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ZOONOTIC PARASITOLOGICAL FINDINGS IN A PUPPY: THE COURSE AND THERAPEUTICAL EFFICACY

Burcáková, Ľ.[1, 2], Štrkolcová, G.[2], Königová, A.[1], Várady, M.[1]

[1]Department of Experimental Pharmacology, Institute of Parasitology
Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice
[2]Department of Epizootiology, Parasitology and Protection of One's Health
University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice
Slovakia

gabriela.strkolcova@uvlf.sk

ABSTRACT

Considering the close contact of companion animals and humans, gastrointestinal parasitic zoonoses are very widespread and represent a high risk of transmission with the potential of severe consequences affecting the digestive tract of both humans and other animals. In this study we focused on enteric zoonoses caused by *Toxocara canis* nematode, *Dipylidium caninum* tapeworm and *Giardia duodenalis* protozoa. Our primary aim was to observe *Toxocara canis* egg excretion within the 27 consecutive days before and after orally treatment (2 Caniverm® tablets) on Day 13 in a naturally infected puppy. An average egg per gram (EPG) of *T. canis* detected by coprological quantitative McMaster method was 4558.33 and 666.66, before and after treatment, respectively. The percentage of faecal egg count reduction (%FECR) in *in vivo* Faecal Egg Count Reduction Test (FECRT) has confirmed an 85.37 % efficacy against *T. canis*. Secondly, the efficacy of Caniverm® against the tapeworm *Dipylidium caninum* was also determined. No *D. caninum* proglottides were detected on Day 14. The data showed 100 % effectiveness of the anthelmintic treatment. Metrobactin® 250 mg has been tested as experimental therapy against *Giardia duodenalis* on Day 3. On day 10, no cysts were observed in the faeces after *per os* ¼ tablet administration twice a day for 7 days.

Key words: *Dipylidium caninum*; efficacy; egg excretion; EPG; FERT; *Giardia intestinalis*; McMaster; *Toxocara canis* treatment; zoonoses

INTRODUCTION

The most prevalent zoonotic gastrointestinal parasites in dogs are the nematodes *Toxocara* spp. and the protozoans *Giardia duodenalis*, and *Cryptosporidium parvum* [5, 29, 40]. Clinical presentation of enteric parasitoses is closely associated with the age and immunological status of the affected animal depending on the intensity of the infection or due to the presence of coinfections with other pathogens. Barutzki, Schaper [4] reported *T. canis* and *G. duodenalis* coinfection in young dogs above 6 weeks

of age. Furthermore, Moskvina, Zheleznova [31] proved, that endoparasites affect the most often puppies and young dogs under 1 year of age. The diseases caused by the enteric endoparasites are mainly manifested by anorexia, anaemia, diarrhoea, emaciation, vomiting and even death. Asymptomatic infections can also develop [3, 13, 47]. The monoxenous protozoa *G. duodenalis* has high pathogenetic effects and orofaecal transmission can occur through contaminated food or water [32]. On the other hand, though one of the most prevalent canine nematode, *T. canis* undergo several infection routes in a host body, such as tracheal, somatic and transplacental migration, and transmammary transmission, its life cycle is direct. In addition, a lactating bitch can be infected as they ingest of immature fourth stage larvae from vomit and faeces of the puppies or transmission through paratenic host may be encountered [34, 36, 37, 52]. The most typical and important infection route for puppies up to 3 months of age is the tracheal migration. Based on this, toxocarosis is primarily an important problem for puppies, which emphasizes the need for control, monitoring and treatment [11, 24].

A rarely occuring zoonotic parasite that also inhabits the alimentary tract is the *D. caninum* tapeworm, known as the cucumber seed or double pore tapeworm. The detection and diagnosis of this parasite is rather challenging, as its life cycle requires an intermediate host (e. g. *Ctenocephalides canis* flea) and irregular excretion of proglottids in the faeces, which makes diagnosis difficult [22, 46, 55, 61]. Dipylidiasis is mainly asymptomatic [16, 56]. If clinical signs occur, they are mostly non-specific, similarly to giardiasis and toxocarosis, apart from the scooting behaviour, that is typical for dipylidiasis [19, 44, 46, 47]. Considering the occurrence, mode of infection and the life cycle of enteric parasites, it is necessary to establish an accurate diagnostics with aimed therapy. In general, the treatment is mainly prophylactic without previous coprological examination, which increases the risk of persistent patent period and resistance with reduced antiparasitic efficacy [39].

