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Estimation of canine intestinal parasites in Córdoba (Spain) and their risk to public health

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Abstract

The prevalence of gastrointestinal parasites in dogs was studied in the province of Córdoba (southern Spain), with special attention to those parasites that can be transmitted to man. The experiment was completed with the examination of soil samples from public parks and city gardens. The study was carried out over a population of 1800 animals entered in the Control Animal Centre (CECA) by coprological methods, and within this group, 300 dogs were sacrificed and necropsied. The prevalence of any intestinal parasitic infection was 71.33%. The following parasites of the gastrointestinal tract were recorded: *Isospora canis* (22%), *Isospora* (*Cystoisospora*) spp. (10.22%), *Sarcocystis* (2.5%), *Hammondia*/*Neospora* (1.94%), *Giardia canis* (1%), *Dipylidium caninum* (13.2%), *Taenia hydatigena* (7.66%), *Taenia pisiformis* (4%), *Uncinaria stenocephala* (33.27%), *Toxascaris leonina* (14.94%), *Toxocara canis* (17.72%) and *Trichuris vulpis* (1.66%). Related to public health, it is important to point out the presence of *T. canis* only in puppies younger than one year and *Uncinaria*, more frequent in adult dogs. Soil samples of parks revealed the presence of eggs of *Toxocara*, and it suggests the existence of real risk for human infection.

Keywords: Dog; Intestinal parasites; Prevalence; Zoonoses; Spain

1. Introduction

Intestinal parasites, both protozoa and helminths, are one of the main enteropathogens of dogs, especially in newly whelped or neonates (Blagburn et al., 1996). Some of these parasites are responsible for important zoonotic diseases; including well-documented diseases such as echinococcosis, and larval migrans (toxocariosis, ancylostomatidosis), as well as to emerging and re-emerging infections, such as cryptosporidiosis and giardiasis. The role of dogs as companion animals and the close relationship between humans and dogs, although offering significant benefits to many people, also represent a potential public health risk, since natural transmission of parasitic infections from dogs to man may occur, directly or indirectly, via environmental factors. In that sense, different studies have demonstrated that the soil contamination of gardens and public grounds by infectious parasitic forms constitutes a significant zoonotic risk (Habluetzel et al., 2003). All kind of dogs (owned and stray dogs) plays a role in that transmission, even if the particular implication of each population is not clearly established (Eguia-Aguilar et al., 2005).

Many studies of canine intestinal parasites have been reported worldwide. However, current information on

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regional prevalences is essential for development and modification of control measures in animal and public health. The aim of the present work is to determine the prevalence of the canine intestinal parasites in our region, with especial attention to potential zoonotic diseases, and an extended survey to study the contamination degree of public gardens and parks of the city.

2. Materials and methods

2.1. Animals

A total number of 1800 dogs were examined for the presence of intestinal parasites, all of them coming from the city of Córdoba and housed in the Animal Control Centre of Córdoba (Spain). They were registered with data of sex, age, size and weight, zone and habitat. These animals were classified according to the origin (homeless or housed) and the habitat (urban, semirural and rural) where they lived (Table 1). All dogs were studied by coprological methods and a group of 300 animals were also studied by necropsy, after being sacrificed according to municipality laws of the city Hall. Samples were collected during two years over all seasons.

2.2. Soil samples

Soil samples were collected from 22 different gardens and public parks of the city of Cordoba, according to recommendations of WHO-OMS. The total number of samples was 347. Each sample consisted of approximately 250 g of material collected from the superficial soil layer (5–7 cm), without vegetation, at five different points in each sampled park or garden, all of them used as game places for children.

2.3. Parasitological procedures

Fecal samples were obtained from all 1800 dogs, collected directly from the rectum and placed in sterile containers labelled with identification data. They were processed by several methods depending on the parasite we were looking for.

All samples were examined by passive flotation methods using sodium chloride solution and zinc sulphate solution, the second one more specific for *Giardia* spp.

