

The frequency of intestinal parasites in puppies from Mexican kennels

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ABSTRACT

The purpose of this investigation was to determine the intestinal parasite prevalence in puppies from six different kennels; four kennels were in Guadalajara and Zapopan cities (Jalisco State) and two kennels were in Mexico City. From October 2006 to November 2007, we collected 441 fecal samples from 147 puppies, both male and female, ranging from 1 to 36 months of age. Three samples from every puppy were analyzed by using the Faust technique. The prevalence found were as follows: *Giardia intestinalis* (genotype A and B) 6.8%; *Cystoisospora* 21.08%; *Uncinaria* 7.48%; *Toxocara canis* 12.29% and multiparasitism (*Giardia*, *Toxocara* and *Uncinaria*) 4.76%. The highest prevalence for both *Giardia* and *Cystoisospora* were found in 2-3-month-old puppies; the highest prevalence for *Toxocara canis* was found in 3-4-month-old puppies. In the kennels of Mexico City we found mainly *Giardia intestinalis*, *Cystoisospora* to be most prevalent in Zapopan and *Toxocara canis* in Guadalajara. The high prevalence of intestinal parasites found in this study demonstrates an increased risk for infection in humans, as these animals are usually a common pet in many homes. This zoonotic phenomenon represents an important health problem for any community.

Keywords: Parasites; *Giardia*; *Cystoisospora*; Dogs

1. INTRODUCTION

The prevalence of intestinal parasites in dogs from kennels depends on several variables, including the kennels' geographic area, number of animals sharing space, and, most importantly, the sanitary conditions in which

the dogs live. Epidemiological studies of intestinal parasites give us an idea of the zoonotic risk for dogs living in kennels as well as dogs living with a family as a domesticated pet.

Several studies from kennels in Australia showed a parasitic prevalence of 23.9%, with 9.3% of the cases testing positive for *Giardia intestinalis* [1]. In Spain, a study showed a prevalence of parasites in 25% of those tested, with 6.0% of the cases testing positive for *Toxocara canis* and 10.0% testing positive for *Cystoisospora* [2]. In Greece, studies found a 26% parasitic prevalence, with 12.8% of the cases testing positive for *Toxocara canis*, 4.3% testing positive for *Giardia duodenalis* and *Cystoisospora*, and 2.8% testing positive for *Uncinaria* [3]. These statistics are important, as multiple parasites are being found in puppies as young as six months; therefore, kennel owners should treat all of their animals for parasites to prevent the continued spread of gastrointestinal diseases [4]. For example, there is a high incidence of *Giardia* in children; this could be explained if the children are living in homes with a contaminated pet [5]. Deworming and treating all kennel animals for parasites would reduce the cases of infection in humans.

When puppies lived in a small, confined area in groups of 12 to 15, especially in cases when they lived together with their mother, tests showed a high prevalence for *Toxocara canis* and *Cystoisospora* (36.4%). In contrast, when puppies were kept in clean, spacious areas, these parasites were found in only 10% of those tested. Finally, some reports show that the prevalence of intestinal parasitism in dogs ranges from 35.5% to 100% [6]. For dogs kept in private kennels, the prevalence range is between 54% and 68% [7].

Intestinal parasitism can be an asymptomatic disease; moreover, there is no a specific symptom that can identify a parasite. Therefore, all puppies kept in a kennel or stored in similar places could receive a prophylactic treatment to prevent intestinal parasitic from spreading

to children who have puppies at home [8]. We therefore conducted this investigation to elucidate the intestinal parasite prevalence in different kennels located in Mexico City and Jalisco State, with the hope of reducing the possible risk factors associated with human-puppy parasitic diseases.

2. MATERIAL AND METHODS

Identification and Prevalence of Parasites: From October 2006 to November 2007, we collected feces from 147 puppies (males and females) ranging from 1 to 36 months in age and obtained from six kennels, four of which were in Guadalajara and Zapopan cities (Jalisco State) and two of them were in Mexico City. The dogs were fed *ad libitum* and they received no special care in their environment. We collected three samples from each animal on different days. Identification of the parasites was performed via the Faust technique [9], and the results were analyzed in relation to the dogs' ages, sexes, breed sizes and kennels. We considered data statistically significant when $p < 0.05$ with 95% confidence interval (CI), using SPSS 10-0 (Copyright 1999, SPSS Inc. program).

