

1 **High prevalence and diversity of zoonotic and other intestinal parasites in dogs**  
2 **from Eastern Spain**

3

4 Paula Sánchez-Thevenet<sup>1,\*</sup>, David Carmena<sup>2,\*</sup>, Manuel Adell-Aledón<sup>1</sup>, Elena Dacal<sup>2</sup>,  
5 Eva Arias<sup>2</sup>, José María Saugar<sup>2</sup>, Esperanza Rodríguez<sup>2</sup>, María Auxiliadora Dea-  
6 Ayuela<sup>3</sup>

7 <sup>1</sup> Department of Medicine, Cardenal Herrera-CEU University, CEU Universities,  
8 Castellón de la Plana, Castellón, Spain

9 <sup>2</sup> Parasitology Reference and Research Laboratory, National Centre for  
10 Microbiology, Health Institute Carlos III, Majadahonda, Madrid, Spain

11 <sup>3</sup> Faculty of Health Sciences, Cardenal Herrera CEU University, CEU Universities,  
12 Alfara del Patriarca, Valencia, Spain

13 \*Corresponding author

14

15

16

17

18

19

20

21

22

23

24

25       **Abstract**

26           The diversity and frequency of enteric parasites in dog populations in the  
27       Castellón province (Eastern Spain) was assessed by means of a prospective cross-  
28       sectional epidemiological survey. A total of 263 canine faecal samples were  
29       collected between July 2014 and July 2016. Detection of intestinal parasites was  
30       conducted by routine coprological methods. In addition, identification of *Giardia*  
31       *duodenalis* and *Cryptosporidium* spp. was carried out by direct immunofluorescence  
32       microscopy, whereas the presence of *Strongyloides* spp. was assessed by real-time  
33       PCR in a selected number of specimens. Based on conventional and/or  
34       immunofluorescence microscopy examination, 65.8% (95% CI: 59.7%–71.5%) of  
35       the investigated dogs were found infected by at least one gastrointestinal parasite.  
36       *Giardia duodenalis* (35.4%) and members of the family Ancylostomatidae (27.0%)  
37       were the most prevalent protozoan and helminth parasites found, respectively. Other  
38       pathogens potentially infective to human included *Toxocara canis* (8.0%),  
39       *Cryptosporidium* spp. (6.8%), and *Strongyloides* spp. (1.1%). Frequency of  
40       occurrence of helminthic, but not protozoan, enteroparasites was geographical  
41       origin-dependent ( $P = 0.02$ ), with dogs living in coastal areas presenting higher  
42       infection rates than those living in inland regions. Similarly, rural dogs were  
43       significantly more infected than urban dogs ( $P < 0.001$ ). Our results revealed that  
44       zoonotic agents were common in dogs from the Castellón province. Animals from  
45       rural areas and sheltered dogs were particularly at risk of these infections.

46

47       *Keywords:* Intestinal parasites; Dogs; Prevalence; Zoonoses; Spain.

48

49

## 50        **Introduction**

51    Intestinal parasites including a wide range of helminth and protozoan species are  
52    common infectious agents of dogs. Some of them can be transmitted to humans causing  
53    diseases such as hydatidosis by *Echinococcus granulosus* sensu lato (s.l.), giardiasis by  
54    *Giardia duodenalis*, and cryptosporidiosis by *Cryptosporidium* spp. (Soriano et al.  
55    2010; Deplazes et al. 2011; Overgaauw and Knapen 2013; Otranto et al. 2017).

56        Many studies on canine intestinal parasites have been conducted worldwide with  
57    heterogeneous results. Dogs, primarily stray and semi-domesticated animals, living in  
58    poor-resource settings with favourable environmental features for pathogen  
59    transmission harbour greater diversity and higher prevalences (above 80%) of parasitic  
60    infections (Dantas-Torres and Otranto 2014). In developed countries, where domestic  
61    dogs are generally well-cared and under adequate sanitary conditions, several surveys  
62    have revealed rates of intestinal parasitic infections in pet dogs typically ranging from  
63    20% to 30%, although large variations may occur among different dog populations and  
64    geographical areas (Dubná et al. 2007; Claerebout et al. 2009; Zanzani et al. 2014). In  
65    Spain, the presence of intestinal parasites in canine populations has been investigated in  
66    a limited number of epidemiological surveys conducted in the autonomous regions  
67    (ARs) of Andalusia (Martínez-Moreno et al. 2007), Aragon (Causapé et al., 1996), the  
68    Basque Country (Benito et al. 2003), Cataluña (Gracenea et al., 2009; Ortuño et al.  
69    2014), Madrid (Miró et al., 2007), and Murcia (Martínez-Carrasco et al. 2007) during  
70    the period 1992–2014. No relevant reports in the field have been published in the  
71    country since then.