Determination of *Cryptosporidium* was made by biphasic method of Ritchie (Allen and Ridley, 1970), and stained with modified Ziehl Neelsen.

Table 1
Total results

	Group 1 ($n = 1500$)		Group 2 ($n = 300$)		Total ($n = 1800$)		χ^2 ($d.f. = 3$)
	Samples	*Positive* (%)	Samples	*Positive* (%)	Samples	*Positive* (%)	
Age							
<4 months	241	*74.27a*	58	*93.10b*	299	*77.93a*	*9.63***
4–12 months	570	*70.35a*	80	*86.25b*	650	*72.31a*	*8.85**
12–36 months	380	*68.95a*	66	*92.42b*	446	*72.42a*	*15.51****
>36 months	309	*60.19a*	96	*76.04b*	405	*63.95ab*	*7.98**
Sex							
Males	965	*70.16a*	187	*83.96b*	1152	*72.40a*	*14.93***
Females	535	*65.61a*	113	*88.50b*	648	*69.60a*	*23.01****
Habitat							
Urban	698	*56.59a*	143	*77.62b*	841	*60.17a*	*21.89****
Rural	497	*78.47a*	96	*91.67b*	593	*80.61a*	*8.96**
Semirural	305	*79.67a*	61	*95.08b*	366	*82.24a*	*8.26**
Origin							
Homeless	1130	*70.18a*	164	*90.24b*	1294	*72.72a*	*29.07****
Housed	370	*63.51a*	136	*80.15b*	506	*67.98a*	*12.64***
Total	1500	*68.53a*	300	*85.67b*	1800	*71.39a*	*35.93****

Prevalence by groups and variables. Samples studied and overall prevalences according to epidemiological variables in each group. Results of multiple comparison test. Same letter in rows indicate no significant differences ($p > 0.05$) between categories according to the multiple comparison of proportion (Marascuillo's procedure).

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Sacrificed animals were necropsied and observation for enteric parasites was carefully done. The cranial end of the oesophagus and the end of the rectum were tied off for removing the entire gastrointestinal tract. The gastrointestinal tract was opened for a thorough examination. Contents were flushed out with saline solution, and the resulting solution was observed. Macroscopical and microscopical analysis of the mucosa was done, examining the digestive content and scrapings of the mucosa.

Soil samples were analysed according to the modified method of Dunsmore et al. (1984).

2.4. Analysis of results

For the statistical analysis of the data, the animals were grouped by age (up to 4 months, 4–12 months, 12–36 months, over 36 months), gender (male and female), status (housed and homeless) and habitat (urban, semirural and rural). The general prevalence for all parasites and the particular prevalence of each parasite was determined in each case. Statistical analyses were performed by using the computer software Statistica 6.0. The differences between two groups were analysed by the chi-square test for two independent proportions and p-values of ≤ 0.05 were considered significant. The comparisons between three or more groups were made with the chi-square test for multiple independent proportions, performing the Marascuillo's procedure to identify significant differences between pairs of proportions (groups).

3. Results

The overall prevalence of infection with parasites was 71.33%, with 1285 infected dogs of 1800 animals tested by coprological methods, and also by necropsy (Table 1).

Protozoa were observed in 31.83% of total samples, 574 animals of 1800. Five species of protozoa were identified in these animals, with different level of infection. Cestodes were present in 15.77% of dogs, both *Dipylidium caninum* and eggs of members of the family Taenidae spp. (necropsy suggested that these eggs belonged to *Taenia hydatigena* and *Taenia pisiformis*) were found. Nematodes were found in 53.27% of animals, identifying four species of this taxonomic group. Prevalence of each parasitic specie found by coprological methods and necropsy are expressed in Table 2.

The most frequently observed parasite was *Uncinaria stenocephala*, present in 33.28% of examined animals, followed by *Isospora* (*Cystoisospora*) *canis* (22%), *Toxocara canis* (17.72%) and *Toxascaris leonina* (14.94%).

