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Article

Cryptosporidium infection frequency in dogs on dairy farms and in urban areas of the state of Aguascalientes, Mexico

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Abstract

The intestinal parasite *Cryptosporidium* spp. is highly infectious in wild and domestic animals and humans. Infection frequency in dogs can vary between rural and urban environments. *Cryptosporidium* spp. infection frequency was quantified in dogs on dairy farms and in an urban area in the state of Aguascalientes, Mexico, and some possible risk factors analyzed. Feces samples were collected from 168 dogs at 30 dairy farms distributed among the state's ten municipalities (rural), and from 144 dogs at the Aguascalientes municipal Animal Control, Care and Welfare Center (urban area). Fecal smears were stained with Kinyoun to identify and count parasite oocysts. A questionnaire was applied to gather information on factors that could increase infection risk, and a risk analysis run using logistic regression. Overall infection frequency was 20.5 % (64/312; CI95% 16-25). In farm dogs it was 30 % (51/168; 95% CI 23-38) and in urban dogs 9 % (13/144; 95% CI 5-15). Seventy percent (70 %) of the dairy farms had positive dogs, average number of dogs per farm was 5.6, and dog density per farm was 2 to 12. Diarrheic feces was the only identified risk factor for *Cryptosporidium* infection, in both urban dogs (OR, 3.2; 95% CI 1.06-9.79 *P*<0.03) and farm dogs (OR, 2.7; CI95% 1.36-5.49 *P*<0.001). Infection frequency was highest in farm dogs, suggesting a consequently higher probability of cross-infection in this type of environment.

Key words: *Cryptosporidium*, Frequency, Dogs, Risk factors.

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Introduction

Cryptosporidiosis is an intestinal parasitic infection caused by protozoans of the genus *Cryptosporidium* (Apicomplexa: Cryptosporiidae). Unlike other coccidia, *Cryptosporidium* spp. oocysts are infectious from the moment excreted by an infected individual and can infect another host through ingestion. They have a cosmopolitan distribution and are considered opportunistic parasites, causing severe digestive disorders in young individuals. They affect many domestic and wild animals, as well as humans(1).

Dogs and cats are common pets because they can develop a bond with humans. However, they are also exposed to numerous parasite infections, representing a risk of transmission to other animals and humans(2). Dogs may be naturally infected by *C. canis*(3), *C. parvum*(4), *C. meleagridis*(5), and *C. muris*(6). Infection is typically asymptomatic, but can cause severe clinical manifestations including watery diarrhea, fever, and pathologies of the respiratory system, liver, and pancreas, especially in immunocompromised animals(7, 8).

Canine cryptosporidiosis is widely distributed. It has been reported worldwide in dogs in private homes, in kennels, in shelters and in stray dogs; reports are largely from urban areas but it has been reported in some rural communities(9,10,11). Little data exists in Mexico on the epidemiological traits of this disease in dog populations in urban and rural areas and communities, which would help to better understand the infection transmission and maintenance process in other domestic animals, livestock and humans.

The present study objective was to quantify *Cryptosporidium* spp. frequency and identify risk factors associated with the infection in dogs from rural environments associated with dairy farms and from an urban area in Aguascalientes, Mexico.

