JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Prevalence of zoonotic helminths in Italian house dogs

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Abstract

Introduction: Dogs may act as potential sources of zoonotic parasites, e.g. intestinal helminths like *Toxocara* spp., *Ancylostoma* spp., *Echinococcus* spp. In particular circumstances, the environment contaminated by parasitic elements represents a source of infection for people and animals. The present study has evaluated the presence of zoonotic helminths in house dogs from central and north-eastern Italy.

Methodology: Stool samples from 493 dogs were examined by a qualitative copromicroscopic technique and differences in prevalence of zoonotic parasites were statistically examined in relation to canine individual data.

Results: 48/493 (9.7%) were positive for at least one parasite. Helminths recovered were *Trichuris vulpis* (5.5%), *Toxocara canis* (4.3%), *Ancylostoma* spp. (0.6%) and *Eucoleus aerophilus* (0.4%), while no cestodes were detected. Age and living with other dogs resulted risk factors for *T. canis* infection.

Conclusions: The health risk associated with the occurrence of parasitic nematodes in privately owned dogs, along with the current anthelmintic treatment plans, are discussed.

Key words: Owned dogs; helminths; zoonoses; public health, Italy.

J Infect Dev Ctries 2018; 12(8):666-672. doi:10.3855/jidc.9865

(Received 23 October 2017 – Accepted 18 June 2018)

Introduction

Dogs may be infected by several species of zoonotic helminths, being intestinal nematodes such as ascarids and ancylostomatids, the most globally distributed [1,2]. Importantly, besides these nematodes, cestodes inhabiting the gut of dogs, e.g. *Echinococcus granulosus* or *Dipylidium caninum*, have also the potential to infect people [3]. Infected dogs shed parasitic eggs with their faeces and contaminate the environment, i.e. the most important source of infections for people and animals *via* the faecal-oral route [2,4].

The worldwide distributed roundworm *Toxocara canis* induces intestinal and respiratory diseases in dogs, that become infected through vertical transmissions, or ingesting infectious eggs from the environment or tissues of paratenic hosts [2,5]. A number of clinical syndromes may occur when people inadvertently ingest larvated eggs or raw or undercooked meat of paratenic hosts [6-8]. The most important syndromes are "visceral *larva migrans*" (VLM), involving organs like liver or lungs, "ocular *larva migrans*" (OLM), where damages to eye and optic nerve occur [6,9], and neurotoxocarosis [10], when the central nervous system is involved. In the recent years it became also clear that infection of children with *Toxocara* may be related with reduced cognitive function [11,12]. Thus, the frequent involvement of children and toddlers as subjects at risk of VLM and OLM is of major sanitary relevance [13].

Different hookworms may infect the dog gut, causing a potentially life-threatening disease especially in puppies. Some of them may cause zoonotic skin, enteric and pulmonary infections. The environmental larvae of the globally spread *Ancylostoma caninum*, i.e. one of the most pathogenic parasites of companion animals [14,15], infect a suitable host by actively penetrating the skin and/or *via* the oral route. The role of *A. caninum* as a cause of cutaneous *larva migrans* is still questioned [16], but in humans this parasite is associated to folliculitis, ephemeral and papular/pustular eruptions, penetration of muscle fibers, lung infiltrates and eosinophilic enteritis [17-20].

The canine whipworm *Trichuris vulpis* is another widespread nematode inducing intestinal damages in dogs. The ubiquitous presence of this parasite is due to the high resistance of its eggs in the environment even in harsh conditions [1]. Although cases of VLM syndromes and patent intestinal infections have been hypothesized to occur in people, the zoonotic potential of *T. vulpis* is doubtful [1,21]. A definitive evidence of the zoonotic role of dog whipworms is yet to be provided and *T. vulpis* is currently not included among causes of human intestinal infections and/or VLM syndromes.

