Factors Affecting the Occurrence of Gastrointestinal Parasites and Lungworms in Dogs and Assessment of Antiparasitic Drugs use Patterns

Eva Vernerova (vernerova@uskvbl.cz)
Institute for State Control of Veterinary Biologicals and Medicines

Nela Dvorakova
Institute for State Control of Veterinary Biologicals and Medicines

Vlasta Svobodova
University of Veterinary and Pharmaceutical Sciences Brno

Jiri Bures
Institute for State Control of Veterinary Biologicals and Medicines

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Research

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Abstract

Background

Endoparasites of the canine gastrointestinal and respiratory tract represent a wide range of protozoans and helminths. They are causative agents of different serious diseases of animals and many of them also pose zoonotic risks to human health. The education of dog owners and their families with the principles of human and animal health protection may especially prevent the spread of zoonoses such giardiosis, toxocarosis, echinococcosis and Taenia crassiceps infection. Pulmonary nematods are not zoonotic but they can cause serious and potentially fatal disease in dogs.

Methods

Three hundred ninety-one samples of canine faeces and 398 completed questionnaires were collected. The questionnaires comprised of description of individual dogs and detailed data about the way the dogs were kept and their deworming. Samples were examined by flotation, larvoscopy by Baermann and PCR method.

Results

We confirmed infection of *Giardia* spp., *Isospora ohioensis*, *Neospora caninum*/ *Hammondia heydorni*, *Ancylostoma* spp., *Angiostrongylus vasorum*, *Capillaria* spp., *Crenosoma vulpis*, *Toxocara canis*, *Toxascaris leonina*, *Trichuris* spp. and *Taenia* spp. Sequences were obtained for 3 of them confirming *T. crassiceps*. No deworming treatment was recorded for insignificant number of dogs. Dog owners most often used multicomponent endoparasitics.

Conclusions

Most of the dogs were dewormed irregularly and antiparasitics were administered namely twice a year, so monthly intervals were not performed in the group of dogs at risk for e.g. possibility of predation. Consumption of antiparasitic drugs cannot ensure the protection of dogs, their owners and other people, unless not applied purposefully, with appropriate knowledge and on the basis of the veterinarian's advice.

Background

Endoparasites of the canine gastrointestinal and respiratory tract represent a wide range of protozoans and helminths. Being causative agents of different serious diseases of animals, they are of great importance in veterinary medicine. Many of them also pose zoonotic risks to human health. Currently, the Czech Republic belongs among the countries with the highest numbers of dogs and cats per capita. They are often considered as family members with whom we have a close relationship. Dogs continue to play an important role in the society as working dogs and their social importance is steadily increasing reflecting the changes in the society. This results in frequent and close contacts of humans and dogs and increases the chance of transmission of zoonotic agents. As far as transmission in the direction from animals to humans is concerned, this risk is higher in children, the elderly, pregnant women, and immunocompromised patients [1, 2]. Some awareness about risks related to transmission of parasitic zoonoses from dogs to humans exists in the general public, but the level of knowledge in this area is often incomplete and low. As an example, research conducted in the EU countries using questionnaires discovered that only 35% or even 25% of responders knew the term “zoonosis” [3, 4]. A close and very friendly relationship with dogs implies sleeping together in a bed and it is often accompanied by neglecting hand washing [5], where both practices facilitate transmission of pathogens between dogs and humans. The education of dog owners and their families with the principles of human and animal health protection may especially prevent the spread of zoonoses. Colleagues from many countries have

highlighted the irreplaceable role of veterinarians in such education [3, 6, 7]. The concept of One Health focusing on the interlinks between humans, animals and environmental health requires interdisciplinary communication. Targeted education, including zoonotic diseases, should be integrated into medical education [8]. Parasitic infections in dogs usually compromise their health, irrespective of the fact whether the parasite has a zoonotic potential or not. It turns out, that most dog owners deworm their dogs regularly [9], but not always in accordance with the therapeutic dose for the parasitic species [3].

Giardiosis is a common infection of puppies and young dogs. It may be accompanied by diarrhoea, especially in puppies. In adult dogs, cysts are usually excreted in the faeces asymptomatically. German research study has shown that dogs predominantly host specific Giardia assemblages C and D, but zoonotic assemblage A has also been proved in dogs [10]. Confirmed transmission of specific assemblage C from dog to human pointed out to the immunodeficiency in patients as an important risk factor [11].

