

The Prevalence of Endoparasites in Slovakian Household Dogs and Cats

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Research Article

Keywords: dog, cat, household, endoparasites, zoonotic species, Slovakia

Posted Date: March 10th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-280776/v1>

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Abstract

Pets play a pivotal role as definitive or reservoir hosts for many zoonotic parasites. Dogs and cats without any clinical signs may be a carrier for the infection. In a one-year-study, collected fecal samples of 257 dogs and 50 cats were examined coproscopically for the endoparasite infections. Out of 307 investigated fecal samples 107 (34.9%) were positive for the presence of the propagative stages of endoparasites. In 257 of dogs fecal samples, 12 different species of endoparasites were detected: *Giardia* spp., *Cystoisopsora* spp., *Sarcocystis* spp., *Hammondia/Neospora*-like, *Angiostrongylus vasorum*, *Capillaria aerophila*, *Crenosoma vulpis*, *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, *Strongyloides stercoralis* and eggs from the family Ancylostomatidae. Only 4 different parasitic species were found in 50 domestic cats' fecal samples - *Giardia* spp., *Cystoisopsora* spp., *T. cati* and larvae of *Aelurostrongylus abstrusus*. It was confirmed that a significant differences were found in relation to age for *Giardia* spp., *T. canis*, *S. stercoralis* and family Ancylostomatidae. Close and frequent contact between younger pets and people increases the risks for the transmission of zoonotic diseases.

Introduction

Intestinal parasites are important enteropathogens in dogs and cats. Moreover, several canine/feline intestinal parasites are considered zoonotic and are of great consequence to the public health (Claerebout et al., 2009). Pets play a pivotal role as definitive or reservoir hosts for many zoonotic parasites, especially in low-income countries and also in socio-economically disadvantaged communities (Traub et al., 2002; Traub et al., 2005; Salb et al., 2008). The period of our time is globalization which increases the likelihood of large-scale and intensive imports and exports of parasites, their vectors and disease vectors, what can lead to local endemics worldwide, or even to pandemics that have a significant impact on human and animal health. Thus, there are no longer any tropical diseases that can be avoided by entering these countries. Today we have passenger disease, we have local zoonoses and we have diseases due to imported animals and plants (Melhorn Aspöck et al., 2008). Zoonotic potential of worldwide spread endoparasites is of major importance due to dwelling proximity and common share of the same living space. Although dogs and cats in the households are usually well cared the frequency of antiparasitic treatments may vary and can be irregular in a great number (Becker et al., 2012). This is the reason why animals without any clinical signs may be a carrier for the infection. Some well-documented and typical canine/feline parasites are responsible for the spread of important zoonotic diseases. Those well-known zoonoses include echinococcosis, larva migrans (toxocariosis, ancylostomatidosis), and emerging and reemerging infections, such as cryptosporidiosis and giardiasis (Martínez-Moreno et al., 2007). All these intestinal parasites have an orofecal transmission cycle and most important factor for dissemination of these parasites is triggered by shedding of eggs or (oo)cysts into the environment (Claerebout et al., 2009). Antiparasitic control measures are based on the prevention (anthelmintic treatments) or reduction of worm eggs and (oo)cysts shedding into the environment. Therefore parasite preventive and control programs should be based and focused on active monitoring of endoparasites distributions, analysis of the environmental contamination and identification of potential zoonotic

agents. The most commonly protozoan and helminth species occurring in Europe are *Giardia* spp. and *Toxocara canis*, respectively. Special veterinary interest is focused on *Giardia* spp. where this infection is not always easy to cure and their roles as a zoonotic agent is not entirely clear (Pallant et al., 2015). *T. canis* is zoonotic and poses a health risk especially for the children (Macpherson, 2013). *Strongyloides stercoralis* represents another potentially zoonotic helminth agent (Deplazes et al., 2011). Others important parasitic diseases are represented by hookworm infections caused by *Ancylostoma* spp. and *Trichuris vulpis* (Traversa, 2011).

In contrast with stray dogs and cats, much less data is available for household pets. Despite the increasing numbers of dogs kept as companion animals in Slovakia during the past years, there are no data reporting the situation regarding parasite infections and vector-borne diseases in this group of animals. In Europe, the known works exist regarding parasite fauna in household dogs in Albania (Shukullari et al., 2015), Greek Islands (Diakou et al., 2019), and central Italy (Scaramozzino et al., 2018). Moreover, less information is available on the prevalence of endoparasites in cats. However, some studies about the cats endoparasites were reported from Transylvania (Romania) (Mircean et al., 2010), Netherlands (Nijse et al., 2016), and Poland (Wierzbowska et al., 2020). All above mentioned facts led us to the analysis of endoparasites incidence in domestic animals which were brought to the veterinary clinic by their owners either for preventive examination or examination based on clinical signs of disease.

