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Short Communication

Gastrointestinal parasites in shelter dogs from Belgrade, Serbia

Marie Franziska Sommer [a,*], Nemanja Zdravković [b], Ana Vasić [b], Felix Grimm [c], Cornelia Silaghi [a,1]

[a] Comparative Tropical Medicine and Parasitology, Ludwig-Maximilians-University, Munich, Germany
[b] Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia
[c] Institute of Parasitology, University of Zurich, Zurich, Switzerland

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ABSTRACT

It is well known that dog shelters are a common source for parasitic infections in different countries worldwide. The present study was conducted in order to determine the prevalence of intestinal parasites and the frequency of polyparasitism in dogs living in two private shelters in Belgrade, Serbia. For this purpose, 134 faecal samples were examined for gastrointestinal parasites with the merthiolate-iodine-formalin concentration (MIFC)-method as well as for *Giardia*-coproantigen with an enzyme-linked-immunosorbent assay (ELISA). Taeniid eggs were identified by PCR and sequence analysis. Overall, at least one of nine different endoparasites was detected in 75.4% (101/134) of the dogs. *Giardia duodenalis* coproantigen was found most frequently (45.5%; 61/134), followed by eggs of Ancylostomatidae (41.0%; 55/134), oocysts of *Hammondia*/*Neospora* (11.2%; 15/134), eggs of *Toxascaris leonina* (9.7%; 13/134), oocysts of *Isospora canis* (8.2%; 11/134), eggs of *Trichuris vulpis* (6.7%; 9/134), cysts of *Sarcocystis* spp. (4.5%; 6/134), eggs of *Toxocara canis* (3.0%; 4/134) and eggs of *Taenia* spp. (1.5%; 2/134). The results of the study confirm a high parasitic burden in the investigated shelter dogs and call for an effective deworming program including an improved hygiene management in the affected facilities.

1. Introduction

Shelter dogs living in overcrowded environments under poor zoohygienic conditions are usually exposed to a wide variety of endoparasites (Bugg et al., 1999; Dubná et al., 2007; Tangtrongsup and Scorza, 2010). Dog shelters may serve as parasite reservoirs since the permanent contamination of the facilities by new or untreated dogs leads to a persistent maintenance of endoparasitic infections (Capelli et al., 2003; Martinez-Carrasco et al., 2007). On the contrary, most dogs living in private households suffer less often from infections with endoparasites compared to shelter and street dogs due to regular appropriate antiparasitic treatments and the higher hygienic standard they live in (Martinez-Moreno et al., 2007; Becker et al., 2012). Effective antiparasitic control programmes are required in order to reduce the parasitic burden of shelter dogs (Ortuño et al., 2014; Simonato et al., 2015). Worldwide, shelter dogs have been found infected with numerous endoparasites like *Giardia duodenalis*, *Isospora canis*, *Hammondia*/*Neospora*, *Sarcocystis* spp., *Trichuris vulpis*, Ascarids, Ancylostomatidae and tapeworms (Bugg et al., 1999; Capelli et al., 2006; Dubná et al., 2007; Martinez-Carrasco et al., 2007; Palmer et al., 2008; Joffe et al., 2011; Ortuño and Castellà, 2011; Simonato et al., 2015). In all these surveys, 30.0% to over 70.0% of the investigated dogs were infected with at least one of the aforementioned parasites.

Information on endoparasitic infections of shelter dogs from South Eastern Europe is limited. For example, data exist for shelter dogs from Romania of which 59.6% were infected with a variety of endoparasites (Sorescu et al., 2014). In comparison to privately owned dogs (50.1%), shelter dogs (73.0%) from rural and urban areas of Hungary were significantly more often infected with gastrointestinal parasites (Fok et al., 2001). Against this background, the aim of the present study was to determine the prevalence of gastrointestinal parasites and the frequency of polyparasitism in shelter dogs from Belgrade, Serbia. Faecal samples were obtained in the framework of a multinational study on multilocus sequence typing of canine *Giardia* from South Eastern European countries (Sommer et al., 2015).