Our primary aim was to monitor the day-dependent *T. canis* eggs excretion before and after treatment in a puppy within and to determine the efficacy of the administered therapy using the FECR test according to Coles et al. [10]. Parasitological examination revealed the presence of other zoonotic endoparasites, namely *G. duodenalis* protozoa and *D. caninum* tapeworm, that we set ourselves to observe and medicate.

MATERIALS AND METHODS

The animal

The 8-week old mixed breed female puppy was selected due to the previous diagnosis of toxocarosis with a medical history of vaccination, deworming, flea treated and no previous severe diseases. The animal was born in a shelter and has been housed indoors with another 6 puppies at about the same age. They had daily access to a garden in a fenced circular enclosure with a diameter of 2 m. The physical examination revealed: the weight of 2.4 kg, anorexia and cough with dyspnoe mainly at night, during exercise or in stressful situations. Watery, mucous diarrhoea with a blood content and abdominal distension with mild pain on palpation in this area were observed. We measured a slightly increased body temperature (38.5 °C) and respiratory rate (34 breaths.min^{-1}) before treatment. The heart rate values were in the normal physiological range. The fleas (*Ctenocephalides canis*) were found scattered along the hair coat mainly around the neck. The inspection of the perianal region dry cucumber seed shaped proglottids attached to the hair coat were noticed. The animal showed scooting behaviour.

Parasitological diagnostics methods

Toxocara canis

Qualitative copromicroscopic flotation method [23] had been conducted to confirm *T. canis* infection. For a more detailed description of the level of infection we applied the coproscopical quantitative McMaster technique according to Taylor et al. [54] using the flotation solution with specific gravity 1.240, where values of eggs *per gram* (EPG) defined the intensity of the infection. Results were evaluated in McMaster chambers via coefficient ×50, which shows sensitivity > 50 EPG for individual faecal samples.

Dipylidium caninum

The canine tapeworm *D. caninum* was diagnosed on the basis of the finding of white or light pink coloured proglottids in a fresh faecal sample (Fig. 1). The latter were separated from the faeces and preserved in 70 % alcohol solution for further microscopic examinations.

D. caninum packs of cells we diagnosed microspopically by pressing proglottides through two microscopic slides in order to push the content out [45] (Fig. 2).

Fig. 1. *Dipylidium caninum* proglottids found in fresh faeces

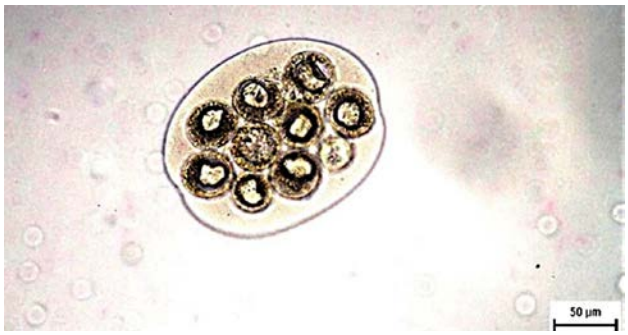

Fig. 2. *Dipylidium caninum* egg packets

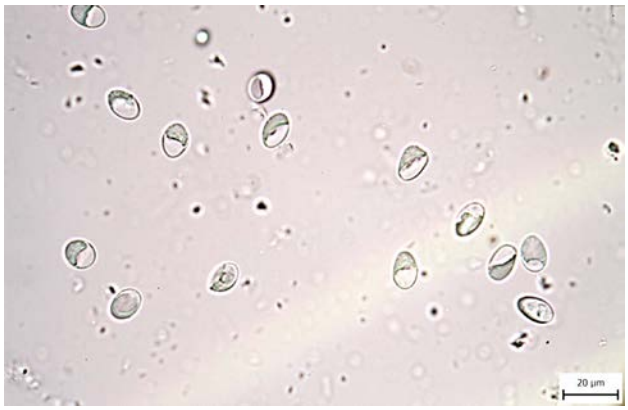

Fig. 3. *Giardia duodenalis* cysts

Giardia duodenalis

In order to identify *G. duodenalis* cysts we used the centrifugal-floatation technique with zinc sulphate solution (1.180 specific gravity) according to Faust (Fig. 3) [15].