Materials And Methods

Fecal samples were collected from 257 dogs and 50 cats at the veterinary medical center in Bratislava within the period of August 2018 up to December 2019. Most of the dog's samples (254) were collected by dog's owners, who came to the veterinary medical center for preventive care or were examined due to some gastrointestinal problems.

Totally 307 feces samples were examined for the endoparasite infection. Animals were sampled by owners during natural emptying for five consecutive days thus obtaining better chance of *Giardia* cysts detection. Owners who sent samples provided information on the animal origin, age and gender. Ethical approval was not required since all activities performed on dogs and cats in this study were performed by routine diagnostic procedures performed by veterinarians at each study site. Consent for the examination and sampling from pet owners of were obtained on a case-by-case basis. The research related to animals complied with all the relevant national regulations and institutional policies for the care and use of animals. Therefore, no additional authorization was required. The proposal was reviewed and approved by the Ethical Commission of Institute of Parasitology SAS in Košice.

All samples were stored without any preservation at 4°C and transferred directly to the laboratory at the Institute of Parasitology SAS in Košice, for further parasitological examination. The entire examination was performed within 24 – 48 hrs. Fecal samples were examined macroscopically for the detection of proglottids and then screened microscopically for the presence of worm eggs and protozoan oocysts. A flotation method with the Shaeter's flotation solution with specific gravity of 1.27 g.ml⁻¹ was used for the

coproscopical examinations. Three grams of fecal sample was mixed with water and centrifuged for 5 minutes at 1200 rpm (Eppendorf 5804 centrifuge, Germany). After the pouring out of supernatant, the Shaefer's flotation solution was added into 2/3 of the test tube and stirred with the sediment, and then centrifuged once again. The Faust flotation solution with specific gravity of 1.18 g.ml⁻¹ was used for the detection of *Giardia* cysts. After the centrifugation, 3 drops were taken from the surface of the flotation solution and put on a slide. All samples were further examined under the light microscope at 200 x and 400 x magnification (Leica Microsystems, DM 5000B light microscope, Germany). Parasitic elements were identified based on morphological characteristics by light microscopy (Mircean et al., 2011). Based on morphological and morphometric characteristics, the oocysts were identified within species: *Cystoisospora* spp.; *Sarcocystis* spp.; and *Hammondia*/*Neospora*-like (*N. caninum* and *Hammondia heydorni*) (Dubey et al., 2002).

Subsequently, all samples positive for *Giardia* spp. cysts were reexamined by the PCR method. DNA was extracted directly from the fresh feces samples. An aliquot sample of 200 mg of feces material was homogenized with Qiagen Tissue Lyser solution (Qiagen, Germany). In next step the DNA was purified by Quick-DNA™ Fecal/Soil Microbe Miniprep Kit. (Zymoresearch, USA) according to the manufacturer's instructions and stored at -20 °C until further use.

Amplification of glutamate dehydrogenase gene (gdh) was performed by semi-nested PCR. The resulting fragments were of 432 bp size. Amplification of beta – giardin gene (βg) was performed by nested PCR. The resulting fragment was of 511bp size. Amplification by semi- nested PCR and nested PCR in 25ml volume for the primary amplification contained 1X CoralLoad PCR Buffer, 1 mM MgCl₂, 200mM of each dNTP, 500nM of GDHef and GDHir primers, 2,5 U TaqPlus DNA Polymerase (Qiagen, Germany) and 5ml of DNA. Towards the secondary amplification (only for gdh gene) 1ml of PCR product from primary reaction and pair of primers GHDif and GDHir were used.

Both reactions were carried on MyCycler™ Thermal Cycler System (Bio-Rad Laboratories, USA). Known isolate of *Giardia* spp. was used as a positive control and PCR water was used as a negative control. Both controls were included into each PCR reaction. Obtained amplicons were separated on 1.5% agarose gel stained by GelRed® Nucleic Acid Gel Stain (BIOTIUM, USA) and TAE buffer (40 mM Tris, pH 7.8, 20 mM acetic acid, 2 mM EDTA).