The influence of the age was different for each parasite group and even for each species. Intestinal protozoa were more common in dogs under 36 months, and the number of parasited animals decreased with age. Cestodes were more prevalent in young animals, with ages between 4 and 36 months. Related to nematodes, *Uncinaria* was more frequent in animals over 12 months, and toxocarosis was more

Table 2
Prevalence by parasitic species and groups

	Group 1 (n = 1500)	Group 2 (n = 300)		Total (n = 1800)	χ^2 (d.f. = 3)
	Coprology	Coprology	Necropsy	Total	Cop. + Nec.	
I. (*Cystoisospora*) *canis*	21.67a	23.67ab	14.00b	23.67ab	22.00a	11.934*
Isospora spp.	9.87	11.67	6.33	12.00	10.22	6.850
Sarcocystis	2.53	2.00	2.00	2.33	2.50	0.585
Hammondia/*Neospora*	1.93	2.00	1.33	2.00	1.94	0.580
Giardia canis	1.07	0.67	0.00	0.67	1.00	3.744
U. stenocephala	33.60	29.00	31.33	31.67	33.28	3.055
T. canis	18.00	14.00	16.33	16.33	17.72	9.395
T. leonine	13.60a	19.33a	21.67b	21.67b	14.94a	24.850**
Trichuris vulpis	1.47	2.00	2.67	2.67	1.67	3.799
Dypilidium caninum	9.67a	8.00a	31.00b	31.00b	13.22ac	175.947***
Taeniidae spp.	7.93a	6.33a	11.67b	11.67b	8.56a	17.011*

Specific prevalences of parasites in groups, by different diagnostic techniques. Results of multiple comparison test. Same letter in rows indicate no significant differences ($p > 0.05$) between categories according to the multiple comparison of proportion (Marascuillo's procedure).

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

Table 3
Prevalence by age

	Total ($n = 1800$)	Age				
		<4 months ($n = 298$)	4–12 months ($n = 650$)	12–36 months ($n = 446$)	>36 months ($n = 406$)	χ^2 (d.f. = 3)
I. (Cystoisospora) canis	22.00	32.2a	22.2b	23.3a	12.8c	38.572***
Isospora spp.	10.22	13.4a	12.8a	8.3ab	5.9b	17.946***
Sarcocystis	2.50	0.0a	2.0b	3.8b	3.7b	13.833**
Hammondia/Neospora	1.94	1.0	2.6	2.2	1.2	4.199
Giardia canis	1.00	4.0a	0.9ab	0.0b	0.0b	35.691***
U. stenocephala	33.28	16.8a	28.9b	42.8c	41.9c	73.902***
T. canis	17.72	31.5a	23.5a	11.9b	4.7c	111.912***
T. leonina	14.94	56.7a	12.2b	2.5c	2.5c	517.351***
Trichuris vulpis	1.67	0.0a	1.5b	3.1b	1.5ab	11.104*
Dypilidium caninum	13.22	7.0a	15.4b	15.5b	11.8ab	15.211**
Taeniidae spp.	6.61	0.0a	5.7b	16.8c	1.7a	112.920***
Overall prevalence	71.39	77.9a	72.3b	72.4ab	64.0b	17.722**

Comparison of overall and specific prevalences by age. Results of multiple comparison test. Same letter in rows indicate no significant differences ($p > 0.05$) between categories according to the multiple comparison of proportion (Marascuillo's procedure).
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

frequently observed in dogs younger than 12 months (Table 3).

Prevalence of intestinal parasites did not show significant differences between males and females, and only infections of *U. stenocephala* showed a statistically significant higher prevalence in females (Table 4).

General prevalence was significantly higher in homeless animals (72.72%) than in housed (67.98%). Most parasites (protozoa, cestoda and nematoda) were also more prevalent in homeless dogs, the higher differences were found in the cestoda (Table 4).

The habitat and location of it was also important for the presence of parasites: the general prevalence was significantly higher in semirural and rural dogs than in urban animals. In this case, urban animals were less parasited with cestodes and nematodes, but there were no differences in protozoan parasitation (Table 5).

