

Research Article

Cite this article: Szydtowicz M, Zajączkowska Ż, Lewicka A, Łukianowski B, Kamiński M, Holubová N, Sak B, Kváč M, Kicia M (2024). Host-specific *Cryptosporidium*, *Giardia* and *Enterocytozoon bieneusi* in shelter dogs from central Europe. *Parasitology* **151**, 351–362. <https://doi.org/10.1017/S003118202400009X>

Received: 4 August 2023

Revised: 14 January 2024

Accepted: 20 January 2024

First published online: 2 February 2024

Keywords:




60-kDa glycoprotein; glutamate dehydrogenase; internal transcribed spacer region of rRNA; intestinal protists; opportunistic pathogens; PCR; small ribosomal subunit rRNA; subtyping; triosephosphate isomerase; β -giardin

Corresponding author:

Magdalena Szydtowicz;

Email: magdalena.szydtowicz@umw.edu.pl

Host-specific *Cryptosporidium*, *Giardia* and *Enterocytozoon bieneusi* in shelter dogs from central Europe

Magdalena Szydtowicz¹ , Żaneta Zajączkowska¹, Antonina Lewicka¹, Błażej Łukianowski^{1,2}, Mateusz Kamiński¹, Nikola Holubová³, Bohumil Sak³ , Martin Kváč^{3,4}  and Marta Kicia¹

¹Department of Biology and Medical Parasitology, Wrocław Medical University, Wrocław, Poland; ²Department of Pathomorphology, 4th Military Clinical Hospital, Wrocław, Poland; ³Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic and ⁴Faculty of Agriculture and Technology, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic

Abstract

Cryptosporidium spp., *Giardia intestinalis* and microsporidia are unicellular opportunistic pathogens that can cause gastrointestinal infections in both animals and humans. Since companion animals may serve as a source of infection, the aim of the present screening study was to analyse the prevalence of these intestinal protists in fecal samples collected from dogs living in 10 animal shelters in central Europe (101 dogs from Poland and 86 from the Czech Republic), combined with molecular subtyping of the detected organisms in order to assess their genetic diversity. Genus-specific polymerase chain reactions were performed to detect DNA of the tested species and to conduct molecular subtyping in collected samples, followed by statistical evaluation of the data obtained (using χ^2 or Fisher's tests). The observed prevalence was 15.5, 10.2, 1 and 1% for *G. intestinalis*, *Enterocytozoon bieneusi*, *Cryptosporidium* spp. and *Encephalitozoon cuniculi*, respectively. Molecular evaluation has revealed the predominance of dog-specific genotypes (*Cryptosporidium canis* XXe1 subtype; *G. intestinalis* assemblages C and D; *E. cuniculi* genotype II; *E. bieneusi* genotypes D and PtEbIX), suggesting that shelter dogs do not pose a high risk of human transmission. Interestingly, the percentage distribution of the detected pathogens differed between both countries and individual shelters, suggesting that the risk of infection may be associated with conditions typical of a given location.

Introduction

Dogs play an important role in human life as companion animals; however, they may be carriers of various pathogens, constituting a potential reservoir of zoonotic infections for their owners. Many of these infectious agents reside in the intestinal tract; therefore, their dispersive forms are excreted with animal stool and may easily be spread to other hosts by fecal–oral transmission through direct contact or indirectly *via* water or food contamination. This includes unicellular enteric organisms such as *Giardia intestinalis* (syn. *duodenalis* or *lamblia*), *Cryptosporidium* spp. or microsporidia from genera *Encephalitozoon* and *Enterocytozoon*, which can be responsible for gastrointestinal symptoms like abdominal pain, diarrhoea or flatulence (Xiao, 2010; Liao *et al.*, 2020). Since these organisms belong to the group of opportunistic pathogens, infection is of particular importance for individuals with impaired immunity (e.g. HIV-infected patients, cancer-treated patients or transplant recipients), in whom it may lead to the development of hazardous, even life-threatening symptoms.