72        The aim of this work was to assess the diversity and frequency of intestinal  
73    parasites in both urban and rural dogs from Castellón Province in the AR of Valencia,

74 an area where the intestinal parasite fauna in the canine host has not been investigated  
75 yet.

76

## 77 **Material and methods**

### 78 **Study area and design**

79 Castellón is a province of the AR of Valencia in Eastern Spain. It has a surface area of  
80 over 6,632 km<sup>2</sup> and has a total population of 582,327 inhabitants. Most of the  
81 population lives in the coastal strip (a third of them in the capital city Castellón de la  
82 Plana), whereas the mountainous interior is practically uninhabited. The province is  
83 divided in eight administrative regions called “*comarcas*” (FIG. 1). Agriculture and  
84 livestock raising constitute the principal economic activities of the province. The  
85 climate of the region is typically Mediterranean, characterized by mild, rainy winters  
86 and warm, dry summers.

87         There were 151,311 domestic dogs officially censused in Castellón in 2015,  
88 21,936 of them belonging to hound-type breeds commonly used in hunting, whereas an  
89 undetermined number of guard or shepherd dogs were used in agricultural exploitations  
90 in rural areas (Registro Informático Valenciano de Identificación Animal 2015).

91 Overall. Stray, abandoned, or surrendered animals in the province were managed by  
92 five private animal shelters, one of them also acting as a licensed breeding kennel.

93 Surveyed animals were categorised as pet dogs, breeding dogs, sheltered dogs, shepherd  
94 dogs and hunting dogs. To achieve the objectives of the present project a cross-sectional  
95 study was carried out in this Spanish province between July 2014 and July 2016.

96

### 97 **Faecal sample collection**

98 Estimated sample size ( $n = 217$ ) was calculated using Open Source Epidemiological  
99 Statistics for Public Health OpenEpi 3.01 software (Dean 2013). Power was set  
100 considering an expected prevalence of 50%, a marginal error of 7% with a 95%  
101 confidence interval (CI), and a loss rate of 10%. A total of 263 faecal dropping samples  
102 from individual dogs were regularly collected during the study period. Faecal specimens  
103 belonged to dogs attended at four of the five animal shelters located at the province ( $n =$   
104 139), breeding dogs for sale ( $n = 18$ ), hunting ( $n = 68$ ), shepherd ( $n = 24$ ) and pet ( $n =$   
105 14) dogs. Faecal specimens were placed in screw-topped specimen containers and  
106 uniquely labelled indicating identification number and date of collection. Data on sex,  
107 age, status, and geographical origin of the dog and consistency of the faecal material  
108 were also recorded.

109

#### 110 **Parasitological procedures**

111 Faeces were stored at 4 °C in 5% (v/v) formaldehyde until further treatment.  
112 Macroscopic examination was firstly performed for detection of proglottids and adult  
113 worms. After homogenization, each faecal sample was divided into two aliquots. In  
114 order to detect parasite eggs, cysts and oocysts, one aliquot was analysed using routine  
115 coprological procedures based on the modified Ritchie formalin-ether and Sheather's  
116 sugar flotation concentration methods (Thienpont et al. 1979). Each sample was  
117 microscopically examined at 10× in triplicate and suspected parasite structures  
118 confirmed at 40× magnification in a Leica DM500 microscope (Wetzlar, Germany).  
119 Parasite eggs, cysts and oocysts were identified according to their morphometric  
120 characteristics. The second aliquot was used to assess the presence of *Giardia*  
121 *duodenalis* cysts and *Cryptosporidium* spp. oocysts by direct fluorescent antibody test  
122 (DFAT) using a commercially available kit (MERIFLUOR® *Cryptosporidium*/*Giardia*,

123 Meridian Bioscience, EE.UU). A sample was recorded as positive if at least one parasite  
124 form was observed by any given method.

125

### 126 **DNA extraction and purification**

127 The presence of *Strongyloides* spp. was assessed by a PCR-based method (see below) in  
128 a limited number of faecal samples from dogs considered at higher risk of being in  
129 contact with the nematode, including shepherd and hunting dogs. Aliquots of selected  
130 faecal samples were stored in 70% ethanol. Total DNA was extracted from ~200 mg of  
131 faecal material using the QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany)  
132 following the manufacturer's instructions. Purified DNA samples (200 µL) were stored  
133 at -20 °C and shipped to the Parasitology Reference and Research Laboratory, Spanish  
134 National Centre for Microbiology (Majadahonda) for further PCR testing.