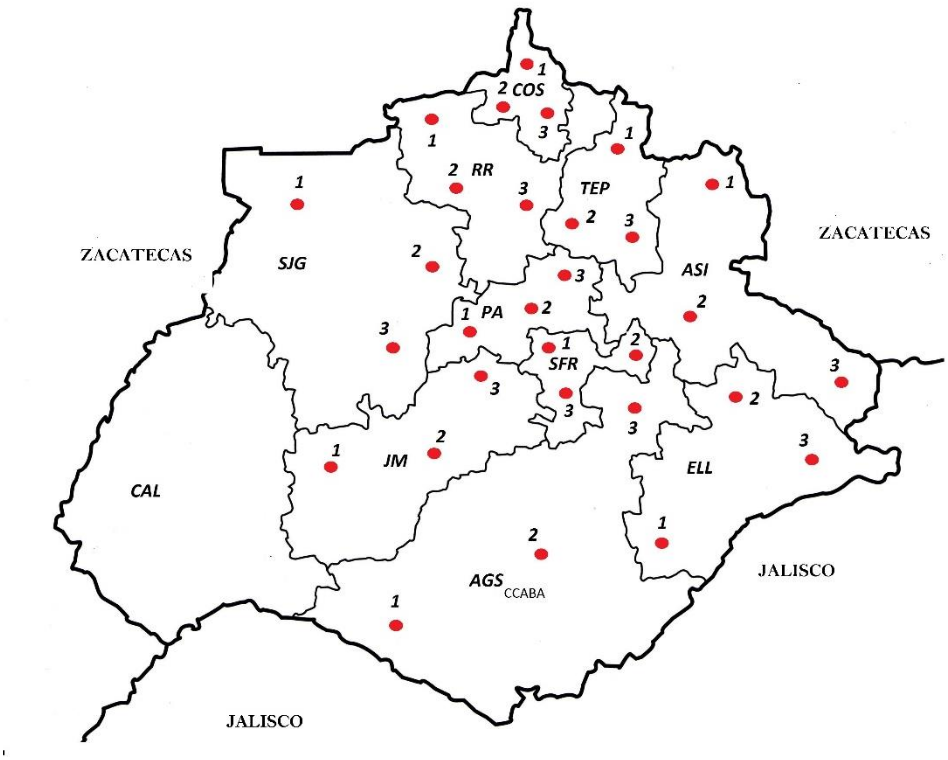
Material and methods

The study was carried out in the state of Aguascalientes, Mexico (21°37', 22°01' N; 101°52', 102°35' W). Altitude in the region ranges from 1,765 and 2,400 m asl, average annual temperature is 17.4 °C, and annual average rainfall is 526 mm, mostly in the summer[12].

Dairy farm sampling sites

Samples were collected at 30 dairy farms in 10 of the state's municipalities. In each municipality, three farms with at least one domestic dog were sampled; only farms where owners could provide the necessary facilities were included.

Figure 1: Location of sampled dairy farms (numbered 1, 2, 3 in each municipality) and CCABA



Municipality codes: Pabellón de Arteaga (PA), Asientos (ASI), San José de Gracia (SJG), Cosío (COS), Tepezalá (TEP), Rincón de Romos (RR), San Francisco de los Romo (SFR), Calvillo (CAL), Jesús María (JM), Aguascalientes (AGS) and El Llano (ELL).

Urban sampling site

Samples were collected from dogs housed at the Aguascalientes Municipal Center for Animal Control, Care and Welfare (Centro de Control, Atención y Bienestar Animal del Municipio de Aguascalientes - CCABA). Dogs here are strays collected from city streets or have been left here. After 72 h, unclaimed dogs are humanely sacrificed following established procedures (NOM-033-SAG/ZOO-2014).

Sample collection

A single visit was made to each dairy farm and a feces sample (approx. 25 g) collected from each clinically healthy dog on site. A total of 168 samples were collected. Weekly visits were made to the CCABA for 3 mo. On each visit, a post mortem feces sample (approx. 25 g) was collected from twelve randomly chosen clinically healthy dogs. A total of 144 samples were collected. Samples were transported to the laboratory under refrigeration and processed the day of collection. Data were recorded on animal sex and age (based on dental evaluation) and sample consistency (firm/diarrheal). For the farm dogs, data were also recorded on food type (dry balanced/prepared at home/combined), water access (exclusively for dog(s)/shared with other species), and any preventive medicine program (vaccination/deworming).

Parasitotic diagnosis

Samples were processed according to Castillo *et al*[13] Briefly, a 10 g feces sample was diluted in oxygenated water (1:1). Six fecal smears were made on a slide, dried for 24 h at room temperature and stained following the Kinyoun acid-alcohol staining technique. Smears were observed under a microscope (LCD Digital, Leica®) at 100x magnification. False positive readings were minimized by classifying a sample as positive when ≥5 *Cryptosporidium* spp. oocysts were identified in at least six smears (dying causes oocysts to appear as pale pink spheres).