Giardia intestinalis is the most common intestinal pathogenic protozoan in humans and animals (Kváč *et al.*, 2017), consisting of 8 distinct assemblages (A–H) differing in host specificities. Assemblages A and B display a broad host range and are most commonly reported in humans, while the remaining 6 seem to be host-specific for non-human species, with assemblages C and D predominantly found in dogs (Bouzid *et al.*, 2015; Ryan and Zahedi, 2019). Among nearly 51 valid *Cryptosporidium* species (Tůmová *et al.*, 2023), *Cryptosporidium hominis* and *Cryptosporidium parvum* represent the major causes of human cryptosporidiosis, whereas *Cryptosporidium meleagridis*, *Cryptosporidium mortiferum*, *Cryptosporidium felis* and *Cryptosporidium canis* are rare causative agents of zoonotic infections, with the latter being the most prevalent in dogs (Xu *et al.*, 2016; Li *et al.*, 2021; Alderisio *et al.*, 2023). Out of over 1200 microsporidian species described so far, *Enterocytozoon bieneusi* and *Encephalitozoon* genus, including *Encephalitozoon intestinalis*, *Encephalitozoon cuniculi* and *Encephalitozoon hellem*, represent the species causing human microsporidiosis (Didier *et al.*, 2000), especially in persons with impaired immunity (Kicia *et al.*, 2014, 2016). These species may also be detected in a broad range of other hosts (livestock, wildlife and domesticated animals) (Dengjel *et al.*, 2001; Mathis *et al.*, 2005). The phylogenetic analysis of *E. bieneusi* allows for distinction of various genotypes differing in a host specificity. Genotypes D, EbpC and type IV are characterized by the widest host range and are also most frequently reported in humans

(Li *et al.*, 2019). In turn, genotype PtEbIX seems to be restricted to canine host population (Li *et al.*, 2019), although dogs can serve as a reservoir of many other zoonotic genotypes as well, including those most often reported in humans (Li *et al.*, 2020). Regarding the genus *Encephalitozoon*, the ability of these 3 species to inhabit a wide variety of organisms has been shown, with *E. cuniculi* having the widest host range, mainly among mammals and birds. Four *E. cuniculi* strains have been identified (I–IV); ‘canine’ strain III has been shown to cause high mortality in dogs, while the recently discovered ‘human’ strain IV has so far been documented in humans, cats and dogs. Although there appears to be some host preference in each strain, this specificity is not exact; humans have been found to be infected with all known strains (though rarely with strain III). In turn, *E. hellem* is the most common species among birds, while *E. intestinalis* is the most prevalent *Encephalitozoon* species in humans (Hinney *et al.*, 2016).

The dispersive forms of the discussed pathogens are very resistant, and many species of wild and domesticated animals, as well as humans, may serve as their hosts which facilitates their spread and maintenance in the environment. Previous research on the occurrence of these species in central Europe, including Poland and the Czech Republic, shows different data depending on the population studied and the detection methods used. According to a review by Plutzer *et al.*, the reported incidence of *Cryptosporidium* spp. and *G. intestinalis* per 100 000 inhabitants is 0.006 and 5.43 for Poland and 0.01 and 0.51 for the Czech Republic, respectively (Plutzer *et al.*, 2018). In Poland, estimates of the prevalence of these 2 species in humans are available based on the results of research limited to specific population groups and regions (Plutzer *et al.*, 2018). However, studies of Czech residents regarding the seroprevalence of *Cryptosporidium* spp. show the frequency of antibodies at the level of approximately 67–72% (Kozisek *et al.*, 2008). In turn, the prevalence of *Cryptosporidium* spp. in dogs in Poland ranges from approximately 3.5 to 12.5% (Bajer and Bednarska, 2007; Piekara-Stepińska *et al.*, 2021a), and *G. intestinalis* from 6 to 36%, with mainly canid-specific genotypes detected, which suggests that they do not represent an important source of *Giardia* infection for humans (Bajer and Bednarska, 2007; Piekarska *et al.*, 2016; Piekara-Stepińska *et al.*, 2021b). A similar prevalence refers to dogs from the Czech Republic, especially those from animal shelters (Zemanová *et al.*, 2005; Dubná *et al.*, 2007). Microsporidia, however, occur in dogs at a low level, only a few per cent, which may nevertheless pose a risk for immunodeficient individuals, as zoonotic genotypes are often detected (Piekarska *et al.*, 2017). Importantly, frequent exposure to microsporidia has been confirmed among immunocompetent people in the Czech Republic (Sak *et al.*, 2011), while in studies conducted in Poland, up to 26% of tested immunocompromised individuals were found to be infected with at least 1 microsporidian species (Kicia *et al.*, 2016, 2019).

