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Abstract

This study aimed to update data on the prevalence of intestinal and lung parasitic infections in owned dogs and cats in Sardinia, Italy. Examinations on fecal samples from 619 dogs and 343 cats routinely referred to the Veterinary Teaching Hospital of the University of Sassari were performed between the years of 2011 and 2015. Individual faecal samples were analysed using the Wisconsin technique for copro-microscopic examination and the Baermann technique for the presence of lungworm larvae.

Endoparasites were found in 34.9% and 43.4% of examined dogs and cats, respectively. Helminthic infections (21.2% in dogs and 32.6% in cats) occurred more frequently than protozoan infections (17.9% in dogs and 17.8% in cats). In both dogs and cats, the most common parasites were ascarids (12.1% and 15.7%), *Cystoisospora* spp. (10.2% and 10.8%), *Giardia duodenalis* (9.4% and 8.5%), and hookworms (7.9% and 5.5%). Evidence of bronchopulmonary nematode infections were found in 0.8% of examined dogs and in 15.8% of examined cats. Age was identified as a risk factor, with animals younger than 6 months more frequently infected than older animals, while no significant association was observed for gender.

This study demonstrated that endoparasites in owned dogs and cats of Sardinia have considerably high prevalence. Veterinary practitioners and pet owners should be more aware of these infections and should adopt more effective and standardized control practices.

Key words: Parasites, lungworms, zoonosis, pets, Sardinia, parasitological updates.

1. Introduction

Sardinia is the second largest island in the Mediterranean sea, with an area of 23,821 square kilometres. It is situated between 38° 51' and 41° 15' latitude north and 8° 8' and 9° 50' longitude east. The island is known as a holiday destination. Statistical data on tourism indicate that more than 1.9 million people visited Sardinia in 2012 (1.9% of tourism in Italy), with a 42% increase in visitors from 2000 to 2012 (Pipia et al., 2014a). The island have also an high numbers of pets; at present 360,406 dogs are listed in the regional canine registries, while no data are available for the cat population (Ministero della Salute, http://www.salute.gov.it/anagcaninapublic_new). This number considerably increases in summer, due to the movement of tourists with their pets, recognised as a potential route for the introduction and spread of endemic and non-endemic pathogens (Otranto et al., 2013; Genchi et al. 2011; Varcasia et al., 2004).

Endoparasitoses caused by intestinal and respiratory helminths are frequently reported in dogs and cats and can lead to severe clinical signs. For this reason, they are regarded as important pathogens in companion animal clinical practice (Riggio et al., 2013; Traversa et al., 2010; Claerebout et al., 2009; Epe, 2009; Sager et al., 2006). Sardinia is endemic for cystic echinococcosis caused by *Echinococcus granulosus* in animals and humans, with 2,111 human hospitalizations between 2001 and 2011 (Centro Nazionale di Referenza per l'Echinococcosi/Idatidosi, "CeNRE"; http://www.izs-sardegna.it/CeNRE_Epu.cfm). Moreover, intestinal and lung parasites of dogs and cats include zoonotic species; *Toxocara* spp. and *Ancylostoma* spp., are responsible for visceral and cutaneous *larva migrans* syndromes in humans, while *Eucoleus aerophilus* is the causative agent of human pulmonary capillariosis (Riggio et al., 2013; Lee et al., 2010; Traversa et al., 2010; Claerebout et al., 2009; Sager et al., 2006; Sommerfelt et al., 2006). In addition, there are suggestions in the medical literature that *Trichuris vulpis* should be added to the list of zoonotic parasitic infections of people (Dunn et al., 2002; Márquez-Navarro et al., 2012). Reports of human infections are few, open to interpretation, and no definitive proof has yet been obtained; the subject, nevertheless, warrants further investigation and consideration. However, no data on these zoonotic parasites in humans are available for Sardinia.

