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Cardiorespiratory and gastrointestinal parasites of dogs in north-west Italy

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Summary

The present study was conducted to evaluate the prevalence of gastrointestinal and cardio-respiratory parasites in dogs in Liguria (northwest Italy) which is a region that has scarcely been studied. A total of 450 dogs (260 males and 190 females) were enrolled in the study from 2009 and 2013. All dogs lived in a rural or semi-rural environment in the provinces of Imperia (n = 352) or Savona (n = 98), Liguria. Coprological examinations showed that 197 dogs (43.8 %, CI 38.7-48.9 %) were infected by parasites. Specifically, 3.3 % of the dogs presented only cardio-respiratory species, 32.4 % only intestinal species, and 8.0 % presented a mixed infection.

The most frequent intestinal parasites were *Toxocara canis* (20 %), *Trichuris vulpis* (17.8 %), Ancylostomatidae (12 %), Coccidia (2.7 %), *Aonchotheca putorii* (1.8 %) and *Toxascaris leonina* (1.8 %). The cardio-respiratory species found were *Eucoleus aerophilus* (9.6 %), *Eucoleus boehmi* (1.6 %), *Angiostrongylus vasorum* (0.7 %), and *Crenosoma vulpis* (0.2 %). A total of 116 dogs (25.8 %) were parasitized by a single species, multiple infections were observed in 81 dogs (18.0 %) up to a six-order infection.

This preliminary study highlighted that the prevalence of intestinal parasites in investigated area is high. Cardio respiratory parasites were detected in an area that has not been investigated before. The creation of a more extensive sampling programme of the area, on a provincial basis in order to build a more detailed map of prevalences for different species of dog parasites throughout Liguria, a more extensive sampling programme of the area needs to be created - ideally for each province.

Keywords: dogs; helminths; epidemiology; Liguria; Italy

Introduction

Dogs can host several helminth species, many of which are of zoonotic importance and may be a threat to human health causing serious diseases (Soriano *et al*., 2010; Mateus *et al*., 2014). In fact, dog parasites represent a potential risk to public health, especially in children or immuno-compromised people, since direct or indirect transmission of parasitic infections from dogs to humans can occur (Martínez-Moreno *et al*., 2007; Xhaxhiu *et al*., 2011). Thus, there is continuing interest in implementing control measures (Traversa, 2012). The number of pets is increasing and dog faeces are an important pollution factor if not properly removed. Parasite eggs can stick to shoes, they can be carried by arthropods, or by wind and rain into homes. The fact that some owners do not remove dog faeces from streets and green areas represents a risk for public health (Deplazes *et al*., 2011).

The most frequent intestinal nematodes of domestic canids are Ascarididae (*Toxocara canis* and *Toxascaris leonina*), Ancylostomatidae (*Ancylostoma caninum* and *Uncinaria stenocephala*), and Trichuridae (*Trichuris vulpis*) (Claerebout *et al*., 2009; Traversa, 2012). The larvae of *T. canis* and, less frequently, of *T. leonina* cause visceral and ocular *larva migrans* in humans, which can be particularly serious for children (Bowmann, 1987; Lee *et al*., 2010). Dog hookworms (Ancylostomatidae) cause cutaneous *larva migrans* (CLM) in humans, by actively penetrating the skin (especially *Ancylostoma* spp.) and/or via the oral route (*Ancylostoma* spp., *Uncinaria* spp.)(Bowmann *et al*., 2010). *A. caninum* can occasionally reach the adult stage and cause eosinophilic enteritis in humans (Bowmann *et al*., 2010). The zoonotic potential of *T. vulpis* is still debated (Traversa, 2011).

Other canine intestinal parasites of zoonotic importance are Taenidae (in particular *Echinococcus granulosus* and *Echinococcus multilocularis*) and Dipylidiidae (*Dipylidium caninum*) (Vervaeke *et al*., 2005; Eckert & Deplazes, 2004).