Table 4
Prevalence by sex and origin

	Total ($n = 1800$) (%)	Sex			Origin		
		Males ($n = 1152$) (%)	Females ($n = 648$) (%)	χ^2	Homeless ($n = 1294$) (%)	Housed ($n = 506$) (%)	χ^2
I. (Cystoisospora) canis	22.00	22.22	21.60	0.092	24.27	16.21	13.772***
Isospora spp.	10.22	10.07	10.49	0.081	10.90	8.50	2.280
Sarcocystis	2.50	3.21	1.23	6.652*	3.01	1.19	4.988*
Hammondia/Neospora	1.94	1.91	2.01	0.020	2.32	0.99	3.376
Giardia canis	1.00	1.04	0.93	0.056	1.16	0.59	1.178
U. stenocephala	33.28	29.69	39.66	18.57***	36.24	25.69	18.243***
T. canis	17.72	17.62	17.90	0.022	12.21	31.82	95.913***
T. leonina	14.94	14.67	15.43	0.189	17.62	8.10	25.920***
Trichuris vulpis	1.67	1.91	1.23	1.153	2.16	0.40	6.942***
Dypilidium caninum	13.22	13.45	12.81	0.151	15.15	8.30	14.860***
Taeniidae spp.	6.61	6.25	7.25	0.676	8.35	2.17	22.446***
Overall prevalence	71.39	70.15	65.61	3.301	72.72	67.98	3.995*

Comparison of specific prevalences by sex and origin. Results of χ^2 test for two independent proportions.
* $p < 0.05$.
*** $p < 0.001$.

Table 5
Prevalence by habitat

	Total (n = 1800)	Habitat			
		Urban (n = 841)	Semirural (n = 593)	Rural (n = 366)	χ^2 (d.f. = 3)
I. (*Cystoisospora*) *canis*	22.00	21.57	22.02	22.95	0.282
Isospora spp.	10.22	9.77	10.92	10.11	0.509
Sarcocystis	2.50	2.15	2.52	3.28	1.344
Hammondia/*Neospora*	1.94	1.67	2.02	2.46	0.859
Giardia canis	1.00	0.95	1.01	1.09	0.051
U. stenocephala	33.28	28.49a	37.48b	37.43b	16.249***
T. canis	17.72	16.45	18.99	18.58	1.776
T. leonina	14.94	11.32a	17.65b	18.85b	16.473***
Trichuris vulpis	1.67	0.95	2.18	2.46	4.981
Dypilidium caninum	13.22	11.92	14.12	14.75	2.406
Taeniidae spp.	6.61	4.17a	7.90b	10.11b	16.940***
Overall prevalence	71.39	60.16a	80.60b	82.24b	97.63***

Comparison of overall and specific prevalences by habitat. Results of multiple comparison test. Same letter in rows indicate no significant differences ($p > 0.05$) between categories according to the multiple comparison of proportion (Marascuillo's procedure).
*** $p < 0.001$.

The prevalence by taxonomical groups and their associations are showed in Table 6. Studies on contamination of parks and gardens revealed the presence of eggs of ascarids in 10 of 22 parks of Cordoba (45.5%): in 5, both *Toxocara* and *Toxascaris* eggs were found, in 3 only *Toxocar*a and in 2, only *Toxascaris*. From the 342 soil samples examined, *Toxocara* was found in 13 (3.8%), from 8 different parks, and *Toxascaris* in 19 (5.6%), from 7 parks. There were also oocysts of *Isospora*, but they were identified as avian parasite (*I. lacazei*).

4. Discussion

The overall prevalence of intestinal parasitosis found in this study is 71.33%, revealing a very high level of infection. In Spain, the highest prevalence found recently was 53.6% (Benito et al., 2003), and studies in other countries also show lower prevalence than we have found: in ''well-cared-for'' dogs, 35.5%, in Venezuela (Ramirez-Barrios et al., 2004) and 34.8% in USA (Kirkpatrick, 1988); on shelter animals, 34.2% in Belgium (Vanparijs et al., 1991), 35.9% in USA (Blagburn et al., 1996) and 52.4% in owned dogs of Argentina (Fontanarrosa et al., 2006).