Many of these intestinal parasites display a direct life cycle, with the oral-faecal route being the most common. A major component of their spread is the high number of eggs or oocysts shed into the environment. Therefore, control measures are based on systematic anthelmintic treatments aimed at preventing or reducing the shedding of eggs or oocysts as well as the adoption of hygienic measures to decrease environmental contamination. However, despite the extensive use of anthelmintic treatments, high prevalence of some intestinal nematode species (*e.g.*, *Toxocara canis*, hookworms, and *Trichuris vulpis*) was recently observed in owned dogs in Switzerland and Belgium (Claerebout et al., 2009; Sager et al., 2006), and the reduced efficacy of anthelmintic treatments was thought to be one of the potential causes for these findings (Riggio et al., 2013; Sager et al., 2006).

Epidemiological studies on endoparasites in pets are important to assess prevalence and to establish appropriate measures of veterinary care and pet-owner education. Even though patent infections caused by several internal helminths can be readily diagnosed by faecal examination, copro-microscopic diagnosis is not routinely performed during general veterinary visits since the control of internal parasites usually is achieved by administering anthelmintic treatments at predetermined intervals.

Thus, a constant surveillance of the epidemiology of the most important or emerging parasitoses, such as those caused by endoparasites, is of paramount importance. Hence, the present survey aimed to update data on the prevalence of intestinal and lung parasitic infections in owned dogs and cats and to identify potential risk factors for infection.

2. Materials and methods

Faecal samples of 619 owned dogs (326 males and 293 females) and 343 owned cats (183 females and 160 males) referred between 2011 and 2015 for routine vaccination or clinical visit at the Veterinary Teaching Hospital of the University of Sassari were examined for endoparasites. For each sampled animal, data on sex, age, and stool consistency were recorded. Stool consistency of each sample was classified as normal (well formed), pasty (soft, not well formed) or diarrhoeic (liquid), as described by Pipia et al. (2014b). Faecal samples were withdrawn from the rectum in accordance with animal welfare standards or from the ground immediately after defecation and stored at 4°C until examination; faecal analyses were performed within 24 hours of collection.

Copro-microscopic examination for internal parasites was performed using the Wisconsin technique. Briefly, approximately 3 grams of the faecal sample were examined with centrifugation/flotation (626 g for 10 minutes) using a zinc sulphate ($ZnSO_4$) flotation solution (specific gravity: 1.2) as described by Pipia et al. (2014b). Faecal samples were also examined for the presence of first-stage larvae (L1) of lungworms using the Baermann technique. Approximately 5 grams of the sample were wrapped in a cheesecloth and placed in a funnel filled with water for 24 hours at room temperature. Afterwards, the sediment obtained with centrifugation (626 g for 10 minutes) was microscopically examined at 100 × magnification for L1 (Pipia et al., 2014a).

Ethical statement

This study was executed following the recommendations of European Council Directive (86/609/EEC) on the protection of animals and all the samples were recovered during routine vaccination or clinical visit at the Veterinary Teaching Hospital of the University of Sassari.

2.1. Statistical analysis

Data were recorded into an electronic spreadsheet (Excel®, Microsoft Corp., Redmond, WA, USA), and difference in prevalence were statistically tested using the Chi-Square for independence (Epi-Info® 6.0, CDC/WHO, Atlanta, GA, USA).

3. Results

Overall, 34.9% of examined dogs and 43.4% of cats tested positive for endoparasites; difference between prevalence for the two host species was statistically significant ($\chi^2 = 6.84$; $P = 0.008$). Among positive animals, 76.8% of dogs and 69.1% of cats were infected with one parasite, while 19% of dogs and 24.8% of cats harboured two different parasites. Few positive animals (2.8% of dogs and 5.4% of cats) were parasitized by three different parasites, and only 1.4% of dogs and 0.7% of cats were infected by more than three parasites.

