Original papers

Internal parasites infecting dogs in rural areas

Maria Michalczyk, Rajmund Sokół, Remigiusz Gałęcki

Department of Parasitology and Invasive Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, ul. M. Oczapowskiego 13, 10-719 Olsztyn, Poland

Corresponding Author: Maria Michalczyk; e-mail: maria.michalczyk@uwm.edu.pl

ABSTRACT. Dogs which spend their lives in rural areas are primarily "sentinels" of animal herds. The dogs' close contact with humans and other animals obligates their caregivers to systematic deworming. The goal is to eliminate potential contamination with parasites which is mostly caused by direct contact or food and water contaminated with dogs' excretions. The aim of this study was to assess internal parasitic invasions on dogs from rural areas which spend most of their lives on farms. In total, 69 samples of fresh stools were collected for the analysis, including 26 from females and 43 from males. Coprological analysis of stool samples was accompanied with a survey where pet owners gave information on the number of visits to the veterinarian in suspicion of their dogs being infected with the parasites, the number of deworming treatments in the previous year and the dogs' behavior towards strangers. Also, the age, sex and body weight of dogs were recorded. Statistical analysis of the obtained results was performed using Statistica 12.5 with a medical set by Statsoft. In total, 29 (46,03%) out of 69 fecal samples contained developmental forms of parasites. Research has shown that animals most vulnerable to invasion were in the 6–10 years old age group, mostly males. In addition, the invasion of *Toxocara canis* roundworms occurred most frequently, as it was found in 12 (41.37%) animals (6 females and 6 males). The analysis of the questionnaires showed that the dog owners' knowledge of the risk of infection and parasites is unsatisfactory. This corresponded with a small number of deworming treatments. It is significant that nearly half of the owners never dewormed their dogs.

Key words: dogs, endoparasites, infection, rural areas

Introduction

Dogs which spend their lives in rural areas are primarily "sentinels" of animal herds and homes [1]. The dogs' close contact with humans and other animals obligates their caregivers to systematic deworming. The goal is to eliminate potential contamination with parasites which is mostly caused by direct contact or food and water contaminated with dogs' excretions [2-6]. Parasite prevention in these areas is especially significant since the dogs live in a limited and defined space for many years, and the threat of parasites is periodic [7]. The major hazard is posed by internal parasites such as: Diphyllobothrium latum, Echinococcus granulosus, Ancylostoma sp., Toxocara canis and Uncinaria stenocephala [8]. The species of the parasite and its developmental form determines the presence of different clinical symptoms in dogs [9-11]. Larval stages of Ancylostoma caninum,

Gnathostoma sp. and *Toxocara* sp. may be the cause of zoonoses such as eosinophilic enteritis or the presence of "larva migrans" in the eye or bowels [12]. Furthermore, dogs are final hosts for many parasites such as *Cryptosporidium* sp., *Giardia duodenalis* and *Ancylostoma* sp. [13–15]. Internal parasites occur both in homeless animals and those in animal shelters, as well as in well-groomed ones and those dewormed on a regular basis [7,16–20].

The aim of the study was to assess the invasions of endoparasites on dogs from rural areas which spend most of their lives on the farm.

Materials and Methods

In July 2015 samples of fresh feces weighing approx. 50 g each were collected from 69 dogs (26 females, 43 males) living in 69 different rural households. The stool samples were examined with flotation method using Darling fluid (saturated NaCl solution and glycerin 1:1). A fecal pellet the size of a hazelnut was poured over with Darling fluid into a sieve and Petri dish, then triturated thoroughly to obtain a homogeneous suspension which was poured into a test tube and centrifuged (800× g for 5 min). Subsequently, 4 drops of suspension were transferred with a glass rod to a glass slide, covered with a coverslip and viewed under a microscope at 10× and 400× magnification in search of developmental forms of parasites. The study was supplemented with a survey where pet owners gave information on the number of visits to the veterinarian in suspicion of their dogs being infected with the parasites, the number of deworming treatments in the previous year and the dogs' behavior towards strangers (degree of aggressiveness) on a scale of 0 to III (0 – aggressive, III – very aggressive). Surveys also contained information on the dogs' age, sex and body weight. Verification of developing forms of parasites was based on the atlas titled Diagnostic Parasitology for Veterinary Technicians, 4th Edition by Charles M. Hendrix and Ed Robinson. Statistical analysis of the obtained results was performed using Statistica 12.5

with a medical set by Statsoft.

