

Endoparasites detected in faecal samples from dogs and cats referred for routine clinical visit in Sardinia, Italy

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1 **Endoparasites detected in faecal samples from dogs and cats referred for routine clinical visit**
2 **in Sardinia, Italy**

3

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13

14 **Abstract**

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

21 Endoparasites were found in 34.9% and 43.4% of examined dogs and cats, respectively. Helminthic
22 infections (21.2% in dogs and 32.6% in cats) occurred more frequently than protozoan infections
23 (17.9% in dogs and 17.8% in cats). In both dogs and cats, the most common parasites were ascarids
24 (12.1% and 15.7%), *Cystoisospora* spp. (10.2% and 10.8%), *Giardia duodenalis* (9.4% and 8.5%),
25 and hookworms (7.9% and 5.5%). Evidence of bronchopulmonary nematode infections were found
26 in 0.8% of examined dogs and in 15.8% of examined cats. Age was identified as a risk factor, with
27 animals younger than 6 months more frequently infected than older animals, while no significant
28 association was observed for gender.

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

32

33

34 **Key words:** Parasites, lungworms, zoonosis, pets, Sardinia, parasitological updates.
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36

37

1. Introduction

38

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

49 Endoparasitoses caused by intestinal and respiratory helminths are frequently reported in dogs and
50 cats and can lead to severe clinical signs. For this reason, they are regarded as important pathogens
51 in companion animal clinical practice (Riggio et al., 2013; Traversa et al., 2010; Claerebout et al.,
52 2009; Epe, 2009; Sager et al., 2006). Sardinia is endemic for cystic echinococcosis caused by
53 *Echinococcus granulosus* in animals and humans, with 2,111 human hospitalizations between 2001
54 and 2011 (Centro Nazionale di Referenza per l'Echinococcosi/Idatidiosi, "CeNRE"; [http://www.izs-](http://www.izs-sardegna.it/CeNRE_Epu.cfm)
55 [sardegna.it/CeNRE_Epu.cfm](http://www.izs-sardegna.it/CeNRE_Epu.cfm)). Moreover, intestinal and lung parasites of dogs and cats include
56 zoonotic species; *Toxocara* spp. and *Ancylostoma* spp., are responsible for visceral and cutaneous
57 *larva migrans* syndromes in humans, while *Eucoleus aerophilus* is the causative agent of human
58 pulmonary capillariosis (Riggio et al., 2013; Lee et al., 2010; Traversa et al., 2010; Claerebout et
59 al., 2009; Sager et al., 2006; Sommerfelt et al., 2006). In addition, there are suggestions in the
60 medical literature that *Trichuris vulpis* should be added to the list of zoonotic parasitic infections of
61 people (Dunn et al., 2002; Márquez-Navarro et al., 2012). Reports of human infections are few,
62 open to interpretation, and no definitive proof has yet been obtained; the subject, nevertheless,
63 warrants further investigation and consideration. However, no data on these zoonotic parasites in
64 humans are available for Sardinia.

65 Many of these intestinal parasites display a direct life cycle, with the oral-faecal route being the
66 most common. A major component of their spread is the high number of eggs or oocysts shed into
67 the environment. Therefore, control measures are based on systematic anthelmintic treatments
68 aimed at preventing or reducing the shedding of eggs or oocysts as well as the adoption of hygienic

69 measures to decrease environmental contamination. However, despite the extensive use of
70 anthelmintic treatments, high prevalence of some intestinal nematode species (*e.g.*, *Toxocara canis*,
71 hookworms, and *Trichuris vulpis*) was recently observed in owned dogs in Switzerland and
72 Belgium (Claerebout et al., 2009; Sager et al., 2006), and the reduced efficacy of anthelmintic
73 treatments was thought to be one of the potential causes for these findings (Riggio et al., 2013;
74 Sager et al., 2006).

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

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

85

86 **2. Materials and methods**

87

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

96 Copro-microscopic examination for internal parasites was performed using the Wisconsin
97 technique. Briefly, approximately 3 grams of the faecal sample were examined with
98 centrifugation/flotation (626 g for 10 minutes) using a zinc sulphate (ZnSO₄) flotation solution
99 (specific gravity: 1.2) as described by Pipia et al. (2014b). Faecal samples were also examined for
100 the presence of first-stage larvae (L1) of lungworms using the Baermann technique. Approximately
101 5 grams of the sample were wrapped in a cheesecloth and placed in a funnel filled with water for 24

102 hours at room temperature. Afterwards, the sediment obtained with centrifugation (626 g for 10
103 minutes) was microscopically examined at 100 × magnification for L1 (Pipia et al., 2014a).

104

105 Ethical statement

106 This study was executed following the recommendations of European Council Directive
107 (86/609/EEC) on the protection of animals and all the samples were recovered during routine
108 vaccination or clinical visit at the Veterinary Teaching Hospital of the University of Sassari.