Several cardio-respiratory nematodes also affect dogs. These include trichuroid nematodes *Eucoleus aerophilus* (syn. *Capillaria aerophila*) and *Eucoleus boehmi* (syn. *Capillaria boehmi*), which are mainly the parasitic nematodes of wild canids, e.g. foxes and wolves, but infections in domestic dogs have also been described (Magi *et al*., 2012; Macchioni *et al*., 2013; Veronesi *et al*., 2013). *E. aerophilus* may be responsible for rare zoonotic infections in humans (Aftandelians *et al*., 1977; Lalošević *et al*., 2008). Similarly, metastrongyloid nematodes *Crenosoma vulpis* and *Angiostrongylus vasorum* infect both wild canids and dogs (Conboy, 2009; Magi *et al*., 2015).

Many surveys have been carried out on dog parasites around the world, and all highlight the importance of studying these parasites both from a veterinary and a public health perspective. This study describes the epidemiology of gastrointestinal and cardio-respiratory helminth infections in dogs living in a rural environment, with possible contact with foxes, in a region that has been scarcely investigated (Liguria, north-west Italy). In fact, foxes can act as a wild parasitic reservoir for pets, with significant implications for public and veterinary health (Deplazes *et al*., 2004; Vervaeke *et al*., 2005; Jenkins *et al*., 2011).

Materials and methods

A total of 450 dogs (260 males and 190 females) were enrolled in the study from 2009 and 2013. All dogs lived in a rural or semi-rural environment in Imperia (n = 352) or Savona (n = 98), Liguria, north-west Italy. Only dogs which had not received any anthelmintic treatment for at least four months prior to the sampling were included in the study. For each dog at least one faecal sample and a blood sample were collected. The results regarding the analysis of the blood samples have already been published (Magi *et al*., 2016). Gender, age, breed, lifestyle and geographical origin were recorded. As for lifestyle, 344 dogs were hunting dogs, 92 dogs were sampled from various kennels of the study area, and 14 were pets (a total of 106 non hunting dogs). Animals were classified into three age classes: 1 year or less (n = 57), between 1 and 7 years (n = 269), and 7 years or more (n = 124).

The dogs' faecal samples were analyzed by flotation in centrifuge, using 50 % zinc sulfate solution (s.g. 1.350) according to the procedure described by Dryden *et al.* (2005) Baermann's technique was also used for the detection of lung nematode larvae according to the procedure described by Taylor *et al*. (2007). Helminths were identified according to the usual keys (Yamaguti, 1959; Campbell, 1991).

As for the statistical analysis, parasitic prevalences with 95 % confidence intervals (CI) were calculated. Multiple parasitic infections were described. Pearson's chi squared test or Fisher's exact test were carried out to compare parasitic prevalences among genders, age classes and groups of hunting and non-hunting dogs. The significance of the tests was confirmed for P values <0.05. Calculations were performed with Microsoft Excel® and R 2.9.1 (R Development Core Team, 2009).

The sample size (450 dogs) was calculated in order to have a 99 % probability of finding at least one infected dog in the examined sample, assuming the prevalence of a certain parasite in this region to be around 1 % (a reasonable value for *Angiostrongylus vasorum*, e.g).

Results

A total of 197 out of 450 dogs (43.8 %, CI 38.7 – 48.9 %) were found to be infected by some kind of parasite. Specifically, 3.3 % of the dogs presented only cardio-respiratory species, 32.4 % only intestinal species, and 8.0 % presented a mixed with both

Table 1. Results of copromicroscopic examination by flotation in centrifuge of the faecal samples of 450 dogs (%: prevalence value; CI: Confidence Interval)

Flotation results of 450 dogs	N tot. pos.	%	CI
Cardio – respiratory parasites			
Eucoleus aerophilus	43	9.6	6.8 – 12.3
Eucoleus boehmi	7	1.6	0.4 – 2.7
Angiostrongylus vasorum	3	0.7	0 – 1.4
Crenosoma vulpis	1	0.2	0 – 0.7
Intestinal parasites			
Toxocara canis	90	20.0	16.3 – 23.7
Trichuris vulpis	80	17.8	14.2 – 21.3
Ancylostomatidae	54	12.0	9.0 – 15.0
Coccidia	12	2.7	1.2 – 4.2
Toxascaris leonina	8	1.8	0.6 – 3.0
Aonchotheca putorii	8	1.8	0.6 – 3.0
Hymenolepis diminuta	5	1.1	0.1 – 2.1
Dipylidium caninum	1	0.2	0 – 0.7
Physaloptera spp.	1	0.2	0 – 0.7