The most important zoonotic tapeworms infecting the intestine of dogs are *Echinococcus granulosus* and *Dipylidium caninum*. Despite the globally distributed taeniid *E. granulosus* has a scarce pathogenic potential in dogs, it is responsible for human cystic echinococcosis (CE), a zoonotic disease of major public health importance [22]. This cestode is primarily transmitted in a synanthropic cycle where dogs are definitive hosts, and livestock, especially sheep, act as intermediate hosts [23]. Therefore, *E. granulosus* is highly prevalent in rural and sub-urban regions where livestock operations are present. In these settings, humans are at risk of CE when ingesting highly resistant eggs of *E. granulosus* shed by infected dogs *via* their faeces, from the environment or from the dog fur. The flea-transmitted tapeworm *D. caninum* also causes a most often subclinical intestinal infection in dogs. This tapeworm is transmitted to people through the accidental ingestion of infected adult fleas. Human infections are rare, but mostly children, one third of them being infants under 6 months old, are at risk of infection and may suffer of non-specific intestinal distress [24].

The prevalence of zoonotic helminths in owned dogs and the potential risks for human health have been worldwide investigated [25-31], but few surveys have been recently carried out in Italy [29,30,32]. Given the merit of a continuous update on parasite occurrence in dogs in contact with people, the present study evaluated the prevalence of zoonotic helminths in privately owned dogs from areas of central and north-eastern Italy where this info is outdated and incomplete [33].

Methodology

Study sites and sample collection

Thirteen veterinarian practices and clinics, six and seven located in Rome (site A, Central Italy) and Padua (site B, North-Eastern Italy) respectively, were enrolled in the study. Veterinarians in each site were asked to randomly collect faecal samples from privately owned dogs referred for routine procedures. In order to estimate the prevalence values, a minimum number of 196 individual faecal samples was foreseen in each city, considering infinite population size, maximum expected copromicroscopic prevalence, 5% maximum error desired, and 95% Confidence Interval (C.I.). The prevalence of 15% detected for *T. vulpis* in previous surveys in north-eastern Italy [33] was chosen as maximum expected value. Stool samples were individually identified, stored at refrigerated conditions and shipped by an express courier as soon as possible to the Parasitology Laboratory of the Department of Animal Medicine, Production and Health (University of Padua), where they were analysed within 48 hours.

Questionnaire

A questionnaire including signalment (e.g. city of provenance, age, gender, breed), anamnestic data (e.g. life-style as indoor or outdoor, cohabitation with other dogs, attendance of green public areas, intestinal distress), and number (from 1 to 4) of annual anthelmintic treatments planned by veterinarians, was filled for each study animal. Dogs that received an anthelmintic treatment within the previous three months were excluded from sampling.

Faecal examinations

All faecal samples were grossly examined for the presence of tapeworm segments or spontaneously expelled nematodes. Then, 2 grams of each stool sample were submitted to a qualitative copromicroscopic technique based on a double-step procedure with sedimentation followed by floatation in sodium-nitrate solution (specific gravity 1.3) [34]. Faeces were stirred in 15 ml of water, and poured into a test tube using a tea strainer. After centrifugation (5 min at 2000 rpm), the sediment was stirred in flotation fluid, and centrifuged again. Then, the test tube was gently topped off with the flotation fluid leaving a convex meniscus at the top of the tube, a coverslip was carefully placed for at least 5 min on the top, and then placed on a clean slide for microscopy. Each sample was examined under 100x and 400x magnifications, and parasitic elements were morphologically and morphometrically identified according to existing keys [35,36].

Statistical analyses

Differences in prevalence of parasites were analysed in relation to individual signalment and anamnestic data (i.e. risk factors) by Pearson's Chi-squared test or Fisher exact test (significance level $p < 0.05$) using SPSS Statistics software, version 22.0.0 (IBM, New York, USA). Parasites with low prevalence values (i.e. below 3.0%) were not included in the statistical analysis.

Results

A total of 493 stool samples was collected, 257 in site A and 236 in site B. All data available on signalment and anamnesis of dogs are reported in Table 1.

No parasitic elements were isolated at the macroscopic examination, while the microscopic analysis revealed at least one parasite in 48 (9.7%) of the 493 examined samples, i.e. 23 (8.9%) in site A and 25 (10.6%) in site B (Table 2). The vast majority (44/48, 91.7%) of the positive animals had a monospecific infection, while four dogs examined in site B had a concurrent infection by *T. vulpis* and *T. canis* (3 dogs) and by *T. vulpis*, *T. canis* and *Ancylostoma* spp./*Uncinaria* spp. (1 dog).