It has been previously shown that specific genotypes and assemblages of these enteric pathogens may be detected in both humans and animals (Xiao *et al.*, 2007; Soliman *et al.*, 2011; Karim *et al.*, 2014a, 2014b; Hinney *et al.*, 2016), suggesting the possible zoonotic route of transmission. One of their sources may be shelter dogs, which often live in poor sanitary conditions and crowded spaces that favour the spread of such microorganisms (Raza *et al.*, 2018). Currently, there are 226 registered animal shelters in Poland (General Veterinary Inspectorate, 2023) and 248 in the Czech Republic (State Veterinary Administration, 2024). Therefore, the aim of the present study was to investigate the occurrence of *Cryptosporidium* spp., *G. intestinalis*, *Encephalitozoon* spp. and *E. bienersi* in dogs living in animal shelters in central Europe (Poland and the Czech Republic) and to assess the host specificity and zoonotic potential of these organisms at the genotype level.

Materials and methods

Samples

Individual fresh fecal samples were collected from dogs in animal shelters in Poland and the Czech Republic. Samples were collected directly from the floor by study research staff immediately after defecation, with care taken to avoid sampling fecal material that came into contact with the ground (concrete surface, without contact with the soil). They were collected in the morning, before daily cleaning routinely performed in each shelter. Each sample was individually placed in a sterile tube with animal ID, refrigerated at 4°C without preservatives and transported to laboratory. None of the collected stool had an apparent diarrhoeal symptom at the time of sampling. Where possible, information about the animal, such as sex and age, was also recorded during material collection (see Supplementary Table 1). Control of intestinal protozoa in dogs and cats in both Polish and Czech shelters is carried out according to current ESCCAP guidelines (ESCCAP, 2018) – in all facilities, pyrantel and fenbendazole were routinely used once every 3 months as part of antiparasitic prophylaxis.

Molecular analysis

Stool samples were stored up to 2 months in 4°C without preservatives until DNA extraction. Initial homogenization of 200 mg of each stool sample was performed by bead disruption for 60 s at 5.5 m s⁻¹ with 0.5 mm glass beads using a Precellys 24 Instrument (Bertin Technologies, Montigny le Bretonneux, France), followed by genomic DNA (gDNA) extraction using a GeneMATRIX Stool DNA Purification Kit (EurX, Gdańsk, Poland). Molecular detection was based on the nested polymerase chain reaction (PCR) protocols for the amplification of the chosen genes of *E. bienersi* (*ITS*), *Encephalitozoon* spp. (*ITS*), *Cryptosporidium* spp. (18S rRNA) and *G. intestinalis* (*TPI*) (Didier *et al.*, 1995; Katzwinkel-Wladarsch *et al.*, 1996; Xiao *et al.*, 1999; Buckholt *et al.*, 2002; Sulaiman *et al.*, 2003). Additional PCRs amplifying selected loci were performed for subtyping in order to assess intra-species genetic diversity in the case of samples positive for *Cryptosporidium* spp. (partial 60-kDa glycoprotein gene – *gp60*) and *G. intestinalis* (β -giardin – *BG* and glutamate dehydrogenase – *GDH*) (see Supplementary Table 2) (Cacciò *et al.*, 2002, 2008; Lalle *et al.*, 2005; Jiang *et al.*, 2021). Each PCR contained 0.25–2.0 μ L of DNA, 200 μ M each of deoxynucleoside triphosphate (dNTP), 1 \times PCR buffer (DreamTaq™ Green Buffer, ThermoFisher Scientific, Waltham, MA, USA), 3.0 mM MgCl₂, 0.125 U of Taq polymerase (ThermoFisher Scientific), 10 μ g of bovine serum albumin (BSA) and 200 nM of each primer in a total of 20–25 μ L reaction. The reactions were performed in a C1000 Bio-Rad thermocycler, with an initial hot start (94°C for 5 min) and a final extension (72°C for 10 min), according to the conditions described in Supplementary Table 2. An aliquot of primary PCR was used as a template for the secondary PCR. Its conditions were identical to the primary PCR, except that BSA was not added to the secondary reaction. Negative (molecular grade water) and positive controls (DNA extracted from *E. bienersi* genotype CZ3, *E. cuniculi* genotype III spores, *Cryptosporidium serpentis* oocysts or *G. intestinalis* assemblage F cysts) were included in each PCR amplification. Secondary PCR products were electrophoresed on a 1% agarose gel containing 0.2 mg mL⁻¹ Midori Green DNA stain in TAE buffer at 75 V for approximately 1 h. Bands of the predicted sizes were visualized using a UV light source, cut from the gel, extracted using a ZymoClean Gel DNA Recovery Kit (Zymo Research, Irvine, CA, USA) and sequenced bi-directionally by a company offering this service commercially (Genomed S.A., Warsaw, Poland). The nucleotide sequences obtained were processed using Chromas Pro 2.4.1 software

