

# Parasites in stray dogs from Italy: prevalence, risk factors and management concerns

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## Abstract

Parasites are among the most common pathogens recorded in dogs. High prevalence rates are usually reported in stray and shelter dogs, as these animals are less likely to be tested and treated. In this study a survey was carried out on intestinal and lung parasites of stray dogs at the moment of the admission in a shelter in Central Italy. In the period June 2014-June 2015, 262 individual faecal samples were examined. Twelve parasitic taxa were detected. Helminths were more prevalent than protozoa (61.8% and 25.6%, respectively). Ancylostomatidae showed the higher prevalence (40.5%), followed by *Giardia duodenalis* (21.4%), *Toxocara canis* (20.6%) and *Trichuris vulpis* (17.6%). *Angiostrongylus vasorum* was the most prevalent lungworm (12.6%), *Crenosoma vulpis* being recorded just in one dog. *G. duodenalis* had a high prevalence, probably because of the diagnostic techniques adopted, actually the most sensitive available for its detection. Nevertheless, the zoonotic potential of this parasite in dogs appears reduced, as all the isolates were identified as C (24.0%) and D (76.0%) dog specific assemblages. Taeniidae constituted a high potential zoonotic risk, as from the eggs it is impossible to exclude they were *Echinococcus granulosus*, the most relevant zoonotic parasite in Europe. The present study underline the importance of stray dogs control, aimed at preventing or minimizing parasitic spread and zoonotic transmission.

## Keywords

Dog, parasites, zoonosis, risk factors, Italy

## Introduction

Intestinal and lung parasites are among the most common pathogens recorded in dogs worldwide (Little *et al.* 2009; Mirzaei and Fooladi 2012; Al-Sabi *et al.* 2013; Riggio *et al.* 2013). Their health impact is significant both for the clinical disorders in dogs (Claerebout *et al.* 2009; Riggio *et al.* 2013) and for the zoonotic potential of some of them (Sager *et al.* 2006; Claerebout *et al.* 2009; Mirzaei and Fooladi 2012; Dopchiz *et al.* 2013; Mateus *et al.* 2014). Parasites are a common occurrence in all dog categories (i.e. household, farm, stray, shelter, hunting, etc.), although with different prevalence (Palmer *et al.* 2008; Claerebout *et al.* 2009; Little *et al.* 2009; Martins *et al.* 2012; Mirzaei and Fooladi 2012; Al-Sabi *et al.* 2013; Dopchiz *et al.* 2013; Traversa *et al.* 2017), higher ones being generally reported in stray and shelter dogs, less likely

to be tested and treated, often exposed to high environmental faecalization and sometimes immunosuppressed because of malnutrition or stress (Palmer *et al.* 2008; Claerebout *et al.* 2009).

Intestinal nematodes and protozoa are usually the most prevalent parasites, given their direct life cycle and the ability of eggs/cysts to remain infective for long periods once shed in soil, while parasites with complex life cycles are generally recorded with lower prevalence (Palmer *et al.* 2008; Martins *et al.* 2012; Zanzani *et al.* 2014; Traversa *et al.* 2017).

Few data regarding parasites in stray dogs are available in literature, in particular concerning the European Union countries. Since stray dogs are not subject to any antiparasitic treatment, can move over long distances and usually live in synanthropic conditions, their environmental faecalization can represent a source of parasitic infection for people, owned dogs and other

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animal species (Martins *et al.* 2012; Mateus *et al.* 2014). Moreover, parasites in stray dogs represent a relevant sanitary and management concern once dogs are captured and transferred to refuge shelters, where high animal density facilitates transmission and spread of parasites with direct life cycles.

In Italy, according to national rules, the sanitary control of captured stray dogs is mandatory, concerning rabies and some zoonotic parasitic infections (e.g. *Echinococcus granulosus* and *Leishmania infantum*). In 2015, in Italy more than 100,000 roaming stray dogs were captured on a total of 600,000 estimated population (Italian Ministry of Health, unpublished data).

