Original Research Article

The prevalence and risk factors of *Giardia duodenalis* infection in cats in Mexico

ABSTRACT

Aims: *Giardia duodenalis* is a globally distributed zoonotic protozoan. It has a variable prevalence. This study determines the prevalence of *Giardia* spp. in cat faecal samples from states of the Mexican Republic.
Place and Duration of Study: Was carried out in 23 of the 32 states of the Mexican Republic, from June to December 2019.
Methodology: Stool samples from 1591 client-owned cats were analysed for the detection of *G. duodenalis (*cysts or trophozoites). Faecal samples were analysed by direct smear techniques with and without staining (Lugol) and centrifugal floatation (faust), and were examined under a light microscope.
Results: Of the cats sampled, 56.94% were positive for *G. duodenalis*. Its prevalence was associated and is a risk factor in cats that live with other animals (Chi2= 21.84, p= 0.0001; OR= 1.61, p= 0.0001), with hunting habits (Chi2= 5.53 p= 0.01, OR= 1.27 p= 0.01), with access to the outside (Chi2= 53.06, p= 0.0001; OR= 2.13, p=0.0001) and with the aqueous faeces (Chi2= 12.30, p=0.03; Chi2= 1.71, p= 0.03). Factors for not presenting Giardia spp. in faeces were, not brushing the cat (OR=0.74, p= 0.006), provenance (OR= 0.42, p=0.02), and median height (OR= 0.78, p= 0.01). Age, gender, hair type, coexistence with other cats and other stool findings were not associated as risk factors for infection.
Conclusion: This study demonstrated a high overall prevalence of *G. duodenalis* in cats in Mexico, in addition to an association of its prevalence with risk factors such as cats living with other animals, hunting habits and access to outdoors.

Keywords: Giardia duodenalis, cats, prevalence, risk factors, Mexico

1. INTRODUCTION

One of the most common parasites in the small animal clinic is *Giardia* spp. [1]. Most infections are usually subclinical; however, there may also be acute or chronic diarrhoea [2]. *Giardia duodenalis*, also called *G. lamblia* or *G. intestinalis*, is a flagellated protozoan parasite with two known forms: the trophozoites and cysts are globally distributed in many vertebrates, including humans and animals (domestic and wild) [1,3,4]. Its zoonotic potential varies and depends on the assembly of the parasite. Based on the genetic analysis of some genetic markers, eight genotypes (A–F) are described, genetically different but morphologically identical, of which A and B are pathogens for humans and have zoonotic potential. The remaining six (C–H) are considered more species-specific. In cats, the zoonotic set A and the specific feline set F tend to predominate [4,5-7].

Transmission occurs through the faecal–oral route, direct from infected individuals or contaminated fomites, or through ingestion of water and/or food contaminated with environmentally resistant cysts [1,8]. The diagnosis of G. duodenalis has been made by microscopic examination of stools for trophozoites or cysts, by direct examination of faecal smears or concentration techniques, direct immunofluorescence assay (IFA), immunoenzymatic methods (ELISA) or the polymerase chain reaction (PCR) has also been used [2,3,9].

There is talk of a variable prevalence between epidemiological studies from different countries, and in cat populations, depending on the age, clinical status, accommodation and geographical region of the surveyed animals and which will also be influenced by the detection method used, prevalence has generally ranged from 1% to 20%; however, some prevalence rates have been reported as high as 50% [2,3,10], these being more common in young animals and refuge populations [5]. Therefore, the objective of this study was to carry out a retrospective survey to determine the prevalence of Giardia spp. in cat faecal samples and the risk factors in the states of Mexico.

2. MATERIAL AND METHODS

2.1 Study area

A cross-sectional study was carried out in 23 of the 32 states of the Mexican Republic, from June to December 2019, 1,591 samples of cats (898 females and 693 males) with owner, regardless of breed, age, gender or state of Health, all owners who agreed to participate were provided with informed consent explaining what the study consisted of, a survey was conducted with epidemiological data and risk factors for *Giardia* spp.

2.2 Animals and sample analysis

All faecal samples were individually analysed by direct smear techniques with and without staining (Lugol) [11] and centrifugal floatation (Faust) using a saturated solution of zinc sulfate 33% (SG 1.18) [12] to detect *G. duodenalis* cysts or trophozoites. The faecal samples were examined carefully under a light microscope at 40x and 100x magnification, field by field, covering the entire slide. Samples were classified as positive when at least one cyst or trophozoite was observed. Any parasitic stage was identified using its previously described morphological characteristics [13]. The samples were analysed by 364 veterinary doctors from 208 veterinary clinics, hospitals and consulting rooms, who were trained in face-to-face workshops, video conferences, webinars or personal communication via email or WhatsApp. All data obtained were recorded on an Excel spreadsheet (Microsoft Office 2010) and verification of the samples analysed was by means of electronic photographs evaluated by the researcher.