Statistical analysis was done using Windows SPSS® (version 11.5) (StatSoft, Inc. STATISTICA, 2007). Cross tables and Chi-square tests were used to calculate possible correlations between age, sex or purity of breed and potential zoonotic endoparasites agents (*Giardia* spp., *Toxocara* spp. *Strongyloides stercoralis* and family Ancylostomatidae). Differences were considered significant at p <0.05 and highly significant at p <0.01.

Results

Total prevalence of endoparasites

The population of examined animals consisted of household owned dogs and cats. Collected fecal samples of 257 dogs and 50 cats were examined coproscopically for the presence of endoparasites. Out of 307 investigated fecal samples 107 were positive for the propagative parasitic stages of what represented the overall prevalence of 34.9%. Examination confirmed that 86 dogs (33.5%) and 21 cats (42.0%) were positive with one or more different parasitic species and genera. However, the majority of animals (25.4%) were infected with only one parasite species. Mixed infections were found in 9.8% of examined samples.

Dogs samples

In 257 of dogs fecal samples, 12 different species of endoparasites were detected. Those found were: *Giardia* spp., *Cystoisospora* spp., and *Sarcocystis* spp., *Hammondia/Neospora*-like, *Angiostrongylus vasorum*, *Capillaria aerophila*, *Crenosoma vulpis*, *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis*, *Strongyloides stercoralis* and eggs from the family Ancylostomatidae (Table 1). The household dogs were infected most frequently with *Giardia* spp. (20.2%). This was followed by *Cystoisospora* spp. (9.3%), *Toxocara canis* (7.4%), and eggs from the family Ancylostomatidae (4.3%), what is also a potential zoonotic agent. *Capillaria aerophila* eggs were observed in 4.3% of examined samples. Eggs of *Trichuris vulpis*, *Strongyloides stercoralis* larvae were detected in 1.2% of samples. Pulmonary worm larvae of *Angiostrongylus vasorum* was also detected during the coproscopical examination in 2 samples. The prevalence of *Toxascaris leonina*, *Crenosoma vulpis*, and *Sarcocystis* spp. was 0.8%. The lowest prevalence was recorded in *Hammondia/Neospora*-like cysts infection which was found in 0.4% of all samples. Out of the 257 examined dogs 59 (23.0%) feces samples contained only one type (family, genus or species) of parasite. Twenty seven samples (10.5%) contained two or more endoparasites of various origins.

Table 1
The prevalence of parasitic species in dogs (n – 257)

Parasite	P	Prevalence (%)
<i>Giardia</i> spp.	52	20.2
<i>Cystoisospora</i> spp.	24	9.3
<i>Toxocara canis</i>	19	7.4
<i>Capillaria aerophila</i>	11	4.3
<i>Trichuris vulpis</i>	3	1.2
<i>Strongyloides stercoralis</i>	3	1.2
<i>Toxascaris leonina</i>	2	0.8
<i>Crenosoma vulpis</i>	2	0.8
<i>Sarcocystis</i> spp.	2	0.8
<i>Hammondia/Neospora</i> - like	1	0.4
f. Ancylostomatidae	11	4.3
P – positive samples, n- the total number of samples; f – family		

Giardia spp. cysts were confirmed not only microscopically, but also by the PCR method. Out of the 52 *G. duodenalis* isolates confirmed by coproscopical examinations, 59.6% (31/52) were successfully amplified only at the β giardin loci. Twenty one isolates (40.4%) were amplified at the *gdh* and β giardin loci.

The examined dogs were divided by age into two groups. 89 (34.6%) of them were within the age of 1 year. Dogs up to 1 year were infected more often than older dogs. Distribution of endoparasites according the age, sex and breed is shown on the Table 2.

Table 2

The occurrence of endoparasites in household dogs according to the age, gender and breed.

