Factors affecting the occurrence of gastrointestinal parasites and lungworm in dogs and assessment of antiparasitic drug use patterns

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Abstract

Dogs are hosts of parasites with different pathogenicities and zoonotic risks. The aim of our study was to assess factors that could affect the occurrence of endoparasitic infections in dogs using questionnaires for owners and examining canine faeces. Based on the responses of dog owners, we evaluated ways of the preventive and therapeutic use of antiparasitic drugs. One faecal sample was obtained from each dog (391 samples total), and then examined by flotation, larvoscopic, and polymerase chain reaction (PCR) methods. We focused on zoonotic tapeworms *Echinococcus multilocularis* and *Taenia crassiceps*. The answers in the dog owners' questionnaires showed that owners most often dewormed their dogs irregularly (96.4%), namely, twice a year (24.3%). In coprological examinations the following parasites were detected: *Giardia* spp., *Cystoisospora ohioensis complex*, *Neospora caninum*/*Hammondia heydorni, Toxocara canis*, *Toxascaris leonina*, *Capillaria* spp., *Trichuris* spp., *Angiostrongylus vasorum*, *Crenosoma vulpis*, *Ancylostoma* spp. and *Taenia* spp. A total of 4 out of 391 PCR investigated samples of faeces were positive for DNA of *Taenia* species. Sequences were obtained for 3 of them, confirming 100% identity with *T. crassiceps*. The zoonotic helminths *Toxocara canis* were the more prevalent species (9.3%, 4.3%, 1.9% group A, B, C). We consider the presence of *T. crassiceps* in 3 dog faecal samples to be serious due to its zoonotic potential. Data collected within this study showed that the prevention against endoparasites carried out at the breeders' discretion is insufficient to ensure the health of their dogs or to cover the risk of zoonotic transmission.

Endoparasites, Taenia, Echinococcus, zoonosis

Endoparasites of the canine gastrointestinal and respiratory tract represent a wide range of protozoans and helminths. Being causative agents of different serious animal diseases, they are of great importance in veterinary medicine. Many of them also pose zoonotic risks to humans. Dogs are often considered family members with whom humans have a close relationship. The risk of transmission from animals to humans is higher in children, the elderly, pregnant women, and immunocompromised patients (Stull and Stevenson 2015; Gurry et al. 2017). While there is some awareness of risks related to transmission of parasitic zoonoses from dogs to humans in the general public, the level of knowledge of this issue is often low. For example, research conducted in the EU countries using questionnaires discovered that only 35% (Pereira et al. 2016) or even 25% (Matos et al. 2015) of responders knew the term "zoonosis".

Giardiosis is a common infection in puppies and young dogs. A German study has shown that dogs predominantly host specific Giardia assemblages C and D, but zoonotic assemblage A has also been found in dogs (Sommera et al. 2018). Confirmed transmission of specific assemblage C from dog to human pointed to the immunodeficiency in patients as an important risk factor (Štrkolcová et al. 2015).

Toxocarosis is another zoonosis of global significance. *Toxocara* spp. eggs are extremely resistant, with environmental contamination being the most important source of infection. In many countries, public parks and playground sandpits have been investigated for

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environmental contamination with *Toxocara* spp. eggs (Gawor et al. 2015; Vanhee et al. 2015; Oteroa et al. 2018).

Dogs that can move around freely and are prone to predation resulting in subsequent infection, excretion, and possible transmission of eggs of dangerous parasites such as the tapeworm *Echinococcus multiocularis*, pose a significant risk of human infection (Svobodová and Lenská 2002; Kolářová et al. 2017; Svobodová 2018; Beranová 2019). The spread of *E. multiocularis* eggs to other countries is facilitated by unrestricted travel with dogs within the European Union (Fooks and Johnson 2015). Another cestode requiring canids as definitive hosts and posing a zoonotic risk for humans is *Taenia crassiceps*. It is associated, i.a., with neurocysticercosis in immunodeficient patients (Arocker-Mettinger et al. 1992; François et al. 1998), but also in immunocompetent individuals (Ntoukas et al. 2013).

In recent years, the pulmonary nematode *Angiostrongylus vasorum* has spread from Southern to Central Europe, causing serious and potentially fatal disease. Another nematode recently identified in the Czech Republic, *Crenosoma vulpis,* also causes severe bronchopneumonia. Both nematodes find favourable conditions for their spreading in the Czech Republic nowadays (Hajnalová et al. 2017).