The aim of this paper is to evaluate the prevalence of intestinal and lung parasites in stray dogs and the associated risk factors, as well as providing veterinarians managing shelters useful information for parasite prevention and control.

## Materials and Methods

### Study area and sampling

In the period June 2014-June 2015, 262 stray dogs were captured from an area of Lazio Region (Central Italy) covering 1,814 square kilometres with a population of 476,586 inhabitants (<https://www.aslroma5.info/web/>) (population density: 262.7 per sq. Km). The study area is characterized by agricultural-rural areas with small towns and villages. The 58.8% of the land is covered by forest, 32.5% is used for agricultural activities, 2.5% is represented by urban areas and 6.2% is classified as "other use" (Corine Land Cover). After capture, all stray dogs were admitted in a refuge shelter.

### Coprological examination

Each dog was checked for parasites by collecting an individual faecal sample at the moment of its first admission in the shelter, prior to any contact with the other recovered animals. Each faecal sample was examined by a set of analysis, able to detect most of the intestinal and lung parasites: a) the wet mount Lugol's iodine staining, b) the flotation test in a sucrose 1,300 density solution (Taylor *et al.* 2007), c) the Baerman technique for the detection of lungworms (Bowman 1995; Taylor *et al.* 2007) and d) a commercial immunofluorescence kit (MERIFLUOR® *Cryptosporidium/Giardia*, Meridian Bioscience, Inc.) for the detection of *Giardia duodenalis* and *Cryptosporidium* spp. For laboratory safety, particularly dealing with the risk due to *E. granulosus*, endemic in Central Italy (Dakkak 2010), macroscopic examination of faecal samples was not performed; hence, data regarding tapeworm infections must be considered underestimated.

Identification of parasites cysts, oocysts and eggs was morphological (Zajac and Conboy 2012). Lungworms first stage larvae were identified according to McGarry and Morgan (2009). Samples positive for *G. duodenalis* at microscopy

(wet mount Lugol's iodine and/or immunofluorescence) were analysed by molecular tools for genotyping.

### *G. duodenalis* genotyping

DNA extraction was performed from a group of randomly selected *Giardia*-positive samples using the QIAamp DNA Stool Mini Kit (Qiagen, Milan, Italy). Samples with less than 130 mg of feces were excluded from the analysis. A nested endpoint PCR was carried out to amplify a 120 bp fragment of the 18S rDNA region, using the specific primers RH11 – RH4 for the first round and the internal primers GIAR-F-GIAR-R for the second round (Read *et al.* 2002). All PCRs were carried out in a 25 µL volume containing 12.5 µL PCR master mix 2X (Promega, Milan, Italy), 4–2 µL template DNA (first and second round, respectively), and 0.6 mM of each primer and performed in a TProfessional Basic Thermocycler (Biometra GmbH, Göttingen, Germany). The PCR products were visualized by electrophoresis on 1% agarose gel stained by SYBR Safe DNA gel stain (Invitrogen, Monza MB, Italy). PCR amplicons were purified using the mi-PCR Purification Kit (Metabion international AG, Planegg, Germany) according to the manufacturer's instructions and directly sequenced by the Bio-Fab Research (Rome, Italy). To assign each isolate to the Assemblage, a comparison of the obtained 18S sequences by multiple alignments against sequences retrieved from GenBank database (Accession number: AF199446-Assemblage A; AF199447-Assemblage B; AY775200-Assemblage C; AY775199-Assemblage D; AF113902-Assemblage E; AF199444-Assemblage F; AF199450-Assemblage G) was performed using Clustal Omega.

### Risk factors and statistical analysis

Data regarding the exposure to potential risk factors were collected through a questionnaire filled by Veterinary Authorities at the moment of dog's capture. For each animal, the following data were recorded: date of catch, geographical coordinates and kind of habitat (urban, suburban, agricultural/rural or natural), where the dog was caught, gender, estimated age class (<1 year old, between 1 and 5 years old, >5 years old), breed (pedigree or crossbreed), presence/absence of diarrhoea.