Results

Infected animals

In 29 (37.7%) out of 69 animals (100%), including 14 females and 15 dogs, various developmental forms of parasites were found. In this group there were seven females aged 0-5 years, three females between 5-10 and 4 above 10 years old. Out of 15 infected dogs 5 males were 0-5 years old, 8 (5-10 years) and 2 above 10 years old. Three infected females had a body weight of 5 kg, 8 (5-10 kg) and 3 above 10 kg, whereas 12 dogs had a body weight of above 10 kg, one (0-5 kg) and 2 (5-10 kg). Eight out of 14 infected females had never visited a veterinarian to detect parasites, and 6 had once. Ten out of 15 infected males had never visited a veterinarian, and 5 of them had only once. Twelve infected females had never been dewormed, two had been dewormed once per year, and out of 15 infected males 14 had never been dewormed and one was dewormed once per year. The analysis of the

Table 1. A summary of the studied dogs and data collected in the surveys

					Nı	umber	of studie	d anima	ls (n=0	69)						
	Nur	nber o	f infected	1 animal	s			Number of uninfected animals								
29									40							
							Se	ex								
	14♀				158			12♀				28				
	Age of animals (years)															
0-5	5-	10	>10	0-5	5-	10	>10	0-5	5-	-10	>10	0-5	5-10		>10	
7		3		5	8	3	2	2	3		7	12	7		9	
Body weight (kg)																
0-5	5-10		>10	0-5	5-	10	>10	0-5	5-	-10	>10	0-5	5-10		>10	
3	8	8		1	2		12	12	_		_	20	5		3	
	The number of visits to the veterinarian (per year)															
0	1x	2x	>	0	1x	2x	>	0	1x	2x	>	0	1x	2x	>	
8	6	_	_	10	5	_	_	_	3	2	7	6	7	10	5	
				Th	e numł	per of	dewormi	ng proce	edures	(per ye	ear)					
0	1x	2x	>	0	1x	2x	>	0	1x	2x	>	0	1 x	2x	>	
12	2	_	_	14	1	_	_	2	3	2	5	1	19	6	2	
					A	nimal	behavior	(aggres	sivene	ss)						
0	Ι	Π	III	0	Ι	II	III	0	Ι	Π	III	0	Ι	Π	III	
7	3	2	2	3	8	_	4	3	3	4	2	6	11	7	4	

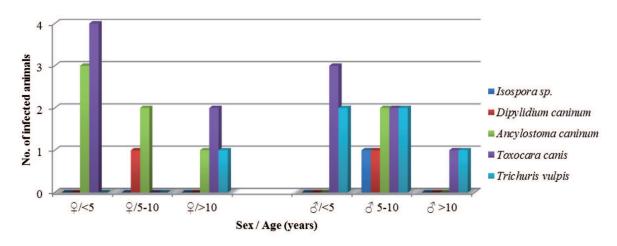


Fig. 1. Species, type and number of internal parasites depending on age and sex of infected animals

animals' behavior (aggressiveness) in this group showed that in seven females aggressiveness was at "0" level, in three females at "I", in two females at "II" and in two females at "III". In 3 males aggressiveness was at "0", in 8 males at "I" and in 4 at "III".

Uninfected animals

In 40 (57.97%) animals (12 females and 28 dogs), there were no internal parasites (Table 1). Two females were 0–5 years old, 3 (5–10 years old) and 7 above 10 years old. Among male dogs 12 were between 0–5 years old, 7 (5–10 years old) and 10 over 10 years old. All females free of parasites had a body weight of up to 5 kg, and 20 males had a body weight of up to 5 kg, 5 dogs (5–10 kg) and 3 above 10 kg. In this group, 2 females had never been dewormed with antiparasitic agent, three had

been treated once per year, two twice per year and 5 more often than twice per year. Nineteen males were dewormed once per year, six twice per year and 2 more often than twice per year, one male had never been dewormed. All females in this group visited a veterinarian regularly, including 3 at least once per year, 2 twice per year and 7 more than twice per year. Six males had never visited a veterinarian, seven went once per year, ten twice per year and five dogs more often than twice per year. Aggressiveness at "0" level was observed in 3 females, 3 females were at level "I", four at level "II" and 2 at level "III". Six males showed "0" aggressiveness level, eleven level "I", seven level "II" and four level "III" (Table 1).

