

EPIDEMIOLOGICAL STUDY ON INTESTINAL PROTOZOA INFECTIONS IN SHELTER DOGS FROM SOUTH-EASTERN ROMANIA

STUDIUL PRIVIND EPIDEMIOLOGIA INFESTAȚIILOR CU PROTOZOARE INTESTINALE LA CÂINI DIN ADĂPOSTURI DIN SUD-ESTUL ROMÂNIE

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ABSTRACT | REZUMAT

Intestinal protozoa are ubiquitous parasitic species in carnivores, some of them with a significant zoonotic potential. Little is known on their prevalence in Romanian shelter dogs. Therefore, an epidemiological study was carried out in order to assess the occurrence and prevalence of digestive protozoa infections in shelter dogs, in South-eastern Romania. Dogs (n=420) originating in four shelters, one in Bucharest (n=105) and three from two neighbouring counties, two in Ilfov (n=166) and one in Giurgiu (n=149), were included in the study. Dogs were of mixed breed, aging from two months to 16 years (average=4.8 years; SD=3.8). To assess the risk factors for protozoan infections, animals were categorized in three age groups: <4 months, 4-12 months and >12 months. Fresh faecal samples collected from individual animals have been subjected for parasitological examination. Overall, 54.05% (95% CI: 49.14-58.89) of the animals were found positive for at least one parasitic species, protozoan and/or helminths. 11.9% of the dogs were positive for protozoa infections, as follows: 8.57% (36/420) for *Isoospora* spp. and 3.33% (14/420) for *Giardia duodenalis*. Infection with *Isoospora* showed a higher and statistically significant prevalence of 40.9% in the age group up to 4 months comparing with the other two age groups in which prevalence of 19.64% and 2.19%, respectively, was registered. The prevalence of *G. duodenalis* infection among these age groups varied from 5.36% to 3.44%. Of the 36 *Isoospora*- and 14 *Giardia*-positive animals, 21 and 5 respectively were single infections, while 15 and 9, respectively, has mixed infections with one (n=5 and n=6), two (n=8 and n=2) or three (n=2 and n=1) different helminth species, as follows: *Ancylostoma caninum*, *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, *Dipylidium caninum*. These findings show the occurrence and epidemiology of protozoa infections and a high diversity of parasitofauna in shelter dogs, including species with zoonotic potential emphasizing the urgent need for appropriate measure to be taken for the parasitological control.

Keywords: intestinal protozoa, epidemiology, shelter dogs

Carnivorele pot fi parazitare de numeroase specii de protozoare, unele dintre ele cu potențial zoonotic.

Prezentul studiu a avut ca scop investigarea prezenței și prevalenței infestațiilor cu protozoare digestive la câini din adăposturi din sud-estul României.

În studiu au fost incluși câini (n=420) de rasă comună, cu vârsta cuprinsă între două luni și 16 ani (media=4,8 ani; Deviația Standard=3,8) proveniți din 4 adăposturi: unul în București (n=105) și trei în județe învecinate, două în Ilfov (n=166 și unul în Giurgiu (n=149). Pentru a evalua factorii de risc pentru infestațiile cu protozoare, animalele au fost împărțite în trei grupe de vârstă: <4 luni, 4-12 luni și >12 luni. Probe proaspete de fecale colectate individual de la animale au fost examinate parazitologic.