Table 2. Statistical results of the comparisons among gender, lifestyle and age groups of 450 dogs with P values of Pearson's or Fisher's tests (ns: not significant, - means that the test was not performed due to very low number of positive cases)

Tests results on 450 dogs	Gender			Lifestyle			Age			
	F (n=190)	M (n=260)	P values	hunting (n=344)	non hunting (n=106)	P values	≤1 year (n=57)	1 – 7 years (n=269)	≥7 years (n=124)	P values
Cardio-respiratory parasites										
Eucoleus aerophilus	25	18	0.0263	43	0	0.0001	12	23	8	0.005
Eucoleus boehmi	1	6	ns	2	5	0.0093	2	2	3	ns
Angiostrongylus vasorum	1	2	ns	2	1	-	0	1	2	ns
Crenosoma vulpis	1	0	ns	1	0	-	0	1	0	ns
Intestinal parasites										
Toxocara canis	36	54	ns	80	10	0.0019	13	53	24	ns
Trichuris vulpis	32	48	ns	51	29	0.0032	13	53	14	ns
Ancylostomatidae	26	28	ns	51	3	0.0009	12	31	11	ns
Coccidia	4	8	ns	9	3	ns	3	6	3	ns
Toxascaris leonina	3	5	ns	8	0	ns	4	4	0	0.009
Aonchotheca putorii	4	4	ns	8	0	ns	0	5	3	ns
Hymenolepis diminuta	4	1	-	3	2	-	1	1	3	-
Dipylidium caninum	1	0	-	1	0	-	0	1	0	-
Physaloptera spp.	0	1	-	1	0	-	0	1	0	-

cadiorespiratory and intestinal species. Prevalences found by flotation examination are summarized in Table 1. The most frequent intestinal parasites were *Toxocara canis* (20.0 %), *Trichuris vulpis* (17.8 %), Ancylostomatidae (12 %), Coccidia (2.7 %), *Aonchotheca putorii* (1.8 %) and *Toxascaris leonina* (1.8 %). The cardio-respiratory species found were *Eucoleus aerophilus* (9.6 %), *Eucoleus boehmi* (1.6 %), *Angiostrongylus vasorum* (0.7 %) and *Crenosoma vulpis* (0.2 %). Larvae of the two last species were detected in the same dogs also using Baermann's technique. No Taenidae eggs were found.

Table 2 reports the results of the statistical comparison of parasitic frequencies among gender, lifestyle and age groups. As for gender, a significant statistical difference was found for *E. aerophilus* (P value= 0.0263), where female dogs were more frequently infected (13.2 %) than males (6.9 %). As for lifestyle, significant differences were found for some parasitic species. Hunting dogs were more frequently infected than non hunting dogs in the case of *E. aerophilus* (the frequencies were 12.5 % and 0 % respectively), *T. canis* (hunting 23.3 %, non hunting 9.4 %) and Ancylostomatidae (hunting 14.8 %, non hunting 2.8 %), while the opposite was true for *E. boehmi* (hunting 0.6 % and non hunting 4.7 %) and for *T. vulpis* (hunting 14.8 % and non hunting 27.4 %). Concerning age classes, young dogs (21.1 %) were more frequently infected than middle age (8.6 %) or old dogs (6.5 %) in the case of *E. aerophilus*. The same was true for *T. leonina* (young dogs 7.0 %, middle age dogs 1.5 % and old dogs 0 %). These last differences were likely due to the sample.

A total of 116 dogs were parasitized by a single species: of these, 12 dogs harboured *E. aerophilus* and two *E. boehmi*, while the remaining 102 dogs were infected by a single intestinal parasite species, the most frequent being *T. canis* (41 dogs) and *T. vulpis* (37 dogs).

Multiple infections were observed in 81 dogs (18.0 %). One dog presented six different parasite species (*E. aerophilus*, *A. vasorum*, *C. vulpis*, *T. canis*, Coccidia and *D. caninum*) and another dog presented five different species (*E. aerophilus*, *T. canis*, *T. vulpis*, Ancylostomatidae and *T. leonina*). Five dogs presented 4 species of parasites, one cardiorespiratory and three intestinal parasites. Five dogs were infected by 3 species, one cardiorespiratory and two intestinal and 13 were infected by 3 species of intestinal parasites. The infections with 2 species were as follows: 24 infections by one cardiorespiratory and one intestinal parasites, one infection by two cardiorespiratory parasites (*E. aerophilus* and *A. vasorum*), and 31 infections by two intestinal parasites.

