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Original article

Gastrointestinal parasites in shelter cats of central Italy

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ABSTRACT

In Italy, the limited resources and the large number of cats living in feline shelters may facilitate the spread of parasite infections that may affect the health of cats and expose shelter workers and adoptive owners to zoonosis. Prevalence and risk factors of potential zoonotic and hostspecific gastrointestinal parasite infections were assessed in cats living in public and private shelters of central Italy. Individual fecal samples were examined macroscopically and then screened microscopically by fresh and Lugol stained fecal smears and by flotation and the Baermann tests. A commercial rapid immunechromatographic assay was used to detect *Giardia duodenalis* and *Cryptosporidium* spp. All animals were clinically examined to evaluate presence of clinical signs compatible to parasitic infections. Data were statistically analysed by multivariate analysis with logistic regression and chi square testing for bivariate analysis. The overall prevalence of gastrointestinal parasites in the shelter cats surveyed was 22% (29/132), with 26/132 (19.7%) of the animals infected with potentially zoonotic organisms. Upon statistical analysis, parasite infections were identified in significantly more cats younger than 18 months of age ($p < 0.05$), and most often associated with the presence of compatible clinical signs (p < 0.05). A higher prevalence of protozoan infections (18.1%, 24/132) than of helminth infections (12.9%, 17/132) was observed. Identified parasites were *Giardia duodenalis* (10.6%, 14/132), *Toxocara cati* (9%, 12/132), *Cystoisospora felis* (3%, 4/132), *Cystoisospora rivolta* (2.3%, 3/132), hookworms (2.3%, 3/132), *Cryptosporidium* spp. (1.6%, 2/132), *Aonchotheca putorii* (0.75%, 1/132), *Tritrichomonas foetus* (0.75%, 1/132) and *Strongyloides* sp. (0.75%, 1/132). Co–infections were identified in the 7.6% of animals surveyed. Results obtained from this study indicate high gastrointestinal parasite risk to shelter cats and public health in the examined areas and suggest the need for more effective control measures in shelters surveyed.

1. Introduction

In cats, gastrointestinal parasite species may be the cause of clinical disease of variable severity, from subclinical infections or mild gastrointestinal disorders to severe diarrhea, anorexia, anemia, developmental disturbances and even death, particularly in young animals with high intensity infections (Riggio et al., 2013; Beugnet et al., 2014; Traversa, 2012). Moreover, some feline gastrointestinal parasite species have zoonotic potential raising public health concerns (Diakou et al., 2017; Traversa, 2012), such as *Toxocara cati*, which may be responsible for visceral and ocular larva migrans in humans after the ingestion of infective eggs from a contaminated environment (Macpherson, 2013). More rarely, the accidental ingestion of cat fleas infected with cysticercoids of the zoonotic tapeworm *Dipylidium caninum* may result in human infections (Reid et al., 1992). Although reported, the zoonotic potential of cat hookworms (*Ancylostoma tubaeformae* and *Uncinaria stenocephala*) is considered low (Bowman et al., 2010; Waap et al., 2014).

Among the protozoans, oocysts of *Toxoplasma gondii* eliminated by infected cats in the environment with feces can lead to human toxoplasmosis, a disease of major importance in public health (Dabritz and Conrad, 2010). Zoonotic genotypes of the protozoa *Giardia duodenalis* and *Cryptosporidium* spp. may be shared between cats and humans and may be responsible for gastrointestinal signs of variable severity (Hinney et al., 2015; Ryan and Cacciò, 2013).

Compared to pet cats, the prevalence of gastrointestinal parasites is generally higher in shelter and free–roaming cats. In Europe, prevalence rarely exceeds 30% in privately owned and well cared for cats (Mircean et al., 2010; Riggio et al., 2013; Beugnet et al., 2014), while it can reach values of 30–50% or greater in shelter and free roaming cats (Duarte et al., 2010; Becker et al., 2012; Spada et al., 2013; Hinney et al., 2015; Szwabe and Blaszkowska, 2017; Symeonidou et al., 2018).