109

110 2.1. Statistical analysis

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

114

115 3. Results

116

117 Overall, 34.9% of examined dogs and 43.4% of cats tested positive for endoparasites; difference
118 between prevalence for the two host species was statistically significant ($\chi^2 = 6.84$; $P = 0.008$).
119 Among positive animals, 76.8% of dogs and 69.1% of cats were infected with one parasite, while
120 19% of dogs and 24.8% of cats harboured two different parasites. Few positive animals (2.8% of
121 dogs and 5.4% of cats) were parasitized by three different parasites, and only 1.4% of dogs and
122 0.7% of cats were infected by more than three parasites.

123 The parasites detected and their prevalence are summarized in Figure 1. Helminth infections (21.2%
124 in dogs and 32.6% in cats; $\chi^2 = 20.1$; $P < 0.0001$) were more frequent than protozoan infections
125 (17.9% and 17.8% in dogs and cats, respectively). In dogs, the detected parasites were ascarids
126 (12.1%; *T. canis*: 11%, *Toxascaris leonina*: 1.1%), *Cystoisospora* sp. (10.2%), *Giardia duodenalis*
127 (9.4%), hookworms (7.9%), *T. vulpis* (3.9%), taeniid (0.6%), and *Sarcocystis* sp. (0.2%). Among
128 cats, the recovered parasites were ascarids (15.7%; *Toxocara cati*: 15.1%, *T. leonina*: 0.6%),
129 lungworms (15.5%), *Cystoisospora* sp. (10.8%), *G. duodenalis* (8.5%), hookworms (5.5%),
130 *Eucoleus aerophilus* (0.3%) and *Sarcocystis* sp. (0.3%). Interestingly, parasites with known or
131 suggested zoonotic potential (*i.e.*, ascarids, hookworms, *T. vulpis*, *G. duodenalis*, *E. aerophilus*, and
132 taeniids) were found in 34% and 33% of dogs and cats, respectively; the difference between
133 prevalence was not significant ($P > 0.05$), while Taeniid eggs were found in 0.6% of dogs and 3.2%
134 of cats.

135 Bronchopulmonary nematodes were found in 0.8% of examined dogs and in 15.8% of examined
136 cats (Metastrongyloid nematodes represented 15.5% of these infections, while *E. aerophilus* was
137 present in 0.3%). The prevalence of bronchopulmonary infections was significantly different
138 between dogs and cats (χ^2 Yates corrected = 82.95; $P < 0.0001$). The lungworm *Angiostrongylus*
139 *vasorum* was detected in dogs, while among cats, the species of bronchopulmonary nematodes
140 found were *Aelurostrongylus abstrusus* (14.3%), *Troglostrongylus* sp. (1.2%), and *E. aerophilus*
141 (0.3%).

142 The mean age of the sampled dogs was 30.2 months (SD \pm 41.3), while that of cats was 21.5
143 months (SD \pm 31.5). The mean age of dogs with parasites detected in faeces was 18.7 months (CI
144 95%: 15.6 – 21.8) and of cats was 13.5 months (CI 95%: 9.9 – 17.1). In both species, animals
145 younger than 6 months were found to be more frequently infected by endoparasites than were older
146 animals ($P < 0.01$) (Tables 1 and 2).

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

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

156 Of the 619 stool samples from dogs, 78.2% had a normal consistency, while 21.8% were classified
157 as diarrhoeic. A higher frequency of positive samples was found in diarrhoeic stool samples
158 (40.7%; 55/135) than in normal ones (33.3%; 161/484). Among stool samples of cats, 70.6%
159 (242/343) were classified as normal and 29.4% as diarrhoeic. In this species, the lowest frequency
160 of positive samples was found in diarrhoeic stool samples (37.6%; 38/101), while the highest
161 frequency was in normal ones (45.9%; 111/242). No statistical differences were found between
162 positive samples in diarrhoeic and normal stools ($P > 0.05$) in both dogs and cats.

163

164 **4. Discussion**

165

166 The present study constitutes the first comprehensive survey on endoparasites in owned dogs and
167 cats of Sardinia. The prevalence of endoparasites found in dogs and cats in this study are higher
168 than those reported in other studies in Italy and in Europe. In Italy, Riggio et al. (2013) reported a

169 prevalence of 31% in dogs and 35% in cats. In Germany, Barutzki and Schaper (2011) observed a
170 prevalence of 30.4% in dogs and 22.8% in cats. A similar study conducted in owned cats in Sardinia
171 described a lower prevalence of endoparasitoses (Porqueddu et al., 2004). On the contrary, a survey
172 carried out in northern Italy among dogs reported a higher prevalence (55.9%), but the population of
173 examined animals was composed of owned, kennel, and stray dogs (Capelli et al., 2006). In
174 northern Italy, the prevalence of endoparasites in dogs and cats was reported with a high degree of
175 variability in three towns in Lombardy (Milan, Bergamo, and Brescia), with values varying from
176 28.16% to 57.41% in dogs and from 32.58% to 60.42% in cats (Zanzani et al., 2014).

