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THE PREVALENCE AND ASSEMBLAGES OF *GIARDIA DUODENALIS* IN DOGS: A SYSTEMATIC REVIEW IN EUROPE

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ABSTRACT

***Giardiasis* is one of the most frequent causes of diarrhoeic diseases in the world. *Giardia* cysts are most commonly transferred via ingestion of contaminated water or food. On the basis of genetic characteristics *Giardia duodenalis* is classified in eight assemblages A—H. Zoonotic assemblages A and B are increasingly found in isolates from dogs which may constitute the reservoir of human giardiasis. This article presents a brief review of *G. duodenalis* assemblages detected in dogs that were documented in European countries.**

Key words: assemblages; dogs; *Giardia duodenalis*; giardiasis

INTRODUCTION

Giardia duodenalis is a cosmopolitan unicellular parasitic protozoan that infects domestic and free living animals including humans [47]. The World Health Organisation (WHO) includes *G. duodenalis* among the so-called "Neglected Tropical Diseases" [45]. Throughout the world as many as 200 million cases of giardiasis are diagnosed annually [17, 22].

On the basis of genetic differences, *G. duodenalis* is classified in eight assemblages [15]. Assemblages A and B are considered zoonotic and were confirmed in humans but also in a broad range of hosts [6, 7]. The remaining 6 assemblages are mostly host-specific; C and D are typical of dogs and other canines; assemblage E was identified in ungulates including cattle, sheep and goats; F in cats; G in rats, and H in marine mammals [3, 44]. Dogs may be infected also by assemblages A or B and present potential risk of transfer of this infection to humans [15]. In order to guarantee the correct identification of assemblages and confirmation of the potential zoonotic transmission of *G. duodenalis*, the use of molecular methods is recommended. The PCR method for identification of *G. duodenalis* has on average 92 % sensitivity and 100 % specificity [49].

The life cycle of *G. duodenalis* is simple. The host is most often infected by the faecal-oral route through contaminated food, water or direct physical contact [22]. *G. duodenalis* occurs in two forms, as the trophozoite or the cyst. The trophozoite is an actively motile, vegetative, flagellated pear-

shaped stage existing in the small intestinal lumen. Survival of this parasite in the outer environment is ensured by the oval cysts that constitute the infectious stage. After the ingestion of cysts, excystation occurs under the action of digestive enzymes and low pH. Each four-nuclei cyst releases two-nuclei trophozoites. By means of a suction disk, the trophozoites adhere to enterocytes in the proximal part of the duodenum and multiply by longitudinal binary fission. Under the action of bile salts their encystation occurs as they transit toward the colon. The cysts that are eliminated to the outer environment with faeces are fully infectious [1]. Younger age categories are more frequently affected by giardiasis and exhibit more pronounced clinical signs. The signs are observed within 2 weeks after ingestion of infectious cysts. Duodenal microvilli in the affected individuals are shortened and thickened which results in a maladsorption syndrome [16, 53]. The characteristic signs include disorders in resorption of fats and fat-soluble vitamins (steatorrhea). The decreased enzymatic activity in the host causes disorders of absorption of zinc which becomes a part of surface antigens of the parasite. Zinc is an element irreplaceable in immunological reactions and its deficiency can have an immunosuppressive effect on the host organism [53]. Clinical signs are frequently non-specific and the infection may have a latent course. Alternating periods of diarrhoea and constipation are observed and the faeces mostly contain mucus and fat, but no blood. In some patients the disease may be chronic and last several months; even years [14]. Giardiasis has been recognised as an important risk factor for long-term syndromes, for example post-infection irritable bowel and chronic fatigue syndromes. Recent studies indicate that extra-intestinal consequences of giardiasis were also observed including arthritis or hypersensitive skin reaction [11, 33].

The aim of this study was to present a review of the prevalence and assemblages of *Giardia duodenalis* in dogs in European countries with respect to the zoonotic potential of this parasite.

