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COMPARISON OF THE EFFICIENCY OF DIAGNOSTIC TESTS USED TO PROVE GIARDIASIS IN TERMS OF THEIR PRACTICALITY AND USE IN THE VETERINARY CLINICAL PRACTICE

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ABSTRACT

Giardiasis is a protozoan disease that affects the health of animals, as well as other humans all over the world. Based on its host spectrum and genetic variability, *Giardia duodenalis* is classified into 8 assemblages (A–H). The present study was aimed at comparing the efficiency of the three most frequently used methods (the flotation method, the SNAP test and the ELISA assay) for the detection of giardiasis in carnivores in terms of the applicability thereof for the scientific purposes and the practicality of their application in the veterinary clinical practice. In the period from March 2020 to February 2022, a total of 173 faecal samples (141 samples collected from shelter dogs; 28 samples from pet dogs; and 6 samples from working dogs) were examined by applying the flotation method. The prevalence of *Giardia duodenalis* identified by the flotation method was 25 %. The SNAP test conducted with the fresh faecal samples revealed the high-level efficiency of 96 %, whereas the enzyme-linked immunosorbent assay (ELISA) achieved the efficiency of 65 %. By applying the nested PCR method, five samples were positively tested for assemblages C and D (*G. canis*) by the amplification of the *bg* and *tpi* loci. The dogs from shelters which were positive for *G. duodenalis* were also presented with a coinfection caused by other intestinal parasites, such as *Trichuris vulpis* (28.0 %) and parasites from the *Ancylostomatidae family* (8.0 %).

Key words: assemblages; dogs; ELISA assay; flotation methods; *Giardia duodenalis;* giardiasis; nested PCR; SNAP test

INTRODUCTION

Giardia duodenalis (also known as *G. intestinalis* or *G. lamblia*) is an intestinal parasite that occurs in developed, as well as developing countries. It is a cause of about 280 million cases of diarrhoea in humans per year [28]. Giardiasis is a zoonotic opportunistic disease with a cosmopolitan spread, afflicting primarily young and immunocompro-

mised individuals. Its developmental cycle is simple and direct, and it does not require any vector or intermediate host to infect a new host. The cycle is based on the alternation of the vegetative stage – a tropozoite, and an environmentally resistant infectious stage – a cyst. A cyst is the only stage of *Giardia duodenalis* in which it is able to survive for a long time outside a host, and it is responsible for launching a new infection cycle [20]. The disease is transmitted by the faecal-oral route and after the ingestion of food or water contaminated by the cysts. The disease is therefore classified as a waterborne and foodborne disease [11, 22, 30, 41, 42].

The basic method for confirming the giardiasis is a microscopic finding of *G. duodenalis* cysts in animal or human faeces. The prevalence of giardiasis may differ, depending on a selected examination method [39].

The standard technique that is used for the detection of *G. duodenalis* cysts is the flotation microscopic examination with the use of the flotation solution (Faust's solution – 33 % $ZnSO_4$). Since the cysts are excreted intermittently, sometimes with a pause lasting for several days, it is necessary to examine at least 3 separate faecal samples within the period of 5–7 days, or to examine the samples collected on three consecutive days [10, 37, 40]. The other diagnostic techniques that are frequently used include, for example, the rapid SNAP test, ELISA (enzyme-linked immunosorbent assay), IFA (indirect fluorescent antibody test) and DFA (direct fluorescent antibody test), whereas the PCR test is used to confirm the presence of assemblages [34, 38]. However, these techniques must be conducted in laboratories with the special equipment and the personnel with the required expertise, and they require higher financial costs.

The application of the commercial ELISA assay aimed at proving the presence of a coproantigen is a highly-sensitive technique (sensitivity: 99–100 %; specificity: 96–99 %), and its advantage is that it is not dependent on the presence of cysts in the examined samples [27, 33]. Veterinary clinics frequently use the SNAP tests, which are based on the principle similar to that of the ELISA assay; however, they provide very fast results – within 10–15 minutes [31, 33]. The presence of coproantigens is most frequently confirmed by applying the flotation method, while the ELISA assay and the SNAP test are the next two most frequently used techniques. Some authors state that compared to the microscopy, the IFA and DFA tests for the detection of *Giardia* cysts exhibit higher sensitivity and specificity (up to 100 %) [8, 32].