Discussion

The interest in intestinal helminths of domestic dogs and cats has somewhat declined over the few last years in the developed world, because the routine use of certain anthelmintics, especially in puppies, is believed to have reduced their diffusion and impact on animal health and welfare (Robertson *et al*., 2000; Traversa *et al*., 2012). Conversely, more attention has been given to extraintestinal parasites, due to the increased number of cases, mainly of

cardiorespiratory nematodes, described over the last decade (e.g. Caro-Vadillo *et al*., 2005; Staebler *et al*., 2005; Callegari *et al*., 2010; Di Cesare *et al*., 2011). In fact, many epidemiological surveys and reports have shown that these parasites are more common than previously thought, suggesting the potential underestimation of extraintestinal species, or, in some cases, the expansion of their geographical range (Conboy, 2009; Genchi *et al*., 2011).
While their underestimation could be due to the lack of appropriate diagnostic techniques (Conboy, 2009), the expansion of their geographical range can be explained by the increased mobility of pets and the possible interaction among dogs and wild canids, which are the reservoir of such parasites (Genchi *et al*., 2011; Otranto *et al*., 2015). In the present study, 43.8 % of dogs were found to be parasitized. A considerable number of dogs presented a mixed infection (8.0 %), 3.3 % were infected only by cardiorespiratory species and 32.4 % only by intestinal species.
Among cardiorespiratory parasites, of particular interest is *A. vasorum*, which is a metastrongyloid nematode that lives on the right side of the heart and pulmonary arteries of dogs, foxes and other wild carnivores and may be responsible for severe clinical symptoms. Following the first description in southern France in a dog (Serres, 1854), this species has frequently been found in foxes and more rarely in dogs (Koch & Willesen, 2009). In the last decade, the number of reports in dogs has risen, showing that the parasite is widely distributed throughout Europe with prevalences varying from 0.3 to 2.2 (Papazahariadou *et al*., 2007, Greece; Taubert *et al*., 2009, Germany; Al-Sabi *et al*., 2013, Denmark; Shukullari *et al*., 2015, Albania).
Several reports have also been made in Italy. Clinical cases have been described in Tuscany (Della Santa *et al*., 2002), Abruzzo (Traversa *et al*., 2008), Apulia (Sasanelli *et al*., 2008) and Campania (Rinaldi *et al*., 2010). Epidemiological surveys have also been conducted in central Italy, where 0.4 % of 239 client-owned dogs examined were found to be positive (Riggio *et al*., 2013) and in central and southern Italy, where 0.96 % of 534 individual dogs and 2.48 % of 471 kennel dogs were positive (Di Cesare *et al*., 2011).
In the study area investigated in the present survey, the prevalence at necropsy of *A. vasorum* in 165 foxes was very high (78.2 %)(Magi *et al*., 2015). Conversely, among the 450 examined dogs, only three were found positive for the larvae of this parasite by faecal examinations (0.7 %). Although the prevalence values are not directly comparable since different diagnostic techniques were used in dogs and foxes, it is known that dogs appear to be far less frequently infected by this parasite than wild canids (Koch & Willesen, 2009), as observed in the study area. In fact, some authors consider canine angiostrongylosis as an epiphenomenon of the cycle "gasteropoda-fox" (Bolt *et al*., 1994). This is likely due to the low palatability for dogs of the intermediate hosts of *A. vasorum* (mainly slugs and snails), and only partially