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Table 1

Prevalence and 95% Confidence Interval of gastrointestinal parasites identified in 132 shelter cats from Latium and Tuscany.

Parasite	Overall prevalence	Prevalence Latium	Prevalence Tuscany
Giardia duodenalis	10.6% (14/132)	3.4% (3/87)	24.4% (11/45)
	95% CI 5.9–17.2	95% CI 0.7–9.7	95% CI 12.9–39.5
Toxocara cati	9% (12/132)	8% (7/87)	11.1% (5/45)
	95% CI 4.8–15.3	95% CI 3.3–15.9	95% CI 3.7–24.1
Cystoisospora felis	3% (4/132)	1.1% (1/87)	6.6% (3/45)
	95% CI 0.8–7.6	95% CI 0.0–6.2	95% CI 1.4–18.3
Cystoisospora rivolta	2.3% (3/132)	1.1% (1/87)	4.4% (2/45)
	95% CI 0.5–6.5	95% CI 0.0–6.2	95% CI 0.5–15.1
Ancylostomatids (*A. tubaeforme* and *U. stenocephala*)	2.3% (3/132)	2.3% (2/87)	2.2% (1/45)
	95% CI 0.5–6.5	95% CI 0.7–9.7	95% CI 0.1–11.8
Cryptosporidium spp.	1.5% (2/132)	0% (0/87)	4.4% (2/45)
	95% CI 0.2–5.4		95% CI 0.5–15.1
Aonchotheca putorii	0.7% (1/132)	0% (0/87)	2.2% (1/45)
	95% CI 0.0–4.1		95% CI 0.5–15.1
Tritrichomonas foetus	0.7% (1/132)	1.1% (1/87)	0% (0/45)
	95% CI 0.0–4.1	95% CI 0.0–6.2	
Strongyloides sp.	0.7% (1/132)	0% (0/87)	2.2% (1/45)
	95% CI 0.0–4.1		95% CI 0.1–11.8
Total Prevalence	22% (29/132)	13.8% (12/87)	37.8% 17/45
	95% CI 15.2–30	95% CI 7.3–22.9	95% CI 23.8–53.5

According to the current Italian legislation, free–roaming cats should be housed in public and private shelters where they remain throughout their lives, unless private owners adopt them. Therefore, the limited resources and the large number of animals often living in these facilities may affect the health of maintained cats and expose shelter workers and adoptive owners to zoonotic agents including some gastrointestinal parasites (Schurer et al., 2015). Nevertheless, few and sporadic data are available regarding the prevalence of gastrointestinal parasites in shelter cats in Italy (Papini et al., 2007). The main aim of present study was to evaluate the prevalence and species of gastrointestinal parasites in shelter cats of central Italy.

2. Materials and methods

Between November 2011–November 2014, individual fecal samples from 132 randomly selected shorthair cats of different gender and age (55 males and 77 females; 64 cats ≤18 months, 68 cats > 18 months), living in public and private shelters of Latium (*n* = 87 cats from 3 shelters) and Tuscany (*n* = 45 cats from 3 shelters), central Italy, were collected and examined. Estimation of the approximate age of cats included in the study was based mainly on the examination of teeth, but also of other parameters, including their size and general condition and eye lenses appearance, evaluated during clinical examination performed at the time of sampling.

In these facilities, cats lived in individual or in common cages, or in rooms of about 30 square meters with several animals in each. Cat density averaged 30–50 animals per shelter. In shelters from Latium, all cats are treated with anthelmintic and ectoparasiticide drugs at the time of their intake, while in shelters from Tuscany only cats with clinical signs or those found positive by eventual parasitological exam are treated for parasites.

In this study, all animals were clinically examined to assess the presence of clinical signs consistent with gastrointestinal parasite infection.

Collection of samples and manipulation of animals were authorized by the shelters and the Italian Ministry of Health in the framework of the Italian surveillance programs for potential zoonotic diseases of stray animals (Italian law No. 281–1991).