The parasites detected and their prevalence are summarized in Figure 1. Helminth infections (21.2% in dogs and 32.6% in cats; $\chi^2 = 20.1$; $P < 0.0001$) were more frequent than protozoan infections (17.9% and 17.8% in dogs and cats, respectively). In dogs, the detected parasites were ascarids (12.1%; *T. canis*: 11%, *Toxascaris leonina*: 1.1%), *Cystoisospora* sp. (10.2%), *Giardia duodenalis* (9.4%), hookworms (7.9%), *T. vulpis* (3.9%), taeniid (0.6%), and *Sarcocystis* sp. (0.2%). Among cats, the recovered parasites were ascarids (15.7%; *Toxocara cati*: 15.1%, *T. leonina*: 0.6%,), lungworms (15.5%), *Cystoisospora* sp. (10.8%), *G. duodenalis* (8.5%), hookworms (5.5%), *Eucoleus aerophilus* (0.3%) and *Sarcocystis* sp. (0.3%). Interestingly, parasites with known or suggested zoonotic potential (*i.e.*, ascarids, hookworms, *T. vulpis*, *G. duodenalis*, *E. aerophilus*, and taeniids) were found in 34% and 33% of dogs and cats, respectively; the difference between prevalence was not significant ($P > 0.05$), while Taeniid eggs were found in 0.6% of dogs and 3.2% of cats.

Bronchopulmonary nematodes were found in 0.8% of examined dogs and in 15.8% of examined cats (Metastrongyloid nematodes represented 15.5% of these infections, while *E. aerophilus* was present in 0.3%). The prevalence of bronchopulmonary infections was significantly different between dogs and cats (χ^2 Yates corrected = 82.95; $P < 0.0001$). The lungworm *Angiostrongylus vasorum* was detected in dogs, while among cats, the species of bronchopulmonary nematodes found were *Aelurostrongylus abstrusus* (14.3%), *Troglostrongylus* sp. (1.2%), and *E. aerophilus* (0.3%).

The mean age of the sampled dogs was 30.2 months (SD ± 41.3), while that of cats was 21.5 months (SD ± 31.5). The mean age of dogs with parasites detected in faeces was 18.7 months (CI 95%: 15.6 – 21.8) and of cats was 13.5 months (CI 95%: 9.9 – 17.1). In both species, animals younger than 6 months were found to be more frequently infected by endoparasites than were older animals ($P < 0.01$) (Tables 1 and 2).

In dogs, helminth infections were higher in young animals (19%) than in adults (15%), but this was not statistically significant ($P > 0.05$). Protozoan infections were statistically significantly higher in young animals (22%) than in adults (7%) ($\chi^2 = 29.83$; $P < 0.05$). In cats, helminth infections were found more frequently in young animals (28%) than in adults (24.5%); similarly, protozoan infections were higher in young animals (13%) than in adults (9%), but in both cases, differences were not statistically significant ($P > 0.05$).

Parasite prevalence in female dogs (36.5%) was slightly higher than in male dogs (33.4%), while, in cats the prevalence was higher in males (44.4%) than in females (42.6%). However, these differences were not statistically significant ($P > 0.05$).

Of the 619 stool samples from dogs, 78.2% had a normal consistency, while 21.8% were classified as diarrhoeic. A higher frequency of positive samples was found in diarrhoeic stool samples (40.7%; 55/135) than in normal ones (33.3%; 161/484). Among stool samples of cats, 70.6% (242/343) were classified as normal and 29.4% as diarrhoeic. In this species, the lowest frequency of positive samples was found in diarrhoeic stool samples (37.6%; 38/101), while the highest frequency was in normal ones (45.9%; 111/242). No statistical differences were found between positive samples in diarrhoeic and normal stools ($P > 0.05$) in both dogs and cats.