177 In the present investigation, monospecific infections were more common than polyspecific
178 infections and helminths were recovered more commonly than protozoa. These findings are
179 consistent with results from previous studies (Riggio et al., 2013; Savilla et al., 2011; Mircean et al.,
180 2010; Ramírez-Barrios et al., 2004). Ascarids were the most frequent parasite species detected in
181 both dogs and cats, and our finding was consistent with studies from other countries (Zanzani et al.,
182 2014; Riggio et al., 2013; Becker et al., 2012; Joffe et al., 2011; Clearebout et al., 2009; Gates and
183 Nolan, 2009; Dubnà et al., 2007; Papazahariadou et al. 2007; Pullola et al., 2006; Sommerfelt et al.,
184 2006).

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

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

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

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

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

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

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

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

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

223

224 **5. Conclusion**

225

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

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

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

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381 **Figure captions**

382 **Figure 1.** Prevalence of parasites detected in dogs and cats.
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385 **Tables**

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

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

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

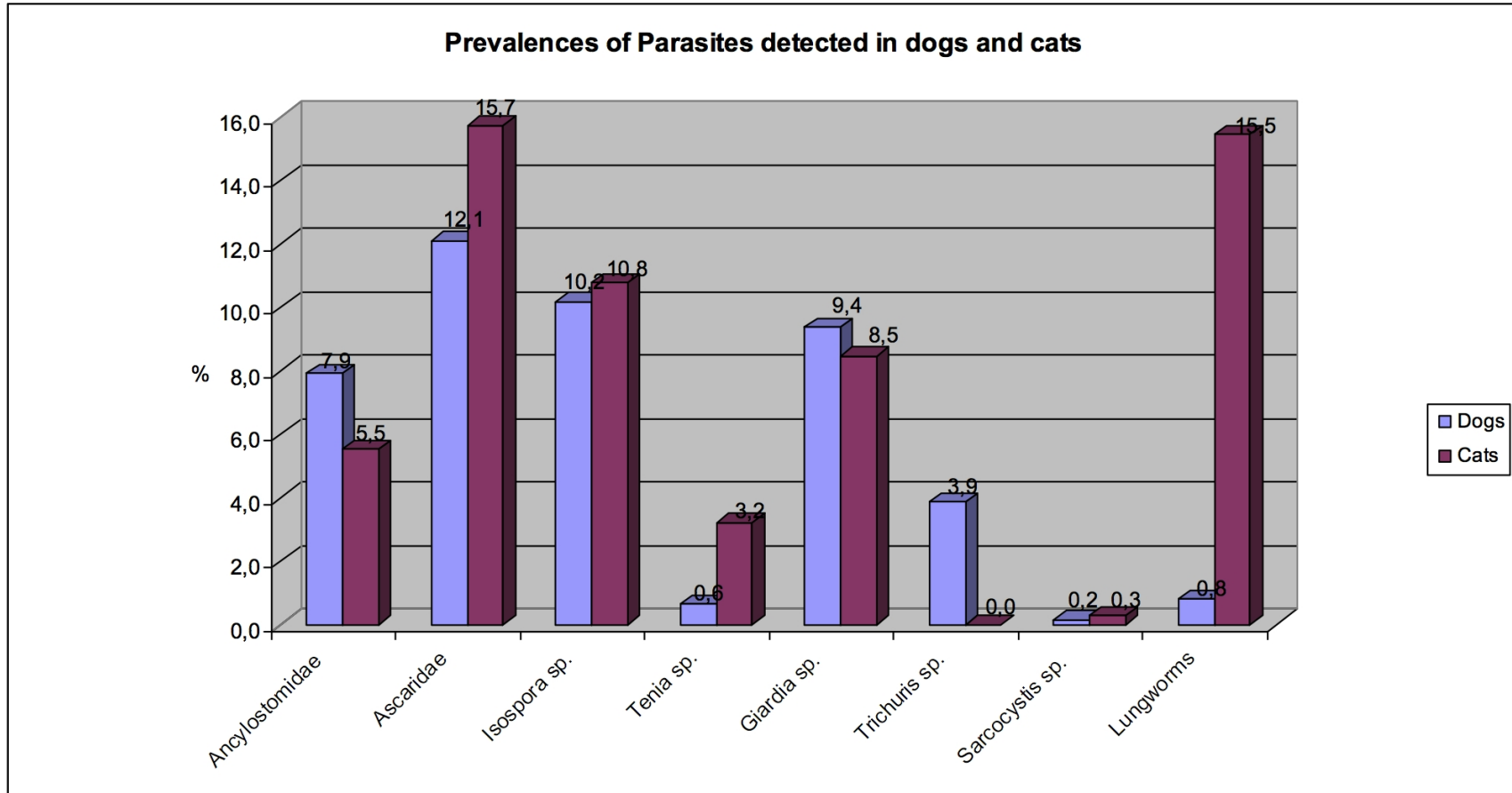


Table 1. Overall prevalence of endoparasitic infections according to host species and class of age.

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

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

	Examined	Positives (%)	Hookworms (%)	Ascarids (%)	<i>Cystoisospora</i> sp (%)	Taeniid (%)	<i>Giardia</i> sp. (%)	<i>Trichuris vulpis</i> (%)	<i>Eucoleus</i> sp (%)	<i>Sarcocystis</i> sp (%)	Metastrongyl es* (%)
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