to limitations concerning the coprological diagnosis. The Baermann technique for the detection of larvae L1 in faeces is currently the elective diagnostic method. However, it has a low sensitivity in terms of intermittent larval excretion, and a low parasite burden or prepatent period (Oliveira-Junior *et al*., 2006; Taubert *et al*., 2009). Serological methods have thus been developed. Testing a subset of dog serum samples (n=347) belonging to this study with an ELISA test for *A. vasorum* antigens and antibodies, a higher prevalence was found in the area (0.3 % with Baermann method, 0.6 % with the ELISA tests) (Guardone *et al*., 2013).
C. vulpis was found only in one dog (0.2 %). This species is endemic in the red fox in Europe and North America and was found with a prevalence of 15.8 % in foxes in the study area (Magi *et al*. 2015). It appears to be rarer in dogs. The first report in a domestic dog occurred in Great Britain (Cobb & Fisher, 1992), followed by a few cases in Ireland (Reilly *et al*., 2000), Switzerland (Unterer *et al*., 2002) and Germany (Barutzki & Schaper, 2003). The prevalence in dogs found in this study is slightly lower than the values found in Germany (0.9 % of 8438 dogs examined, Barutzki & Schaper, 2003; 2.4 % of 958 dogs Taubert *et al*., 2009) and in Denmark (1.4 % of 4151 dogs Taubert *et al*., 2009). In Italy *C. vulpis* was reported for the first time in a dog in Campania by Rinaldi *et al*., (2007), and was recently found in kennels (18.4 % of the examined kennels) in the same region (Rinaldi *et al*., 2012).
Respiratory capillariosis by *E. aerophilus* and *E. boehmi* are considered to be sporadic in pets, although the results of many studies in Italy and in other countries suggest that the presence of these two nematodes in domestic animals is most likely an underestimation (Burgess *et al*., 2008; Madeira de Carvalho *et al*., 2009; Di Cesare *et al*., 2011; Clark *et al*., 2013; Riggio *et al*., 2013; Veronesi *et al*., 2013). This could be due to various difficulties in coprological diagnosis, since, without a careful morphometrical and morphological analysis, eggs of *E. aerophilus* and of *E. boehmi* can be confused with those of the better known intestinal parasite, *Trichuris vulpis* (Magi *et al*., 2012; Macchioni *et al*., 2013). One of the most reliable distinctive characteristics is the external structure of the shell (Zarnowsky & Patyk 1960), which can be clearly visualized using SEM (Traversa *et al*., 2011; Magi *et al*., 2012).
In this study *E. aerophilus* was found with a prevalence of 9.6 %, which is slightly higher than the values reported in the south of Italy by Traversa *et al*., 2009 (2.8 % of 569 dogs examined) and lower than those reported by Di Cesare *et al*., in 2011 (20.22 % of 534 dogs examined). This parasite, like the preceding ones, is generally found more frequently in foxes. In Liguria its prevalence in foxes was 41.8 % at necropsy, and 25.5 %. at copromicroscopy (Magi *et al*., 2015).
The finding of *E. boehmi* in dogs in the study area represents one of the first reports of this parasite in Italy (De Liberato *et al*., 2009; Veronesi *et al*., 2013). The prevalence (5.3 %) was similar to the value found by De Liberato *et al*., 2009 (6.8 % out of 619 dogs examined). To the best of the authors' knowledge, reports of nasal capillariasis in European dogs have only been found in Poland (Gajewska *et al*., 2004) and in Italy (De Liberato *et al*., 2009; Veronesi *et al*., 2013). Three adult worms of *E. boehmi* were collected from the nasal cavities of a fox examined in the study area (Magi *et al*., 2015).