Results are presented as absolute frequencies and percentages (%). Associations between presence/absence of each parasite and the other categorical variables (age, gender, breed, caught area) were evaluated by Chi square test ( $\chi^2$ ). When more than one risk factor was associated with the presence of a parasite, they were included in a multivariate logistic model to estimate odds ratio (OR) with 95% confidence interval (CI 95%). Hosmer and Lemeshow (H-L) test was used to evaluate the model goodness of fit. A p value <0.05 was considered statistically significant. All analyses were performed by StataSE v.12.1 for Windows (StataCorp LP, TX, USA).

## Results

Among the 262 examined dogs, 181 (69.1%) were positive for at least one parasite. Ninety-four dogs (51.9%) were poly-parasitized. Two, three, four or five taxa were detected in the 27.6%,

**Table I.** – Prevalence of parasites detected in stray dogs (n = 262) from Central Italy

Parasite	No of positive dogs	Prevalence %
Ancylostomatidae	106	40.5
<i>Giardia duodenalis</i>	56	21.4
<i>Toxocara canis</i>	54	20.6
<i>Trichuris vulpis</i>	46	17.6
<i>Angiostrongylus vasorum</i>	33	12.6
<i>Cystoisospora</i> sp.	16	6.1
<i>Eucoleus aerophilus</i>	15	5.7
Taeniidae	11	4.2
<i>Sarcocystis</i> sp.	3	1.1
<i>Dipylidium caninum</i>	2	0.8
<i>Toxascaris leonina</i>	2	0.8
<i>Crenosoma vulpis</i>	1	0.4

13.3%, 7.7% and 3.3% of positive dogs, respectively. Overall, twelve parasitic taxa were detected, three of protozoa and 9 of helminths (Table 1), the helminths being more prevalent than protozoa, 61.8% and 25.6% respectively (162 dogs with at least one helminth vs 67 dogs with at least one protozoan).

Concerning protozoa, *G. duodenalis* was the most prevalent parasite reported (21.4%). *Giardia* genotyping obtained by the alignment of the 25 positive samples assigned all isolates to the dog-specific assemblages C (24.0%) and D (76.0%).

Concerning intestinal helminths, hookworms of the family Ancylostomatidae were the overall most common recorded parasites (40.5%), followed by *Toxocara canis* (20.6%) and *Trichuris vulpis* (17.6%). Among lungworms, *A. vasorum* was the most prevalent (12.6%), while *Crenosoma vulpis* was recorded just in one dog (0.4%). Tapeworms of the family Taeniidae were reported with prevalence of 4.2% (Table I).

None of the sampled dogs showed diarrhoea at the moment of the introduction in the shelter.

The associations between risk factors and the parasite presence/absence are reported in Table II. No significant association was found between the number of parasite species infecting each dog and the considered variables (age  $\chi^2 = 6.56$  p = 0.77, gender  $\chi^2 = 9.35$  p = 0.11, breed  $\chi^2 = 6.31$  p = 0.10 and capture area  $\chi^2 = 22.30$  p = 0.10). Taeniidae were associated

**Table II.** Significant associations between parasites and risk factors

Parasites	N = 262 dogs		Univariate analysis	Multivariate analysis OR (CI 95%)
	No of sampled dogs	No of positive dogs (Prevalence)		
<b>Taeniidae</b>				
Capture area	Natural	22	$\chi^2 = 14.62$ p<0.01	–
	Agricultural/Rural	28		
	Suburban	126		
	Urban	86		
<b><i>Trichuris vulpis</i></b>				
Capture area	Natural	22	$\chi^2 = 14.54$ p<0.01	0.99 (0.30–3.32)
	Agricultural/Rural	28		
	Suburban	126		
	Urban	86		
Age in years <sup>o</sup>	<1	57	$\chi^2 = 7.76$ p<0.01	4.89 (1.42–16.77)*
	1–5	196		
	>5	8		
Breed	Pedigree	227	$\chi^2 = 5.37$ p<0.05	–
	Crossbreed	35		
<b><i>Toxocara canis</i></b>				
Age in years <sup>o</sup>	<1	196	$\chi^2 = 31.64$ p<0.001	–
	1–5	57		
	>5	8		
<b><i>Angiostrongylus vasorum</i></b>				
Gender <sup>o</sup>	Male	147	$\chi^2 = 5.65$ p<0.05	–
	Female	82		