Fecal samples (of about 3 g each) were screened for parasites by macroscopic examination to detect the presence of proglottids, nematodes and/or fragments of parasites. Then, samples were analysed microscopically by a centrifugal–flotation method using saturated NaCl solution (specific gravity 1.2), to evaluate the presence of helminth eggs and/or protozoan (oo)cysts (Riggio et al., 2013; Taylor et al., 2010; Zajac and Conboy, 2012). Fresh and Lugol stained fecal smears were also performed to detect *G. duodenalis* and *Tritrichomonas foetus* trophozoites. For the isolation of *Strongyloides* larvae from fecal samples, the Baermann technique was also used. Parasites were identified based on morphologic and morphometric features (Bowman et al., 2002; Taylor et al., 2010). In addition, a commercial rapid immune-chromatographic assay (RIDA® QUICK *Cryptosporidium/Giardia* Combi, RBiopharm, Darmstadt, Germany) was used to detect *G. duodenalis* and *Cryptosporidium* fecal antigens.

Data analysis was performed using the statistical software Epi Info Version 3.5.3. Gender, age, provenance and presence of clinical signs compatible with parasitic infection were the putative risk factors considered in the study. The prevalence of gastrointestinal parasites was estimated as the number of positive animals/total number of examined animals. For the evaluation of risk factors, multivariate analysis with logistic regression and Chi square tests for bivariate analysis was performed. The Fischer's exact testing was used when the percentages were small. A significance level of $p < 0.05$ was used in the statistical analyses.

3. Results

Except for a single cat of 4 months in age, all examined cats were adults (≥ 12 months, *n* = 131). Overall, the age ranged from 4 to 120 months, with a mean of 29.7 months ± 26.89 SD and a median of 24 months. The length of time in which the cats were housed in the shelters ranged between 3 and 24 months.

Gastrointestinal parasites were identified in surveyed cats with an overall prevalence of 22% (29/132, 95% CI 15.2–30). Prevalence of identified parasite species and shelter location are listed in Table 1. Potentially zoonotic parasites were identified in 26/132 (19.7%; 95% CI 13.3–27.5) of examined animals and in 26/29 (89.7%; 95% CI 72.6–97.8) of positive cats. Statistical analysis indicated parasite infection prevalence was significantly higher in cats younger than 18 months of age ($p < 0.05$) (Table 2) and overall significantly associated with the presence of compatible clinical signs (p < 0.05). The main clinical signs found in fecal positive animals were diarrhea (23.5%), polyphagia (19.3%) and poor body condition (15.3%).

A higher prevalence of protozoal infections (18.1%, 24/132; 95% CI 12–25.8) than of helminth infections (12.9%, 17/132; 95% CI 7.7–19.8) was observed. Similar results were observed when considering shelters from Latium (protozoa: 6.9% (6/87), 95% CI 2.6–14.4; helminths

Table 2

Age distribution of gastrointestinal parasites identified in 132 shelter cats from Latium and Tuscany.

Parasite	Age	
	19 months–10 years	4–18 months
Giardia duodenalis	2 (2.9%)	12 (18.8%)
	95% CI 0.4–10.2	95% CI 10.1–30.5
Toxocara cati	1 (1.5%)	11 (17.2%)
	95% CI 0.0–7.9	95% CI 8.9–28.7
Cystoisospora felis	1 (1.5%)	3 (4.7%)
	95% CI 0.0–7.9	95% CI 1.0–13.1
Cystoisospora rivolta	–	3 (4.7%)
		95% CI 1.0–13.1
Ancylostomatids (*A. tubaeforme* and *U. stenocephala*)	1 (1.5%)	2 (3.1%)
	95% CI 0.0–7.9	95% CI 0.4–10.8
Cryptosporidium spp.	–	2 (3.1%)
		95% CI 0.4–10.8
Aonchotheca putorii	–	1 (1.6%)
		95% CI 0.0–8.4
Tritrichomonas foetus	–	1 (1.6%)
		95% CI 0.0–8.4
Strongyloides sp.	–	1 (1.6%)
		95% CI 0.0–8.4

10.3% (9/87), 95% CI 4.8–18.7) and from Tuscany (protozoa: 40% (18/45), 95% CI 25.7–55.7; helminths: 17.7% (8/45), 95% CI 8–32.1), separately.