The aim of our study was to assess factors that could affect the occurrence of endoparasitic infections (gastrointestinal parasites and lungworms) in dogs, using questionnaires for owners and examining canine faeces. Questionnaires were used to collect information on the use of antiparasitics in accordance with the approved package leaflet. These antiparasitics are veterinary medicinal products (VMPs) for companion animals which are frequently available without veterinary prescription in the Czech Republic. Based on the dog owners' responses, we evaluated the use of antiparasitic drugs for preventive and therapeutic purposes.

Materials and Methods

Dataset

In the period between 2015 and 2018, we approached veterinarians from 15 practices from different regions in the Czech Republic who engaged dog owners for cooperation. Totals of 391 samples of canine faeces and 398 completed questionnaires were collected. The questionnaires comprised of a description of the dogs and details of the way the dogs were kept, their outdoor movement, possibility of rodent hunting/scavenging, their health, what medical product was used, frequency of deworming and whether the drugs used were administered based on the results of a parasitological examination.

The consumption of endoparasitic VMPs reported in the questionnaires was compared with the total of VMPs containing antiparasitic active substances sold for dogs in the Czech Republic. For clarity, recorded values of the consumed active ingredients contained in sold VMPs are related to dogs weighing 10 kg and the generally recommended therapeutic dose according to the composition and type of VMP is taken into account.

Examination of samples

Sampling was organized by individual veterinarians who collected questionnaires and samples of faeces brought by breeders. The obtained faecal samples were shipped to our laboratory. Their examination included flotation with a saturated sugar solution of specific gravity 1.3. Species determination of oocysts of the genus *Cystoisospora* was performed on the basis of micrometric measurements. All samples were examined also by larvoscopy according to Baermann technique.

All samples were subsequently analysed by polymerase chain reaction (PCR) for presence of tapeworm *Echinococcus granulosus*, *E. multilocularis* and *Taenia crassiceps*. These samples were stored at −20 °C until processing. Total genomic DNA was isolated from individual faecal samples using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol and then stored at −20 °C. Single target PCRs were performed for the amplification of DNA fragments of the mitochondrial gene (*nad1*) of *E. granulosus* and the small subunit of ribosomal RNA (rrnS) of *E. multiocularis* and *Taenia* spp. (Trachsel et al. 2007) using PCRBIO HS Taq Mix Red (PCR Biosystems Ltd, London, United Kingdom). All PCRs were carried out in a total volume of 25 µl with 2 µl of extracted DNA. Amplicons were visualised on 1.2% agarose gel using Midori Green (Elisabeth Pharmacon, Brno, Czech Republic) under UV light. Positive samples were purified using the Invisorb Fragment CleanUp (Stratec Molecular GmbH, Berlin, Germany) directly from the amplicons. Sequencing was provided by a private service company (SEQme s.r.o., Dobříš, Czech Republic) on an automatic ABI 3730 DNA analyser and the obtained sequences were identified by BLAST analysis.

Table 1. The occurrence of endoparasites in dog faeces.

Group	Age in years	Results of flotation	Number of positive samples	Results of larvoscopy	Number of positive samples	Results of PCR	Number of positive samples	Number of examined samples
A	0–1	*Capilaria* spp.	1					54
		Giardia spp.	1					
		Cystoisospora ohioensis complex	1					
		Taenia spp.	1			*Taenia crassiceps*	1	
		Toxocara canis	5					
		Toxascaris leonina	1					
B	> 1–7	*Cystoisospora ohioensis complex*	1	*Angiostrongylus vasorum*	4			232
		Neospora caninum/Hammondia heydorni	1	*Crenosoma vulpis*	2			
		Taenia spp.	1			*Taenia crassiceps*	1	
		Toxocara canis	10					
		Toxascaris leonina	2					
		Trichuris vulpis	4					
C	> 7	*Ancylostoma* spp.	1	*Crenosoma vulpis*	3			105
		Taenia spp.	2			*Taenia* spp., *Taenia crassiceps*	1+1	
		Toxocara canis	2					
Total								391

Statistical analysis

Chi-square test was used for comparing the differences between individual categories and in cases where multiple factors had a *P* value lower than 0.3, the multifactor logistic regression analysis was added. The relationship between the observed phenomenon and the factors that could be affected were investigated by the multifactor logistic regression analysis.