Data analysis

Cryptosporidium spp. infection frequency in the sampled dogs was calculated based on total sample parasitosis results and characteristics of the two sampled populations. A logistic regression risk analysis was done[14], in which the dependent variable was parasitic infection condition and the independent variables were selected by the "backward step-by-step" method and a χ^2 test; non-significant variables were excluded (P<0.05). Odds ratios (OR) were estimated for independent variables shown to be significant in the multivariate analysis (*P*<0.05). Statistical analyses were run with the Statistics Data Analysis v. 9.1 program (STATA).

Results

Overall *Cryptosporidium* spp. infection frequency in the sampled dogs was 20.5 % (64/312; CI95% 16-25). Frequency in urban dogs was 9 % (13/144; CI95% 5-15), and in farm dogs it was 30 % (51/168; CI95% 23-38).

Infection frequency among farm dogs was highest in Jesús María municipality (58 %) and lowest in Cosío municipality (15 %) (Table 1). All the municipalities contained positive animals, whereas 70 % of the farms did (21/30). The average number of dogs per farm was 5.6, and density was 2 to 12 dogs.

Table 1: *Cryptosporidium* spp. infection distribution in farm dogs in ten municipalities in the state of Aguascalientes, Mexico

Municipality/Farm	n	Positives	Frequency (%)	CI 95%*
Aguascalientes	18	4	22	7-48
1	9	4		
2	5	-		
3	4	-		
Asientos	14	3	21	5-51
1	7	2		
2	2	-		
3	5	1		
Cosío	13	2	15	2-46
1	2	1		
2	6	1		
3	5	-		
El Llano	31	12	39	22-57
1	12	6		
2	10	4		
3	9	2		
Jesús María	19	11	58	33-78
1	2	2		
2	8	6		
3	9	3		
Pabellón de Arteaga	22	4	18	6-41
1	11	2		
2	6	2		
3	5	0		
Rincón de Romos	11	4	36	12-68
1	5	4		
2	4	-		
3	2	-		
San Fco. de los Romo	16	4	25	8-52
1	5	-		
2	4	1		
3	7	3		
San José de Gracia	12	2	17	3-49
1	4	-		
2	4	2		
3	4	-		
Tepezalá	12	5	42	16-71
1	3	3		
2	5	-		
3	4	2		
Total	168	51	30	23-38

* CI: 95% Confidence interval.

Most of the studied dogs were young (≤18 mo of age), and infection frequency was highest in this age group (Table 2). Farm dogs exhibited a higher frequency (37 %) than urban dogs (12 %). In both populations, females were more frequently positive than males. Feces samples described as diarrheal had a higher frequency (31 %) than those described as firm (11 %). In farm dogs, frequency by food type was 22 % for dry food, 32 % for prepared food and 36% for a combination of these. Farm dogs that had their own water had a higher frequency than those that shared water with other animals. Frequency was nearly the same in vaccinated (usually against rabies) and unvaccinated dogs, but those that had not been dewormed exhibited a higher frequency.

Table 2: *Cryptosporidium* spp. frequency as diagnosed by microscope in dogs in the state of Aguascalientes, Mexico, by different variables

	Urban dogs			**Farms dogs**			**Total**		
Variable	**n**	**Posit.**	**%**	**n**	**Posit.**	**%**	**n**	**Posit.**	**%**
Age:									
0-6 mo	58	7	12	67	25	37	125	32	26
7-18 mo	41	3	7	49	15	31	90	18	20
19-66 mo	28	1	3	20	3	15	48	4	8
> 66 mo	17	2	12	32	8	25	49	10	20
Sex:									
Male	74	6	8	78	20	25.6	152	24	16
Female	70	7	10	90	31	34	160	40	25
Feces texture:									
Firm	90	5	5	77	14	18	167	19	11
Diarrheal	54	8	15	91	37	41	145	45	31
Food:		na							
Dry				37	8	22	37	8	22
Prepared				28	10	36	28	10	36
Combined				103	33	32	103	33	32
Water:		na							
Exclusive				98	42	43	98	42	43
Shared				70	9	13	70	9	13
Preventive care:		na							
Vaccination				121	36	30	121	36	30
No vaccination				47	15	32	47	15	32
Deparasitization				37	7	19	37	7	19
No deparasitization				131	44	33	131	44	33

na: not applicable.