Statistical analysis was performed for *T. vulpis* and *T. canis*, that showed prevalence values of 5.5% (27/493) and 4.3% (21/493), respectively. Other recorded intestinal helminths were ancylostomatids (3/493, 0.6%), while no samples were positive for cestodes. Eggs of the respiratory nematode *Eucoleus aerophilus* were isolated in two dogs from site B (Table 2).

No significant differences in prevalence were found between dogs from site A and B, both for *T. vulpis* and *T. canis* (5.1% vs. 5.9% and 3.1% vs. 5.5%, respectively). The analysis of risk factors showed prevalence differences for *T. vulpis* and for *T. canis*. In particular, *T. vulpis* was significantly ($\chi^2 = 8.043$; $p < 0.01$) more present in crossbreed dogs (9.1%) than in pure-breed dogs (3.1%), while *T. canis* was more frequently ($\chi^2 = 6.059$; $p < 0.05$) detected in younger dogs (< 12 months, 8.7%) than in dogs belonging to the other two age-classes (1-5 years: 3.6%; > 5 years: 2.7%). Moreover, the infection prevalence value for *T. canis* was significantly ($\chi^2 = 5.244$; $p <0.05$) higher in dogs living with other dogs (6.5%) than in animals living alone or with other animal species (2.2%). No other significant differences were recovered. With regard to worm control programs, 307 questionnaires were filled in, and annual (n = 170, 55.4%) or twice (n = 119, 38.8%) yearly anthelmintic treatments were indicated in most cases.

Table 1. Signalment and anamnestic data of examined dogs.

	Rome (site A)		Padua (site B)		Total	
Signalment data	**n**	**%**	**n**	**%**	**n**	**%**
Gender						
Male	120	47.6	112	48.9	232	48.2
Female	132	52.4	117	51.1	249	51.8
Total	252	100.0	229	100.0	481	100.0
Breed	n	%	n	%	n	%
Pure-breed	144	56.7	144	62.3	288	59.4
Cross-breed	110	43.3	87	37.7	197	40.6
Total	254	100.0	231	100.0	485	100.0
Age classes	n	%	n	%	n	%
<1 year	36	15.5	68	30.4	104	22.8
1-5 years	93	39.9	74	33.0	167	36.5
>5 years	104	44.6	82	36.6	186	40.7
Total	233	100.0	224	100.0	457	100.0
Anamnestic data						
Life-style	n	%	n	%	n	%
Indoor	170	69.1	62	26.6	232	48.4
Outdoor	76	30.9	171	73.4	247	51.6
Total	246	100.0	233	100.0	479	100.0
Cohabitation with other dogs	n	%	n	%	n	%
No	98	39.2	133	58.1	231	48.2
Yes	152	60.8	96	41.9	248	51.8
Total	250	100.0	229	100.0	479	100.0
Attendance of green public areas	n	%	n	%	n	%
No	26	10.5	63	27.2	89	18.6
Yes	221	89.5	169	72.8	390	81.4
Total	247	100.0	232	100.0	479	100.0
Intestinal distress	n	%	n	%	n	%
Yes	55	21.6	34	14.7	89	18.3
No	200	78.4	197	85.3	397	81.7
Total	255	100.0	231	100.0	486	100.0

Table 2. Intestinal helminth eggs in examined dogs.

	Rome (site A)		Padua (site B)		Total	
Copromicroscopic results	**n**	**%**	**n**	**%**	**n**	**%**
Total samples	257	*100.0*	236	*100.0*	493	*100.0*
Positive samples	23	*8.9*	25	*10.6*	48	*9.7*
Trichuris vulpis	13	*5.1*	14	*5.9*	27	*5.5*
Toxocara canis	8	*3.1*	13	*5.5*	21	*4.3*
Ancylostomatids	2	*0.8*	1	*0.4*	3	*0.6*
Eucoleus aerophilus	0	*0*	2	*0.8*	2	*0.4*

Discussion

Companion animals are usually considered members of the family and the consequent psycho-physical well-being of humans is widely described [7,37,38]. However, next to the benefits, there are potential health risks associated, for instance, to some canine zoonotic helminths of great significance for both veterinary medicine and public health [39]. The presence and distribution of canine intestinal helminths strictly depend by many factors, primarily by the investigated population, e.g. stray, kennelled or shelter, breeding, "neighbourhood" pets (i.e. free-ranging animals belonging to people living in the same neighbourhood), or privately owned dogs. In fact, dog lifestyle may significantly increase the risk of acquiring different diseases, including helminthoses, despite a generally acceptable veterinary care. This aspect also represents a key epizootiological factor in privately owned dogs that may contribute to the environmental contamination of recreational, public and urban areas with infective parasitic elements [5].