Gastrointestinal parasite co–infections were identified in the 34.5% (10/29, CI 95% 17.9–54.3) of positive cats and the associations *G. duodenalis* + *C. felis* (30%, 3/10) and *G. duodenalis* + *Cryptosporidium* sp. (20%, 2/10) were more frequently observed. The majority of cats identified with co–infections were 12 months old and male, with approximately 50% positive animals were diarrheic. Furthermore, the overall prevalence of protozoan infections ($p < 0.05$) and co–infections were significantly higher ($p < 0.001$) in the shelters of Tuscany than Latium.

4. Discussion

Gastrointestinal parasite infections are of great concern in cat shelters (Hurley, 2005). Highly contaminated, stressful and crowded environments are considered the main reasons why in cat shelters these infections are prevalent and often responsible for clinical disease (Hurley, 2005; Kostopoulou et al., 2017).

A high prevalence of gastrointestinal parasite infections (22%) was observed in shelters examined in this study. However, the rate of infection was significantly lower in shelters of Latium (17%) than in those of Tuscany (about 58%), where it reached values higher than that generally reported in shelter cats from Europe (33–56%) (Becker et al., 2012; Symeonidou et al., 2018).

Many cat endoparasites are potentially zoonotic and may be a threat to public health (Beugnet et al., 2014). In this study, potentially zoonotic parasites were identified in the 19.7% of examined animals.

Among these parasites, *G. duodenalis* (10.6%) and *T. cati* (9%) were found prevalent in the examined cats, in agreement with previous European studies in different cat populations (Becker et al., 2012; Symeonidou et al., 2018).

Prevalence of *T. cati* here observed is similar to that recorded in shelter cats from Greece and Portugal (Duarte et al., 2010; Kostopoulou et al., 2017), although higher prevalence rates have been observed in stray and fostered cats from Italy and other European countries (Becker et al., 2012; Spada et al., 2013; Szwabe and Blaszkowska, 2017). Considering the severity of health problems that *T. cati* may cause in humans and in cats (Becker et al., 2012; Macpherson, 2013), effective control measures aimed at reducing the prevalence of this parasite are needed in the shelters surveyed.

About *G. duodenalis*, this protozoan is considered the most common enteric parasite of cats in developed countries (Ballweber et al., 2010; Kostopoulou et al., 2017), especially in younger, free–roaming or sheltered animals (Tangtrongsup and Scorza, 2010). Results obtained in this study confirm these previous observations. In most European studies, both the zoonotic assemblages A and the cat specific assemblage F have been reported in cats, the latter considered an unlikely cause of human infection (Paoletti et al., 2011; Kostopoulou et al., 2017; Ryan and Cacciò, 2013; Sommer et al., 2018). Due to the low immunity to re–infections and to the continuous exposure to the parasite, repeated *G. duodenalis* infections are frequently observed in facilities with high animal density, as shelters (Tangtrongsup and Scorza, 2010).

High housing density and increased contact with other cats have been identified as risk factors also for other feline intestinal parasites, including the potential zoonotic *Cryptosporidium* spp., and the cat-specific protozoans *T. foetus* and *Cystoisospora* spp. This may possibly reflect the common fecal-oral route of infection shared by these parasites and the high environmental contamination frequently observed in shelters (Riggio et al., 2013; Paris et al., 2014; Waap et al., 2014; Yang et al., 2015; Gil et al., 2017; Kostopoulou et al., 2017). These factors may also represent the main reasons for the high frequency of co–infections, especially of the associations *G. duodenalis* + *C. felis* and *G. duodenalis* + *Cryptosporidium* spp., as observed in this study.