Further disease, toxocarosis, is zoonosis of global importance. Increasing of its seroprevalence in humans correlates with the occurrence of toxocarosis in dogs, frequency of human and animal movements and climate change [12]. *Toxocara* spp. eggs are extremely resistant and environmental contamination accounts for the most important source of infection. In many countries, public parks and playground sandpits were investigated for environmental contamination with *Toxocara* spp. eggs. For instance, *Toxocara* was confirmed in 53% of soil samples with the average density 4.2 eggs per 100 g of soil in Greater Lisbon, Portugal [13]. Similarly in Belgium, Flanders, *Toxocara* eggs were found in 14% of the public sandpits and in 2% of the sandpits of kindergartens [14]. In Poland, the number of clinical cases of human toxocarosis in children is increasing, what is associated with soil contamination with eggs [15].

Dogs with free movement and predation possibilities of subsequent infection, excretion and possible transmission of eggs of dangerous parasites as *Echinococcus multiocularis* pose significant risk of human infection. In the Czech Republic, 8.1% of fecal samples from herding dogs were positive to *Echinococcus* spp. [16]. *E. multiocularis* has been reported in red foxes and racoon dogs in all territory of the Czech Republic [17]. Fatal alveolar echinococcosis was diagnosed in ZOO kept apes in the Czech Republic [18]. Unfortunately the occurrence of human alveolar echinococcosis is increasing in the Czech Republic [19]. The spread of tapeworm eggs *E. multilocularis* into other countries is facilitated due to unlimited travel with dogs within the European Union [20]. Another cestode requiring canids as definitive hosts and posing zoonotic risk for humans is *Taenia crassiceps*. It is associated, inter alia, with neurocysticercosis in immunodeficiency patients [21, 22], but also in immunocompetent individuals [23]. In the Czech Republic, *T. crassiceps* tapeworm cysticercosis was also atypically found in naturally infected dogs as cases of subcutaneous cysticercosis and cysticercosis of thorax cavity as well as in ZOO animals [24]. This indicates the versatility of the parasite and the potential to cause severe health problems not only in humans, but also in other species.

In recent years, the pulmonary nematode *Angiostrongylus vasorum* has spread from South 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 spreading in the Czech Republic today [25].

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. We used also owners' questionnaires to collect information of using of antiparasitics for dogs 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 responses of dog owners, we evaluated ways of preventive and therapeutic use of antiparasitic drugs.

Methods

Dataset

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

The consumption of endoparasitic s VMPs resulting from questioners was compared with total sold of VMPs for dogs containing antiparasitic active substances in the Czech Republic. For the sake of clarity, the recorded values of the consumed active ingredients contained in sold VMPs are related to a “consensual” dog weighing 10 kg body weight and the general recommendation therapeutically dose according to composition and type of VMP is taken into account.

Examination of samples

Collected faecal samples were examined by flotation with a saturated sugar solution of specific gravity 1.3 and larvoscopy by Baermann. All samples were subsequently analysed by PCR for presence of tapeworm *Echinococcus granulosus*, *E. multilocularis* and *Taenia crassiceps*. 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 amplification DNA fragments of the mitochondrial gene (*nad1*) of *E. granulosus* and the small subunit of ribosomal RNA (rrnS) of *E. multiocularis* and *Taenia spp.* [26] using PCRBIO HS Taq Mix Red (PCR Biosystems Ltd, London, United Kingdom). All PCRs were carried out in the 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 private service company (SEQme s.r.o., Dobříš, Czech Republic) on an automatic ABI 3730 DNA analyzer and obtained sequences were identified by BLAST analysis.

Results

Coprological examinations

Achievement coprological examinations of investigated dogs were divided into three groups (Group A – C) according to age of dogs due to some specificity of parasitosis. Of total of 391 investigated dogs, 13.8% were dogs up to one year of age (group A). In this age category, *Toxocara canis* infection was confirmed in 9.3% and *Giardia* spp., *Isospora ohioensis*, *Toxascaris leonina*, *Taenia* spp. and *Capillaria* spp. in 1.9% of cases equally. Dogs over the age of one to seven years (group B) were 59.3%. In this group, *T. canis* eggs were detected in 4.3% of dogs, *Trichuris* spp. (1.7%), *Toxascaris leonina* (0.9%), *Taenia* spp., *Isospora ohioensis* and *Neospora caninum*/ *Hammondia heydorni* (0.4%). Larvoscopy showed infection with *Angiostrongylus vasorum* (1.7%) and *Crenosoma vulpis* (0.9%). In the group (C) of

dogs over seven years of age (26.9% of all examined individuals), 1% was infected *Ancylostoma* spp., 1.9% *T. canis* and *Taenia* spp. and 2.9% *C. vulpis*. The results of coprologic examination are shown in Table 1.