Genotype of *Giardia*: DNA was isolated from each puppy's fecal sample by using the fast DNA kit and the fast prep instrument (QBiogene, inc., CA). Then B-giardin gene from *Giardia intestinalis* was amplified using the nested-PCR method [10]. After PCR, electrophoresis in 1.5% agarose gel was performed. Next, in a 10 μ L reaction volume, restriction digest reaction contained 7 μ L of amplicon obtained from the nested-PCR reaction, 10 mg/mL BSA and 10 U of HaeIII enzyme [11]. We incubated this reaction mix at 37°C for 3 hours, and then analyzed the product using 2% agarose gel electrophoresis to determine the fragments obtained.

3. RESULTS

Identification and prevalence of parasites: The prevalence of *Giardia intestinalis* in dogs at different ages was 4.69%-6.8%, (95% CI, 2.11 to 11.49), as is seen in **Table 1**. The highest prevalence occurred in dogs that were 2-3 months old. A statistical difference was observed between groups: those groups with dogs older than 3 months and those groups with dogs older than 11 months ($p < 0.05$). In the same table, we have demonstrated that in Mexico City, kennel-housed animals had the highest rates of *Giardia intestinalis* ($p = 0.003$). Other analyses did not show any statistical differences between breeds or breed size, while the location of kennel housing was important. The prevalence of both *Cystoisospora* and *Toxocara* in the puppies of Mexico City kennels was

Table 1. Prevalence of *Giardia intestinalis* in dogs from different ages and kennels.

<i>Giardia intestinalis</i>				
Age months	Total	N (+)	95% CI	p-value
Dogs				
< 1-2	56	1	1.78-16.75	
> 2-3	48	6	12.50-9.35	0.029
> 3-4	23	1	4.34-8.33	N.S.
> 4-5	8	0	0	-
> 5-11	5	1	20-35.06	0.04
> 11	7	1	14.28-25.92	N.S.
Total	147	10	2.11-11.49	
Kennel				
Guadalajara, Jal	47	2	4.24 \pm 5.77	
Zapopan, Jal	28	0	-	-
Mexico City	72	8	11.11-7.25	0.003
Total	147	10	6.80-4.07	
Breed size				
Small	76	3	3.94-4.37	N.S.
Medium	43	5	11.62-9.58	N.S.
Large	28	2	7.14-9.53	N.S.
Total	147	10	6.80-4.07	

N (+): Number of positives cases; 95% Confidence interval; P > 0.05; N.S. Non significant

statistically unique. As seen in **Table 2**, we analyzed dogs 2-3-month old dogs and 3-4 month-old dogs. Multiple parasites were isolated in dogs older than 2-3 months old ($p < 0.002$). Finally, **Table 3** shows what parasites were prevalent in each kennel. The highest infestation rate in Mexico City was for *Cystoisospora*, and *Toxocara* was highest in Zapopan ($p < 0.001$). In Guadalajara, most cases showed a high prevalence for multiparasite infections. Notably, other analyses did not show statistical differences in breed and sex.

The Genotype of *Giardia*: as shown in **Figure 1** one *Giardia* isolated was Genotype A2 and nine isolates were Genotype A1.

4. DISCUSSION

The prevalence of *Giardia intestinalis* in puppies from Mexican kennels was 6.8%, with the highest value in dogs younger than 6 months old. Similar results have been published by others [6,12-14] However, a good

Table 2. Prevalence of *Cystoisospora*, *Toxocara* and *Uncinaria* isolated from puppies in different ages.

Age (months)	<i>Cystoisospora</i>			<i>Uncinaria</i>			<i>Toxocara</i>			Multiparasites: (<i>Giardia</i> , <i>Toxocara</i> and <i>Uncinaria</i>).			Neg/T	%
	(+)/T	95% CI	p	(+)/T	95%CI	p	(+)/T	95% CI	p	(+)/T	95% CI	p		
< 1-2	3/56	5.35 ± 5.89	NS	0/56	0	-	7/56	12.5 - 8.66	NS	0	0	-	46/56	82.16
> 2-3	14/48	29.16 ± 12.85	0.003	8/48	16.66 ± 10.54	NS	8/48	16.66 - 10.54	NS	5/48	10.41 - 8.64	0.002	13/48	27.08
> 3-4	11/23	47.82 ± 20.41	NS	2/23	8.69 ± 11.51	NS	2/23	8.69 - 11.51	0.01	-	-	-	8/23	34.78
> 4-5	2/8	25 ± 30	NS	1/8	12.5 ± 22.91	NS	2/8	25 -30	NS	2/8	25 - 30	NS	1/8	12.5
Total	30/135	21.08 ± 6.59		11/135	7.48 ± 4.25		19/135	14.07 - 5.42		7/56	12.5 -3.44		68/135	50.37

(+): positives cases; T: total; 95% Confidence interval; P > 0.05; N.S. Non significant

Table 3. Prevalence of multiparasites found in each kennel.