Parasite	Age		Gender		Breed	
	≤ one year	≥ one year	Female	Male	Mix breed	Purebred
	(n = 89)	(n=168)	(n = 152)	(n = 105)	(n = 122)	(n = 135)
	p	P	P	p	P	P
<i>Giardia</i> spp.	26	25	28	24	24	28
<i>Cystoisospora</i> spp.	20	4	17	7	10	14
<i>Toxocara canis</i>	10	9	12	7	8	11
<i>Capillaria aerophila</i>	7	4	4	7	8	3
<i>Trichuris vulpis</i>	0	3	2	1	2	1
<i>Strongyloides stercoralis</i>	2	1	2	1	1	2
<i>Toxascaris leonina</i>	2	0	2	0	0	2
<i>Crenosoma vulpis</i>	1	1	1	1	0	2
<i>Sarcocystis</i> spp.	1	1	1	1	1	1
<i>Hammondia/Neospora</i> -like	1	0	0	1	0	1
f. Ancylostomatidae	4	7	8	3	5	6
P – positive samples, n – the total number of samples, f-family						

Correlations between potential zoonotic parasites (*Giardia* spp., *Toxocara* spp., *Strongyloides stercoralis* and eggs from family Ancylostomatidae) infection and age were determined. The chi-square statistic was 13.8876 and the p-value was 0.003062. The result was significant at $p < 0.05$ and $p < 0.01$. The dogs were between 2 months up to 16 years of age were divided into two groups. Dogs up to 1 year ($n = 89$; 34.6%) and dogs older than 1 year ($n = 168$; 65.4%). The younger dogs up to 1 year (47.2%) were infected considerably more ($p < 0.01$) than the older ones (25.0%). However, we have to state that the dogs older than 1 year were in numbers nearly twice as much than the younger ones.

Cats samples

Out of 50 examined cat feces samples, a simple infection was found in 19 cats (38.0%), and multiple infections were found in 2 cats (4.0%). Among of 21 positive cases 57.1% ($n = 12$) were male and 42.9% ($n = 9$) were female cats. Regarding the cat breed 47.6% ($n = 10$) were mixed cat breeds, 38.1% ($n = 8$) were European shorthair, and 14.3% ($n = 3$) were Ragdoll. The cat age ranged between 2 months up to 9

years. Cats younger than 1-year old were infected more frequently what represented 61.9% of all cases in this study. Parasites detected were *Giardia* spp., *Cystoisospora* spp., and *T. cati*. The first stage larvae of pulmonary lungworm *Aelurostrongylus abstrusus* was detected during the coproscopical examination as well. Though, it was found only in 2% of all examined samples. The most prevalent parasite was *Giardia* spp. (36.0%). This was followed by *Cystoisospora* spp. (4.0%). Helminth *T. cati* was found in 6.0% of samples. Cats up to 1 year of age were likewise dogs infected more significantly (53.3 %) than the older animals (20.0%; $p < 0.01$).

Out of the 18 cat feces samples, which were positive for *Giardia* cysts during coproscopically examination and submitted to the nested and semi - nested PCR, 16 were confirmed to be positive at the β giardin loci. Only two isolates were successfully amplified at both the *gdh* and β giardin markers.

Correlation between potential zoonotic parasite infection and gender/breed

Gender in dogs was distributed unevenly and were composed of 105 males (40.9 %) and 152 females (59.1 %). Gender distribution in cats was more even and consisted of 30 males (56.0 %) and 20 females (44.0 %). There was no significant correlation found between breed (pure breed vs. mixed-breed) and gender. It appears those two examined factors do not belong to the risk factors for potential zoonotic endoparasitism spread involving household dogs and cats population.

Discussion

A key objective for the control of dogs and cats parasites is reduction the risk of transmission for zoonotic diseases. Parasitic fauna and endoparasites prevalence in household pets depend on many abiotic and biotic factors such as geographic location, climate, demographic factors, sampling protocols, and diagnostic techniques, status of animal ownership, veterinary facilities, antihelmintic usage and public awareness (Katagiri and Oliveira-Sequeira, 2008). Our study detected high parasitic prevalences in dogs and cats of which most of them are of zoonotic importance. The diversity in contamination of canine and feline feces with different groups of parasites points out on the substantial potential of public spaces contamination and presents a constant and non-diminishing hazard to the public health. The occurrence of relatively high infection intensities can be result of deworming programs absence or bad selection of the antiparasitic drug administered without adequate copro-parasitological examination. Recent study is the first who provides data on gastrointestinal endoparasites in household dogs and cats in Slovakia. Almost 30.7% of dogs and 36% of cats were positive for the protozoan infections (mainly *Giardia* cysts) and nematode endoparasites. So far the known endoparasites occurrence in Slovakia is known from coproscopical feces examinations from parks and playgrounds in urban settlements (Antolová et al., 2004; Ondriska et al., 2013; Papajová et al., 2014; Bystrianska et al., 2019).