OCCURRENCE OF *G. DUODENALIS* IN EUROPE

Giardia spp. is a well-known agent of one of the most extensively spread intestinal infections in temperate, tropical and subtropical zones. It has been assessed that it causes annually 280 million of diarrhoeal diseases throughout the world [58]. *G. duodenalis* is capable of infecting a broad range of hosts including humans and domestic and wild living animals. Investigations of various populations of dogs have revealed a different prevalence dependent on the age. The highest prevalence (46—50 %) was observed in dogs under 1 year of age [13, 24]. In some European countries the prevalence of giardiasis in dog breeding centres reached 100 %. On the other hand, only 10 % positivity was detected in dogs kept under good hygiene conditions [26]. The previous studies that involved groups of dogs of different age and breeding conditions confirmed differences in the prevalence of giardiasis. The following text and Table 1 provides a summary of results obtained in various European countries including Slovakia.

In 1999, Letková et al. [32] reported 10.1 % prevalence of *G. duodenalis* in dogs in Košice, eastern Slovakia. In 2007, Szabová et al. [54] detected the occurrence of *G. duodenalis* in 5 districts of eastern Slovakia (Michalovce, Trebišov, Košice, Poprad and Bardejov). Samples of examined faeces originated from dogs living in households, dog shelters, quarantine stations and public grounds in Košice and Bardejov. Cysts were detected in the faeces of dogs in the district Trebišov (1.1 % positive samples) and Košice (1.7 %). Goldová et al. [19] confirmed 69.1 % and 36.9 % prevalence of giardiasis in dogs from a dog shelter; younger than 7 months and older than 7 months, respectively. Štrkolcová et al. [50] found a 33.3 % prevalence of *G. duodenalis* in dogs from shelters in the Košice district and 4.2 % in dogs from a Roma settlement. The studies cited above were based on the microscopic examination of faecal samples and molecular methods were not used.

Giardiasis in dogs as human companions can have serious consequences due to the zoonotic potential of this parasite [15]. The first infection of humans in Europe caused by specific canine genotype C was confirmed by the study conducted in Slovakia. This supports the potential role of dogs in zoonotic transfer of *G. duodenalis* assemblage C to humans [51].

In 1994, Hořejš and Koudela investigated the occurrence of giardiasis in dogs in the Czech Republic and detected an overall prevalence of 5—6 % [23]. In 2001, Svobodová and Doležil detected the presence of giardia cysts in 41.6 % of the dogs in a dog shelter in Ostrava [52]. Dubná et al. [12] investigated in 2007 the prevalence of parasites in dog faeces in the Prague city

Table 1. Review of assemblages of *G. duodenalis* diagnosed in dogs in Europe

Country	Assemblages	Source
Slovakia	–	Letková 1999 [32]; Szabová 2007 [54]; Goldová 2011 [19]; Štrkolcová 2014 [50], 2015 [51]
Czech Republic	-–	Hořejš and Koudela 1994 [23]; Svobodová and Doležil 2001 [52]; Dubná et al. 2007 [12]
Austria	A/AII/B/BIV	Joachim and Prosl 2005 [25]; Hinney et al. 2017 [21]; Lee et al. 2017 [30]
Poland	A/B/C/D/C + D	Bajer 2008 [2]; Stojecki et al. 2015 [49]; Piekarska et al. 2016 [43]
Hungary	C/D	Szénási et al. 2007 [55]
Germany	C/D/A + C/ C + D	Cirak and Bauer 2004 [8]; Leonhard et al. 2007 [31]; Pallant et al. 2015 [42]; Sommer et al. 2018 [48]
Serbia	–	Nikolić et al. 1993 [39], 2002 [36], 2008 [37], 2011 [38]
Romania	C/D/E/C + D	Mircean et al. 2012 [34]; Györke et al. 2016 [20]
Italy	C/D	Zanzani et al. 2014 [57]; Scaramozzino et al. 2018 [46]
Belgium	A/B	Claerebout et al. 2009 [9]
Great Britain	AIII/C/D	Upjohn et al. 2010 [56]
Bosnia	–	Omeragić et al. 2014 [40]
Estonia	–	Lassen et al. 2009 [28]
Sweden	A/C/D/C + D	Lebbad et al. 2010 [29]
Holland	A/C/D/C + D	Overgaauw et al. 2009 [41]
Croatia	C/D	Beck et al. 2012 [5]
Spain	AII/BIII/BIV/C/D	deLucio et al. 2017 [10]; Gill et al. 2017 [18]
Portugal	–	Neves et al. 2014 [35]
Greece	C/D	Kostopoulou et al. 2017 [27]

centre, agricultural areas and two shelters. They observed that the prevalence of *Giardia* spp. in Prague reached 0.1 %. The examination of 540 samples from rural areas showed a prevalence of 2.2 % and the *Giardia* spp. were one of the most frequently detected parasites.