2.3 Statistical analysis

Due to the nature of the data, non-parametric tests were used for statistical analysis of the association between prevalence and the variables gender, age, habits and physical characteristics of the felines and faecal characteristics, using the Chi-square test. Odds Ratio analysis with an α-value of 0.05 was applied to determine the risk factor of the aforementioned variables; the statistical software JMP 0.8 was used.

3. RESULTS

Of the 1591 cats included in this study, 898 were females and 693 males, 714 adults (13 months), 338 young (7–12 months) and 539 kittens (6 months) of the following breeds: Abyssinian, American Shorthair, Turkish Angora, Russian Blue, Bengali, Burmese, British Shorthair, Burmese, Norwegian Forest, Domestic Shorthair, Domestic Longhair, Exotic, Himalayan, Maine Coon, Manx, Persian, Siamese, Siberian, Scottish Fold, Persian Calico, Orange Tabby. The point prevalence of Giardia spp. was 56.94%. Age and gender were not associated with the presence of Giardia spp. cysts, nor were they a risk factor (Table 1).
The discussion should not repeat the results, but provide detailed interpretation of data. This should interpret the significance of the findings of the work. Citations should be given in support of the findings. The results and discussion part can also be described as separate, if appropriate.

Table 2 shows the results of the analysis of feline habits. Living with other cats was not associated or considered a risk factor, living with other animals is associated (Chi2 = 21.84, p = 0.0001) with prevalence of *Giardia* spp. and it is a risk factor (OR = 1.61, p = 0.0001), just as cats that had hunting habits had an association with *Giardia* spp. and it was a risk factor (Chi2 = 5.53 p = 0.01, OR = 1.27 p = 0.01), animals with access to the outside presented a strong association (Chi2 = 53.06, p = 0.0001) with the presence of *Giardia* spp. and it is considered as a risk factor (OR = 2.13, p = 0.0001) in this group of animals, on the other hand, never brushing the feline had no association, but it is a factor for not presenting *Giardia* spp. in stool (OR=0.74, p= 0.006).

Cat provenance was not associated with prevalence of *Giardia* spp. however, it was a factor for not presenting *Giardia* spp. in faeces (OR = 0.42, p = 0.02) as it can be seen in table 3, the type of hair was not associated nor was it a risk factor, the median size in the feline if it was associated with being negative (Chi2 = 6.12, p = 0.04) and was a factor for not presenting *Giardia* spp. (OR = 0.78, p = 0.01). Table 4 shows the characteristics of the stool, the light brown colour in the

stool had no association, but it was a risk factor (Chi2 = 2.74, p = 0.0001) to present cysts of *Giardia* spp., Aqueous stool were associated (Chi2 = 12.30, p = 0.03) with prevalence and was a risk factor (Chi2 = 1.71, p = 0.03) in cats.

4. DISCUSSION

Among the 1591 faecal samples analysed in this study, a point prevalence of *G. duodenalis* of 56.94% was obtained, this result being comparable to the estimate obtained in other studies in which it is reported to be one of the most widely observed parasites in the samples analysed, with prevalence rates from 50% (Portugal), 42.1% (Australia), 36.84% (Italy), 27.9% (Romania), 20.5% (Greece), 19.1% (Japan) and 16% (Canada) [14-20]. However, other studies differ, as they have shown other parasites to be the most prevalent, finding *Giardia* spp. to be less frequent, mentioning prevalence rates of 0.7% (Romania), 2% (Egypt), 3.2% (Finland), 4.2% (Brazil and Spain), 5.7% (Milan), 9.9% (Canada) and 10.7% (Iran) [21-28]. The prevalence of *Giardia* spp. in the various studies carried out worldwide varies according to the cat population, geographical location and sensitivity of the diagnostic test used, among other factors such as the analysis of only a single faecal sample, early infections and intermittent detachment of cysts that in many of the cases can lead to an underestimation of the actual prevalence, complicating the comparison between results.

In shelters or catteries there are usually high population densities of animals and unhygienic conditions. In addition, both diagnosis and treatment can be complicated, laborious and unsuccessful in these places, so *G. duodenalis* presents a great challenge [29]. Therefore, there would be expected to be a high prevalence of *G. duodenalis* in animals kept in these conditions. In this study, it was identified that the percentage of positives was higher in adopted cats (54.47%) than in those from a cattery or those purchased. Taking into account that the cats adopted in this study came from the street or from shelters, this coincides with a study carried out in Greece, where the prevalence was higher in cats in shelters (39.0%) than in domestic cats (15.6%) [20]. Cats from a street environment have probably never received deworming treatment, in addition to being able to access various sources of parasitic infection, and the conditions may play an important role in the transmission of these parasites through faecal environmental contamination (soil, food or water) [28]. However, in Thailand a higher prevalence of *G. duodenalis* was found in cats from catteries (76.9%) compared to those living at home (15.1%), with significant difference (p = 0.01) [30].