The overall helminth prevalence recorded in the present survey (9.7%) is in agreement with data recently reported in restricted areas of central and north-western Italy [29,30,32]. Moreover, single infections were more frequent than mixed infections, as already reported in other studies [26,40,41]. The higher prevalence rates of *T. vulpis* and *T. canis* are probably related to the high resistance of their infectious eggs in the environment, and to the transplacental and transmammary transmission of ascarid larvae [2]. On the other side, the lower prevalence values detected for ancylostomatids may be due to the limited resistance of their infectious stages, i.e. filariform larvae present in the soil. This is the primary reason for why hookworms are spread in warm countries, especially where hygiene measures are poor and animals usually defecate on humid and unpaved soils. Therefore, prevention of human infection with both canine roundworms and hookworms is dependant from adequate hygiene measures (e.g. contact with the soil, hand and feet washings), and removal of dog faeces in areas where animals usually defecate. Serological findings (seroprevalence of 1.6-14.5%) indicate that human infection with *T. canis* also occurs in Italy, with highest values in young subjects, adult epileptics, *Strongyloides stercoralis*-infected patients, and outdoors or soil related workers [42-44]. It is worthy of note that the presence of pets at home is not correlated with seroprevalence [45], while the most important factor that increases the seropositivity in human beings is represented by the habit of frequenting public soils contaminated with a high load of infectious *Toxocara* eggs [46]. These observations highlight that *Toxocara* infections in people mainly originate through a sapro-zoonotic route, i.e. accidental ingestion of embryonated eggs from the soil [47], while the presence of ascarid eggs on animal coats is a less likely source of infection. In fact, *Toxocara* eggs need about 2-6 weeks to become infective, they are strongly adhesive on the fur and difficult to ingest, most of them are not viable, and several fur grams should be swallowed to cause infection [2,7,8].

In general, *T. vulpis* is among the most frequent parasitic helminth in dog populations, including privately owned animals. The zoonotic role of this trichuroid whipworm is debated among the scientific community, because there is no an ultimate microscopic and molecular evidence on the ability of *T. vulpis* in causing intestinal infections in humans [1]. On the contrary, in this survey the zoonotic lungworm *E. aerophilus* (syn. *Capillaria aerophila*) was detected in two dogs from the site B. This respiratory parasite may cause subclinical infections or various respiratory signs in cats and dogs [48], while bronchitis, pulmonary carcinoma-like masses and several symptoms (coughing, mucoid sputum, presence of blood in the mucus, fever, dyspnoea) have been described in humans [49].

The absence of tapeworms should be considered in relation to the investigated dog population. In fact, the typical "prey-predator" life cycle of the majority of cestodes (except the flea-borne *D. caninum*) is very unlikely to occur in privately owned animals living in metropolitan areas. Furthermore, a single copromicroscopic examination has very low sensitivity to detect tapeworms, due to the inconstant elimination of proglottids and the undistributed eggs in the faeces [50].

The association between *T. vulpis* and breed may be due to other factors that have not been investigated, e.g. by different management between cross and pure-breed dogs. The association between *T. canis* and age is not surprising [51] and likely due to the immature immune system and the vertical transmissions in puppies [52]. Moreover, the higher prevalences of *T. canis* in co-living dogs confirms the higher risk of exposure for these animals due to the limited area contaminated by *Toxocara* eggs and the high environmental resistance.