The genetic characterisation of *G. duodenalis* is conducted by applying the conventional nested PCR method with the exact identification of assemblages and sub-assemblages. There are several different protocols for the study of various gene loci (*gdh, tpi* and bg) with the use of specific primers [6, 18]. At present, the most frequently used protocol is the MLST (Multilocus Sequence Typing), which is based on multiple (up to five) genes. It is aimed at studying the genetic variability, the ability to divide the individual isolates into groups and detect the mixed infections. The real-time PCR method is also one of the methods that are used to detect the assemblages, primarily from human isolates. There are many research projects that are currently investigating into the confirmation of cysts in the environment and water [4, 31].

Based on the genetic differences, *G. duodenalis* is categorised into eight assemblages. Assemblages A (*G. duodenalis*) and B (*G. enterica*) are regarded as zoonotic and have been confirmed not only in humans but also in a wide spectrum of hosts [5, 9]. The remaining 6 assemblages are host-specific. Assemblages C and D (*G. canis*) are typical of dogs and other canids. In domestic mammals, such as cattle, sheep and goats, the assemblage E (*G. bovis*) was detected. The assemblage F was found in cats (*G. cati*), the assemblage G (*G. muris*) in rats, and the assemblage H (*G. simondi*) in marine mammals [14, 42].

The disease may be asymptomatic, acute or chronically causing catarrhal gastroenteritis. The symptomatology may vary, depending on the assemblage, the virulence of the infecting strain of *Giardia*, the current state of the host's immunity, the host's food, a coinfection with other enteropathogens, the composition and the function of the resident microbiota and the immunity modulation of *Giardia* (Variant-specific Surface Protein), while the environmental factors also play an important role [3, 4, 7, 8, 10, 14]. The interrupted excretion of cysts, a low count of cysts in faeces, and asymptomatic infections may complicate the diagnostics [23]. Farm animals, particularly the beef cattle, may suffer from diarrhoea, retarded growth, loss of weight (thus exhibit decreased productivity) and even death; all that results in significant economic losses [5, 18]. One of the main clinical symptoms of giardiasis in carnivores is diarrhoea, which is caused by the malabsorption combined with the hypersecretion [33]. The faeces are

characterised by a strong smell and the presence of mucus; they should never contain blood [13]. Giardiasis is more frequently detected in dogs from shelters that in pet dogs; this is primarily caused by the poor hygiene conditions in shelters, a higher concentration of individuals at one place, shared enclosures and the subsequent contamination of the environment with faeces containing the infectious stages of parasites in shelters [12, 16, 17].

MATERIALS AND METHODS

In the period from October 2020 to February 2022, a total of 173 samples of dog faeces were examined. The samples were collected from the dogs kept in the shelter of the Union of Mutual Help of People and Dogs (UVP) near Košice. The other examined groups included the dogs from households in Košice and the surrounding regions, and the working dogs from Bratislava. There were two age groups: the dogs younger than 6 months and the dogs older than 6 months. All the samples were examined by applying the coprological flotation technique, based on the differences in the specific weights of the propagation stages and the flotation solution that is used. The post-flotation positive samples were subjected to the commercial diagnostic tests: SNAP, ELISA and PCR. The IDEXX SNAP *Giardia*® testing kit (IDEXX Laboratories Inc., Westbrook, Maine, USA) is used as a rapid enzyme immunoassay for the detection of *Giardia* antigen in the faeces of carnivores. A positive test result confirms the presence of the coproantigen and a high probability of the presence of cysts excreted with faeces into the environment. According to the manufacturer's instruction manual, it is possible to examine samples made of fresh faeces, faeces stored for a maximum of seven days at 2–8 °C, or frozen faeces. Therefore, the assay efficiency was tested using the fresh samples, as well as the samples that were frozen and then defrosted after 6, 9 and 12 months. A similar principle is used in the *Giardia lamblia* Antigen ELISA kit (EAGLE BIOSCIENCES, INC., Nashua, USA), which is intended for the qualitative detection of the *Giardia duodenalis* antigen in faeces. In our present study, the ELISA assay was conducted with the frozen samples only. The 26 positive samples with moderate to high intensity of infection were included in SNAP and ELISA detection. For the molecular identification of the assemblages, we used Nested – PCR method, amplifying the genes sequences *triose-phosphate isomerase* (*tpi*) and *β-giardin* (*bg*), following the protocols published by Sulaiman et al. 2003 and Feng, Xiao 2011 [13, 36].