În total, 54,05% (95% CI: 49,14-58,89) dintre animale au fost găsite pozitive pentru cel puțin o specie parazitară, protozoare și/sau helminți. 11,9% dintre câini au fost pozitivi pentru infestații cu protozoare: 8,57% (36/420) pentru *Isoospora* spp. și 3,33% (14/420) pentru *Giardia duodenalis*. Infestația cu *Isoospora* a înregistrat o prevalență mai mare (40,9%) și statistic semnificativă la grupa de vârstă de până la 4 luni. Prevalența infestației cu *G. duodenalis* pe grupe de vârstă a variat de la 5,36% la 3,44%, fără semnificație statistică. Din cele 36 de animale pozitive pentru *Isoospora* și cele 14 pozitive pentru *Giardia*, 21 și respectiv 5 au avut infestații unice, în timp ce 15 și respectiv 9 au avut infestații mixte cu una (n=5 și n=6), două (n=8 și n=2) sau trei specii de helminți (n=2 și n=1), după cum urmează: *Ancylostoma caninum*, *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, *Dipylidium caninum*. Aceste rezultate arată prezența infestațiilor cu protozoare și o mare diversitate a parazitofaunei la câinii din adăposturi, inclusiv specii cu potențial zoonotic evidențiind necesitatea aplicării cu regularitate de măsuri specifice de control parazitologic.

Cuvinte cheie: protozoare intestinale, epidemiologie, câini din adăposturi

Intestinal protozoa are ubiquitous parasitic species in dog caused by different species including *Giardia duodenalis*, *Isoospora* spp., but also other species (*Cryptosporidium* spp., *Neospora caninum*, *Hammondia heydorni*, *Sarcocystis* spp.), some of them with zoonotic po-

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tential (17). Clinical diseases can evolve acutely, especially in puppies and debilitated animals, with digestive symptoms such as varying degrees of diarrhoea, anorexia, abdominal pain, vomiting, weight loss and lethargy (9). Additionally, *N. caninum* infection causes a nervous syndrome affecting the hind limbs, with paresis and ataxia which becomes progressively more severe (7, 18).

Dog shelters represent a special environment characterized by a limited space, organized for the coexistence of a large number of animals, for variable periods of housing. The animal population is not stable, there is a permanent circulation of dogs that can carry different endo- and ectoparasites. Generally, there are strict regulations for the building and functioning of these shelters (1, 2, 13, 19). The efficiency of the periodic disinfections relies heavily on the shelter housing design and construction materials that facilitate the disinfection or make it more difficult (20). The differences can arise from the type of floor, cage, and paddock material, where the animals stay together part of the day: ground or gravel, sawdust, cement or wood with or without pores, painted with light washable materials. Spaces in which animals are kept most of their time are organized for 3-4 individuals, but in many cases their number is higher. Thus, shelter dogs can play an important epidemiological role as carriers and source of infection, parasites included, given the co-existence of multiple associated risk factors (10).

Little is known about the eco-epidemiology of parasitic protozoa in shelter dogs in Romania. Therefore, an epidemiological study was carried out in order to assess the occurrence and the prevalence of digestive protozoa infection in shelter dogs from South-eastern Romania.

MATERIALS AND METHODS

Study area

The study was conducted between February 2015 - December 2016, in four private shelters located in Bu-

charest and the neighbouring counties: one in Bucharest (n=105 dogs), two in Ilfov county (n=166 dogs) and one in Giurgiu county (n=149 dogs).

The four shelters are isolated, placed at the outskirts or outside the localities. Two of the shelters have concrete flooring, one has mixed ground-concrete floor and one ground and gravel floor (Table 1). All the shelters have wooden cages, with straw bedding for the cold season. Dogs spend most of their time sharing the same pens, and two of the shelters (IF2 and GR) have playgrounds where dogs have access separately at different hours.

Animals

A total of 420 dogs of mixed breed, aging from 2 months to 16 years (average=4.8 years; standard deviation=3.8) were included in the study. To assess the risk factors for protozoan infections, animals were classified into three age categories: ≤4 months; >4-12 months and >12 months (Table 2).

The dogs were first subjected for a routine physical and clinical examination and clinical signs were recorded. According to the shelters' owners, the dogs were not dewormed with at least 6 weeks before sampling.

Investigations

Fresh faecal samples were collected from individual animals and have been subjected for parasitological examination in the Laboratory of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine of Bucharest.

The faecal samples have been first analysed grossly (macroscopically) and thereafter microscopically for the presence of *Giardia* cysts, protozoan oocysts, and helminths eggs, by using a flotation method with 33% zinc sulphate solution and Lügol's iodine stain (11, 17).