FECRT (Faecal egg count reduction test) [10]

The FECRT was conducted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) instructions. The animal was treated by the recommended dose of fenbendazole 37.5 mg-pyrantel embonate 36.0 mg-praziquantel 12.5 mg combination/1 tbl. (Caniverm® mite tbl 6 × 1.75 g, Bioveta, Czech Republic) following the instructions for use of the corresponding product depending on the weight and age. The percentage of faecal

Table 2. Drug schedule in chronological order within the experimental period

Parasite	Day	Drug/active substance	Drug administration
Giardia duodenalis	3	Metrobactin® 250 mg/ metronidazole	*per os*
Ctenocephalides canis	4	Frontline Combo Spot-on on dogs/ fipronil, s-methoprene	spot-on
Toxocara canis	13	Caniverm® mite tbl 6 × 1.75 g/ fenbendazole-pyrantel embonate-praziquantel	*per os*
	18	Caniverm® mite tbl 6 × 1.75 g/ fenbendazole-pyrantel embonate-praziquantel	
Dipylidium caninum	13	Caniverm® mite tbl 6 × 1.75 g/ fenbendazol-pyrantel embonate-praziquantel	*per os*

egg count reduction (%FECR) of *T. canis* eggs was counted using the method: %FECRT = 100 – [(post-treatment EPG count/pre-treatment EPG count) × 100], where a shortage of treatment efficacy was presumed if %FECRT > 90 %.

Drug schedule

The puppy carried three different zoonotic diseases caused by three cathegories of parasites (*G. duodenalis* protozoa, *T. canis* nematode, *D. caninum* tapeworm). *C. canis* flea infestation also occured. The administered drugs are summarized in Table 2.

Ethical considerations

The research was approved by the UVLF Ethics Committee in accordance with applicable national and international animal welfare legislation.

The authors declare that there is no conflict of interest.

RESULTS

Toxocara canis

Our study lasted 27 days and a total of 84 individual faecal samples had been examined as part of the daily monitoring of the dynamics of *T. canis* infection before and after *per os* administration of Caniverm® deworming therapy. In order to verify the efficacy of the antihelmintic treatment, egg shedding during the experimental trial, followed by the evaluation of the results using the *in vivo* FECR test [10] were used.

Monitoring of the intensity of infection before and after treatment using the modiffied McMaster method [54]

The experimental period was divided into two phases, before and after the treatment. The first phase took 12 days, where *T. canis* eggs were detected using the McMaster method and egg counts assesed. *T. canis* egg excretion before treatment is represented in Fig. 4. The irregular egg excretion is clearly seen. The first 7 days the chart shows a slightly fluctuating tendency, while Day 8 indicated a significant decrease in the production with the lowest count on the Day 9. On Day 10, egg shedding increased markedly with the peak of 6800 EPG on Day 11. The mean (arithmetic) value 4558.33 EPG ranging from 900 epg to 6800 EPG was determined in the first 12 days of the experimental period.

The animal was treated with a single dose (2 tablets) based on a fenbendazole 37.5 mg-pyrantel embonate 36.0 mg-praziquantel 12.5 mg combination (Caniverm® mite tbl 6 × 1.75 g, Bioveta, Czech Republic) on Day 13. The patent period persisted for another 2 days after antihelminthics administration (Fig. 5). Egg shedding dropped sharply from 2500 EPG to 700 EPG without egg production on the Day 15 directly after treatment. The following day, egg shedding reappeared and was on the uptrend over the next few days. Due to persistent patent period, we repeated the therapy on Day 18 and Day 13.

The EPG counts decreased gradually with no finding of *T. canis* eggs on Day 23. The assessment was extended by 4 days for post-therapeutic control. No eggs were found