Kennel	<i>Cystoisospora</i>			<i>Uncinaria</i>			<i>Toxocara</i>			Multiparasites: (<i>Giardia</i> , <i>Toxocara</i> and <i>Uncinaria</i>).			Neg/T	%
	(+)/T	CI ± 95%	p ¹	(+)/T	CI ± 95%	p ¹	(+)/T	CI ± 95%	p ¹	(+)/T	CI ± 95%	p ¹		
Guadalajara Jalisco	10/17	21.27 ± 11.7	NS	0	-	-	0	-	-	1/47	2.12 ± 4.1	0.03	36/47	76.59
Zapopan Jalisco	15/28	53.57 ± 18.4	NS	2/28	7.14 ± 9.53	NS	1/28	3.57 ± 6.8	0.001	2/28	7.14 ± 9.5	NS	8/28	28.57
Mexico City	6/72	8.33 ± 6.38	0.001	9/72	12.5 ± 7.63	NS	18/72	25 ± 10	NS	4/72	5.55 ± 5.2	NS	35/72	48.61
Total	31/147	21.08 ± 6.5		11/147	7.48 ± 4.25		19/147	12.92 ± 5.4		7/147	4.76 ± 3.4		79/147	53.74

(+): positives cases; T: total; 95% Confidence interval; P > 0.05; N.S. Non significant

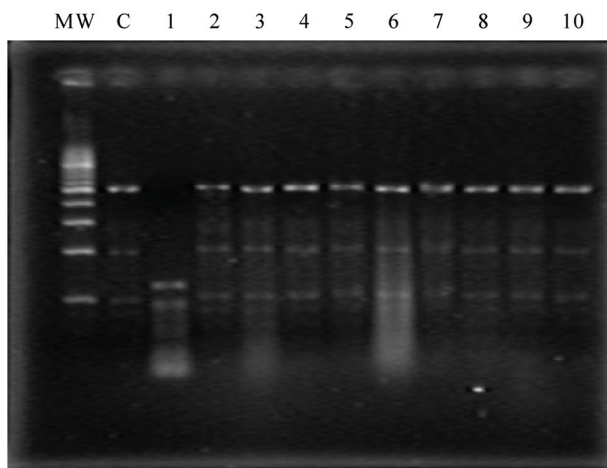


Figure 1. Genotype of *Giardia intestinalis* using *B-giardina* gene. MW - Molecular weights; CA Amplification control; Lane 1.- Genotype A2; Lanes 2-10 Genotype A1.

analysis should consider the following factors at least: geographic area; whether the kennel is private, public, or a business; number of animals living in the same space; hygiene standards; and feeding conditions. Determination of a *Giardia* genotype in the present investigation was important, as genotypes A and B are found in humans. Because the genotype found in nine samples was A1, whereas only one sample contained A2, and the fact

that these samples were all collected from puppies living in different kennels, we can assume that we are facing a potential zoonotic problem in communities that house dogs taken from kennels [15].

The presence of several parasites (such as *Giardia*, *Cystoisospora* and *Toxocara*) that were observed in the present study have been studied and published by others [4,5,16,17]. This multiple parasitism could be the result of puppies becoming contaminated by their mother when they were very small or contracted from living in small, private spaces and/or lack of treatment due to the cost of preventive medication. In addition, Nikolić [18] reported that even inside private homes, there is a distinct prevalence of puppies with intestinal parasites. However, less than 50% of the animals we tested showed positive results for infection.

Toxocara canis infection is both an animal and a human health problem that has been described in the last decade, since it became possible to isolate *Toxocara* from dog's hair, public parks, gardens, and even indoor homes [17,19,20]. Furthermore, it is an important zoonotic problem [21-23]. In humans, the disease is referred as *larva migrans*, and it is considered an epidemiological disease [24].

This investigation demonstrates the importance of treating parasites in dogs, and recommends community programs be offered on pet handling and immunization

to prevent further human infection, as some of the parasites have been isolated from both animals and humans, suggesting a problem stemming from veterinary-human interaction.

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