Results

Coprological examinations

Few parasite infections are age-related. Due to the investigated dogs were divided into three groups (Group A–C) according to age. Of the total of 391 investigated dogs, 13.8% were 0–1-year-old dogs (group A). In this age category, *Toxocara canis* infection was confirmed in 9.3% and *Giardia* spp., *Cystoisospora ohioensis complex*, *Toxascaris leonina*, *Taenia* spp. and *Capillaria* spp. in 1.9% of cases equally. 59.3% of the dogs were age > 1–7 years (group B). In this group, *T. canis* eggs were detected in 4.3% of dogs, *Trichuris* spp. (1.7%), *Toxascaris leonina* (0.9%), *Taenia* spp., *Cystoisospora ohioensis complex* and *Neospora caninum*/*Hammondia heydorni* (0.4%). Larvoscopy showed infection with *Angiostrongylus vasorum* (1.7%) and *Crenosoma vulpis* (0.9%). In group (C) of over seven years old dogs (26.9% of all examined individuals), 1.0% was infected with *Ancylostoma* spp., 1.9% with *T. canis* and *Taenia* spp. and 2.9% with *C. vulpis*. The results of the coprologic examination are shown in Table 1.

Polymerase chain reaction

A total of 4 out of 391 PCR investigated samples of faeces were positive for DNA of *Taenia* species. Sequences were obtained for 3 of them, confirming 100% identity with *T. crassiceps*.

Breeding of dogs and risk factors

The answers in the dog owners' questionnaires showed that dogs (although many of them were kept inside the house) always have the opportunity to eat grass or lick anything during a daily walk and more than half of dogs (67.7%) hunt rodents, eat carrion or the internal organs of game animals.

Frequency of deworming

Owners most often dewormed their dogs irregularly (96.4%). Antiparasitics were administered twice a year (24.3%). Once, three or four times a year, endoparasitics are administered by 15.9, 9.2 or 9.5% of owners, respectively. No deworming treatment was recorded for 1.8% of dogs. Some of the owners did not answer all questions.

Deworming based on laboratory test

The administration of antiparasitics based on laboratory test results was declared by 6.1% of owners.

Use of VMPs

Most owners indicated that they administer antiparasitics in accordance with the approved package leaflet. Off-label use of veterinary medicinal products was mentioned by 2.8% questionnaire respondents.

Assortment of VMPs used in treatment

For treatment, dog owners most often used multicomponent endoparasitics (93%), namely a combination of praziquantel (against cestodes) and active ingredients (AIs) against nematodes or against nematodes and some ectoparasites. These AIs were pyrantel embonate, fenbendazole, febantel, oxantel, emodepside or milbemycin oxime. Praziquantel, pyrantel embonate and fenbendazole/febantel was the most common combination of the above-mentioned AIs (38% and 22.4% treatments, respectively). The combination of AIs such as pyrantel embonate and febantel, niclosamide and oxibendazole, emodepside and toltrazuril or AIs with an ectoparasitic effect such as imidacloprid and moxidectin or afoxolaner and milbemycin oxime were used less frequently (0.4–12% of treatments). The frequency of administration of mono-component veterinary medicinal products was 6.7%. These were veterinary medicinal products containing febantel, pyrantel embonate, flubendazole or selamectin. The most commonly administered mono-component VMPs was pyrantel embonate drug (69.8% of all mono-component VMP administered in this study). Most VMPs were administered to treat endoparasites only (91.4% of all antiparasitic treatments). Endectoparasitics were used in 8.6% of cases. Orally used VMPs came in the form of a tablet (81.6%), oral paste (12.7%) or oral suspension (1.6%). Veterinary medicinal products in the form of a spot-on solution (4.1%) were administered topically. In 2% of cases, adverse reactions were reported in association with VMP administration to dogs. Their manifestations were not beyond the expected adverse reactions listed in the approved package leaflet such as salivation, vomiting, diarrhoea or itching.

Statistical analysis of the data

The multiple logistic regression analysis in the two tested datasets, the deworming schedule and the deworming frequency, did not show a conclusive relationship between the investigated phenomenon and factors that could affect it ($P > 0.05$).

Discussion

The occurrence and prevalence of parasites in dogs can be influenced by many factors. The results of age group are summarised in Table 1. Toxocarosis (*T. canis*) occurs especially

in young dogs. In our group of 391 coprological examined individuals, 9.3% of dogs aged < 1 year, 4.3% of dogs aged 1–7 years, and 1.9% of dogs > 7 years of age were infected with this parasite. By informing and educating dog owners, veterinarians play a key role in reducing the amount of *T. canis* eggs in the environment (Overgaauw and van Knapen 2013). Currently *Toxocara* seroprevalence in the Czech human population is at 3.6%, a rate similar to or even lower than those in some other Central European countries (Skulinová et al. 2020).