4. Discussion

The present study constitutes the first comprehensive survey on endoparasites in owned dogs and cats of Sardinia. The prevalence of endoparasites found in dogs and cats in this study are higher than those reported in other studies in Italy and in Europe. In Italy, Riggio et al. (2013) reported a prevalence of 31% in dogs and 35% in cats. In Germany, Barutzki and Schaper (2011) observed a prevalence of 30.4% in dogs and 22.8% in cats. A similar study conducted in owned cats in Sardinia described a lower prevalence of endoparasitoses (Porqueddu et al., 2004). On the contrary, a survey carried out in northern Italy among dogs reported a higer prevalence (55.9%), but the population of examined animals was composed of owned, kennel, and stray dogs (Capelli et al., 2006). In northern Italy, the prevalence of endoparasites in dogs and cats was reported with a high degree of variability in three towns in Lombardy (Milan, Bergamo, and Brescia), with values varying from 28.16% to 57.41% in dogs and from 32.58% to 60.42% in cats (Zanzani et al., 2014).

In the present investigation, monospecific infections were more common than polyspecific infections and helminths were recovered more commonly than protozoa. These findings are consistent with results from previous studies (Riggio et al., 2013; Savilla et al., 2011; Mircean et al., 2010; Ramírez-Barrios et al., 2004). Ascarids were the most frequent parasite species detected in both dogs and cats, and our finding was consistent with studies from other countries (Zanzani et al., 2014; Riggio et al., 2013; Becker et al., 2012; Joffe et al., 2011; Clearebout et al., 2009; Gates and Nolan, 2009; Dubnà et al., 2007; Papazahariadou et al. 2007; Pullola et al., 2006; Sommerfelt et al., 2006).

Although *Taeniid* eggs prevalence observed was quite limited, the presence in Sardinia of zoonotic species such as *E. granulosus* cannot be excluded or confirmed, as specie specific diagnosis cannot be performed morphologically (Varcasia et al., 2004; Varcasia et al., 2011)

With respect to bronchopulmonary infections, the prevalence found in cats was higher than that found in a previous study carried out in Sardinia, and higher than that reported from other European countries (Beugnet et al., 2014; Porqueddu et al., 2004). The vast majority of cats examined in this study had constant outdoor access with intense predatory activity on paratenic hosts (*e.g.*, rodents, birds, and lizards); this may account for the high prevalence reported (Giannelli et al., 2015).

Among protozoa, the parasite found most frequently was *Cystoisospora* sp., with a prevalence higher than 10% in both dogs and cats, in accordance with what has been observed in other studies (Barutzki and Schaper, 2011; Mircean et al., 2010; Sommerfelt et al., 2006; Porqueddu et al., 2004; Ramírez-Barrios et al., 2004). *G. duodenalis* was the most frequently diagnosed zoonotic protozoa in dogs and cats, but with prevalence lower than that reported previously in dogs from Sardinia (Pipia et al., 2014b). The prevalence was potentially underestimated in our study since the sensitivity of single faecal examinations can be low in chronic infections (Capelli et al., 2006). Another explanation for the difference is that in the study by Pipia et al. (2014b), the dogs examined were from animal shelters where transmission of *Giardia* could be higher.

Giardia has a genetic heterogeneity with eight distinct assemblages (A-H) two of which, A and B, are infective for humans and animals (Feng and Xiao, 2011). Though no molecular characterization of this parasite was carried out in this survey, the zoonotic potential of *Giardia* cannot be ignored in relation to the presence of assemblage A, subtype A2, reported by Pipia et al. (2014b).

Neospora caninum was never isolated in sampled animals, even it seems widespread in livestock of Sardinia (Varcasia et al., 2006; Tamponi et al., 2015).

In the present study, there was no significant difference in prevalence for any parasite species by host gender. This finding is consistent with results of other European studies (Zanzani et al., 2014; Riggio et al., 2013; Savilla et al., 2011), and indicates that gender does not play a role as a risk factor for endoparasites.

Age was identified as a risk factor. Results of the study indicate that young dogs (< 6 months) were significantly more susceptible to ascarids, *Giardia*, and coccidian infections than were adults. This is consistent with findings of other studies (Mircean et al., 2010; Clearebout et al., 2009; Gates and Nolan, 2009; Palmer et al., 2008; Martínez-Carrasco et al., 2007; Pullola et al., 2006). On the other hand, whipworms, lungworms, and cestodes were more prevalent in adults, as also observed by Riggio et al. (2013), Clearebout et al. (2009), and Gates and Nolan (2009).