<sup>o</sup>Age: 1 missing, Gender: 33 missing. \*p<0.05 vs baseline

with the capture area ( $p < 0.01$ ), being natural areas where it is most likely to find positive dogs (18.2%). *T. canis* prevalence was significantly lower ( $p < 0.001$ ) in age class  $< 1$  (13.3%) than 1–5 years old (47.4%). The presence of Ancylostomatidae was significantly associated with *T. vulpis* ( $p < 0.05$ ) and with *A. vasorum* ( $p < 0.001$ ), being *T. vulpis* and *A. vasorum* also mutually associated ( $p < 0.001$ ). *A. vasorum* was associated with gender ( $p < 0.05$ ), being females more infected (20.7%) than males (9.5%). *T. vulpis* was significantly associated with capture area, age and breed, as detailed in Table II. Considering *T. vulpis* presence/absence as outcome variable, the first two exposure variables were confirmed as protective/risk factors using a multivariate logistic model ( $H-L = 4.14$ ,  $p = 0.39$ ). In fact, suburban ( $OR = 0.26$ ) and urban ( $OR = 0.25$ ) areas resulted significant protective factors ( $p < 0.05$ ) respect to natural areas, whereas the age class 1–5 years was a significant risk factor, respect to the  $< 1$  class ( $OR = 4.89$ ,  $p < 0.05$ ). In the multivariate model the influence of breed resulted not significant ( $p = 0.126$ ) and it was excluded by the final estimates.

## Discussion

Overall, our results agree with those of previous studies conducted in Italy and abroad. Depending on study area, dog category and adopted coprological techniques, roundworms, hookworms, whipworms and *G. duodenalis* are reported to be the dominant parasites (Capelli *et al.* 2006; Mohamed *et al.* 2009; Riggio *et al.* 2013; Zanzani *et al.* 2014; Traversa *et al.* 2017), other ones (*Cystoisospora* sp., tapeworms, lungworms, *Eucoleus* spp., etc.), being usually reported with lower prevalence. In our study, we found a higher overall prevalence of helminths than protozoa, similarly to what reported by Riggio *et al.* (2013).

*G. duodenalis* prevalence observed in the present study (21.4%) is high and confirms the findings of other recent studies both from Italy (Capelli *et al.* 2006; Paoletti *et al.* 2015) and from other European countries (Claerebout *et al.* 2009; Epe *et al.* 2010; Upjohn *et al.* 2010). Probably, until recently, the real prevalence of this parasite in dogs was often underestimated (Capelli *et al.* 2006), because of the low level of awareness of veterinarians through this parasite and the common use of less sensitive diagnostic techniques (Szwabe and Błaszowska 2017), compared to the immunofluorescence kit adopted in the present study. According to our results, the relevance of stray dogs as possible source of *G. duodenalis* zoonotic infection to humans would be low, as only the specific assemblages C and D were identified. Previous surveys on dogs from the same study area reported a 76.5% (Berrilli *et al.* 2004) and a 69.3% (Scaramozzino *et al.* 2009) of dog-specific genotypes. Other studies in different Italian areas did not report any zoonotic assemblage (Paoletti *et al.* 2015; Simonato *et al.* 2015).

Regarding tapeworms of the family Taeniidae, it is impossible to rule out that some of our findings were *E. granulosus* as eggs in the Taeniidae family are morphologically

undistinguishable. The area where the study was carried out is considered endemic for ovine echinococcosis (Dakkak 2010), hence stray dogs are far suspected to be infected.

Nematodes of the family Ancylostomatidae were the most common parasites reported in the present study, with a very high prevalence. Indeed, the study area has environmental conditions particularly favourable to these parasites. The predominant natural-agricultural environment provides microhabitats where free-living larvae are protected from direct sun light and dehydration. Moreover, another possible route of transmission of Ancylostomatidae – the trans-mammary route – could have been favoured by the lack of controls and treatments in the stray dog population.