Among zoonotic parasite infections, in European countries feline cryptosporidiosis has been reported with prevalence rates ranging from 0% to about 16%, with higher values found in shelter or stray cats or in cats with clinical manifestations (Paoletti et al., 2011; Hinney et al., 2015; Mancianti et al., 2015; Kostopoulou et al., 2017). In this study, *Cryptosporidium* was identified with an overall prevalence of 1.5%. Although several *Cryptosporidium* species have been reported in cats, *Cryptosporidium felis* is considered the main cause of feline infections and a rare cause of human infections (Lucio-Forster et al., 2010; Hinney et al., 2015; Yang et al., 2015).

Nematodes of the genus *Strongyloides* sp. were identified in a single cat in this study. Among species of this genus, *Strongyloides tumefaciens*, *Strongyloides planiceps* and *Strongyloides felis* have been reported in feline natural infections (Thamsborg et al., 2017). However, these species have never been reported in Europe (Thamsborg et al., 2017) and further molecular studies are needed to identify the species infecting the cat found positive in this investigation.

Oocysts of *T. gondii* and eggs of tapeworms were not detected in the present study. However, in cats the prevalence of *T. gondii* oocyst shedding is generally low, in Europe about 0.1–0.4%. Therefore, larger sample size and more sensitive and specific methods are probably required for the detection of this important zoonotic parasite in cat fecal samples (Berger-Schoch et al., 2011; Beugnet et al., 2014; Hinney et al., 2015; Symeonidou et al., 2018).

About tapeworms, the prevalence of these parasites in shelter and stray cats is generally low in Europe, about 1–2% (Mircean et al., 2010; Barutzki and Schaper, 2011; Spada et al., 2013). Moreover, the sensitivity of copromicroscopic examination for the diagnosis of these parasites is also very low (Beugnet et al., 2014; Simonato et al., 2015).

According to the age, in cats prevalence and intensity of gastrointestinal parasite infections are generally higher in younger animals (Beugnet et al., 2014; Riggio et al., 2013; Paris et al., 2014). The lack of anamnestic responses against previously seen parasites and the incomplete ability of the immune system of very young animals to generate long-term immunity to parasites, are considered possible explanations for the association of younger ages with parasite infections (Gates and Nolan, 2009). In agreement with previous data (Beugnet et al., 2014; Riggio et al., 2013; Paris et al., 2014), also in this study the overall prevalence of gastrointestinal parasite infections and that of each identified parasite species, were higher in animals younger than 18 months in age.

Although in feline shelters and colonies asymptomatic animals may play an important epidemiological role in the transmission of several

parasitic infections (Hurley, 2005; Becker et al., 2012; Symeonidou et al., 2018), data from this study showed that a significant ($p < 0.05$) number of positive cats (about 90%) showed clinical signs, with most of them being diarrheic. Indeed, most of parasites identified in this study, as *G. duodenalis*, *Cryptosporidium* spp., *Cystoisospora* spp. and *T. foetus*, are included among the most frequent causes of diarrhea in infected cats of all ages (Dubey et al., 2009; ESCCAP, 2018; Scorza and Tangtrongsup, 2010; Epe et al., 2010; Holliday et al., 2009; Tolbert and Gookin, 2009). Clinical disease caused by these protozoans is generally observed mainly in younger or immunocompromised cats and in animals living in colonies, since stress caused by overcrowding and nutritional deficiencies can exacerbate the effects of these infections (Riggio et al., 2013; Paris et al., 2014; Waap et al., 2014; Yang et al., 2015; Gil et al., 2017; Kostopoulou et al., 2017). Diarrhea is also frequently observed in animals with multiple infections, mainly in the cases in which two or more of these intestinal protozoan species are associated (Scorza and Tangtrongsup, 2010; Paris et al., 2014). About feline coccidiosis, data observed in this study are in agreement with most of previous reports in Europe indicating a prevalence of about 3–9% and *C. felis* as the most frequently identified species (Riggio et al., 2013; Waap et al., 2014; Hinney et al., 2015). The protozoan species *T. foetus* has been reported as a primary causative agent of feline diarrhea worldwide, with a prevalence of 32% found in diarrheic shelter cats in Italy (Holliday et al., 2009; Tolbert and Gookin, 2009). Although *T. foetus* was detected only in a single cat in the present study, prevalence was presumably underestimated considering the low sensitivity of direct microscopy for the detection of *T. foetus* and the intermittent shedding of this parasite with feces of infected cats (Holliday et al., 2009).