Occurrence of internal parasites depending on:

Sex and age. D. caninum eggs were detected in

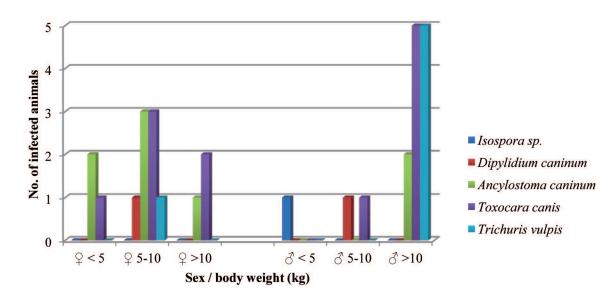


Fig. 2. Species, type and number of internal parasites depending on sex and body weight (kg) of infected animals

2 samples (3.44%) in one bitch and one dog at the age of 5–10 years, and the eggs of *A. caninum* in 8 samples (27.58%) in 6 bitches and in 2 dogs, most frequently at the age of 5–10 years (2 females and 2 dogs). *T. canis* eggs were found in the studied samples most often: 12 positive samples (41.37%): in 6 bitches and 6 dogs, including animals aged <5 years 7 positive samples (17.39%): in 4 females and 3 dogs. Eggs of *Trichuris vulpis* were detected in 6 samples (20.68%) including one female and 5 males, especially in males aged <5 and 5–10 years (two dogs in each age group). Protozoa oocysts of the genus *Isospora sp.* were detected in one dog (3.44%) aged 5–10 years. (Fig. 1).

Sex and body weight. With regard to the animals' body weight, *A. caninum* eggs were found in 3 females (10.34%), in the same number of females *T. canis* eggs were detected (10.34%). In one bitch eggs of *D. caninum* (3.44%) and in one female eggs of *T. vulpis* (3.44%) were detected. 3 females (10.34%) weighing 0–5 kg and 3 females (10.34%) of over 10 kg were infected with *T. canis* nematodes (10.34%) or *A. caninum* (10.34%) (Fig. 2). In 5 males (17.24%) the eggs of *T. canis* were detected, also in 5 males (17.24%) the eggs of *T. vulpis* and in 2 males the eggs of *A. caninum* (2.89%) (Fig. 2.)

The number of visits to the veterinarian. The analysis of the surveys showed that 18 (62.06%) (8 females and 10 males) out of 29 animals infected with endoparasites were not in veterinary care, and 11 (37.93%) (6 females and 5 males) had visited a veterinarian once. In animals which had visited a

veterinarian twice or more times, parasites were not found. Regardless of sex, *T. canis* infection was detected most frequently: in 12 animals, including 6 males and 6 females. (Fig. 3).

Number of deworming treatments. It has been shown that 26 (89.65%) out of 29 infected animals had never been dewormed (12 females and 14 dogs) and three dogs (10.34%) (2 females and 1 male) once per year (Fig. 4). Only one dog (3.44%), which had never had antiparasitic protection, had Isospora sp. oocysts, similarly D. caninum eggs were detected in one female (3.44%) and one male (3.44%). T. canis infection was found most frequently: in 12 animals (41.37%) (including 6 females and 6 dogs). In this group 4 females (13.79%) with diagnosed roundworms had never been dewormed and 2 females (6.89%) were dewormed 1×/year, while as many as 6 males (20.68%) with diagnosed roundworms' eggs had never had deworming treatments. In 6 females (20.68%), which had never been dewormed, eggs of A. caninum were found and in 2 dogs (6.89%) (one that had never been dewormed and one that was dewormed 1x/year). T. vulpis eggs were detected in one female (3.44%) and 5 dogs (17.24%) which had never been given any antiparasitic preparations (Fig. 4)

Animals' behavior. The dogs' reaction to the appearance of strangers on the farm, expressed with loud barking and intense running along the fence of the property or in a playpen, was evaluated. The collected information shows that in 29 dogs infected with parasites the level of aggressiveness was different. In 10 animals (34.48%) (7 females and 3