Previous studies in Spain showed high level of infection in homeless animals, 95.12% in Barcelona (Gállego and Pumarola, 1952), 88% in La Rioja (González-Castro et al., 1962) and 72.90% in Zaragoza (Martínez-Gómez and López-Vivas, 1969). Results were 52.4% twenty years later (Ares-Mazas et al., 1987) in some parts of Spain, considering animals coming from rural and city habitats, but still were high in stray dogs, 70.97% (Illescas-Gómez et al., 1989).

The results observed in stray dogs, with the highest prevalences in all parasites, are in agreement with other studies, such as those of South Africa (Minnaar et al., 2002), that found that 76% of the stray dogs studied were infected with intestinal parasites, and Morocco (Pandey et al., 1987), that determined that 100% of studied animals were infected. These results can be easily explained, as these animals have no health control measures and, because of their habits, they are exposed to natural infection more than owned dogs. Although the exact role of these populations in the transmission of parasites to man is not clearly determined (Eguia-Aguilar et al., 2005), they may be an important source of

Table 6
Number of animals and prevalence by taxonomical groups

	Necropsy	Coprology	Total	Percentage
Negative	43	472	515	28.61
Positive	257	1028	1285	71.33
Protozoa	33	185	218	12.11
Cestodes	15	59	74	4.11
Nematodes	41	473	514	28.53
prot. + nema.	78	191	269	14.94
nema. + cest.	41	82	123	6.83
prot. + cest.	14	20	34	1.83
prot. + cest. + nema.	35	18	53	2.94
Total protozoa	160	463	574	31.83
Total cestodes	105	179	284	15.77
Total nematodes	195	592	959	53.27

infection for humans and constitute a relevant public health problem.

The most prevalent parasites in our study were *U. stenocephala* (33.2%), *I.* (*Cystoisospora*) *canis* (22.0%), *T. canis* (17.7%), *T. leonina* (14.9%) and *D. caninum* (13.2%). *Isospora* and *Dipylidium* have been usually considered the most frequent protozoa and cestode in dogs (Benito et al., 2003; Ramirez-Barrios et al., 2004). The nematodes were present in more than 50% of the dogs and *U. stenocephala* was most prevalent parasite in the study. It is noteworthy that only one species of the family Ancylostomatidae has been found, as two species had been previously reported in Spain: Gállego and Pumarola (1952), González-Castro et al. (1962), Ares-Mazas et al. (1987) and Illescas-Gómez et al. (1989). Anyway, this same fact of only one species has been previously recorded for *Uncinaria* (Rupérez et al., 1993) and also for *Ancylostoma* spp. (Asano et al., 2004; Oliveira-Sequeira et al., 2002). Most Ascarids (*Toxocara* and *Toxascaris*) have been found mainly in dogs younger than one year, and it was expected in *Toxocara* according to the transmission pattern of these parasites (Overgaauw, 1997; Robertson and Thompson, 2002).

These results indicate that the main risks to public health are *Toxocara* and *Uncinaria*, responsible for the production of larva migrans syndromes in man who come into contact with infecting larvae or eggs. *Giardia* has been detected in a low percentage of cases. No other zoonotic parasites were recorded in this study, and it is important to remark the absence of *Echinococcus granulosus*, undetected in necropsied animals, a quite representative number of animals (300) to assess the total prevalence of this parasite.

Contamination of soil from public gardens with *Toxocara* eggs were observed in 3.8% of soil samples and in 36.4% of the public gardens. This is a moderate contamination level, similar to that previously described in Spain by Garcia et al. (1989), and slightly lower than those described in Japan (Shimizu, 1993), Italy (Giacometti et al., 2000) and also Spain (Ruiz de Ybanez et al., 2001), with more than 50% of public gardens and parks contaminated. Although the contamination level is not high, there is clear risk of human infection, since the soil samples were taken from areas frequently used by children in their games.

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