135

### 136 **Molecular detection of *Strongyloides* spp.**

137 Genus-specific (F: 5'–GAATTCCAAGTAAACGTAAGTCATTAGC–3' and R: 5'–  
138 TGCCTCTGGATATTGCTCAGTTC–3') primers were used to amplify a partial  
139 sequence of the small subunit ribosomal RNA (*ssu* rDNA) gene of *Strongyloides* spp.  
140 (Verweij et al. 2009) by a qualitative real-time PCR (qPCR) assay as described  
141 elsewhere (Saugar et al. 2015). qPCR reactions (25 µL) contained 1× Quantimix  
142 EasyMaster Mix (Biotools B&M Laboratories, Madrid, Spain), 0.2 µM of each specific  
143 primer, 0.5 µL of 50× SybrGreen (Invitrogen, San Diego, CA, USA), and 10 µL of total  
144 DNA extracted from faecal specimens. Purified genomic DNA from *Strongyloides*  
145 *venezuelensis* L3 was used as positive control. All DNA isolates were assayed in  
146 duplicate. An internal inhibition control including 10 ng of *S. venezuelensis* DNA was  
147 used for each sample. Negative and no template controls were included in each run.

148 Cycling conditions were 15 min at 95 °C followed by 50 cycles of 10 s at 95 °C, 10 s at  
149 60 °C and 30 s at 72 °C. DNA amplification and detection of fluorescence at the end of  
150 each amplification cycle were performed on a Corbett Rotor Gene™ 6000 real-time  
151 PCR system (Qiagen). Data were analysed with Rotor Gene 6000 Series software  
152 version 1.7.

153

## 154 **Data analyses**

155 Infection rates of total and individual intestinal parasites were determined. The Chi-  
156 square ( $\chi^2$ ) test was used to compare total and individual parasite infection rates in the  
157 canine population under study by sex, status, and geographical origin of the animals.  
158 Prevalence risk ratios (PRR) with 95% confidence intervals (CI) were also calculated to  
159 assess the association between the above mentioned variables and the occurrence of  
160 enteric parasite infections. A probability ( $P$ ) value  $< 0.05$  was considered evidence of  
161 statistical significance. Data were analysed with the free software RStudio Version  
162 1.0.44 (<https://www.rstudio.com/>) using the EpiTools library.

163

## 164 **Results**

165 The study included dogs from six of the eight administrative regions of the Castellón  
166 Province (FIG. 1). Intestinal parasites were found in 65.8% (173/263; 95% CI: 59.7%–  
167 71.5%) of the faecal samples analysed. Frequencies of appearance of each individual  
168 parasite species are shown in Table 1.

169 Helminth (38.4%; 101/263) and protozoa (43.0%; 113/263) infections were  
170 observed in the examined samples with no significant differences ( $P > 0.05$ ). In  
171 addition, co-infections involving protozoan and helminth species were detected in  
172 15.6% (41/263) of the samples, whereas no parasite infections were found in 34.2%

173 (90/263) of the faecal specimens examined. Overall, seven species of protozoa, three of  
174 cestodes, and seven of nematodes were identified. Intestinal protozoa were the most  
175 frequent type of enteric parasites identified in all dog groups excepting shepherd and  
176 hunting dogs, which were primarily infected by nematodes (Table 1).

177 The most frequently observed parasite species were *Giardia duodenalis* (35.4%),  
178 followed by hookworms (27.0%), *Toxocara canis* (8.0%), and *Trichuris vulpis* (6.8%).  
179 However, these figures varied largely depending on the dog's status considered (Table  
180 1). Out of the 93 *Giardia*-positive samples, 90 were detected only by DFAT, whereas  
181 the remaining three tested positive both by microscopy examination and DFAT.  
182 Additionally, two shepherd dogs carried sporulated *Eimeria* spp. oocysts. Because this  
183 coccidia does not naturally infect dogs this finding very likely reflects events of  
184 coprophagy or predation of other species. In samples with a positive result to any given  
185 intestinal parasite, single and multiple (two or more) infections were identified in 36.5%  
186 (96/263) and 29.3% (77/263) of the cases, respectively. Double ( $n = 48$ ), triple ( $n = 21$ ),  
187 quadruple ( $n = 4$ ), and quintuple ( $n = 4$ ) infections were recorded. Co-infection by *G.*  
188 *duodenalis* and *Ancylostoma caninum* was the most frequent association identified  
189 (9.4%; 33/173). The distribution of single and multiple parasite infections according to  
190 the status of the investigated dogs is shown in Table 2. Interestingly, urban (pet and  
191 breeding) dogs were significantly less likely ( $\chi^2 = 24.8$ ;  $P < 0.001$ ) to harbour parasitic  
192 infections than rural (shepherd and hunting) dogs.