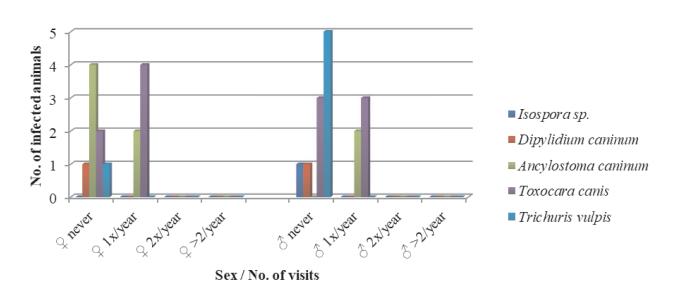


Fig. 3. The species, type and number of internal parasites depending on sex and the number of visits of infected animals to a veterinarian

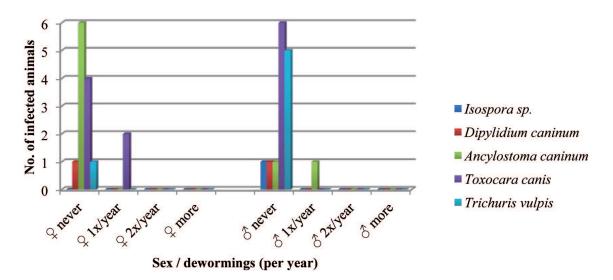


Fig. 4. Species, type and number of internal parasites depending on sex and the number of infected animals' dewormings

dogs) aggressiveness levels were "0", in 10 animals (34.48%) (3 females and 7 dogs) the aggressiveness was at level "I", 3 animals (10.34%) (2 females and 1 male) showed the level of aggressiveness "II", in 6 dogs (20.68%) (2 bitches and 4 dogs) "III" level of aggressiveness was observed (Fig. 5). Most of the parasites were found in animals with the level of aggressiveness "I": in 4 patients (13.79%) *T. canis* eggs, in 3 (10.34%) *A. caninum*, in 3 patients (10.34%) *T. vulpis* and in 1 (3.44%) *D. caninum*, in 6 subjects (20.68%) at the third level of aggressiveness, eggs of *T. canis* were found (in 2

bitches and 2 dogs) and in 2 animals (6.89%) *T. vulpis* eggs. In dogs with the level of aggressiveness rated at 0: oocysts of *Isospora sp.* were detected in one dog (3.44%), *A. caninum* eggs in 4 females (13.79%) and 1 dog (3.44%) and eggs of *T. canis* in 3 females (10.34%).

Statistical evaluation of the research results: statistical evaluation was based on the multivariate logistic regression model, where the dependent variable is dichotomous (0 or 1, absence/presence of endoparasites) and independent variables were based on the dogs' sex (female/male), veterinary care

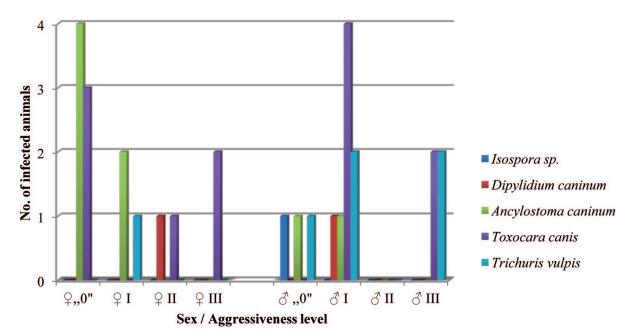


Fig. 5. Species, type and number of internal parasites depending on sex and the level of aggressiveness of infected animals

(none/provided), deworming (none/ administered at least once a year), body weight (0–5kg/5–10kg />10kg), age (0–5 years/5–10 years/>10 years) and aggressiveness level (lack of aggression/signs of aggression). To examine the relationship between visits to the veterinarian and deworming treatments, Cramer's V was used.

It has been shown that characteristics such as sex ($\chi 2 = 2.81$, df = 1, P = 0.09), age ($\chi 2 = 0.005$, df = 1, P = 0.94) and aggressiveness ($\chi 2 = 0.28$, df = 1, P = 0.60) were not statistically significant.