The epizootological studies conducted in Austria in 1990–2005 showed that prevalence of giardiasis in dogs ranged between 22 and 36 % [25]. An extensive research conducted in 2015 in Vienna included the examination of 1001 samples of dog faeces. The diagnostic methods included the flotation method according to Faust and a rapid SNAP test for the proof of coproantigen. The flotation method detected cysts in 5.2 % of the samples and the 6.5 % of the samples were positive in the SNAP test [21]. Lee et al. [30] confirmed by molecular analysis the occurrence of assemblages A and B in Austria. The most frequent assemblages were AII and BIV.

Several studies investigated the prevalence of giardiasis in dogs in Poland. The total prevalence ranged from 1.9 to 36 % [2]. The prevalence of 5.6 % was determined in dogs in eastern Poland using a specific fluorescent staining and genotyping revealed the zoonotic assemblage A [49]. Piekarska et al. analysed isolates from domestic dogs in Wroclaw and identified assemblages D (9 %) and B (4.5 %), and assemblages C + D (4.5 %) in one mixed infection [43].

Szénási et al. investigated the prevalence of giardiasis in dogs from shelters in Hungary in relation to the method used. Microscopic examination indicated positivity in 14 samples (prevalence 7.5 %), while the ELISA test for the proof of coproantigen determined *G. duodenalis* in 58.8 % of the dogs. On the basis of sequencing the positive samples, they were classified in the assemblages C and D [55].

In Germany Cirak and Bauer observed 9.5 % occurrence of *G. duodenalis* cysts in domestic dogs [8]. Another study in south Germany investigated samples of faeces from asymptomatic dogs that were brought to veterinary clinics. *G. duodenalis* was detected in 55 of the 60 examined samples. Assemblage A was detected most frequently followed by mixed infections A + C and C + D. Individual assemblages C and D were proved only in 2 samples [31]. In a retrospective study, the results of parasitological examinations of faecal samples from 8,560 cats and 24,677 dogs between January 2003 and December 2010 in Germany were analysed. The examination of the faecal samples from dogs revealed stages of *Giardia* spp. (18. %). Dogs in the age groups up to 3 months and >3 up to 6 months of age showed significantly higher infection rates with *Giardia* spp. (37.5 % and 38.2 %, respectively) [4].

In the study by Sommer et al. [48] the prevalence of *Giardia duodenalis* infections in dogs and cats living in Germany were investigated using different diagnostic tests and the *Giardia* assemblages of infected animals were identified. All samples were investigated by enzyme-linked immunosorbent assay (ELISA), merthiolate-iodine-formalin concentration technique (MIFC) and zinc chloride flotation. ELISA-positive samples were additionally screened with a direct immunofluorescence assay (IFA). The samples from dogs tested positive for *Giardia* coproantigen (ELISA) in 30.6 %. The MIFC technique revealed *Giardia* cysts in 33.9 % of canine ELISA-positive samples, while using IFA, cysts were present in 90.4 % of the canine ELISA-positive samples. Dog-specific *Giardia* assemblages C and D were detected in 42 and 55 canine isolates, respectively. Two canine samples harboured the zoonotic assemblage A. According to the results of the study, *Giardia* is a common endoparasite in dogs and cats from Germany.

Publications from 1993 until 2011 [36—39] have confirmed that *G. duodenalis* was the most common intestinal protozoan parasite in dogs from the Belgrade area. Faecal samples from household, stray, farm and military working (kennel) dogs were investigated in three different studies. Significantly higher infection rates were found in stray, farm and military working dogs. With the intention to evaluate the correlation of *Giardia* infections in household dogs and their owners, faecal samples of all family members of households accommodating *Giardia* positive dogs were also screened for *Giardia* cysts in two of the three studies. Two people living in one household with an infected dog carried an infection with *G. duodenalis* as well. The finding supports a possible transmission of *Giardia* infections between human and canine cycles. However, a molecular analysis of the concerned samples would have been essential for a further statement on the zoonotic potential and the transmission dynamics arising from the investigated dog population. Human giardiasis was spread throughout Serbia with a higher incidence in the Northern part of the country [38].