The risk factors of cats that lived with other animals, that had hunting habits or had access to the outdoors were the most significant, showing a significant differences of p = 0.0001, 0.01 and 0.0001, respectively, compared to cats that lived with others of the same species; the latter only showing a higher percentage of positives (42.24%). This susceptibility to *Giardia* spp. in cats that had access to the outdoors has also been reported in another study [31]. Tangtrongsup et al. [30] showed that households with multiple cats showed higher prevalence (43.2%) and also that the number of animals (5–10) showed a significant difference (p = 0.02), although, there are others who differ from this, since the raising of a single cat showed greater prevalence than multiple cats [17]. Considering that cats with more access to the outdoors are more likely to hunt and be in contact with intermediate hosts than are indoor cats, this could be one reason why these cats showed greater prevalence in this study. Households with cats living with more animals are likely to share environmental conditions that expose them to sources of *G. duodenalis* infection or even to infected animals that act as a source of reinfection for the other members. Living with other cats in the same residence could lead to stress, which could be a reason for high prevalence.

It is known that many cats can persist asymptomatically, but that *Giardia* spp. can generate a variety of signs, such as diarrhoea [32]. In this study, an association (Chi^2 = 12.30, p = 0.03) was found to prevail and aqueous stool was a risk factor (Chi^2 = 1.71, p = 0.03) when comparing stool consistencies (watery, soft, pasty and hard and dried); however, *Giardia* spp. Was found in greater numbers (31.05%) in firm stool. The light brown colour in the stool was associated as a risk factor (Chi^2 = 2.74, p = 0.0001) for the presence of cysts of *Giardia* spp., but when dealing with structures found in the stool, no association was found with the presence of *G. duodenalis*. Other studies have previously reported that of cats positive for *G. duodenalis*, 90% did not present diarrhoea [31] and even 100% were asymptomatic [16]. This also coincides with a survey carried out in a shelter, where a higher number (40%) cats without diarrhoea had *G. duodenalis*, while house cats with diarrhoea were more prevalent (16.4%) [20]. Other studies coincide with the latter, since a higher prevalence of *G. duodenalis* has been found in diarrheal cats than in cats with normal stools, with no significant difference [29,30,33] and with significant difference [15]. These cats that do not develop clinical signs play an important role in the transmission of *G. duodenalis*, since they act as carriers [31].

According to the gender analysis, females were more positive (32.56%) than males (24.39%); however, the sex was not a significant risk factor in this study (p = 0.50), which coincides with another study by Tangtrongsup et al. [30] where females (32.1%) showed a higher percentage of positives than males (23.7%), but the difference was not statistically significant. Another study differed from this, since males showed a higher prevalence (36.8%) than females (32.2%) [34], although, as in this study, but the difference was not statistically significant. Until now it is unknown whether sexual

orientation is due to behavioural reasons (affiliative or gender) or intrinsic biological reasons, so this factor would need to be studied further [35]. For example, pregnancy can generate an immunosuppressive effect contributing to the rates of excretion of parasites [36].

In 2018, in a study in Mazovia evaluating the status of parasitic infections, *G. duodenalis* cysts were recovered in 30.4% of faeces from younger cats (<1 year) and 38.8% of those from adult cats (>1 year) but the difference was not statistically significant [34]. This was the case in this study, where an age-related risk of *G. duodenalis* infection was not observed, since, although the age group ≥13 months showed higher prevalence (25.33%) than the group of ≤6 months (18.86%) and from 7 to 12 months (12.76%), no significant difference was found (p = 0.41). This coincides with other studies where no relationship with age was seen [35,37]. However, other studies have found higher prevalence in younger cats than in adults [17,18,31,39], even finding it to be a risk factor, especially in cats of <6 months, they being more likely to become infected with *G. duodenalis* [15,31-33,39]. Age-related risk for *G. duodenalis* infection has been associated in puppies with a poor immune response [32]. However, in this study it is likely that other unidentified factors are contributing to the presence of *G. duodenalis* in older cats.

No significant associations were found for the presence of *G. duodenalis*, between hair type (p = 0.81) and brushing habits (p = 0.35), although cats with long hair were found to be more positive (44.40%) to Giardia than those with short hair. Furthermore, *G. duodenalis* was found to be more prevalent (37.15%) in cats that never received hair brushing compared to cats that had a brushing habit. When feline size was compared, the association was found to be negative (Chi^2 = 6.12, p = 0.04) and was a factor for not presenting *Giardia* spp. (OR = 0.78, p = 0.01) in cats that were medium in size. This contrasts with a previous study [17] where no significant difference related to size was found.