2. Material and methods

In September 2013, 134 faecal samples were collected immediately after defecation from 134 dogs living in two privately owned dog shelters located in the outskirts of Belgrade. All investigated dogs were mixed-breeds older than one year. In the shelters, up to 200 and 400 dogs were accommodated in several kennels separated by fences, respectively. In both shelters, several dogs were kept together in one kennel. The main part of the kennel ground was plain soil and one quarter to one third was covered with concrete. The concrete part of the shelters was cleaned with water at least once a week and excrements on the

* Corresponding author at: Leopoldstr. 5, 80802 Munich, Germany.
E-mail address: Franziska.Sommer@tropa.vetmed.uni-muenchen.de (M.F. Sommer).
[1] Present address: National Centre for Vector Entomology, Institute of Parasitology, University of Zurich, Zurich, Switzerland.

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other areas of the kennels were removed on a regular basis. All dogs were vaccinated against rabies and were treated orally against helminths with pyrantel and praziquantel once yearly.

All 134 faecal samples were investigated with the merthiolate-iodine-formalin concentration (MIFC)-method, which is suitable for all kinds of faecal parasites (Allen and Ridley, 1970; Pfister et al., 2013). In order to screen the samples for the presence of *Giardia* antigen, the enzyme-linked-immunosorbent assay (ELISA) ProSpecT™ Giardia Microplate assay (Remel, Lenexa, USA) was used according to the manufacturer's instructions. Samples containing taeniid eggs were differentiated by a multiplex PCR (Trachsel et al., 2007). Taenia-positive samples were further analysed by amplifying and sequencing of a fragment of the cox 1 gene (Bowles et al., 1992).

The correlation of a *Giardia* and Ancylostomatidae infection determined by MIFC was evaluated with χ^2-test. For parasitic infections occurring less than five times, a Fisher's exact test was performed additionally to determine their correlation (calculation of both tests: http://graphpad.com/quickcalcs/contingency1.cfm). For either test, p values < 0.05 were considered significant.

3. Results

At least one gastrointestinal parasitic infection was detected in a total of 101 out of the 134 dogs (75.4%) with MIFC or ELISA. MIFC-positive samples contained developmental stages of different protozoans and eggs of nematodes and tapeworms (Table 1). Cysts of *G. duodenalis* and eggs of Ancylostomatidae were identified most frequently with 42.5% and 41.0%, respectively. *Giardia* antigen was detected in four additional samples even though the presence of *Giardia* cysts could not be confirmed with MIFC. Two samples contained taeniid eggs, which were confirmed to be eggs of *Taenia* spp. by PCR. The subsequent sequencing of the partial cox 1 gene revealed *Taenia hydatigena* in one case. No sequencing result was obtained for the second *Taenia* spp.-positive sample.

A total of 41.0% of the investigated dogs were infected with more than one and up to four different parasite species (Table 2). There was no statistically significant correlation of *Giardia* infection with any of the other parasitic infections (p values from 0.146 to 0.946). The correlation between Ancylostomatidae infection and either *Trichuris vulpis*, *Toxascaris leonina* or *Sarcocystis* spp. was considered statistically significant (p values of 0.0002, 0.002 and 0.042, respectively). The presence of *Toxocara canis* and *Toxascaris leonina* eggs correlated significantly ($p = 0.047$).

4. Discussion

The current study confirms the hypothesis of a high parasitic burden in shelter dogs (Martinez-Carrasco et al., 2007; Simonato et al., 2015).

Table 1
Prevalence of intestinal parasites in 134 shelter dogs from Belgrade, Serbia.

	Parasite	All dogs (n = 134)	
		Positive (n)	Prevalence (%)
Protozoa	*Giardia* (ELISA)	61	45.5
	Giardia (MIFC)	57	42.5
	Hammondia/Neospora	15	11.2
	Isospora canis	11	8.2
	Sarcocystis spp.	6	4.5
Helminths	Ancylostomatidae	55	41.0
	Toxascaris leonina	13	9.7
	Trichuris vulpis	9	6.7
	Toxocara canis	4	3.0
	Taenia spp.	2	1.5
	Total[a]	101	75.4

[a] Number of dogs, which were infected with at least one parasite.