193 Table 3 shows the assessment of risk factors that may influence the occurrence  
194 and transmission patterns of canine intestinal parasites in the present study. In order to  
195 increase statistical power, shepherd and hunting dogs were grouped together and  
196 categorised as rural animals, whereas pet and breeding dogs were regarded as urban  
197 animals. Similarly, and based on their region of origin, dogs were allocated between



198 two categories: inland (municipalities of Alcaatén, Alto Palancia, and Alto Maestrazgo)  
199 and coastal (Bajo Maestrazgo, Plana Alta, and Plana Baja) areas (see also FIG. 1). The  
200 male/female ratio was 1.5. Although intestinal parasites were more prevalent in female  
201 (69.1%; 65/94) than in male (58.1%; 79/136) dogs, sex was not a risk factor for  
202 infection ( $P = 0.09$ ). As expected, rural dogs were at higher risk of harbouring  
203 enteroparasites than urban dogs (PRR: 1.51;  $P < 0.001$ ). However, no statistically  
204 significant differences in the occurrence of enteric pathogens were demonstrated  
205 between dogs living in coastal areas and those living in inner regions of the Castellón  
206 province (PRR: 1.24;  $P = 0.12$ ).

207       Importantly, surveyed dogs were demonstrated to frequently harbour intestinal  
208 protozoan and helminth species potentially infective to humans including *G. duodenalis*  
209 (35.4%), hookworms (27.0%), *T. canis* (8.0%), *Cryptosporidium* spp. (6.8%),  
210 *Dipylidium caninum* (1.9%), members of the family Taeniidae (1.5%), and *Blastocystis*  
211 spp. (1.1%). Of note, three dogs (1.1%) were initially found positive for *Strongyloides*  
212 spp. at microscopy. Considering that Castellón has been long regarded as an endemic  
213 area for *S. stercoralis*, and that human strongyloidosis cases are still sporadically  
214 detected in the province (Martinez-Perez and Lopez-Velez 2015), we further expanded  
215 this preliminary finding. Thus, the presence of *Strongyloides* spp. was assessed by  
216 molecular methods in selected faecal samples ( $n = 87$ ) from dogs considered at higher  
217 risk of being infected by the parasite, including shepherd ( $n = 16$ ) and hunting ( $n = 19$ )  
218 dogs. A number of sheltered dogs ( $n = 52$ ) were also tested based on recently published  
219 literature (Paradies et al. 2017). Interestingly, PCR-positive results for *Strongyloides*  
220 were obtained in 13.8% (12/87) of the dogs investigated. The infection was more  
221 prevalent in shepherd (25.0%, 4/16) than in sheltered (15.4%, 8/52) dogs, but was not

222 detected in hunting dogs. Neither sex nor origins of the animals were significantly  
223 associated to higher *Strongyloides* spp. infection rates.

224

## 225 **Discussion**

226 This study provides the first description of the diversity and frequency of intestinal  
227 parasites in dogs from the Castellón province. The overall canine infection rate (66%)  
228 recorded here is one of the highest reported in Spain to date, only behind of those  
229 (~70%) previously identified primarily by microscopy in southern (Martínez-Moreno et  
230 al. 2007) and north-eastern Spain (Ortuño et al. 2014). Additionally, a high diversity of  
231 intestinal parasites was also identified, including seven protozoa, three cestode, and  
232 seven nematode species. Taken together, these data depict an epidemiological scenario  
233 characterised by elevated prevalences leading to high infection and (very likely) re-  
234 infection rates.

235 In the European context, our results are in agreement with those documented in  
236 Belgium (Claerebout et al. 2009), Czech Republic (Dubná et al. 2007), France (Osman  
237 et al. 2015), Germany (Barutzki and Schaper 2003), Greece (Kostopoulou et al. 2017),  
238 Italy (Zanzani et al. 2014), and Portugal (Mateus et al. 2014). In these surveys *G.*  
239 *duodenalis*, *A. caninum*, and *T. canis* were demonstrated to be the most common  
240 endoparasite species infecting dogs, although variations in parasite diversity and  
241 frequency rates were often reported among different dog populations and geographical  
242 areas. Of note, the *G. duodenalis* infection rates observed in the present study (up to  
243 43.2% in sheltered dogs), together with that (43.9%) previously reported in Belgium  
244 also by DFAT (Claerebout et al. 2009) are among the highest documented in Europe to  
245 date. This fact is probably associated to the superior diagnostic sensitivity of DFAT  
246 compared to conventional microscopy, and the high infection pressures and crowded

247 conditions commonly seen in kennelled dogs (Gil et al. 2017; Adell-Aledón et al. 2018).  
248 Indeed, sheltered dogs harboured the highest parasite diversity (15 species) detected in  
249 the present survey.