The higher occurrence of parasites in young dogs advocates the need for more effective deworming schemes, especially against ascarids, and highlights the importance of protozoan infections in the health of dogs, particularly in the first year of life (Little et al., 2009).

No correlation was found between faecal consistency and presence of parasites and suggest that, in clinical practice, parasites should not be suspected or investigated only in animals with diarrhea.

5. Conclusion

This study showed considerably high prevalence of endoparasites in owned dogs and cats of Sardinia, compared to similar surveys carried out in other European countries.

Our findings may indicate a potential zoonotic risk, especially in the case of *Toxocara* spp.and *Ancylostoma* spp. that in this study were the most frequently isolated parasites.

Veterinary practitioners and pet owners should be aware of these infections and should adopt more effective and standardized control practices, as recommended by the European Specialist Counsel Companion Animal Parasites, which suggests that parasitized animals should be treated to minimise environmental contamination and monitored, where necessary, by faecal examination to confirm treatment efficacy (http://www.esccap.org).

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Figure captions

Figure 1. Prevalence of parasites detected in dogs and cats.

Tables

Table 1. Overall prevalence of endoparasitic infections according to host species and age class.

Table 2. These data refer to prevalence of different endoparasites according to host species and age class.

Table 1. Prevalences of parasites detected in dogs and cats.