Table 1. Risk factors and associations of the prevalence of *Giardia* with the age and gender of cats

	Positives n= 906	%	Negatives n= 685	%	Chi^2	P	OR	P	CI
Age									
≤6 months	300	18.86	239	15.02	1.77	0.41	0.92	0.45	0.749-1.139
7 - 12 months	203	12.76	135	8.49					
≥13 months	403	25.33	311	19.55					
Gender									
Female	518	32.56	380	23.88	0.45	0.49	1.07	0.49	0.87-1.30
Male	388	24.39	305	19.07					

Chi-square, OR, odds ratio, 95% CI, 95% confidence interval, * Significative

Table 2. Habits and their association with the presence of *Giardia* and risk factors in cats

	Positives n= 906	%	Negatives n= 685	%	Chi^2	P	OR	P	CI
Live with other cats									
Yes	672	42.24	499	31.36	0.35	0.55	1.07	0.55	0.85-1.34
No	234	14.71	186	11.69					
Live with other animals									
Yes	549	33.53	334	21.01	21.84	0.0001*	1.61	0.0001*	1.31-1.96
No	357	22.45	350	22.01					

Hunting habit									
Yes	559	35.16	383	24.09	5.53	0.01*	1.27	0.01*	1.04-1.55
No	346	21.76	302						
Outside access									
Yes	472	29.09	231	14.53	53.06	0.0001*	2.13	0.0001*	1.73-2.61
No	434	27.30	453	28.49					
Brushed									
Daily	46	2.89	30	1.89					
Weekly	196	12.32	155	9.74					
Monthly	73	4.59	71	4.46					
Never	591	37.15	429	26.96	3.28	0.35	0.74	0.006*	0.597-0.917

Chi-square, OR, odds ratio, 95% CI, 95% confidence interval, * Significative

Table 3. Variables associated with the presence of *Giardia* and risk factors in cats

	Positives n= 906	**%**	**Negatives n= 685**	**%**	**Chi^2**	**P**	**OR**	**P**	**CI**
Origin									
Adopted	866	54.47	647	40.69					
Bought	10	0.63	18	1.13	6.86	0.07	0.42	0.02*	0.19-0.91
Hatchery	19	1.19	15	0.94					
Unknown	11	0.69	5	0.25					
Hair type									
Long	706	44.40	531	33.40	0.05	0.81	0.97	0.81	0.76-1.23
Short	199	12.52	154	9.69					
Size									
Big	133	8.36	84	5.28					
Medium	388	24.39	335	21.05	6.12	0.04*	0.78	0.01	0.641-0.955
Small	385	24.20	266	16.72					

Chi-square, OR, odds ratio, 95% CI, 95% confidence interval, * Significative

Table 4. Stool characteristics and association with *Giardia* prevalence and risk factor

	Positives n= 906	**%**	**Negatives n= 685**	**%**	**Chi^2**	**P**	**OR**	**P**	**CI**
Color									
Yellow	108	6.79	85	5.34					
White	3	0.19	0	0					
Light brown	508	31.93	378	23.76	6.29	0.39	2.74	0.0001*	1.995-3.768
Out of classification	9	0.57	5	0.31					
Brown	155	9.74	103	6.47					

Black	99	6.22	90	5.66					
Green	24	1.51	24	1.51					
Consistency									
Aqueous	51	3.21	23	1.45	12.30	0.03*	1.71	0.03*	1.03-2.83
Soft	80	5.03	56	3.52					
Hard and dry	46	2.89	57	3.58					
Firm	494	31.05	382	24.01					
Out of classification	4	0.25	1	0.06					
Pasty	231	14.52	166	10. 43					
Findings									
Unidentified structures	82	5.15	58	3.65					
Mucus	119	7.48	75	4.71	3.25	0.51	1.22	0.18	0.90-1.67
Parasites	78	4.90	51	3.21					
Blood	49	3.08	37	2.33					
No findings	578	36.33	464	29.16					

Chi-square, OR, odds ratio, 95% CI, 95% confidence interval, * Significative

5. CONCLUSION

This study demonstrated a high prevalence of *G. duodenalis* (56.94%) in cats from different states of Mexico using three different diagnostic techniques. Cats that live with other animals, with hunting habits and with access to the outdoors are associated with the prevalence of *G. duodenalis* and are risk factors. It has been reported that there are other sources of human infection more relevant than the risk of infection by pets. However, subsequent genotyping studies would be necessary for a realistic estimate of the zoonotic risk of *G. duodenalis* in cats in Mexico.

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