Risk analysis identified the variable of diarrheal feces in urban dogs (OR, 3.2; CI95% 1.06-9.79 $P<0.03$) and farm dogs (OR, 2.7; CI95% 1.36-5.49 $P<0.001$) as related to infection frequency diagnosed by parasitological analysis. No other analyzed variable exhibited a significant association.

Discussion

The prevalence of *Cryptosporidium* infection in dogs, be they pets, street dogs or farm dogs, ranges from 1 to 45 %, and is higher in puppies than adults[9-11,15-21]. Results can vary in response to characteristics such as experimental design, diagnostic test, geographic region and environmental conditions. The 9 % infection frequency observed here in urban dogs is relatively low, and similar to the 11.5 % reported in pet dogs in Mexico City as determined by FRECU Gum Infection[22]. The farm dogs exhibited a frequency of 30 %, which is significantly higher than among the urban dogs. In addition, infected farm dogs were widely distributed among the sampled farms, highlighting this parasite's cosmopolitan nature. Higher frequencies have been previously reported in street dogs and pet dogs in rural communities than in urban dogs[9]. The present study is the first report of *Cryptosporidium* spp. in dogs at dairy farms. Of note is that cryptosporidiosis is widely distributed in dairy cattle in Aguascalientes, with a significant presence in replacement heifers (40 – 67 %). The only species identified to date is *C. parvum*[13,23].

In the present results, *Cryptosporidium* spp. infection frequency was highest in young dogs; this agrees with previous studies[15,22]. Infection prevalence tends to decrease in naturally infected puppies as they grow[24]. However, age is not considered a risk factor for contracting this parasite[25,26], which is also supported by the present data.

Sex had no effect on frequency in the studied populations, although females exhibited higher values than males. The same has been reported elsewhere[25, 27, 28].

Presence of diarrhea is considered the principal clinical sign if canine cryptosporidiosis[8]. The present results support this in that dogs with diarrheal feces had a higher infection frequency than those with firm feces; it was identified as a risk factor in both urban dogs (OR 3.2) and farm dogs (OR 2.7). Samples came from clinically healthy animals, that is, asymptomatic carriers. Dogs with diarrhea are known to excrete more oocysts than those lacking clinical signs[29], making it a recognized risk factor[15,25,27]. Asymptomatic dogs or those with sporadic episodes of diarrhea are the source of soil and water contamination in the environments they inhabit. Because oocysts are highly environmentally resist, they represent a significant risk of transmission to humans, and domestic and wild animals. This is the case in rural, agricultural, urban and peridomestic environments[(1)].

Food type in farm dogs was not identified as a risk factor, which has been reported previously[(25)]. However, the studied farm dogs roamed freely about the dairy facilities, providing them easy access to fetuses and placental waste, and allowing them to sporadically hunt birds and rodents. Drinking water is an effective vehicle for *Cryptosporidium* transmission, especially in humans[(1)]. In the present study, only the use of the recipient form which the dogs drank water was assessed, and this variable was not identified as a risk factor. Another study in which drinking water source was assessed did not find it to be a risk factor[(25)].

Application of a vaccination regime in the farm dogs had no apparent effect on infection frequency since vaccinated and unvaccinated dogs exhibited similar frequencies. In contrast, farm dogs that had received deparasitization treatment had a lower frequency than those that had not received it; nonetheless, this variable was not identified as a risk or a protection factor. The present data suggest that the prevailing environment at dairy farms favors transmission and maintenance of *Cryptosporidium* spp. infection. Contributing factors include dog density at each farm, their high mobility and their interaction with other domestic and wild animals. The studied urban dogs were largely strays but faced a distinct environmental situation and were therefore less exposed to possible *Cryptosporidium* spp. infection.

Conclusions and implications

Cryptosporidium spp. infection was identified in both urban and dairy farm dogs. Infection frequency was relatively low in the urban dogs, but high among the farm dogs. Infection distribution was broad throughout the sampled rural areas, reflecting this protozoan’s high infective capacity. Further research is needed on the possible effects of the dog-cattle relationship on this disease’s epidemiology. Both dog populations represent a transmission risk to humans since they can be carriers of both *C. canis* and *C. parvum*.

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