Three quarters (101/134) of the investigated faecal samples contained developmental stages of at least one endoparasite. All nine different endoparasitic species detected in the investigated canine population have already been reported to occur in dogs from Serbia and other South Eastern European countries. A survey on zoonoses associated with dogs revealed the presence of *G. duodenalis*, *Toxocara canis*, *Trichuris vulpis*, Ancylostomatidae or *Taenia*-type helminths in 75.5% of a mixed population of household, stray and military working dogs from Belgrade, Serbia (Nikolić et al., 2008). In addition to the mentioned parasitic infections, privately owned dogs from Albania and Romania were infected with *Isospora canis*, *Hammondia/Neospora*, *Sarcocystis* spp. or *Toxascaris leonina* (Amfim et al., 2011; Xhaxhiu et al., 2011; Shukullari et al., 2015).

In the investigated shelter dogs, the intestinal protozoan *G. duodenalis* was detected most often, not only by the indirect coproantigen ELISA (45.5%) but also by the direct MIFC-method (42.5%). Compared to privately owned dogs, *Giardia* infections are more prevalent in shelter dogs (Huber et al., 2005; Tangtrongsup and Scorza, 2010). Dubná et al. (2007) detected an 11-fold increase of the prevalence for *Giardia* infections in dogs during the time of their stay at a shelter in the Czech Republic. This result is not surprising considering that the direct life cycle and the short prepatent period of *Giardia* facilitate a permanent infection cycle through contaminated food, water or hair coats in shelters with a high concentration of dogs (Papini et al., 2005).

Faecal forms of Ancylostomatidae had the second highest frequency of occurrence (41.0%) in the present study. Nikolić et al. (2008) asserted that hookworms belong to the most prevalent endoparasites of dogs in Belgrade. In a worldwide context, the prevalence for infections with Ancylostomatidae in comparable canine populations varies remarkably. For instance, 0.8% of shelter dogs were tested positive for hookworm eggs in the Czech Republic (Dubná et al., 2007), 0.9% in Northern Germany (Becker et al., 2012), 9.3% in Italy (Simonato et al., 2015), 26.8% in the Slovak Republic (Szabová et al., 2007), 62.5% in Mexico (Eguia-Aguilar et al., 2005) and 64.9% in Poland (Borecka, 2005).

Compared to *Giardia* and hookworms, faecal forms of other endoparasites were detected considerably less often in the present study. The prevalence rates for infections with *Toxascaris leonina* (9.7%), *Isospora canis* (8.2%) and *Taenia* spp. (1.5%) are similar to previously reported values (Bugg et al., 1999; Martinez-Carrasco et al., 2007; Miro et al., 2007). The low prevalence for tapeworms might be explained by the fact that a single faecal sample per dog was examined. Proglottids of tapeworms are shed inconsistently and eggs are often erratically distributed in the faeces, which might be the reason for a low sensitivity of single coprological examinations (Simonato et al., 2015). However, the finding of two *Taenia*-positive dogs indicates that that the shelter dogs have access to meat containing cysticercoids.

In comparison to other surveys, a higher prevalence for *Hammondia/Neospora*-like oocysts (11.2%) and *Trichuris vulpis* eggs (6.7%) was found in the present study (Bugg et al., 1999; Dubná et al., 2007; Martinez-Carrasco et al., 2007). Even though oocysts of *Hammondia* and *Neospora* share several phenotypical characters, those two members of the Sarcocystidae family are morphologically indistinguishable (Mugridge et al., 1999). *Neospora* is present in Serbia in both dogs as definite hosts (15.4%) and in cows as intermediate hosts (4.6–17.2%) according to previous seroprevalence studies from Vojvodina and South Banat (Gavrilović et al., 2013; Kuruca et al., 2013).

Shelter dogs from other countries harboured *Toxocara canis* (3.0%) more often than the dogs in the present study (Szabová et al., 2007; Simonato et al., 2015). However, prevalence data in the present study especially for ascarids should be evaluated with care since the habit of coprophagia is not unusual in dogs and may induce the presence of ingested immature ascarid eggs (Houpt, 1991). This behaviour might be even boosted by the hygienic situation in shelters with multiple dogs sharing one kennel.