250 Interestingly, shepherd and hunting dogs (both categories linked to rural  
251 activities) were significantly more infected by helminth species than dogs from urban  
252 areas such as pet and breeding dogs. Thus, infections by hookworms (50–76%) and *T.*  
253 *canis* (7–17%) were particularly abundant among the former dog categories. Similar  
254 prevalence rates have been previously reported in farm and hunting dogs for *A. caninum*  
255 (70%) in neighbour Portugal (Mateus et al. 2014), and for *T. canis* (13%) in Greece  
256 (Papazahariadou et al. 2007). These findings are indicative of failure of dog owners to  
257 comply with prescribed deworming protocols.

258 Data presented here are also relevant from a public veterinary health perspective.  
259 Among the recovered protozoa, *G. duodenalis* was the most prevalent species.  
260 Importantly, *G. duodenalis* was present in 36% and 28% of the pet and breeding dogs  
261 analysed, respectively. Because of their close contact with their owners, these animals  
262 may act as potential sources of human giardiasis. In this regard, it should be noted that  
263 zoonotic sub-assemblages AII, BIII, and BIV of the parasite have been previously  
264 described in sheltered dogs in northern Spain, although the genotypes found seemed  
265 primarily transmitted within canine cycles and posed therefore limited risk to humans  
266 (Gil et al. 2017). Furthermore, no evidence of zoonotic (or anthroponotic) transmission  
267 of *G. duodenalis* was demonstrated between humans and pet dogs sharing households in  
268 the geographical area (de Lucio et al. 2017). Similar results and conclusions were  
269 reached for the molecular characterization of the *G. duodenalis* samples generated in the  
270 present survey, as described elsewhere (Adell-Aledón et al. 2018). Taken together, all  
271 these molecular data indicate that domestic dogs do not play a relevant role as natural

272 source of human giardiasis in Spain. Other zoonotic protozoan parasites including  
273 *Blastocystis* spp. and *Cryptosporidium* spp., were found at lower rates.

274 *Toxocara canis* represents an important public health concern not only in  
275 developing countries but also in industrialized settings with adequate sanitary facilities  
276 (Stolk et al. 2016; Salas-Coronas et al. 2018). Over the last few years, toxocariasis has  
277 gained an increasing international attention and was listed among the five most  
278 neglected parasitic infections according to the US Centers for Disease Control and  
279 Prevention (Chen et al. 2018). Human toxocariasis has been described in more than 100  
280 countries, with Spain ranking first among the European countries reporting cases of the  
281 visceral form of the disease. Humans acquire the infection via contact with soil  
282 contaminated with *Toxocara* eggs. *Toxocara* worms have a tendency to cause extra-  
283 intestinal pathologies including four clinical (visceral larva migrans, ocular toxocariasis,  
284 covert toxocariasis, neurotoxocariasis) forms which can lead to serious health  
285 consequences. Due to the non-specific symptoms of this disease, its medical and public  
286 health impact might be underestimated (Chen et al. 2018). The high prevalence of  
287 *Toxocara* in dogs poses also a considerable public health risk as the eggs are  
288 environmentally resistant. Considering that the latest available treatment protocols have  
289 improved the control of the disease (Rehbein et al. 2017), it should be emphasized that  
290 regular pet deworming would be a useful tool to reduce this problem.