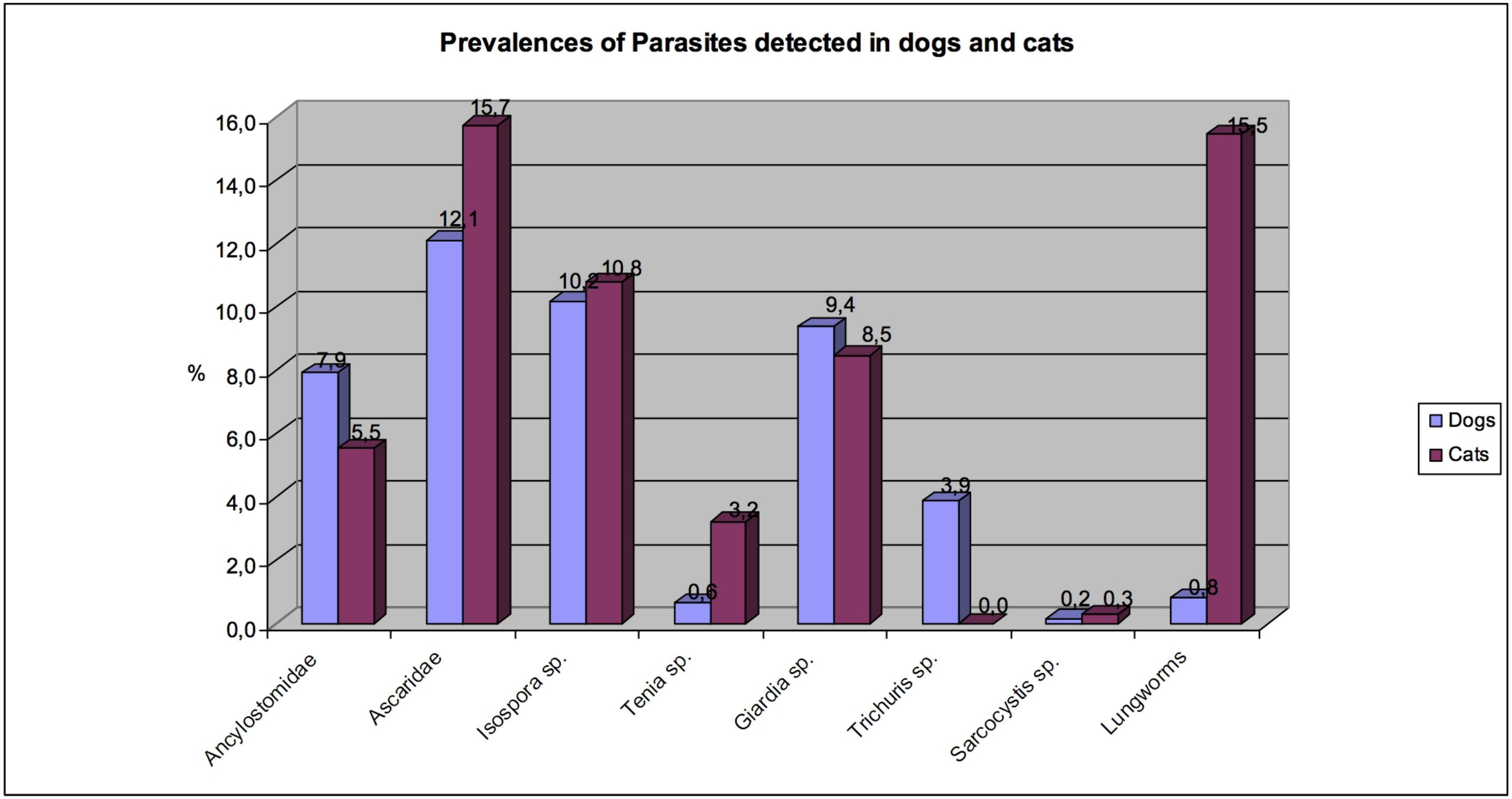

Table 1. Overall prevalence of endoparasitic infections according to host species and class of age.

Species	Age Class (Months)	Examined	Positives	Prevalence (%)	Odds Ratio	χ^2 for linear trend
Dogs	≤ 6	275	131	47.6	1.00	
	> 6 ≤ 12	68	18	26.5	0.40	$\chi^2 = 30.347$
	> 12 ≤ 24	90	23	25.6	0.38	P < 0.00001
	> 24	186	44	23.7	0.34	
Cats	≤ 6	139	63	45.3	1.00	
	> 6 ≤ 12	72	26	36.1	0.68	$\chi^2 = 16.066$
	> 12 ≤ 24	68	16	23.5	0.37	P = 0.00006
	> 24	64	13	20.3	0.31	

Table 2. Prevalence of endoparasites according to host species and class of age.

	Examined	Positives (%)	Hookworms (%)	Ascarids (%)	*Cystoisospora* sp (%)	Taeniid (%)	*Giardia* sp. (%)	*Trichuris vulpis* (%)	*Eucoleus* sp (%)	*Sarcocystis* sp (%)	Metastrongyles* (%)
Dogs											
Total	619	34.9	7.9	12.1	10.2	0.6	9.4	3.9	0.0	0.2	0.8
≤ 6 months	275	47.6	6.9	20.0	17.8	0.4	13.8	0.4	0.0	0.4	0.0
> 6 ≤ 12 months	68	26.5	8.8	5.9	8.8	0.0	11.8	1.5	0.0	0.0	0.0
> 12 ≤ 24 months	90	25.6	10.0	8.9	2.2	0.0	5.6	6.7	0.0	0.0	0.0
> 24 months	186	23.7	8.1	4.3	3.2	1.6	3.8	8.6	0.0	0.0	2.7
Cats											
Total	343	43.4	5.5	15.7	10.8	3.2	8.5	0.0	0.3	0.3	15.5
≤ 6 months	139	45.3	4.3	23.7	18.0	2.2	9.4	0.0	0.0	0.0	12.2
> 6 ≤ 12 months	72	36.1	9.7	15.3	11.1	2.8	8.3	0.0	0.0	1.4	18.1
> 12 ≤ 24 months	68	23.5	5.9	8.8	2.9	5.9	8.8	0.0	1.5	0.0	22.1
> 24 months	64	20.3	3.1	6.3	3.1	3.1	6.3	0.0	0.0	0.0	12.5

** This data referred to first-stage larvae recovered by the Baermann technique*