The nematodes infections identified in the dogs and cats feces may cause zoonotic diseases and pose a risk for human health. *T. canis* and *T. cati* may lead to the visceral and ocular larva migrans, which may trigger blindness. Similarly to hookworm *A. caninum* which also may set off cutaneous larva migrans

(Heukelbach and Hengge, 2009). The study of Fahrion et al. (2011) showed that a high percentage of *Toxocara* eggs found in the dogs feces identified by PCR analysis were actually *T. cati* where coprophagy of cat feces is quite common in dogs. However, *Toxocara* eggs in the present study have not been distinguished by PCR. We do not know how many of the *Toxocara* eggs found coproscopically were actually only contaminants from ingested cat feces. Since there is no difference in the zoonotic potential between *T. canis* and *T. cati* (Cardillo et al., 2009) this finding deserves special attention.

The overall endoparasites prevalence in household pets in this study was 34.9%. Detected prevalence was comparable to that obtained in studies conducted in Albania and Brazil (Shukullari et al., 2015; Curia et al., 2017) where the majority of animals were infected with only one parasite species (25.4%). In our study the majority of infection was represented by protozoa (31.6%) and remaining (17.9%) were helminthic infections. Combined infections were found in 9.8% of all examined cases.

The first stage larvae of *Angiostrongylus vasorum* were diagnosed in two dog fecal samples. This cardiopulmonary nematode, which can be found in dogs and several wild canines has been reported by identification of first-stage larvae from fecal examination what was similar to Greece findings (Diakou, 1995; Founta et al., 2000).

Our study showed that endoparasites prevalence in domestic cats (42.0%) is higher than the prevalence reported in a study from Canada, where only 6.0% of samples were positive for the parasite developmental stages (Hoopes et al., 2015). Similar lower infectivity levels (10.1%) were reported in study conducted in Japan (Itoh et al., 2016) and Germany (22.8%) (Barutzki and Schaper, 2013).

The prevalence's observed in this recent work was comparable to those observed in Greece and Hungary (Shukullari, et al., 2015; Kostopoulou et al., 2017; Capari et al., 2013). In contrast to dogs less data is available for the cats. In our study, the prevalence of *T. cacti* was found to be about 6.0%, *Cystoisospora* spp. 4.0%, and *A. abstrusus* 2.0%. The most common parasite was *Giardia* spp. with prevalence of 36.0%.

Helminth *T. cati* was found in 6.0% of examined samples and when compared with results stray cats results in Iran (44%) and Germany (27.1%) (Becker et al., 2012; Sharif et al., 2010), the prevalence in our study was significantly lower. This observation points out on the importance of adequate treatment and animals deworming. Work of Barutzki and Shaper (2013) found that the prevalence of *T. cati* in Germany was 4.7%. This is comparable to our results. In contrast to our data Capari et al. (2013) study found that the prevalence of endoparasites infection in Hungarian domestic cats was 17.4%. The data is comparable with studies from Brazil and Cyprus where *T. cati* was found to be prevalent in 16.67% and 12%, respectively (Diakou et al., 2017; Ramos et al., 2020).

Parasite *Aelurostrongylus abstrusus* is the most well-known nematode infecting cat respiratory tract as its natural host (Traversa et al., 2010). In our study, the larvae of *A. abstrusus* feline lungworm, were found only in one of fecal sample, but the Baermann technique was not used. Nematode larvae were detected by the floatation technique; and therefore, the actual infection with *A. abstrusus* is presumably underestimated.

In general canine and feline feces samples were coinfecting largely with protozoan cysts of *Giardia* spp. Since *Giardia* is a common cause of diarrheal disease in humans the companion animals are able to transmit it on the owners. Depending on the investigated animal population the prevalence data of *Giardia* infections in dogs and cats may vary extremely. As a result, the utilized diagnostic method is the most important factor affecting the prevalence rate (Bouzid et al., 2015). Our study demonstrated that the overall prevalence of *Giardia* spp. in domestic dogs and cats was 22.1% what is similar to study from Albania, where *Giardia* spp. prevalence was 26.4% (Shukullari et al., 2015). The overall *Giardia* spp. prevalence in cats was 36.0% and in dogs 20.2% this indicates that every third cat and every fifth dog were infected with this potentially zoonotic protozoan parasite - *Giardia* spp. Because of various *Giardia* hosts-specific genotypes/species in dogs and cats more studies are required on genotypisation of *Giardia* isolates in order to better understand the giardiasis epidemiology and transmission ecology. The infection prevalence in this study in household kept animals was much more higher when compared with the study of Bouzid et al. (2015), where the *Giardia* spp. occurrence was 15.2% in dogs and 12% in cats. We assume that the difference between those studies was due to use of different quantitative and qualitative diagnostic methodology.