291 Also noteworthy was the finding of taenid eggs in faecal specimens belonging to  
292 sheltered and hunting dogs. The family Taeniidae comprises cestodes of the genus  
293 *Taenia* and *Echinococcus*, important (and neglected) zoonotic helminths of dogs whose  
294 eggs are morphologically indistinguishable at microscopy examination. Although we  
295 did not conduct any molecular test for the specific detection of *E. granulosus* s.l. (the  
296 causal agent of human CE or hydatid disease), the possibility that some of the

297 investigated dogs were naturally infected by this cestode cannot be completely ruled  
298 out. Indeed, an *E. granulosus* infection rate of 0.5% (5/1,040) by necropsy has been  
299 previously described in sheltered dogs in Northern Spain (Benito et al. 2003).  
300 Therefore, more studies are required to investigate the current epidemiological situation  
301 of canine equinococcosis in this geographical area. One of the most intriguing  
302 contributions of this paper was the detection of *Strongyloides* spp. in a significant  
303 number of shepherd and sheltered (but not hunting) dogs. Members of the family  
304 Canidae and Felidae are considered suitable hosts for a number of *Strongyloides* species  
305 including *S. stercoralis*, the etiological agent of human strongyloidosis (Thamsborg et  
306 al. 2017). Whether domestic dogs can act as suitable reservoirs of human infections  
307 remains a matter of intense debate, but a recent molecular survey conducted in rural  
308 Cambodia has demonstrated that humans and their dogs can be infected by the same  
309 genetic variant of *S. stercoralis* (Jaleta et al. 2017). Arguing in favour of the occurrence  
310 of zoonotic transmission, the authors suggested that in order to reduce the exposure of  
311 humans to infective *S. stercoralis* larvae, dogs should be treated against the infection  
312 along with their owner. In Europe there are few studies on the prevalence of this  
313 parasite in dogs. The infected animals were usually asymptomatic and when signs and  
314 symptoms appeared they were unspecific. However, the increase of human  
315 strongyloidiasis cases diagnosed globally has lead the scientific community to  
316 reconsider the role of domestic dogs as potential natural reservoirs of human infections  
317 (Paradies et al. 2017). Imported human strongyloidiasis associated to immigrant  
318 populations and returning travellers from endemic areas is increasingly reported in  
319 Spain (Martinez-Perez et al. 2018, Belhassen-García et al. 2017), although in Castellón  
320 Province sporadic autochthonous cases of the disease are still recorded. The fact that  
321 these cases correspond to individuals of older age has been interpreted as evidence of

322 successful interruption of the transmission cycle of the parasite (Martinez-Perez and  
323 Lopez-Velez, 2015). Still, it would be very interesting to isolate *Strongyloides* larvae  
324 from fresh faecal material of canine origin in order to identify the species involved and  
325 assess the associated zoonotic risk.

326

## 327 **Conclusions**

328 This is the first coprological, microscopy-based study targeting different dog  
329 populations conducted to date in the Castellón Province. Investigated dogs were  
330 infected at high rates by a wide range of protozoa and helminth species, some of them  
331 with zoonotic potential. Dogs from rural areas (mainly shepherd and hunting dogs) were  
332 more exposed. Simple measures, such as periodic deworming, prompt removal of faeces  
333 from kennels, and improving owner's education on zoonotic transmission are all cost-  
334 effective methods to limit the risk of animal and human infections by enteric parasites.  
335 People at higher risk of infection (e.g. veterinarians, slaughterhouse workers, animal  
336 husbandry workers, kennel personnel, and hunters) should be provided with accurate  
337 information on the potential risks associated to dog handling and management. Finally,  
338 data provided here are expected to be of interest for public veterinary health authorities  
339 and decision makers in order to design and implement effective control measures  
340 against these infections.

341

## 342 **Acknowledgements**

343 This study was funded by grants from CEU-Santander Program under project Zoocan  
344 Study, Universidad CEU Cardenal Herrera, Spain. The authors are grateful to Sandra  
345 Diago Porcar for her technical assistance during sampling and methodological  
346 procedures.

347

348 **Author Disclosure Statement**

349 No competing financial interests exist.

350

351 **References**

352 Adell-Aledón M, Köster PC, de Lucio A, Puente P, et al. Occurrence and molecular

353 epidemiology of *Giardia duodenalis* infection in dog populations in Eastern

354 Spain. BMC Vet Res 2018;14:1-11.

355 Barutzki D, Schaper R. Endoparasites in dogs and cats in Germany 1999-2002. Parasitol

356 Res 2003;90 (Suppl 3):148-150.

357 Benito A, Carmena D, Postigo I, Estíbalez JJ, et al. Intestinal helminths in dogs in

358 Alava, North of Spain. Res Rev Parasitol 2003;63:121-126.

359 Belhassen-García M, Alonso-Sardón M, Martínez-Perez A, Soler C, et al. Surveillance

360 of strongyloidiasis in Spanish in-patients (1998-2014). PLoS One

361 2017;28:e0189449.