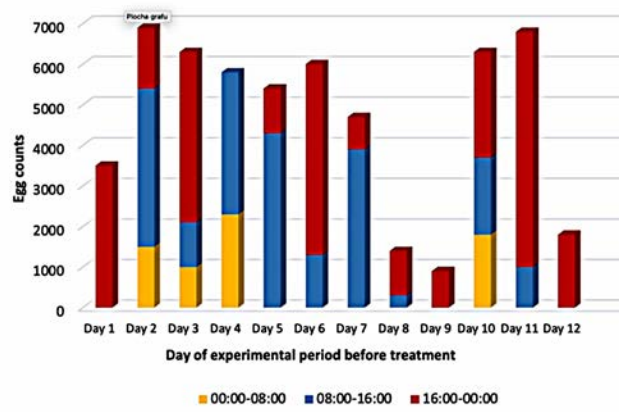

Fig. 4. *Toxocara canis* egg excretion before treatment in the course of time

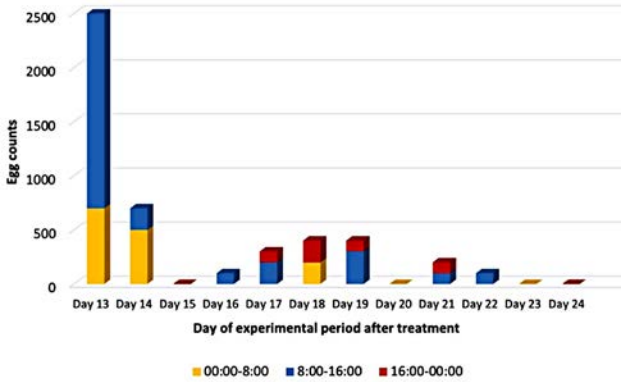

Fig. 5. *Toxocara canis* egg excretion after treatment in the course of time

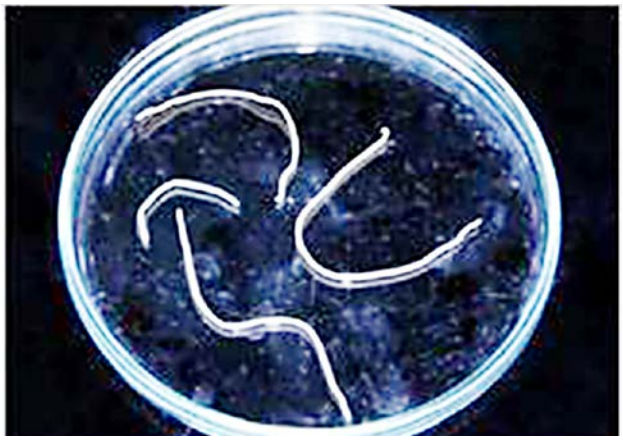

Fig. 6. *Toxocara canis* larvae found in the faeces samples after treatment

in the faeces samples during the control days. The mean (arithmetic) value 666.66 EPG ranging from 100 EPG to 2500 EPG was quantified in the post-treatment phase including Day 13 to Day 18.

Table 1. Egg counts within the trial and mean values before treatment *Toxocara canis* egg excretion. Mean (a) value is comprised of the egg excretion within the before treatment (Day 1—12) phase. Mean (b) includes range of Day 13—18 after treatment

Before treatment		After treatment	
Day	**n**	**Day**	**n**
1	3500	13	2500
2	5800	14	700
3	6300	15	0
4	5800	16	100
5	5400	17	300
6	6000	18	400
7	4700	19	400
8	1400	20	0
9	900	21	200
10	6300	22	100
11	6800	23	0
12	1800	24	0
Mean (a)	**4558.33**	**Mean (b)**	**666.66**

n—summary of egg counts per day; a—mean (arithmetic) egg counts during pre-treatment trial; b—mean (arithmetic) egg counts during post-treatment trial

Toxocara canis larvae finding after treatment

We found 14 *T. canis* larvae in the faeces sample directly after the first drug administration in ranged 1.9—11.7 cm in size (Fig. 6). Larvae with the size of 10.2 cm and 11.7 cm we diagnosed as adult females. After the second Caniverm® drug administration, we detected 4 larvae ranged from 2.4—5.8 cm.

Determination of antihelmintic efficacy using *in vivo* Faecal egg Count Reduction (FECR) analysis [10]

To evaluate the antihelmintic efficacy, we considered the mean (arithmetic) values (Table 1) during pre-treatment (from Day 1 to Day 12) and post-treatment (from Day 13 to Day 18) phase. The efficacy of Caniverm® against *T. canis* was 85.37 %, which means the shortage of drug efficacy. This finding is also substantiated by the fact of persistent patent period of *T. canis* eggs after the first drug administration. The irregular curve of egg shedding within the whole experimental period is showed in Fig. 7.

Dipilidium caninum

Faecal proglottids excretion and therapeutical approach

We have confirmed *D. caninum* infection by the positive finding of proglottides in a faeces (Fig. 1). Microscopic examination using the pressing method to extrude the proglottides content revealed typical egg packets (cocoons). Fleas *C. canis* (n = 15) were found and examined due to the presences of larvocyst cysticercoids, which showed a negative result. In total, 39 gravid tapeworm segments were found in the faecal samples before treatment. Fig. 8 shows the course of proglottides excretion within the pre-treatment phase. A sharp drop of excretion on the sixth day is rather significant with interruption from Day 7 to Day 12 included. Caniverm® was chosen as a treatment against *D. caninum* on Day 13. After drug administration we detected 3 proglottides in the faeces. Within the deworming against dipylidiasis it is also necessary to eliminate ectoparasites. Thus, the flea infestation was treated by the spot-on application pipette based on fipronil 67 mg, s-methoprene 60.30 mg (Frontline Combo Spot on on dogs 2—10 kg, 1 × 0.67 ml, MERIAL, France) on Day 4 of the experimental period.