Potential zoonotic agents represented by *T. canis* and eggs from family Ancylostomatidae were found in 7.4% and 4.3% of samples, respectively. Our study revealed that *T. canis* is the most common nematode in examined domestic dogs (7.39%). The prevalence of *T. canis* was comparable with studies where values were 8.7% (Katagiri and Oliveira-Sequeira, 2008; Klimpel et al., 2010), and 5.5% (Oliveira-Sequeira et al., 2002).

In general, the eggs from family Ancylostomatidae had higher prevalence than found in our study. In Brazil the hookworm prevalence ranged from 47–95.7% (Curia et al., 2017; Klimpel et al., 2010). In Romania it was 33% (Mircean et al., 2010). Dogs and cats up to 1 year of age were infected significantly more often than older animals ($p < 0.01$). The reason behind is that immune system of puppies and kittens is developing and not able yet to generate sufficient immunity (Gates and Nolan, 2009). Obviously a primary infection leads to an acquired immunity and does can reduce the parasite prevalence in older dog. Another possibility is that females may infect their puppies inside the uterus or via milk (Melhorn Aspöck et al., 2008).

Results of the present study indicate that animal gender does not play an important role as a risk factor for the endoparasites occurrence. This finding is similar to results from the other European studies (Riggio et al., 2013; Zanzani et al., 2014; Savilla et al., 2011). However, the age was identified as an important risk factor. The results suggest that young dogs (< 1 year) are significantly more susceptible to the ascarid and coccidial infections than adults. This is consistent with the findings from other studies (Claerebout et al., 2009; Mircean et al., 2010) where parasites incidence in young dogs is higher. This is also a justification for utilization of more effective deworming protocols, especially against ascarides. Another study emphasizes the importance of protozoan infections for dog health, especially in their first year of life [Claerebout et al., 2009; Mircean et al., 2010; Little et al., 2009]. Testing of canine and feline fecal samples contributes to knowledge on the prevalence of various parasitic infections in companion

animals. This survey is the first which evaluated the endoparasite status of client-owned, veterinary cared dogs and cats in Slovakia. The data presented in this study indicate the presence of a wide variety of endoparasites and demonstrate relatively high prevalence of potentially zoonotic agents. Therefore, the veterinary doctors and pets owners need to step up their interest on periodical endoparasites examination and antihelminthic treatment. Only these approaches will reduce the parasitism in pets and consequently lower the potential for transmission of zoonotic agents to humans.

Conclusions

Close and frequent contact between pets and people increases the risks for the transmission of zoonotic diseases. The contamination of the environment by dogs and cats feces represents an everyday serious health hazards. It is due to the spread of parasite developmental (cysts, eggs and larval) stages which can survive in the ecosystems for a very long time. We recommend the use of appropriate fecal and molecular diagnostic techniques to detect the spread of gastrointestinal parasites. The proper and timely diagnosis of zoonotic agents is very important. The utilization of the proper treatments and control strategies will lessen the risk of infection spread to the other household animals, and also reduce the subsequent exposure of humans to zoonotic parasites.

Declarations

Ethics declarations

Ethics approval and consent to participate

The research related to animals complied with all the relevant national regulations and institutional policies for the care and use of animals. The proposal was reviewed and approved by the Ethical Commission of Institute of Parasitology SAS in Košice. Consent for the examination and sampling from pet owners of were obtained on a case-by-case basis.

Consent to Publish

Yes

Authors' contributions

Conceptualization - IP and JŠo; methodology - JŠ and IP; formal analysis - JŠ, IP, JT and LT; investigation - JŠ, IP and LŠ; resources - IP; data duration - JB and IP; original draft preparation - JŠ and IP; review and editing - JŠ, IP, JŠo and VŠ; supervision - IP; project administration - IP; funding acquisition - IP. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the scientific Grant Agency of the Ministry of Education of the Slovak Republic, by support from the Slovak Academy of Sciences, VEGA no. 2/0138/21 and The Slovak Research and Development Agency under the contract no. APVV-18-0351.

Competing Interests

The authors declare no conflict of interest.

Data availability

The datasets in this study are available from the corresponding author on reasonable request.

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