For the statistical analysis the Quantitative Parasitology Program 3.0 (21) was used.

Table 1

Dog originating shelters in the study
(location, type of floor, surface and number of dogs in each shelter are presented)

Shelter ID	Location (county/town)	Constructive shelter features			Number of dogs
		Floor material	Average area (m ²) / dog	Drainage	
IF1	Ilfov	Ground + Gravel	1.3	No	151
IF2	Ilfov	Concrete, access to playground with sand	2	Yes	15
GR	Giurgiu	Concrete, access to playground with sand	1.1	Yes	149
B	Bucharest	Concrete inside, Ground + Gravel outside	1.5	Yes	105

m²: square meter

Table 2

Data on shelter dog population included in the parasitological study
(dogs are stratified by age, gender, and originating shelter)

Originating shelter*	Number of dogs included in the study							Total number of dogs
	Dog gender and age categories						Dog's average age (years) [SD]	
	≤4 months		4 - 12 months		>12 months			
male	female	male	female	male	female			
IF1	7	7	14	21	49	53	3.11 [2.52]	151
IF2	-	-	-	1	6	8	2.86 [2.63]	15
GR	14	16	13	7	48	51	5.29 [3.84]	149
B1	-	-	-	-	49	56	9.79 [2.42]	105
Total	21	23	27	29	152	168	4.78 3.8	420

*: IF1 and IF2: Ilfov county, GR: Giurgiu county, B: Bucharest. SD=standard deviation

RESULTS AND DISCUSSIONS

For assessing the epidemiology of parasitic protozoan infections in shelter dogs a parasitological survey was conducted on four dog shelters located in South-eastern Romania. A total of 420 dogs were copro-parasitologically investigated. Of them, 12.6% (53/420) showed digestive clinical signs (soft stools, diarrhoea, melena etc.) and the remainder (367/420; 87.4%) were apparently healthy.

Subsequently to the parasitological testing, overall, 54.05% (95% CI: 49.14-58.89) of the animals were found positive for at least one parasitic species, protozoan and/or helminths, as single (32.38%; 136/420) or mixed infections with two (16.19%; 68/420), three (4.52%; 19/420), and four (0.95%; 4/420) parasitic species, respectively.

Of the dogs tested positive for parasitic infection, 10.6% (24/227) showed digestive disorders of varying

degrees of soft stools, green diarrhoea, intermittent diarrhoea to watery diarrhoea, while the rest (89.4%; 203/227) were apparently clinically healthy.

Regarding the protozoa infections, 11.9% (50/420) of dogs were positive, as follows: 8.57% (36/420) for *Isoospora* spp. and 3.33% (14/420) for *G. duodenalis*. No mixed protozoa infection with *Isoospora* spp. and *G. duodenalis* were found. Details about the animals infected with *Isoospora* spp. are presented in Tables 3 and 4, and with *Giardia* in Tables 5 and 6.

Out of the 36 dogs infected with *Isoospora* spp., 58.33% (21/36) were as single infection, while 5 (13.89%), 8 (22.22%) and 2 (5.56%) dogs were co-infected with one, two, and three, respectively, other parasitic species (Table 4). Digestive clinical signs were present in 13 (36.11%) out of the 36 dogs, 7 dogs (53.84%) with single *Isoospora* infection, 1 (7.7%) co-infected with *T. canis* and 5 (36.5%) co-infected with *A. caninum* plus *T. canis*.