362 Causapé AC, Quílez J, Sánchez-Acedo C, del Cacho E. Prevalence of intestinal

363 parasites, including *Cryptosporidium parvum*, in dogs in Zaragoza city, Spain. Vet

364 Parasitol 1996;67:161-167.

365 Claerebout E, Casaert S, Dalemans AC, De Wilde N, et al. *Giardia* and other intestinal

366 parasites in different dog populations in Northern Belgium. Vet Parasitol

367 2009;161:41-46.

368 Chen J, Liu Q, Liu GH, Zheng WB, et al. Toxocariasis: a silent threat with a progressive

369 public health impact. Infect Dis Poverty 2018;7(1):59.

370 Dantas-Torres F, Otranto D. Dogs, cats, parasites, and humans in Brazil: opening the

371 black box. Parasit Vectors 2014;7:1-25.

372 de Lucio A, Bailo B, Aguilera M, Cardona GA, et al. No molecular epidemiological  
373 evidence supporting household transmission of zoonotic *Giardia duodenalis* and  
374 *Cryptosporidium* spp. from pet dogs and cats in the province of Álava, Northern  
375 Spain. *Acta Trop* 2017;170:48-56.

376 Dean AG, Sullivan KM, Soe MM. OpenEpi: Open source epidemiologic statistics for  
377 public health, Version 3.01. [www.OpenEpi.com](http://www.OpenEpi.com). (Accessed 18 February 2019).

378 Deplazes P, van Knapen F, Schweiger A, Overgaauw PA. Role of pet dogs and cats in  
379 the transmission of helminthic zoonoses in Europe, with a focus on  
380 echinococcosis and toxocarosis. *Vet Parasitol* 2011;182:1-53.

381 Dubná S, Langrová I, Nápravník J, Jankovská I, et al. The prevalence of intestinal  
382 parasites in dogs from Prague, rural areas, and shelters of the Czech Republic. *Vet*  
383 *Parasitol* 2007;145:120-128.

384 Gil H, Cano L, de Lucio A, Bailo B, et al. Detection and molecular diversity of *Giardia*  
385 *duodenalis* and *Cryptosporidium* spp. in sheltered dogs and cats in Northern  
386 Spain. *Infect Genet Evol* 2017;50:62-69.

387 Gracenea M, Gómez MS, Torres J. Prevalence of intestinal parasites in shelter dogs and  
388 cats in the metropolitan area of Barcelona (Spain). *Acta Parasitologica*  
389 2009;54:73-77.

390 Jaleta TG, Zhou S, Bemm FM, Schär F, et al. Different but overlapping populations of  
391 *Strongyloides stercoralis* in dogs and humans-Dogs as a possible source for  
392 zoonotic strongyloidiasis. *PLoS Neglect Trop Dis* 2017;11:1-21.

393 Kostopoulou D, Claerebout E, Arvanitis D, Ligda P, et al. Abundance, zoonotic  
394 potential and risk factors of intestinal parasitism amongst dog and cat populations:  
395 The scenario of Crete, Greece. *Parasit Vectors* 2017;10:43.



396 Martínez-Carrasco C, Berriatua E, Garijo M, Martínez J, et al. Epidemiological study of  
397 non-systemic parasitism in dogs in southeast Mediterranean Spain assessed by  
398 coprological and post-mortem examination. *Zoonoses Public Health* 2007;54:195-  
399 203.

400 Martínez-Moreno FJ, Hernández S, López-Cobos E, Becerra C, et al. Estimation of  
401 canine intestinal parasites in Córdoba (Spain) and their risk to public health. *Vet*  
402 *Parasitol* 2007;143:7-13.

403 Martínez-Perez A, Lopez-Velez R. Is strongyloidiasis endemic in Spain? *PLoS Neglect*  
404 *Trop Dis* 2015;9:e0003482.

405 Martínez-Perez A, Roure Díez S, Belhassen-Garcia M, Torrús-Tendero D, et al.  
406 Management of severe strongyloidiasis attended at reference centers in Spain  
407 *PLoS Neglect Trop Dis* 2018;23:e0006272.

408 Mateus TL, Castro A, Ribeiro JN, Vieira-Pinto M. Multiple zoonotic parasites identified  
409 in dog feces collected in Ponte de Lima, Portugal-a potential threat to human  
410 health. *Int Jf Environ Res Public Health* 2014;11:9050-9067.

411 Miró G, Mateo M, Montoya A, Vela E, et al. Survey of intestinal parasites in stray dogs  
412 in the Madrid area and comparison of the efficacy of three anthelmintics in  
413 naturally infected dogs. *Parasitol Res* 2007;100:317-320.