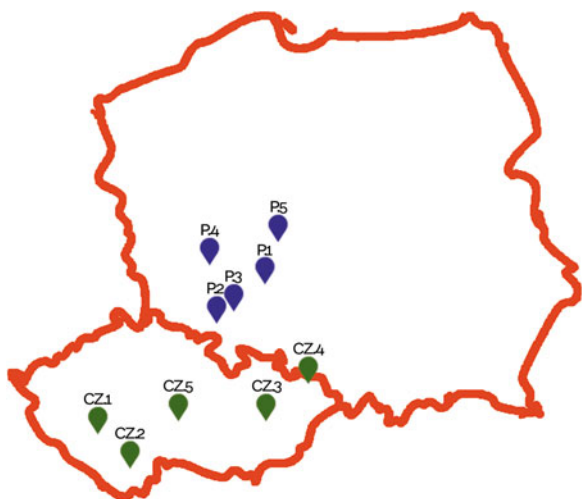


Figure 1. Schematic arrangement of shelters in Poland (P.1–P.5) and the Czech Republic (CZ.1–CZ.5) from which samples were collected.

(Technelysium, Pty, Ltd., South Brisbane, Australia). Subsequently, BLAST analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was performed to verify the identity of the sequences. The edited and aligned sequences were further processed using BioEdit v.7.0.5 (Hall, 1999). To align the obtained sequences with reference sequences from GenBank, the online server MAFFT version 7

was used (<http://mafft.cbrc.jp/alignment/software>). The best model for DNA/protein phylogeny for each alignment was selected based on the Bayesian information criterion in MEGA 7 (Kumar *et al.*, 2016). Tamura's 3-parameter model + G + I was used for the alignments. The maximum likelihood (ML) approach was carried out in MEGA7 software. Bootstrap support was calculated based on 1000 replications to evaluate the robustness of tree branching. Finally, the resulting trees were visualized using Corel Draw X7 software (<https://www.corel.draw.com>). Representative nucleotide sequences of all loci used as markers for subtyping of isolates obtained in the current study were deposited in GenBank with the accession numbers OR791083, OR791084, OR791770, OR791659, OR791771–OR791785, OR807726 and OR807727.

Statistical analysis

Statistical analysis was performed using χ^2 or Fisher's tests to compare the frequency of occurrence of the tested pathogens between Polish and Czech shelters (Statistica software, TIBCO Software Inc., USA). A $P < 0.05$ was considered significant.

Results

A total of 187 apparently healthy dogs from 10 shelters (Fig. 1), 5 in Poland (101 dogs) and 5 in the Czech Republic (86 dogs), were studied (Table 1). Specific DNA of targeted parasites was detected in 50 of 187 animals (26.7%), with higher occurrence observed in

Table 1. Occurrence of *Cryptosporidium* spp., *Giardia intestinalis*, *Encephalitozoon cuniculi* and *Enterocytozoon bieneusi* in individual dogs kept in animal shelters in Poland and the Czech Republic

Country	Location	No. of tested animals	Dogs ID	No. of dogs positive for screened parasites				
				<i>Cryptosporidium</i> spp.	<i>G. intestinalis</i>	<i>E. cuniculi</i>	<i>E. bieneusi</i>	
Poland	P.1	39	2806	1	–	–	–	
			2803, 2807	–	2	–	–	
			2839	–	–	1	–	
			2808, 2834	–	–	–	2	
	P.2	32	2728, 2732, 2733, 2742, 2744, 2747, 2748, 2751, 2753, 2757	–	10	–	–	
			2735	1	–	–	–	
			2727	–	–	–	1	
	P.3	13	2768, 2771, 2772, 2773, 2775, 2778, 2779, 2780	–	8	–	–	
	P.4	11	2783	–	–	1	–	
			2787	–	1	–	–	
			2789	–	–	–	1	
	P.5	6	2696	–	1	–	–	
			2699	–	–	–	1	
	Czech Republic	CZ.1	20	2966	–	1	–	1
				2965, 2973, 2977	–	–	–	3
CZ.2		7	2985, 2986, 2987, 2989	–	–	–	4	
CZ.3		6	–	–	–	–		
CZ.4		30	3010, 3027	–	2	–	–	
CZ.5		23	3031	–	1	–	1	
			3030, 3036, 3041	–	3	–	–	
			3032, 3044, 3047, 3048, 3049	–	–	–	5	
Total		187		2 (1.1%)	29 (15.5%)	2 (1.1%)	19 (10.2%)	