RESULTS

Flotation method

Out of the total number of 173 samples, 141 faecal samples collected from the shelter dogs were examined during the study period. The positivity was confirmed in 32 samples, representing the 23 % prevalence. Out of 28 tested samples of faeces from the pet dogs, 9 were positive (32 %). The presence of *Giardia duodenalis* cysts in the working dogs was detected in 2 samples, out of the total number of 6 samples.

SNAP test

The SNAP test was used to analyse 43 samples identified as positive by applying the flotation method. Out of 26 samples of fresh faeces, the presence of the *Giardia duodenalis* coproantigen was confirmed in 25 samples. The efficiency of the SNAP test with the fresh faecal samples was 96 %.

Twenty six frozen samples were tested after 6, 9 and 12 months. After 6 months, the test revealed positivity in 17 samples, representing the 65 % efficiency. After another period of 3 months, the samples were tested again, while 18 of the samples were found positive; the efficiency of the SNAP test was 69 %. After 12 months, the samples were tested for the third time. The SNAP test efficiency was identical to that identified after 9 months. There were 18 positive samples (Table 1). Figure 1 shows that the results of the individual tests were identical.

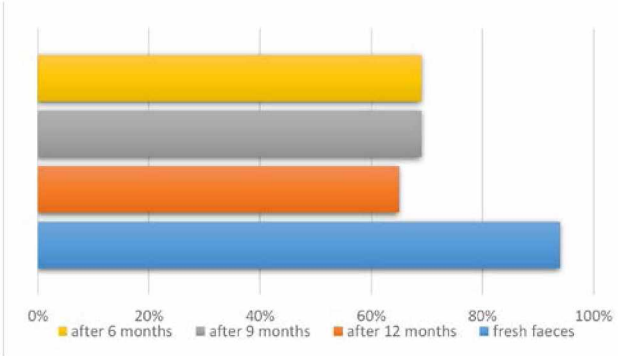

Fig. 1. Comparison of positivity in samples after 6, 9 and 12 months

Table 1. Examination of faeces by the SNAP test

Sample number	Fresh faeces	Frozen faeces after 6 m	Frozen faeces after 9 m	Frozen faeces after 12 m
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	-	-	+
6	+	-	-	+
7	+	-	-	+
8	+	+	+	+
9	+	-	-	-
10	+	-	-	+
11	+	+	+	+
12	+	-	+	+
13	+	+	+	-
14	+	+	+	+
15	+	+	+	+
16	+	+	+	+
17	+	+	+	+
18	+	-	-	-
19	+	+	+	+
20	-	+	+	+
21	+	+	+	+
22	+	-	-	-
23	+	+	+	-
24	+	+	+	-
25	+	-	-	-
26	+	+	-	-

m – months

ELISA assay

The ELISA assay was used to evaluate 26 selected samples that were identified as positive by applying the flotation method. The presence of coproantigen was detected in 17 samples (65 % efficiency).

Nested PCR method

The amplification of *bg* and *tpi* genes was carried out in 5 microscopically positive samples collected from the pet dogs. In the both cases, the amplification of 1 of the samples was unsuccessful. The genetic identification of the *G. duodenalis* species was confirmed by the direct sequencing of the PCR product. In 4 samples, the amplification of the *tpi* gene confirmed the presence of assemblage C, while the amplification of the *bg* gene identified, in addition to assemblage C, also assemblage D in one of the samples. Assemblages C and D are the specific assemblages that occur in dogs and other canids.

Coinfection with other endoparasites in the examined samples

Based on all 173 samples analysed by applying the flotation method, the dogs were found to be infected not only by *G. duodenalis* but also by other endoparasites. Their occurrence was only detected in the dogs kept in shelters. The frequency of the presence of parasites other than *G. duodenalis* was 26 %. The percentages of the occurrence of the individual endoparasites were as follows: *Trichuris vulpis* (38 %), followed by parasites from the *Ancylostomatidae* family (35 %), *Cystoisospora* spp. (15 %), and *Toxocara canis* (12 %). In 23 samples (13 %), various coinfections were detected. Out of them, 11 samples were found to be presented with a coinfection by *G. duodenalis* and other parasites. In these samples, the most frequently

identified coinfection was the coinfection by *G. duodenalis* and by *T. vulpis* (28 %).

A monoinfection by *G. duodenalis* was detected among the shelter dogs in 25 samples, representing 18 % of the total amount.