We confirmed *Giardia* spp. cysts only in dogs aged < 1 year, where the percentage reached 1.9%. The zoonotic potential of *Giardia* is limited, but the confirmed transmission of specific assemblage C from dogs to humans indicates a risk to immunodeficiency patients (Štrkolcová et al. 2015). Using molecular biological methods, we focused on dangerous zoonotic tapeworms. *Echinococcus multilocularis* eggs were not found in any of the investigated dogs, nevertheless, it is necessary to educate owners through veterinarians about the need for prevention, especially in dogs with the potential for hunting rodents. The finding of the other zoonotic tapeworm *Taenia crassiceps* was surprising and alarming. Taeniid eggs were detected by PCR in 4 dogs, in one of them eggs were revealed microscopically by flotation. Out of the 4 dogs DNA of *T. crassiceps* was not confirmed in only one dog. Other species of genus *Taenia* was not specified further. *Taenia crassiceps* was confirmed by PCR in three dogs 12 years, 3 years, and 7 months of age. The 12-year-old dog was regularly dewormed 2 × per year. It lived in a house and could be taken for walks. Dog owners reported their dog frequently running away uncontrolled, with the potential of predation. A fixed combination of active substances praziquantel, pyrantel, and fenbendazol was used for deworming this dog and coprological examination was performed after the last deworming one month prior. The owner of a 3-year-old dog reported regular deworming 4 × per year. A combination of active substances praziquantel and fenbendazol was used for the last deworming. The owner did not mention the exact date of deworming, only providing the same year as when the coprological examination was performed. A seven-month-old dog was kept freely moving around the garden and treated 5 × at a one-month interval. However, a non-tapeworm product (selamectin) was used for the last treatment. The confirmed occurrence of *T. crassiceps* underscores the importance of choosing a suitable VMP and performing a coprological examination regularly.

Excreted eggs of *T. crassiceps* may cause cysticercosis, especially in individuals with weakened immunity, and endanger the health of not only animals (Hofmannova et al. 2018) but also humans (François et al. 1998; Trachsel et al. 2007). Dog owners could underestimate the risk of zoonosis and therefore, the risk of parasitosis should be mitigated by regular laboratory tests and corresponding therapy. A rapid screening test for taeniid eggs could be of great benefit to veterinary practices because it could stop their excretion. Parasites pathogenic for dogs without a zoonotic potential were also diagnosed in the investigated dogs. Lungworms *Angiostrongylus vasorum* (1.7%) and *Crenosoma vulpis* (0.9%) were detected by larvoscopy testing. Similar results were obtained by Hajnalová et al. (2017). As lungworm was rarely identified in dogs in the Czech Republic in the past (Husnik et al. 2010), the awareness of lungworm disease should be reinforced among animal owners and veterinarians (Roesel et al. 2014). Based on the findings from the examined group, the vast majority of dogs may occasionally become infected during a walk outside. The owner responses show that the frequency of administration of endoparasitics is chosen randomly regardless of the way of life. Endoparasitics are usually administered one to four times a year, most often 1–2 times a year. The frequency of administration does not take into account any risk factors, such as the dog's age or the possibility to move around outdoors freely and hunt rodents, which significantly increase the risk of infection. A survey in Germany (Strube et al. 2019) found that the frequency of dog deworming was 2.07 × a year although the questionnaire responses showed that 62% of the animals were at

risk because of their age and behaviour. For this risk group, it is recommended to perform a coprological examination or to administer an anthelmintic at monthly intervals according to the ESCCAP (European Scientific Counsel Companion Animal Parasites).

Adequate choice of antiparasitics and appropriate administration frequency are essential to prevent excretion of parasite eggs and subsequent environmental contamination. A wide range of freely accessible antiparasitic VMPs is available to dog owners without a prescription. However, the intended effect will not occur if VMPs are used without consulting a veterinarian (e. g. puppies and geriatric animals are at greater risk than healthy adults) or the dog's living conditions including the possibility of predation. Stipulating which VMPs should be prescription-only is one of the risk management tools of the National Agency that influence the handling of VMPs in practice.

As for the results presented, the actual prevalence of endoparasitosis in dogs will probably be higher considering that we can only examine one sample of faeces per dog. It should be noted that the dogs studied belong to owners that are more likely to co-operate with veterinarians.

In conclusion, the recorded frequency of dog deworming showed that the animals were at risk of infection because of their age and behaviour. Most of the dogs were dewormed irregularly and antiparasitics were administered twice a year. The administration of an anthelmintic at monthly intervals was not performed in the group of dogs at risk (for example the animals with possibility of predation). A wide range of antiparasitic VMPs is available, nevertheless, the intended effect will not be achieved if VMPs are used without adequate knowledge.

Dogs are hosts of parasites with different pathogenicities and zoonotic risks. Friendly relationship with humans requires consistent antiparasitic care that protects the health of both dogs and humans. Not even a high consumption of antiparasitic drugs can ensure the protection of dogs and their owners, unless they are administered purposefully, with appropriate knowledge and on the advice of a professional.

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