Among intestinal parasites, *T. canis* (prevalence 20 %), *T. vulpis* (17.8 %) and Ancylostomatidae (12 %) were the most prevalent species in dogs in this survey. Their presence in the study area is not surprising, since these intestinal nematodes are frequently found in dogs in Europe (see Claerebout *et al*., 2009 and references therein).

In analogous recent studies: in northern Italy *T. canis*, Ancylostomatidae and *T. vulpis* were found with prevalences of 9.7 %, 8.2 % and 29.2 % respectively among 318 kennel dogs (Simonato *et al*., 2015). A study of 592 dogs found *Toxocara* spp. in 1.35 % of the environmental samples, in 5.64 % of the farm dogs and in 2.97 % of the hunting dogs; Ancylostomatidae in 19.59 % of the environmental samples, in 14.87 % of the farm dogs and 20.79 % of the hunting dogs, and *T. vulpis* in 11.49 % of the environmental samples, in 16.41 % of the farm dogs, and in 5.94 % of the hunting dogs (Mateus *et al*., 2014).

In the same study area of the present survey, the prevalence of *T. canis* in foxes was 26.7 % at necropsy and 18.3 % at coproscopy (Magi *et al*., 2015), similar to the prevalence found in dogs. Also *T. vulpis,* with a prevalence of 21.1 % at necropsy and 18.3 % at coproscopy in foxes, showed similar values to the prevalence in dogs. Conversely, Ancylostomatidae appeared to be more frequent in foxes, with 70 % by necropsy and 47.2 % by coproscopy (Magi *et al*., 2015).

Toxascaris leonina was rarer in dogs than the above-mentioned intestinal parasites, most likely because the transmission occurs mainly with the ingestion of third stage larvae (L3) in infected paratenic hosts. In fact, the positive cases in this study were found only in hunting dogs, which are more likely to ingest paratenic hosts (however the difference was not statistically significant). The prevalence of these nematodes in Liguria adds to the data reported by other authors in other Italian regions such as in central Italy (1.7 % of 239 client-owned dogs examined, Riggio *et al*., 2013) and in northern Italy (10.02 % of 253 client-owned dogs, Zanzani *et al*., 2010).

In eight hunting dogs, capillarid eggs of *A. putorii* were identified by accurate morphological analysis according to the description given in Campbell *et al*. (1991). The presence of this parasite in dogs has not been reported before in Italy, however its presence in foxes in the same area (Magi *et al*., 2015) and the broad host range of the parasite suggests that it could also be present in dogs. SEM and biomolecular analysis are still ongoing for this trichuroid.

Physaloptera spp. was found in the faecal examination of one hunting dog. *Physaloptera* spp. have rarely been reported in domestic canids as being responsible for gastric disease (Burrows, 1983). Reports concern mainly the Middle East and South America (Clark, 1990; Theisen *et al*., 1998). However, the presence of the parasite in foxes in the same area (8.9 % at necropsy, Magi *et al*., 2015) may be the source of infection in the hunting dog.

As widely recognized, coprological flotation only has low sensitivity for the diagnosis of cestodes. The only cestodes found in dogs in this survey were *Hymenolepis diminuta* in five dogs (1.12 %) and *Dipylidium caninum* in one dog (0.22 %). *D. caninum* is frequently found in domestic carnivores, while the presence of *H. diminuta* is rare (Guardone *et al*., 2010).

In fact, *H. diminuta* is a common parasite of rodents, which can sometimes infect humans, monkeys and dogs (Neveu-Lemaire, 1936). There are very few data in the literature on the presence of *H. diminuta* in dogs. The only confirmed case of intestinal infection was found at necropsy in a dog in Africa (Gomes *et al*., 2000). While in many cases *H. diminuta* eggs have been attributed to spurious infections (Miterpakova *et al*., 2009), in the present study the positivity of one kennel dog was confirmed in different faecal samples over a 3-week period, suggesting true infection, at least in this case, and not the ingestion of an infected rodent (Guardone *et al*., 2010). The main intermediate hosts of *H. diminuta* are grain beetles, flour beetles and fleas while the definitive hosts are dogs, cats and wild carnivores. Humans acquire the parasite in case of accidental ingestion of intermediate hosts, possibly with contaminated flour, cereal products, or dried fruits. Human infections are sporadic but occur worldwide and mainly occur in children (Hamrick *et al*., 1990). In Italy it was recently diagnosed in a child from Sicily (Patamia *et al*., 2010) and previously in Rome (Marangi *et al*., 2003) and Alessandria (Scaglione *et al*., 1990).

Conclusions

This study revealed a significant presence of parasites of zoonotic importance in dogs living in rural and semirural environments in an area of Liguria. The sample population was chosen in order to examine dogs with potential close contact with foxes, considering that close contact between humans, domestic and wild canids may have significant public health implications.

Understanding and studying the epidemiology of zoonotic parasitic infections is important in order to minimize the risks to humans (Mateus *et al*., 2014). Health education should therefore be developed in order to defend the population against the risks resulting from infections especially in children. In rural areas, farmers and hunters should be addressed in particular. Of particular interest is the detection of cardio respiratory parasites in an area that has never previously been investigated. The extension of the sampling area, on a provincial basis, is needed in order to build a more detailed map of prevalences for different species of dog parasites throughout Liguria.

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