DISCUSSION

Endoparasitic intestinal infections affect the health not only of animals but also of humans, since many of them are transmitted zoonotically. In human populations, dogs are used for guarding, working, hunting and rescue purposes, but primarily as pets that are important companions in many households.

Despite the fact that they play an important role in human lives, they may be sources of parasites that infect humans. Giardiasis ranks among the most intensively spread parasitic diseases that occur in various regions all over the world. In the present study, the presence of cysts and the coproantigen was confirmed in the population of shelters dogs, pet dogs and working dogs. The prevalence of giardiasis varies and often depends on a number of factors, including an animal's age and health, its living conditions, a selected diagnostic method and a test group [39].

The application of the flotation method revealed the prevalence of giardiasis in 23 % (32/141) of the dogs kept in the shelter of the Union of Mutual Help of People and Dogs (UVP), while the prevalence in the pet dogs amounted to 32 % (9/28). The studies that included various groups of dogs, which lived in different living conditions, have confirmed that there were differences in the prevalence of giardiasis. A higher occurrence was identified in the dogs kept in shelters and in other groups. The factors which have negative effects on the spread of infections may include, in particular, an excessive concentration of dogs at one place and poor hygiene conditions. In the study conducted in 2014, authors A d a m o v á and Š t r k o l c o v á confirmed a 10.3 % higher prevalence in the very same shelter, while the frequency of the occurrence among the pet dogs amounted to 25.1 % (6/23) [1]. Three years later, M r a v c o v á et al. confirmed the 16.6 % (9/54) occurrence of this disease in the UVP [21]. In years 2010–2012 in Wroclaw, out of 128 examined faecal samples collected from the pet dogs, 27 were positive – the prevalence amounted to 21.1 % [24]. In Germany, the 9.5 % occurrence was identified in the pet dogs [8]. In a study conducted in Romania in the years 2008–2009, authors investigated into the occurrence of parasitic infections in pet dogs, shelter dogs, herding dogs and breeding dogs. The positivity rate for the shelter dogs amounted to 16.5 % (27/164); as for the dogs kept in breeding stations, it was 7.2 %; 4.8 % for the pet dogs; and 4.3 % for the herding dogs [19]. In a study conducted by U i t e r j w i j k and N i j s s e in 2018, significant differences were found between the different populations in the prevalence of *G. duodenalis*, while the highest prevalence of *G. duodenalis* was identified for the hunting dogs (64.9 %) and the lowest one for the pet dogs (7.9 %) [40].

In our present study, after the coproantigen was confirmed, the both tests (SNAP and ELISA) were used with the same samples in the same quantities. The IDEXX SNAP *Giardia*® test exhibited the highest efficiency – as much as 96 % (25/26) with the use of the fresh faecal samples. The tested samples also included the frozen faecal samples, stored at temperatures below -20 °C. In this present study, for the faecal samples that were defrosted after 6 months of the execution of the flotation method, the efficiency was proved to amount to 65 % (17/26). The efficiency of the SNAP test after 9 and after 12 months was 4 % higher than the efficiency after 6 months.

In a study conducted by U i t e r w i j k and N i j s s e in 2018, out of 4 applied diagnostic tests (IDEXX SNAP *Giardia*®, qPCR, CSF and DFA), the highest specificity (99.6 %) was identified for the IDEXX SNAP *Giardia*® [40]. In the present study, the Antigen ELISA assay for faecal *Giardia duodenalis* was used with the defrosted faecal samples, and the confirmed efficiency amounted to 65 % (17/26). The same percentage was achieved in the evaluation of the samples defrosted after 6 months by applying the SNAP test. In an investigation in Iraq, authors applied the ELISA assay and identified the 76.4 % sensitivity, compared to that of the microscopy, while the specificity amounted to 100 % [3]. In a 2018 study by S o m m e r, the prevalence of 30.6 % was identified by applying the ELISA assay for *Giardia* coproantigen [35].

In terms of the efficiency, the flotation method ranks among the most efficient techniques that are used in the diagnostics of the disease, considering the obtained results. The SNAP test is mostly used in private clinics, as it facilitates fast diagnostics of the disease. The use of the ELISA method is adequate primarily in laboratories that conduct

the epidemiological or clinical research. In the case the ELISA test is conducted with fresh faeces, the resulting percentage of positivity might be higher.