Table 2. Results of genotyping of *Cryptosporidium* spp., *G. intestinalis*, *E. cuniculi* and *E. bieneusi* in all tested samples

Country	Dog ID_ Location	<i>G. intestinalis</i> assemblage			<i>Cryptosporidium</i> spp. species/ subtype		<i>E. cuniculi</i> genotype ITS	<i>E. bieneusi</i> genotype ITS
		<i>TPI</i>	<i>BG</i>	<i>GDH</i>	18S rRNA	<i>gp60</i>		
Poland	2803_P.1	C [OR791776]	C [OR791779]	C				
	2806_P.1				<i>Cryptosporidium canis</i> [OR791083]	XXe1 [OR791770]		
	2807_P.1	C [OR791774]	C	C [OR791782]				
	2808_P.1							PtEbIX
	2834_P.1							PtEbIX [OR807727]
	2839_P.1						II [OR791659]	
	2728_P.2	C	D	D [OR791785]				
	2732_P.2	C	D	D				
	2733_P.2	C	D [OR791780]	D				
	2735_P.2				<i>Cryptosporidium proliferans</i> ^a [OR791084]			
	2742_P.2	C	C [OR791778]	C				
	2744_P.2	C	C	C				
	2747_P.2	C	D	D				
	2748_P.2	C	D	D [OR791784]				
	2751_P.2	C [OR791775]	C	D				
	2753_P.2	C	D	D [OR791783]				
	2757_P.2	C	C	C				
	2768_P.3	C	C	C				
	2771_P.3	C	C	C				
	2772_P.3	C	N/A ^b	N/A ^b				
	2773_P.3	C	C	C				
	2775_P.3	C	C	C				
	2778_P.3	C [OR791772]	C	C				
	2779_P.3	C	C	C				
	2780_P.3	C	C	C				
	2783_P.4						II	
	2787_P.4	C	C	C				
	2789_P.4							PtEbIX
	2696_P.5	C	N/A ^b	N/A ^b				
	2699_P.5							D [OR807726]
Czech Republic	2965_CZ.1							PtEbIX
	2966_CZ.1	C [OR791771]	D	D				PtEbIX
	2973_CZ.1							PtEbIX
	2977_CZ.1							PtEbIX
	2985_CZ.2							PtEbIX
	2986_CZ.2							PtEbIX
	2987_CZ.2							PtEbIX
	2989_CZ.2							PtEbIX
	3010_CZ.4	C	D	N/A ^b				
	3027_CZ.4	C [OR791777]	C	D				
3030_CZ.5	C	D	D					

(Continued)

Table 2. (Continued.)

Country	Dog ID_ Location	<i>G. intestinalis</i> assemblage			<i>Cryptosporidium</i> spp. species/ subtype		<i>E. cuniculi</i> genotype ITS	<i>E. bieneusi</i> genotype ITS
		<i>TPI</i>	<i>BG</i>	<i>GDH</i>	18S rRNA	<i>gp60</i>		
	3031_CZ.5	C	D	D				PtEbIX
	3032_CZ.5							PtEbIX
	3036_CZ.5	C [OR791773]	C	D				
	3041_CZ.5	C	C	C [OR791781]				
	3044_CZ.5							PtEbIX
	3047_CZ.5							PtEbIX
	3048_CZ.5							PtEbIX
	3049_CZ.5							PtEbIX

Accession numbers in square brackets indicate the isolates deposited in GenBank as representative nucleotide sequences derived from the present study.

TPI, triosephosphate isomerase; *BG*, β -giardin; *GDH*, glutamate dehydrogenase; 18S rRNA, small ribosomal subunit rRNA; *gp60*, 60-kDa glycoprotein; *ITS*, internal transcribed spacer region of rRNA.