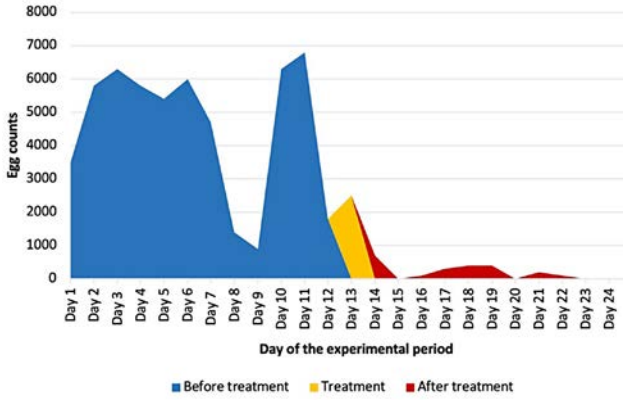

Fig. 7. *Toxocara canis* egg excretion during experimental period

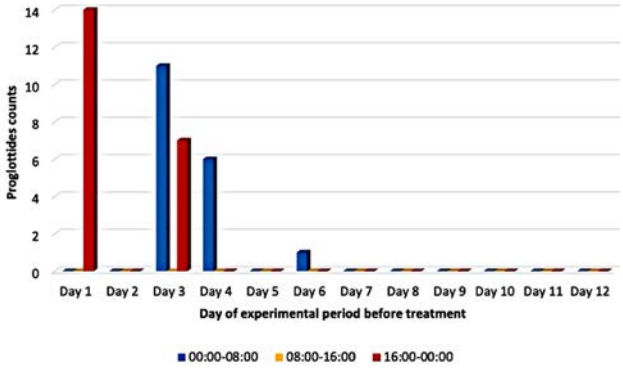

Fig. 8. *Dipylidium caninum* proglottides excretion before treatment

Giardia duodenalis

Therapeutical approach

The protozoan parasitic species *G. duodenalis* was detected using the concentration flotation method [15]. The puppy was orally given Metronidazole 250 mg (Metrobactin® 250 mg, Le Vet Beheer B. V., Holand) at the dose of ¼ of tablet twice a day for 7 days on the third day of the experimental period. During seven days of metronidazole administration, we performed one follow-up examination, where a significant decrease in a number of cysts was detected. The last control microscopic assesment we performed as a confirmation of the successful treatment. No evidence of *G. duodenalis* cysts on Day 10 was found.

DISCUSSION

The gastrointestinal tract of dogs may be affected by a variety of endoparasites with consequences encompassing no clinical signs up to loss of appetite, diarrhoea and even death. Zoonotic enteric diseases are particularly a serious threat due to their pathogenicity and possible transmission to humans [39]. Basic microscopic parasitological assessment of faecal samples using the flotation method remains still crucial in the identification of intestinal parasitic disorders [27, 30]. Due to the increased need for more accurate and precise diagnostics, assorted modifications of the McMaster method have been obtained [10, 18, 39, 41, 58, 60]. Based on this, quantification of egg counts per gram in the faeces by the McMaster method played a key role in determining the level or phase of parasitic infections. This knowledge enhanced the treatment of the patients and antihelmintic resistance were reliably determined [55] using FECRT to determine drug efficacy [10]. Pereckiene et al. [39] reported higher sensitivity of FECs (Faecal Egg Counts), assuming that the McMaster method procedure involved a centrifugation. He also confirmed that more accurate results are acquired using solutions with a higher specific gravity (range of 1.200-—1.270) and counting in a minimal of two grids of the McMaster chamber. Although the McMaster method is very accurate, it does not give a real picture of worm burden at a certain time, as eggs are excreted only by adult females, thus adult males and immature worms are not embraced. The other factors influencing the FECs are: female egg productivity and fecundity, nematode species and its biology, females and males ratio, the amount of faeces daily passed or egg concentration in a faeces sample. We must take into consideration age, immune and physiological state of the host or other ongoing diseases. As regards all these aspects, the results of McMaster method may differ [6, 7, 43, 57]. During an experimental period of our study, a circadian rhythm of *T. canis* eggs excretion using the McMaster method has been conducted. The technical procedure included centrifugation and flotation solution with 1.240 specific gravity and multiplication coefficient ×50 for more accurate EPG values, as mentioned above. It is well known that various nematode parasites evince diverse egg productivity within the day and each species of adult female ascarids excretes different amounts of unembryonated eggs in the faeces [7, 53]. To our knowledge, several investigations for egg excretion in roundworms have been performed and the results varied depending on the ascarid species. The following EPG values produced by females per day at the peak