Deworming procedures had a significant statistical impact on parasitic invasion ($\chi 2 = 10.15$, df = 1, P = 0.001). Dewormed dogs were 2.3 times less likely to have endoparasites than dogs without such treatment (Odds Ratio = 0.023, 95% Confidence Interval: 0.0022 -0.23).

Also, the number of visits to the veterinarian had a statistically significant effect on the invasions ($\chi 2$ = 3.83, df = 1, P = 0.047). Dogs with provided veterinary care were 1.5 times less prone to parasitic infections than dogs without veterinary care (Odds Ratio = 0.015, 95% Confidence Interval: 0.011 -0.96).

Statistical analysis revealed that the body weight of the animals had a statistically significant influence on the parasitic invasions ($\chi 2 = 5.00$, df = 1, P = 0.025). Dogs weighing 0–5 kg were 1.78 times less affected by internal parasites than dogs weighing 5–10 kg, and 3.57 times less vulnerable than dogs weighing >10kg (Odds Ratio = 0.023, 95% Confidence Interval: 0.0022 -0.23).

Furthermore, the statistical analysis revealed moderate correlation between visits to the veterinarian and the number of deworming procedures ($\chi 2 = 16.42$, df = 1, P = 0.00005, V = 0.49).

Discussion

In the literature there are many reports regarding the prevalence of intestinal parasites in dogs [21– 25]. Some of them demonstrated prevalence between 4 and 40% and others more than 60% [25]. The divergence of research results may have been caused by many factors, e.g. the size of the collected samples, the place of collection, as well as the type of diagnostic techniques used [21,22]. It has been shown that intestinal parasites in dogs often occur in developing countries [24], as dogs in these regions are rarely examined and treated, and antiparasitic prophylaxis is limited [23]. Our research revealed that animals most vulnerable to infection were in the age group 5–10 years and most commonly males: 8 positive samples (27.58%). Similar research was performed by Amissah-Reynolds et al. [25], in which 154 dogs were examined for the presence of intestinal parasites, and it showed that 53% of the animals were infected, out of which the biggest group was 0–6 years old (86.7%), and the smallest 6–12 years old (41.5%), with more males (55.1%) than females (48.2%). During the study *T. canis* (18.8%), *Ancylostoma* spp. (16.9%), *Troglotrema salmincola* (7.8%) and *D. latum* (7.1%) were identified.

In our study, the most often diagnosed parasite was T. canis which was reported in 12 dogs (41.37%), including 6 females and 6 males. Similarly, Kimura et al. [26] in their study confirmed that the most frequently observed parasite in dogs was T. canis. In northern Greece the dominating parasite in hunting dogs was also T. canis (12.8%) and T. vulpis (9.6%), with a marginal invasion of A. caninum (2.8%) and Isospora spp. (3.9%) [27]. Michalczyk et al. [7] studied stool samples from dogs and cats, and evaluated the correlation between internal parasites infection and the owners' veterinary and breeding knowledge. It was found that animals which stayed in an animal shelter were the most infected ones, with the most common parasite eggs of T. canis and T. cati. In animals under the care of the employees and students of the Department of Veterinary Medicine low levels of infection (16.6%) were found, which confirms good and regular veterinary care provided for the animals. Schar et al. [8] investigated the occurrence and the variety of parasites in humans, dogs and pigs. They confirmed that in rural areas in Cambodian intestinal parasites are common, and some of them may be zoonoses, e.g. G. duodenalis, Entamoeba spp., T. canis. The examined dogs often went outside, had access to vegetable gardens and crops.

In our research internal parasites were found in 29 (43%) samples. Parasites were diagnosed regardless of age or sex. The number of infected animals was dependent on the frequency of visits to the veterinarian and antiparasitic prevention. However, the level of aggressiveness did not affect the occurrence of parasites. The presented research results show that pet owners should be aware of systematic deworming of their dogs. Additional analysis of the questionnaires showed that the knowledge of the owners of the risk caused by

invasive diseases was very small, and thus also the number of deworming treatments was low. It is significant that nearly half of the dog owners never dewormed their dogs, which, combined with the low awareness of the threat of zoonoses, is very dangerous to human health.

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