Not negligible is the prevalence reported for *A. vasorum* (12.6%), a parasite considered in geographical expansion in the last ten years (Traversa *et al.* 2010) and increasing in both wild canids (foxes and wolves) and dogs in the study area (Eleni *et al.* 2013, 2014). The report of *C. vulpis* is also remarkable, as this parasite was previously detected only once in our country from the dog (Rinaldi *et al.* 2007). Foxes are the natural reservoirs of *A. vasorum* and *C. vulpis* (Barutzki and Schaper 2009), hence their report is not surprising, when considering the mainly rural/natural environments of the sampling area, probably sustaining a high fox population.

In central Italy, two species of the genus *Eucoleus* are found in dogs, *Eucoleus aerophilus* and *Eucoleus boëmi* (De Liberato *et al.* 2009). The report of *E. aerophilus* is not surprising, as this species is the most commonly recorded. Regarding observed prevalence, our results are in the range of those reported in a recent paper by Traversa *et al.* (2017), ranging from 3.0% to 8.0%.

Despite the overall high parasite prevalence and the report of dogs harboring up to 5 parasitic taxa, no apparent clinical signs were observed in anyone of the tested animals. A high tolerance of dogs to many of the more common parasites is well known from literature (Palmer *et al.* 2008, Claerebout *et al.* 2009). However, Riggio *et al.* (2013) found a positive correlation between the presence of clinical signs and parasitic infection.

The systematic collection of information on dogs and the standardized lab procedures allowed us to explore the association between parasites and dog-environmental variables. Although *T. canis* is usually reported to be more frequent in young dogs respect to older ones (Nijssen *et al.* 2015), in the present study it resulted significantly more prevalent in dogs 1–5 years old than  $< 1$ . This apparently surprising result could be related to the different dog categories considered. The owned and well-cared dogs are usually controlled and treated against *T. canis* when few weeks old, while in the present study it is assumable that most part of dogs involved in the present study never received an anthelmintic treatment, hence the prevalence of this nematode would not drop down in adult class of age (Sager *et al.* 2006). Both Taeniidae and *T. vulpis* were significantly more frequent in dogs captured in natural areas. This result was expected for Taeniidae, due to their com-

plex life cycle, but it was quite unexpected for *T. vulpis*. This nematode was also significantly more common in dogs 1–5 years old, when compared with younger ones, confirming the majority of comparable studies. A possible explanation can be a cumulative effect during dog lifetime because of the lack of anthelmintic treatments, joined with the high resistance of the eggs in the environment. The observed association between the presence of Ancylostomatidae and *T. vulpis* is probably ascribable to their common infection route via soil contamination. Despite in published papers no association of *A. vasorum* and host gender was reported, in the present study a significant difference was found between females and males. As it does not seem to be any biological plausibility, we cannot exclude a selection bias due to the passive recruitment of the sampled dogs.

Standing the high parasite prevalence reported at the moment of their introduction in shelters, if not properly managed (control-treatment-post treatment control), stray dogs can constitute a source of infection for dogs already present in the structures and a hazard for zoonosis for people working in shelters. Elsewhere, parasite control in shelters can be very hard to chase, for the high costs for testing and treatments and, in some case the need of repeated treatments (ESCCAP 2010). In general, treatment failures are a common occurrence when dealing with dog parasites, due to re-infection or incorrect application of therapeutic protocols and to the possible appearance of parasites lineages resistant to the most common drugs, as reported for *G. duodenalis* in human cases (Leitsch 2015).

Parasitized dogs treated at the moment of their introduction in shelters should not be considered parasite-free after a single drug administration and should be tested again before introducing the animal in the shelter in order to minimise environmental contamination (ESCCAP 2010). Parasite control programs are also important to minimize the risk for zoonosis transmission when the animals are adopted by families. The present study provides updated information on the occurrence of parasites in stray dogs from central Italy, confirming that these dogs are a source of environmental contamination with protozoa and helminths of zoonotic concern. Competent authorities should focus efforts on the reduction of the stray dog population and to standardize the sanitary protocols in the dog shelters.

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