Among helminths, hookworms, i.e. *A. tubeformae* and *U. stenocephala*, are considered responsible mainly for subclinical infections, stunted growth and failure to thrive. Nevertheless, in heavy infections these parasites may cause more severe lesions and clinical signs in cats, as hemorrhagic enteritis, anemia, and sometimes death (Symeonidou et al., 2018). In this study, hookworms were detected at a lower prevalence (2.3%) when compared to data reported in stray and shelter cats in other European areas (about 9–16%) (Spada et al., 2013; Kostopoulou et al., 2017; Symeonidou et al., 2018) but similar to that found in other cat populations in Europe (Riggio et al., 2013; Giannelli et al., 2017). *Aonchotheca putorii* is a gastrointestinal capillariid nematode unfrequently recorded in cats, but it may cause gastric ulcers and secondary anemia in infected animals (Taylor et al., 2010). Although recently this parasite has been reported with a prevalence of about 7% in Danish stray cats (Takeuchi-Storm et al., 2015), in this study *A. putorii* was detected with a very low prevalence (0.7%).

Differently from what was previously observed in shelter cats in other European studies, protozoan infections were overall prevalent in animals examined in this study (Beugnet et al., 2014; Symeonidou et al., 2018). Nevertheless, the prevalence of protozoan infections here observed is in agreement with previous European data in cats with clinical signs or asymptomatic (Hinney et al., 2015; Mancianti et al., 2015). More specifically, the prevalence of protozoan infections was higher in cats of Tuscany (40%) while in cats from Latium this prevalence was significantly lower (6.7%). Also the prevalence of helminth infections was higher in the shelters from Tuscany, but these results may depend at least in part on the anthelmintic treatments to which all newly entered cats were subjected in the shelters from Latium. Nevertheless, these results may depend also on a more effective management of these latter shelters, mainly in terms of reduced environmental fecal contamination. This may suggest that structural and/or management factors may favor the spread of gastrointestinal protozoa in the shelters from Tuscany, indicating the need to improve the control of gastrointestinal parasite infections mainly in the facilities of this area.

5. Conclusion

Results from the present study may help to increase the knowledge on the prevalence and species composition of gastrointestinal parasite infections in shelter cats in Italy. Considering the zoonotic potential and the clinical importance of most identified parasite species, obtained results show that in cat shelters of the examined area gastrointestinal parasites are prevalent and may be responsible for high cat and public health risks. The finding of a lower rate of gastrointestinal parasite infections in those facilities in which cats are treated for helminths at the entry may be indicative of the effectiveness of this procedure, although treatments for protozoan infections would be also needed. Nevertheless, obtained data suggest the need for more effective control measures in all examined shelters. These should include effective management procedures, improved hygiene measures and, mostly, more frequent and regular parasitological surveillance and adequate treatment of positive cats. Moreover, in shelters examined in this study it is also necessary to control the presence of other animals, as mice, rats and arthropods, considering that the life cycle of most gastrointestinal parasite species identified in this study can be both direct and indirect, by the ingestion of paratenic hosts (Taylor et al., 2010; Macpherson, 2013; Lindsay et al., 2014).

Authorship

Stefania Perrucci conceived and designed the study; all authors contributed to the acquisition of data, analysed and interpreted the results; Stefania Perrucci wrote the article and Claudio De Liberato revised it critically.

Ethical statement

Collection of fecal samples and manipulation of animals were authorized by the shelters and the Italian Ministry of Health in the framework of the Italian surveillance programs of potential zoonotic diseases of stray animals (Italian law No. 281-1991).

Declaration of Competing Interest

The authors do not have any potential conflicts of interest to declare.

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