Table 1
The occurence of dog's endoparasites in faeces

Group	Age [Year]	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					
		Giardia spp.	1					
		Isospora ohioensis	1					
		Taenia spp.	1			*Taenia crassiceps*	1	
		Toxocara canis	5					
		Toxascaris leonina	1					
								54
B	>1 - 7	*Isospora ohioensis*	1	*Angiostrongylus vasorum*	4			
		Neospora caninum/ Hammondia heydorni	1	*Crenosoma vulpis*	2			
		Taenia spp.	1			*Taenia crassiceps*	1	
		Toxocara canis	10					
		Toxascaris leonina	2					
		Trichuris vulpis	4					
								232
C	> 7	*Ancylostoma* spp.	1	*Crenosoma vulpis*	3			
		Taenia spp.	2			*Taenia* spp., *Taenia crassiceps*	1+1	
		Toxocara canis	2					
								105
Total								391

PCR

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

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

Frequency of deworming

Owners most often dewormed their dogs irregularly (96.4%). Antiparasitics were administered namely 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 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.

Using 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 the treatment, dog owners most often used multicomponent endoparasitics (93%), namely 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 combinations of the AIs such as pyrantel embonate and febantel, niclosamide and oxibendazole, emodepside and toltrazuril or the AIs with ectoparasitic effect as imidacloprid and moxidectin or afoxolaner and milbemycin oxime were used less frequently (0.4–12% treatments). 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's treatments). Endectoparasitics were used in 8.6% of cases. Orally used VMPs were in the form of a tablet (81.6%), oral paste (12.7%) or oral suspension (1.6%). VMPs 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 as salivation, vomiting, diarrhoea or itching.

Discussion

The occurrence and prevalence of parasites in dogs can be influenced by many factors. Toxocarosis (*T. canis*) occurs especially in young dogs. In our group of 391 coprological examined individuals, 9.3% of dogs less than 1 year, 4.3% of dogs aged 1–7 years and 1.9% of dogs over 7 years were infected with this parasite. Dangerous accumulation of *Toxocara* eggs occurs even during limited excretion because these are extremely resistant and survive in the

environment for a long time. Environmental contamination and transmission of *T. canis* eggs to humans is an internationally known epidemiological risk [12]. Informing and educating dog owners through veterinarians play a key role in reduction the amount of *T. canis* eggs in the environment [6]. We cannot leave out the risk of resistance to pyrantel in ascarids and hookworm in dogs and cats [27, 28]. Although mono-component VMPs are used less commonly (6.7%) due to using of pyrantel embonate in 69.8% cases from using of mono-component VMPs the responsible vet should give recommendations on how to limit further selection for resistance to anthelmintics. We confirmed *Giardia* spp. cysts only in dogs less than 1 year, the percentage reached to 1.9%. Zoonotic potential of *Giardia* is limited, but confirmed transmission of specific assemblage C from dog to human indicates a risk for immunodeficiency patients [11]. Using molecular biological methods we have focused on dangerous zoonotic tapeworms. *Echinococcus multilocularis* eggs were not found in any of the investigated dogs, nevertheless, it is necessary to familiarize owners through veterinarians with the need for prevention, especially in dogs with the possibility of hunting rodents. The finding of the second 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. *T. crassiceps* was confirmed by PCR in three dogs, 12 years, 3 years and 7 months of age. The dogs, 12 years and 3 years, lived in the house with the possibility of walks. Dog owners reported frequent running away without control with the possibility of predation. The dogs were dewormed twice and four times a year, respectively, with VMP containing combination of praziquantel, pyrantel and febantel or fenbendazole. Seven months old dog was kept freely in the garden and treated five times in one-month interval. However, a non-tapeworm product (selamectin) was used for the last treatment. The confirmed occurrence of *T. crassiceps* alerts to choice suitable VMP and using 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 but also humans [23, 29]. For this reason the owners could underestimate risk of zoonosis and the risk of parasitosis should be suppressed by regular laboratory tests and corresponding therapy. Fast screening test of taeniid eggs could be great benefit for veterinary practice because they could stop shedding of danger eggs.