The results of our present study indicate that the SNAP test, conducted with fresh faeces, exhibited a high degree of efficiency, approaching to that of the flotation method. However, it is necessary to take into account that it is three times cheaper to apply the flotation method than to apply the SNAP test, and that it is more environment-friendly and has lower impact on the environment. One of the advantages of the SNAP test is that it is practical, fast and simple in terms of the required technical skills, compared to the flotation method and the ELISA assay.

All the samples tested for the presence of coproantigen by applying the SNAP test and the ELISA assay were flotation-positive, and the tests were conducted while strictly following the manufacturer's instructions; nevertheless, in 1 fresh sample, the SNAP test failed to detect the coproantigen. However, after 6, 9 and 12 months, the coproantigen was confirmed.

Despite the efficiency values of the SNAP test and the ELISA assay that were identified in the present study, the flotation method still remains to be the most efficient, economical, ecological and efficient method for the diagnostics of giardiasis.

The selected positive samples were used to identify the assemblages of *Giardia duodenalis* by applying the nested PCR. In ten microscopically positive samples, the PCR method was applied and the assemblages C and D (*G. canis*) were detected by the amplification of *bg* and *tpi* loci. In 2020, in a study conducted in Egypt, the PCR method was applied and the same assemblages were detected in dogs [29]. In the same year, the occurrence of assemblages C and D was also confirmed in a Polish study through the amplification of the β-giardin locus [23]. One year later in Columbia, the amplification of *bg*, *tpi* and *gdh* loci was applied and assemblages C and D were confirmed in 6 samples of dogs faeces [14]. A study by M r a v c o v á et al., conducted in 2017, confirmed the presence of assemblage C in 8 samples [21]. In the research conducted in Berlin in 2019, a total of 6 samples were detected, and in addition to the presence of assemblage C, they also confirmed the presence of assemblage A, assemblage D and a combination of assemblages A and B in the faecal samples [26]. In the research in Netherlands, conducted in 2001, authors came to the conclusion that assemblage A is associated with episodic diarrhoea, while persisting diarrhoea was associated with the genotype B [15].

In our present study, in addition to the presence of *Giardia duodenalis* in the population of shelter dogs, the presence of other endoparasite species was also confirmed. The highest occurrence was found for *Trichuris vulpis* (38 %) and parasites from the *Ancylostomidae* family (35 %). Similar results of the detected occurrence were published in Cuba in 2015, where the most frequently detected parasites were particularly *Ancylostoma caninum* (21.4 %) and *Trichuris vulpis* (16.3 %) [25]. In the present study, the presence of *Cystoisospora* spp. (15 %) and *Toxocara canis* (12 %) was also detected. In Romania, the dogs positive for *Giardia* spp. exhibited the coinfections with other intestinal parasites: *Toxocara canis* (26.9 %); *Cystoisospora ohiensis* (23.1 %); *Ancylostoma caninum* (17.3 %); *Uncinaria stenocephala* (13.5 %); *Neospora caninum* (9.6 %); *Sarcocystis* spp. (9.6 %); *Cystoisospora canis* (7.7 %); *Capillaria aerophilla* (5.8 %); *Strongyloides stercoralis* (3.8 %); *Dipylidium caninum* (1.9 %); and *Toxascaris leonina* (1.9 %) [19]. The results of the present research indicated the presence of coinfections by *Trichuris vulpis* (28 %) and by parasites from the *Ancylostomidae* family (8 %), while in 8 % of the samples, the coinfections by *Giardia* spp. and by the both above mentioned parasites were detected. The occurrence of parasites other than *G. duodenalis* exclusively in the shelter dogs may indicate a higher susceptibility to the infection due to the insufficient application of the deworming procedures with the use of efficient antiparasitic preparations, or due to an excessive environmental burden caused by the presence of cysts or eggs in the soil, and in some cases also due to their prolonged survival time.

CONCLUSIONS

The purpose of this study was to confirm the prevalence of Giardia duodenalis in dogs kept under different living conditions by using three methods (flotation method, SNAP and ELISA tests), and to compare the effectiveness of those methods in terms of their practical use, price, and use in veterinary clinical practice. The prevalence of giardiasis varies and often depends on a number of factors, including an animal's age and health, its living conditions, the selected diagnostic method and a test group. The One Health approach plays an important role in the diagnostics of giardiasis as the cause of diarrhoea in animals and

humans, and in the management of cysts spreading in the environment.

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