owners of these animals represents an important step in the prevention of intestinal parasites. (Cortes, 2020). It is also necessary to reinforce the role of veterinary clinicians as educators about animal welfare and human-animal bonds. (Dolby & Litster, 2015).

CONCLUSIONS

The predominance of protozoa in owned dogs compared to helminths is of utmost importance because preventive administration of antiparasitic drugs without previous diagnosis is a common practice in some countries, and the spectrum of administered drugs does not include protozoa such as *Cystoisospora* sp. or *Cryptosporidium* sp. Consequently, diagnosis is fundamental before and after antiparasitic treatment. Regarding risk factors, mongrel dogs presented a significantly higher frequency of infection, which could be attributed to the lower veterinary care related to their low economic value, compared to purebred dogs (Ojo *et al*., 2019).

The presence of *Cryptosporidium* sp. is of great interest, mainly because of its zoonotic potential and lack of treatment options. Further studies are needed in both owned and stray dogs with a larger sample size to identify the factors that contribute to the distribution of these parasites throughout the northwestern regions of Mexico.

STATEMENTS

Conflicts of interest: the authors declare that they have no conflicts of interest.

DECLARATION OF ETHICS

All the samples were collected using standard collection method, without harming the dogs. The research was conducted with the approval of the Ethics Committee of the Veterinary Sciences Research Institute from the Autonomous University of Baja California. The personal data provided by the owners of the pets sampled in this study were handled in accordance with the Federal Law for the Protection of Personal Data in Possession of Private Parties (DOF 05-07-2010).

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