Of the 14 dogs positive for *G. duodenalis* cysts, the

Table 3

Prevalence of *Isoospora* spp. infection in shelter dogs, South-eastern Romania
(animals are stratified by gender, age category, and originating shelter)

Originating dog shelter**	<i>Isoospora</i> - positive dogs: number positive/number tested and percentage (%)						Total
	Dog gender and age categories						
	≤ 4 months		< 4 - ≤ 12 months		> 1year		
M	F	M	F	M	F		
GR	6/14	4/16	2/13	0/7	1/48	0/51	13/149 (8.72%)
B	* n.a	n.a	n.a	n.a	2/49	0/56	2/105 (1.9%)
IF1	3/7	5/7	5/14	3/21	0/49	0/53	16/151 (10.6%)
IF2	n.a	n.a	n.a	1/1	1/6	3/8	5/15 (33.33%)
Total	9/21 (42.86%)	9/23 (39.13%)	7/27 (25.92%)	4/29 (13.79%)	4/152 (2.63%)	3/168 (1.79%)	36/420 (8.57%)
	18/44 (40.91%)		11/56 (19.64%)		7/320 (2.19%)		

$p < 0.05$

* n.a.: not applicable, no dogs in that category; **: IF1, IF2: Ilfov county, GR: Giurgiu county, B: Bucharest

Table 4

Prevalence of *Isospora* spp. and mixed infection with other endoparasites in shelter dogs, South-eastern Romania

(animals are stratified by age category and the presence of clinical signs)

Parasite species (single and/or mixed infection)	Total number of dogs (n=420)		Dog age category						Number of <i>Isospora</i> -positive dogs with (+) and without (-) clinical signs	
	No.	%	≤ 4 months (n=44)		< 4 - ≤ 12 months (n=56)		>1 year (n=320)		+	-
			No.	%	No.	%	No.	%		
<i>Isospora</i> spp. total	36	8.57	18	40.91	11	19.64	7	2.19	13	23
<i>Isospora</i> spp. as single parasite	21	5.0	14	31.82	2	3.57	5	1.56	7	14
<i>Isospora</i> spp. + <i>T. canis</i>	2	0.48	1	2.27	1	1.79	0	0	1	1
<i>Isospora</i> spp. + <i>A. caninum</i>	2	0.48	0	0	2	3.57	0	0	0	2
<i>Isospora</i> spp. + <i>T. leonina</i>	1	0.24	0	0	0	0	1	0.31	0	1
<i>Isospora</i> spp. + <i>A. caninum</i> + <i>T. canis</i>	7	1.67	3	6.82	4	7.14	0	0	5	2
<i>Isospora</i> spp. + <i>A. caninum</i> + <i>T. leonina</i>	1	0.24	0	0	1	1.79	0	0	0	1
<i>Isospora</i> spp. + <i>A. caninum</i> + <i>T. vulpis</i> + <i>T. canis</i>	1	0.24	0	0	1	1.79	0	0	0	1
<i>Isospora</i> spp. + <i>A. caninum</i> + <i>T. vulpis</i> + <i>T. leonina</i>	1	0.24	0	0	0	0	1	0.31	0	1

n= number of tested dogs; No= number of positive dogs; %: percentage

highest prevalence (5.36%; 3/56) was registered among puppies aged between 4 and 12 months, a close value among dogs over one year of age (3.44%; 11/320), and in none of the puppies under 4 months ($p > 0.05$) (Table 5).

Nine of the dogs tested positive for *G. duodenalis* were co-infected with one, two and three helminth species, including: *A. caninum* (n=3), *T. vulpis* (n= 2), and *D. caninum* (n=1), *A. caninum* + *T. vulpis* (n=2), and *A. caninum* + *T. canis* + *T. leonina* (n=1), respectively.

Out of these, just one dog (7.15%; 1/14) showed clinical digestive signs (Table 6).

Overall, the prevalence of total parasites (protozoa and/or helminths) and protozoa infections was higher (68.87% and 16.56%, respectively) in shelters with soil and gravel flooring, showing statistically significant differences for both parasite categories ($p < 0.05$). Prevalence data found in this study correlated with shelters' flooring is presented in Table 7.