The parasites pathogenic for dogs without zoonotic potential were also diagnosed in investigated dogs. Lungworms *Angiostrongylus vasorum* (1.7%) and *Crenosoma vulpis* (0.9%) were detected by larvoscopy testing, similar results were obtained in 2017 [25]. As lungworms in the dogs were rarely identified in the Czech Republic in the past, disease awareness of lungworms among animal owners and veterinarians should be implemented [30]. Based on the investigated group of individuals, the vast majority of dogs may occasionally become infected during a walk. The responses of owners show that the frequency of administration of endoparasitics is chosen randomly regardless of way of life, usually one to four times a year, most often 1–2 times a year. Frequency of administration does not take into account any risk factors, whether dog age or the possibility of rodent hunting and free movement in nature, which significantly increase the risk of infection. By questionnaire action in Germany, frequency of dogs deworming was found 2.07 times a year although questionnaire responses showed that 62% of the animals were at risk for 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 ESCCAP (European Scientific Counsel Companion Animal Parasites) [31]. Proper choice of antiparasitic and appropriate frequency of its administration is essential to prevent excretion of parasite eggs and contamination of the environment with them. Wide range of freely accessible antiparasitic veterinary medicinal products is available without a prescription to be gained and used by dog´s owner. However, intended effect will not occur if veterinary medicinal products are used without consulting the veterinarian, i.e. without adequate knowledge when owners did not have a respect to criteria for deworming age of dogs or type of breeding

including possibility of predation. Stipulating certain VMPs as prescription only, is one of the National Agency risk management tools influencing the handling of VMPs in practice.

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

In the Czech Republic, consumption of VMPs with antiparasitic active substances is monitored similarly as in other EU countries by the National Agency, the Institute for State Control of Veterinary Biologicals and Medicines, as listed below.

Between the years 2015 and 2018 in the Czech Republic, 3,267,186 to 4,699,352 therapeutically doses of antiparasitics were used for treatment of dogs. Most frequently, VMPs were administered in the pharmaceutical form as tablet; approximately 2,898,493 to 4,395,305 doses of endoparasitics were used and approximately 2,763 to 25,149 doses of endectoparasitics. Pharmaceutical form as oral paste, suspension or gel was used in 147,038 to 237,210 therapeutically doses. Endectoparasitic in pharmaceutical form as spot-on was administered to 53,368 to 100,373 therapeutically doses. In terms of the reported total consumption of antiparasitics for dogs in the Czech Republic, the proportion of the pharmaceutical forms corresponds to the pharmaceutical forms in the monitored group, similarly rate of the consumption of combined VMPs to the consumption of mono-component VMPs. Various combinations of active substance as praziquantel with pyrantel embonate, fenbendazole, febantel, oxantel, emodepside or milbemycin-oxime were administered orally in tablets to approximately 2,766,649–4,380,478 therapeutical doses, of which the combination of praziquantel with pyrantel embonate and fenbendazole or febantel was administrated to 182,853–3,668,126 therapeutical doses.

Conclusions

- Recorded frequency of dogs deworming showed that the animals were at risk of infection for their age and behaviour. Most of the dogs were dewormed irregularly and antiparasitics were administered namely twice a year.
- The coprological examination or administration of an anthelmintic at monthly intervals was not performed in the group of dogs at risk for e.g. possibility of predation.
- The decrease of environment contamination was not achieved. Proper choice of antiparasitic and appropriate frequency of its administration is essential to prevent excretion of parasite eggs.
- Wide range of antiparasitic veterinary medicinal products is available, nevertheless, the intended effect will not occur if veterinary medicinal products are used without adequate knowledge. The prescription restriction is one of the National Agency risk management tools influencing the handling of VMPs in practice.

Dogs are hosts of parasites with different pathogenicity and zoonotic risk. Friendly relationship with humans requires consistent antiparasitic care that protects the health of both, dogs and humans. Even high consumption of antiparasitic drugs cannot ensure the protection of dogs, their owners and other people, unless not applied purposefully, with appropriate knowledge and on the basis of the veterinarian's advice.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information file. The consumption of VMPs with antiparasitic active substances data analysed in this study is available from the Institute for State Control of Veterinary Biologicals and Medicines, http://www.uskvbl.cz/.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

EV and VS conceptualized, designed the study and drafted the manuscript. ND performed molecular analyses of samples and edited the manuscript. JB supervised the study and critically reviewed the manuscript. All authors read and approved the final manuscript.

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