have been reported: *Toxocara canis* 980—5.700 EPG.day^{-1} [42], *Toxocara vitulorum* 52.000—168.000 EPG.day^{-1} [43], *Ascaris lumbricoides* 240.000 EPG.day^{-1} [9, 46], and *Toxocara cati* 19.000—24.000 EPG.day^{-1} [12]. The resulting egg counts per day varies significantly, as it was in our case of 900—6.800 EPG.day^{-1} with the peak output during 16:00 — 00:00 hours within the first phase of the experimental period. The same circadian rhythm of egg excretion repeated also in the post-treatment phase. These findings agree with W a t k i n s, H a r v e y [59], who reported that consequent number of excreted eggs varies depending on the timing of the female production and on feeding and defecating mode of the animal. Based on the above-mentioned facts, we can assume that the ascarids egg excretion is irregular, and cannot be predicted and is affected by many factors, whether from the parasite's side or the host's body side. This claim is supported by R i c h a r d s et al. [42]. He stated that there is no direct association between the count of the *T. canis* adult worms per host body and the eggs passed in the faeces. That means, that the fact that the finding of 2 adult females in the faecal sample after deworming may not correspond to the number of eggs. The main goal of veterinarians should be to implement early and efficient treatment, to avoid reinfection and anthelmintic resistance. The broad spectrum of anthelmintics against ascarids is available and clearly described by several authors [1, 14, 25, 26, 28, 35, 38, 51]. According to R o b e r t s [43] pyrantel is very effective against immature *T. canis* developmental stages. Studies show that its efficacy is very high and did not change since it was first introduced [8, 50]. J a c o b s [21] points out that the efficacy of pyrantel pamoate alone is 95.9 %, but on the other hand an effect of 100 % was reported by B e c s k e i et al. [7]. By using febantel-based treatment there has been claims of 94.6—100 % efficacy [20]. A comparative study of S c h e n k e r et al. [50] described the effect of orally administered milbemycin oxime- and febantel-pyrantel embonate-based tablets against *T. canis* in puppies. The combination of febantel, pyrantel embonate and praziquantel shows 84.7—98.1 % efficacy [26, 49]. In our investigation, the result of egg output was found at the bottom boundary of this margin, which was also confirmed by persistent patency after treatment. This fact just confirms that the combinations of febantel and pyrantel embonate with other drugs shows reduced effect as previously mentioned. Praziquantel has been successfully used against tapeworms in dogs [2, 48, 53] as we confirmed in our case of *D. caninum* infection in a puppy. Taking into consideration that not only monoinfection was treated, but 3 parasitic concurrent zoonotic parasites, also the course of *Toxocara* egg shedding could be changed and also physical manifestation of such animal can be different. In the case of giardiasis, the administration of metronidazole markedly changed the consistency of the faeces from watery with blood content to mild solid. Metronidazole is very efficient against cysts excretion as previously published by Z a a t et al. [62], G a r d n e r, H i l l [17], N a s h et al. [33]. As we described, it is very important to consider many factors and aspects, before the inference is deduced. Last but not least, our results are not statistically significant as only a single experimental animal had been treated, which creates the room for further investigations.

CONCLUSIONS

The companion animals are common reservoir of various illnessess with zoonotic potential, which is from the point of view of health protection very alarming. The subject of this study, a puppy, carried three parasitic zoonoses (toxocarosis, dipylidiosis, giardiasis). We specifically investigated the dynamics of *Toxocara canis* egg excretion and efficacy of Caniverm® against *Toxocara canis* and *Dipylidium caninum*. Experimental treatment using Metrobactin® against *Giardia duodenalis* was also conducted. The purpose of our study was to verify that the chosen antiparasitic therapy may not be effective in many cases, especially if animal owners use the same drugs repeatedly without previous parasitological assessment. We wanted to highlight the need of precise parasitological examination not only for general diagnostics, but also to exclude parasite presence after treatment. As advice, closer communication between the veterinary doctors and owners is needed, to explain what are the risks and disadvantages of unproper diagnostics and treatment, mainly if severe zoonosis are involved.

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