The present study carried out to investigate the pre-

Table 5

Prevalence of *Giardia duodenalis* infection in shelter dogs, South-eastern Romania

(animals are stratified by gender, age category, and originating shelter)

Originating dog shelter**	<i>Giardia</i> -positive dogs: number positive/number tested and percentage (%)						
	Dog gender and age categories						Total
	≤ 4months		< 4 - ≤ 12months		> 1 year		
M	F	M	F	M	F		
GR	0/14	0/16	0/13	0/7	1/48	1/51	2/149 (1.34%)
B	*n.a	n.a	n.a	n.a	1/49	2/56	3/105 (2.86%)
IF1	0/7	0/7	0/14	3/21	3/49	3/53	9/151 (5.96%)
IF2	n.a	n.a	n.a	0/1	0/6	0/8	0/15 (0%)
Total	0/21 (0%)	0/23 (0%)	0/27 (0%)	3/29 (10.34%)	5/152	6/168	14/420 (3.33%)
	0/44 (0%)		3/56 (5.36%)		11/320 (3.44%)		

$p > 0.05$

* n.a.: not applicable, no dogs in that category; **: IF1, IF2: Ilfov county, GR: Giurgiu county, B: Bucharest

Table 6

**Prevalence of *Giardia duodenalis* and mixed infection
with other endo-parasites in shelter dogs, South-eastern Romania**
(animals are stratified by age category and the presence of clinical signs)

Parasite species (single and/or mixed infection)	Total number of dogs (n=420)		Dog age category						Number of <i>Giardia</i> -positive dogs with (+) and without (-) clinical signs	
			≤ 4 months (n=44)		<4 - ≤12 months (n=56)		>1 year (n=320)			
	No.	%	No.	%	No.	%	No.	%	+	-
<i>Giardia duodenalis</i> total	14	3.33	0	0	3	5.36	11	3.44	1	13
<i>G. duodenalis</i> as single parasite	5	1.19	0	0	0	0	5	1.56	1	4
<i>G. duodenalis</i> + <i>A.</i> <i>caninum</i>	3	0.71	0	0	2	3.57	1	0.31	0	3
<i>G. duodenalis</i> + <i>T.</i> <i>vulpis</i>	2	0.48	0	0	0	0	2	0.63	0	2
<i>G. duodenalis</i> + <i>Dipylidium caninum</i>	1	0.24	0	0	0	0	1	0.31	0	1
<i>G. duodenalis</i> + <i>A.</i> <i>caninum</i> + <i>T. vulpis</i>	2	0.48	0	0	0	0	2	0.63	0	2
<i>G. duodenalis</i> + <i>A.</i> <i>caninum</i> ,+ <i>T. canis</i> + <i>T. leonina</i>	1	0.24	0	0	1	1.75	0	0	0	1

n= number of tested dogs; No= number of positive dogs; %: percentage

sence of intestinal protozoa in shelter dogs from 4 different shelters located in South-eastern Romania highlighted a diversity of parasitic species and their high prevalence correlated with different risk factors.

With regard to protozoan, the prevalence of *Isospora* infection was double (40.91%) in puppies up to 4 months compared with those aging from 4 months to one-year-old (19.64%), and almost 20 times higher compared to the older dogs (>1 year old) (2.19%) (Table 3). These findings showed statistical significance ($p < 0.05$), and thus confirm that the age of dogs is a risk factor for the *Isospora* infection. There were not found any significant differences regarding the prevalence of different parasitic species in males and females, our results being in agreement with other studies (4, 10). For *Giardia*-infected dogs there were not

found any significant age- or gender-related differences, the number of infected males (20/200; 10%) and females (16/220; 7.27%) being very similar, therefore it cannot be demonstrate a different susceptibility of these dog categories (Table 5). However, it is known that the prevalence of *Giardia* infection can be underestimated due to some factors, such as frequent subclinical course of infection, inconsistency of cyst shedding (22). Additionally, other factors such as season can also impact the occurrence and frequency of shedding cysts in faeces (8, 10). Regarding the zoonotic potential of *Giardia*, recent reports have demonstrated the coexistence of the zoonotic assemblages (A) with those specific of dogs (C and D) (12).