414 Ortuño A, Scorza V, Castellà J, Lappin M. Prevalence of intestinal parasites in shelter  
415 and hunting dogs in Catalonia, Northeastern Spain. *Vet J* 2014;199:465–467.

416 Otranto D, Dantas-Torres F, Mihalca AD, Traub RJ, et al. Zoonotic parasites of  
417 sheltered and stray dogs in the era of the global economic and political crisis.  
418 *Trends Parasitol* 2017;33:813-825.

419 Overgaauw PA, van Knapen F. Veterinary and public health aspects of *Toxocara* spp.  
420 *Vet Parasitol* 2013;193:398-403.

421 Papazahariadou M, Founta A, Papadopoulos E, Chliounakis S, et al. Gastrointestinal  
422 parasites of shepherd and hunting dogs in the Serres Prefecture, Northern Greece.  
423 Vet Parasitol 2007;148:170-173.

424 Paradies P, Iarussi F, Sasanelli M, Capogna A, et al. Occurrence of strongyloidiasis in  
425 privately owned and sheltered dogs: clinical presentation and treatment outcome.  
426 Parasit Vectors 2017;10:345.

427 Rehbein S, Knaus M, Mallouk Y, Breiltgens T, et al. Efficacy against nematode  
428 infections and safety of afoxolaner plus milbemycin oxime chewable tablets in  
429 domestic dogs under field conditions in Europe. Parasitol Res 2017;116:259-269.

430 Registro Informático Valenciano de Identificación Animal. <http://www.rivia.org/>.  
431 (accessed 18 February 2019).

432 Salas-Coronas J, Cabezas-Fernández MT, Lozano-Serrano AB, Soriano-Pérez MJ, et al.  
433 Newly arrived African migrants to Spain: Epidemiology and burden of disease.  
434 Am J Trop Med Hyg 2018;98:319-325.

435 Saugar JM, Merino FJ, Martín-Rabadán P, Fernández-Soto P, et al. Application of real-  
436 time PCR for the detection of *Strongyloides* spp. in clinical samples in a reference  
437 center in Spain. Acta Trop 2015;42:20-25.

438 Soriano SV, Pierangeli NB, Roccia I, Bergagna HF et al. A wide diversity of zoonotic  
439 intestinal parasites infects urban and rural dogs in Neuquén, Patagonia, Argentina.  
440 Vet Parasitol 2010;167:81-85.

441 Stolk WA, Kulik MC, le Rutte EA, Jacobson J et al. Between-country inequalities in the  
442 neglected tropical disease burden in 1990 and 2010, with projections for 2020.  
443 PLoS Neglect Trop Dis 2016;10:e0004560.

444 Thamsborg SM, Ketzis J, Horii Y, Matthews JB. *Strongyloides* spp. infections of  
445 veterinary importance. Parasitology 2017;144:274-284.

446 Thienpont D, Rochette F, Vanparijs OF. Diagnostic De Verminose Par Examen  
447 Coprologique. In: Beerse, Belgique, ed. Belgium: Janssen Research Foundation;  
448 1979:35-36.

449 Verweij JJ, Canales M, Polman K, Ziem J, et al. Molecular diagnosis of *Strongyloides*  
450 *stercoralis* in faecal samples using real- time PCR. Trans R Soc Trop Med Hyg  
451 2009;103:342-346.

452 Zanzani SA, Gazzonis AL, Scarpa P, Berrilli F, et al. Intestinal parasites of owned dogs  
453 and cats from metropolitan and micropolitan areas: prevalence, zoonotic risks, and  
454 pet owner awareness in northern Italy. BioMed Res Int 2014;2014:696508.

455

456 Address correspondence to:

457 Paula Sanchez

458 Department of Medicine, Cardenal Herrera-CEU University, CEU Universities, C/

459 Grecia, 31, 12006 Castellón de la Plana, Castellón, Spain.

460 E-mail: [paula.sanchez@uchceu.es](mailto:paula.sanchez@uchceu.es)

461 Address correspondence to:

462 David Carmena

463 Parasitology Reference and Research Laboratory, National Centre for Microbiology,

464 Health Institute Carlos III. Ctra. Majadahonda-Pozuelo Km 2, 28220 Majadahonda,

465 Madrid, Spain.

466 E-mail: [dacarmena@isciii.es](mailto:dacarmena@isciii.es)

467

## 468 **Figure legends**

469 **FIG.1.** Map of the administrative divisions of the Castellón province. The

470 municipalities where sampling was conducted and the status of the dog sub-populations

471 are indicated. The location of Castellón in Spain is highlighted in red in the upper left  
472 corner. Image reproduced with permission of BioMed Central.

473