A one-year study (2008—2009) in Romania investigated the prevalence of parasitic infections in sheepdogs, domestic and shelter dogs and dogs used for breeding. The prevalence of *G. duodenalis* in shelter dogs determined by the flotation method reached 16.5 % (27/164). Positivity in breeding dogs reached 7.2 %; in domestic dogs 4.8 % and in sheepdogs 4.3 % [34]. The more recent studies that used the PCR-RFLP method identified assemblages C, D and E. In one case, a mixed C + D infection was detected.

In Milano, Italy, of 37 examined samples of dog faeces, 11 were positive for *G. duodenalis*. In these dogs the PCR method identified assemblages C and D [57]. In another 3-year study that was carried out in Rome, *G. duodenalis* was the third most frequently diagnosed parasite with a prevalence of 20.5 %. The genotyping of the positive samples proved the presence of a typical canine assemblages C and D [46].

Studies in Belgium observed giardiasis in various dog categories. Prevalence in domestic dogs reached 9.3 % and dogs with gastrointestinal signs were positive in 18.1 % of cases while sheltered dog showed as high as 43.9 % positivity. In 80 % of the samples zoonotic assemblages A and B were identified [9].

The study conducted by Upjohn et al. investigated samples of faeces from 878 dogs in London and observed a prevalence of 21.0 %. The genotyping used identified assemblages C and D and in one sample a zoonotic subtype AIII [56].

The study conducted in 2013 in Tuzla municipality, Bosna and Hercegovina, determined the prevalence of *G. duodenalis* by the standard flotation method in the faeces from 134 dogs (79 from shelter, 44 with owners; 76 of dogs ≤6 months, 58 > 6 months) and detected the presence of *Giardia* spp. in 9/76 (11.84 %) of the ≤6 months old dogs and in 6/58 (10.34 %) of the dogs >6 months old [40].

Lassen et al. detected *Giardia* cysts in dogs in Estonia. During a 5-year study they determined a prevalence of 14 % in canine faecal samples 14 % [28].

The prevalence of *G. duodenalis* in faecal samples from farm and pet animals was investigated in Sweden. Forty samples from dogs were examined by the flotation method according to Faust and the microscopically positive ones were examined by PCR. The assemblages A, C, and D and one mixed infection caused by assemblages C + D were identified [29].

The examination of samples from 152 dogs in the Netherlands showed 15.2 % prevalence of *G. duodenalis*. The samples originated from veterinary clinics from a town and other rural areas. The ELISA method identified the coproantigen in 16 dogs and genotyping detected assemblages A, C and D and one mixed infection C + D [41].

A 9-year study (2007—2015) aimed at the determination of the prevalence of *G. duodenalis* in dogs was conducted in Zagreb. The flotation method according to Faust indicated the presence of cysts in 25.88 % of the samples. The subsequent study employing sequence analysis confirmed the assemblages C and D [5].

An epidemiological survey was conducted in Álava province in northern Spain with the aim of identifying the cysts of *G. duodenalis* in humans and animals. The fluorescence method was used for the proof of *Giardia* cysts in the samples. The total prevalence in dogs reached 29 % (16/55). Genotyping revealed the typical canine assemblage C [10]. In the same year, G i l l et al. identified assemblage D and sub-assemblages AII, BIII and BIV in sheltered dogs in the south of Spain. The circulating of these assemblages in dogs may present potential zoonotic risk [18].

In a recent study in Portugal, *G. duodenalis* was the most frequently detected parasite in dogs with gastrointestinal signs. The prevalence reached 15.5 %. No molecular methods were used for the identification of the assemblages [35].

Research published in Greece in 2017 revealed that the total prevalence of *G. duodenalis* in a study reached 25.2 %. Samples were examined first by the flotation method according to Faust and the positive isolates were subjected to genotyping. This proved the presence of assemblages C and D [27].

CONCLUSIONS

The diagnostic methods used for the proof of *G. duodenalis* are relatively simple. They include the basic microscopic examination for the presence of cysts in faecal samples; ELISA test for detection of coproantigen or the direct immunofluorescence method. However, only the molecular characterisation of *G. duodenalis* can guarantee the correct identification of assemblages and the confirmation of the potential zoonotic transmission. The PCR method for the identification of *G. duodenalis* has a mean 92 % sensitivity and 100 % specificity.

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