In previous similar studies in dogs from South-eastern areas, including stray, shelter, and owned dogs, a

Table 7

Prevalence of parasitic infections in shelter dogs, in South-eastern Romania, according to the shelter floor type
(number of positive dogs/total number of tested dogs are presented within the brackets)

Floor material of the dog shelter*	Total prevalence (all the parasite species identified)	<i>p</i> - value	Protozoans' prevalence	<i>p</i> - value
Soil + Gravel (IF1)	68.87% (104/151)		16.56% (25/151)	
Concrete, access to playground with sand (GR+IF2)	45.12% (74/164)	$p < 0.05$	12.2% (20/164)	$p < 0.05$
Concrete in interior, Soil + Gravel at exterior (B)	46.67% (49/105)		4.76% (5/105)	

*: IF1, IF2: Ilfov county, GR: Giurgiu county, B: Bucharest

slightly higher prevalence for *Isospora* infection, ranging from 11.3% to 12.23% (24, 25) was reported.

For *Giardia* infection, a higher prevalence was found in owned dogs (21.28%) (25) and quite similar in shelter / stray dogs (5.7%) (24). Also, when comparing the results of the present study with other similar studies in Romania, the prevalence of *Isospora* infection in shelter dog of 8.57% is in agreement with studies in stray dogs (10.71%) from some urban and rural areas in Southern Romania (6), but higher prevalence (31.18%) was reported for dogs in Center and North-western Romania (15).

For *G. duodenalis*, a prevalence rate of 16.5%, by coproscopy, but higher by ELISA (47.7%) has been reported in shelter dogs from Central Romania (14). As showed, although many prevalence surveys on *Giardia* infection in dogs are available, results should be carefully compared since many factors can impact on the values, including the sensitivity of the method used for detection of *Giardia* infections (23).

Overall, the findings of the present study are consistent with similar studies from European countries. For *Isospora* infection in dogs, prevalence values of 5.6% and 5.1% in Germany and Northern Germany, respectively (3, 4), 16.4% in Spain (20) have been recently reported. In Canada and the USA, South-eastern Wisconsin, a prevalence of 10.5% and 5.2%, respectively of *Isospora* infection in shelter dogs was reported (5, 27). For *Giardia* infection close values were found in shelter dogs from the Czech Republic (5%) (26), Canada (3.5%) (27) and South-eastern Wisconsin (USA) (4.5%) (5). In Spain, a high prevalence value, up to 40.6% was found in 12 dog shelters (20).

Despite the parsimonious scarcely published data, shelters' flooring is known as a risk factor for parasite infection in shelter animals (16). In our study, the shelters' type of flooring was found to be relevant for the epidemiology of parasites species, particularly for protozoa infection, but also for other parasitic infections. A higher and statistically significant ($p < 0.05$) prevalence of parasitic infection and protozoa infection respectively was found in dogs from shelters without concrete floor and drainage (IF1) (total parasite infections' prevalence of 68.87%; protozoan infection of 6.56%) compared with dogs from shelters with concrete floor and outdoors pavilions (B) (total prevalence: 46.67%; protozoan infection of 4.76%, respectively), or those with concrete paddocks and dogs with limited access time to the playground (total prevalence: 45.12%; protozoan: 12.2%). Similar data are reported by (20) showing statistically relevant differ-

ences of positive dog numbers when analysed in relation with cage material (concrete, metal, wood) and floor material (concrete, waterproof paint, soil, sawdust, straw). By corroborating all these findings, a need is documented to recommend special measures for improving the shelter flooring in order to minimize the risks for parasitic infections.

CONCLUSIONS

The present study emphasizes the occurrence and epidemiology of protozoan infections in shelter dogs and demonstrates that *Isospora* and *Giardia* protozoan should be considered as common parasites in shelter dogs. Additionally, the findings also highlight a high diversity of parasitofauna in this dog category, including species with relevance for both animal and public health and the urgent need for appropriate measures to be taken for the parasitological control.

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