The absence of differences in parasite prevalence between dogs from site A and site B suggests that privately owned dogs receive in both cities similar worm control programs. At the same time the overall prevalence of zoonotic nematodes recorded in this survey in site B is similar to that (24/156; 15.4%) observed for the same parasites about 10 years ago [33]. To the Authors' knowledge, no previous data on prevalence of zoonotic helminths in owned dogs have recently been generated for site A, thus no any comparison is possible. Anyway, with regard to worm control programs, questionnaires filled by veterinarians showed that one or two anthelmintic treatments per year were suggested in the majority of cases. These findings are not consistent with guidelines by the European Scientific Counsel Companion Animal Parasites (ESCCAP) [53]. Specifically, ESCCAP Guideline 1 (2017) advises that 1-2 treatments/year for *Toxocara* spp. do not reduce the risk of patent infections while a treatment frequency of at least 4 times per year is a general recommendation. This suggestion might be applied also for *T. vulpis*, whose eggs, similarly to ascarids, have a very high resistance in the environment, thus favouring re-infections. When no regular anthelmintic treatments are applied, monthly or 3 monthly faecal examinations are proposed by ESCCAP Guideline 1 (2017) as a feasible alternative. In a recent survey (Traversa and Frangipane di Regalbono, unpublished results), performed in the city of Rome and Padua by a questionnaire delivered to the dog owners, a very few percentage of respondents declared to bring their dog more than once a year to the veterinarian for faecal examinations. This means 3.7% (3/82) and 14.1% (31/220) for each town, respectively, while the majority affirmed to perform a faecal examination once a year (36.6% and 21.8%) "only if required" (42.7% and 45.9%) or never (17.1% and 18.2%). Moreover, out of a total of 469 questionnaires administered in the same survey to both dog- and non-dog owners, only 51 (10.9%) were aware on the potential transmission of parasites *via* animal faeces, with no significant differences between the two categories of respondents. These findings prove that people often have a low awareness of the health risks associated with canine zoonotic helminths, along with a limited willingness of pet owners to engage correct collaborations with veterinarians, towards regular anthelmintic therapy and copromicroscopic examinations. It is crucial to emphasize that the close-contact with dogs infected by intestinal helminths should not be considered as a relevant risk for human beings, and there is no association between dog ownership and infection occurrence in humans [13,32,54]. Nonetheless, infected owned dogs could contaminate the environment and act as an indirect source of infection for children or other categories (e.g., retarded mentally patients, adult epileptics, co-infected or immunodeficient patients, soil related workers), that are more exposed to the risk of infection mainly due to their behaviour [55].

Conclusion

In this study the 9.7% of the examined dog faeces was positive for at least one helminth species, i.e. *T. vulpis*, *T. canis*, *Ancylostoma* spp. and *E. aerophilus*, and all these species, except for *T. vulpis*, are potentially zoonotic. On the other hand, administered questionnaires revealed that number of anthelmintic treatments per year usually performed are lower than what recommended by ESCCAP and not sufficient to reduce the risk of patent infections. Dogs infected by intestinal helminths contaminate the environment and act as an indirect source of infection for people, in particular children and immunocompromised subjects. Prevention of dog and human infections necessitates regular check-up of animals and adoption of adequate hygienic measures including the removal of faeces from soil, to avoid the environmental dispersion of parasitic elements.

In conclusion, it is mandatory to trust that ownership of companion dogs is beneficial and safe as long as animals are healthy. However, there is still much to do in terms of continuing education to enhance awareness of pet owners and the general public, and collaboration with veterinary practitioners in constant epidemiological vigilance and appropriate control measures, to prevent intestinal helminth infections in dogs and minimize the risk of their transmission to people.

Acknowledgements
The authors are grateful to all veterinarians who have collaborated in the study. Rome: Francesca Carocci, Marco Colaceci, Angelo De Luca, Fabio Di Natale, Costantino Melis, Ludovico Pozzi, Sylvie Michele Renaud. Padua: Stelvio Boaretto, Silvia Bortolini, Edoardo La Cava, Andrea Mazzocchin, Lucia Morosin, Ferdinando Zanin, Maria Antonia Zerbetto.

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Conflict of interests: The study was financed by Novartis Animal Health (nowadays Elanco), of which FLT was employee and PhD student at the University of Teramo during the study. FLT is currently employed at Zoetis Italia srl. AFdR and DT are the scientific responsible for the unpublished results reported in the study, and obtained within the research project n. CPDA110843 financed by the University of Padua.