^a*Cryptosporidium* sp. isolate 2735_P.2, phylogenetically clustered near the gastric *Cryptosporidium* spp., differed from *C. proliferans* in 3 SNPs with 99.6% sequence identity of the 18S rRNA region.

^bN/A, assemblage not available (subtyping failed).

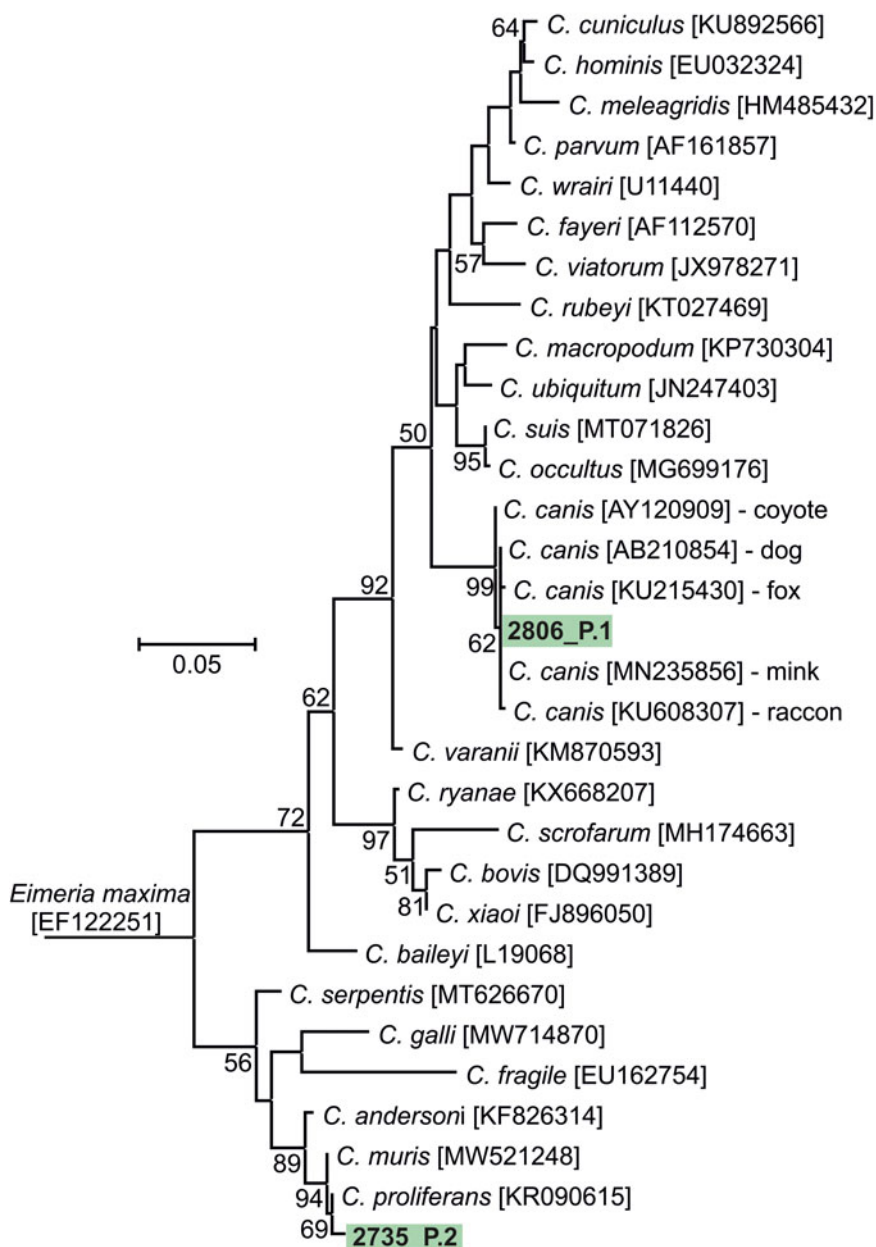


Figure 2. Phylogenetic relationships between *Cryptosporidium* spp. detected in dogs in this study (highlighted in green) and other *Cryptosporidium* available in GenBank using an ML analysis of partial sequences of 18S rRNA (sequence alignment length: 820 bp). Percentage supports (>50%) from 1000 pseudoreplicates are indicated next to the supported node. The branch length scale bar indicates the number of substitutions per site. Sequences from this study are identified by an isolate number (e.g. 2806) followed by region and location (P.1, Poland location 1).

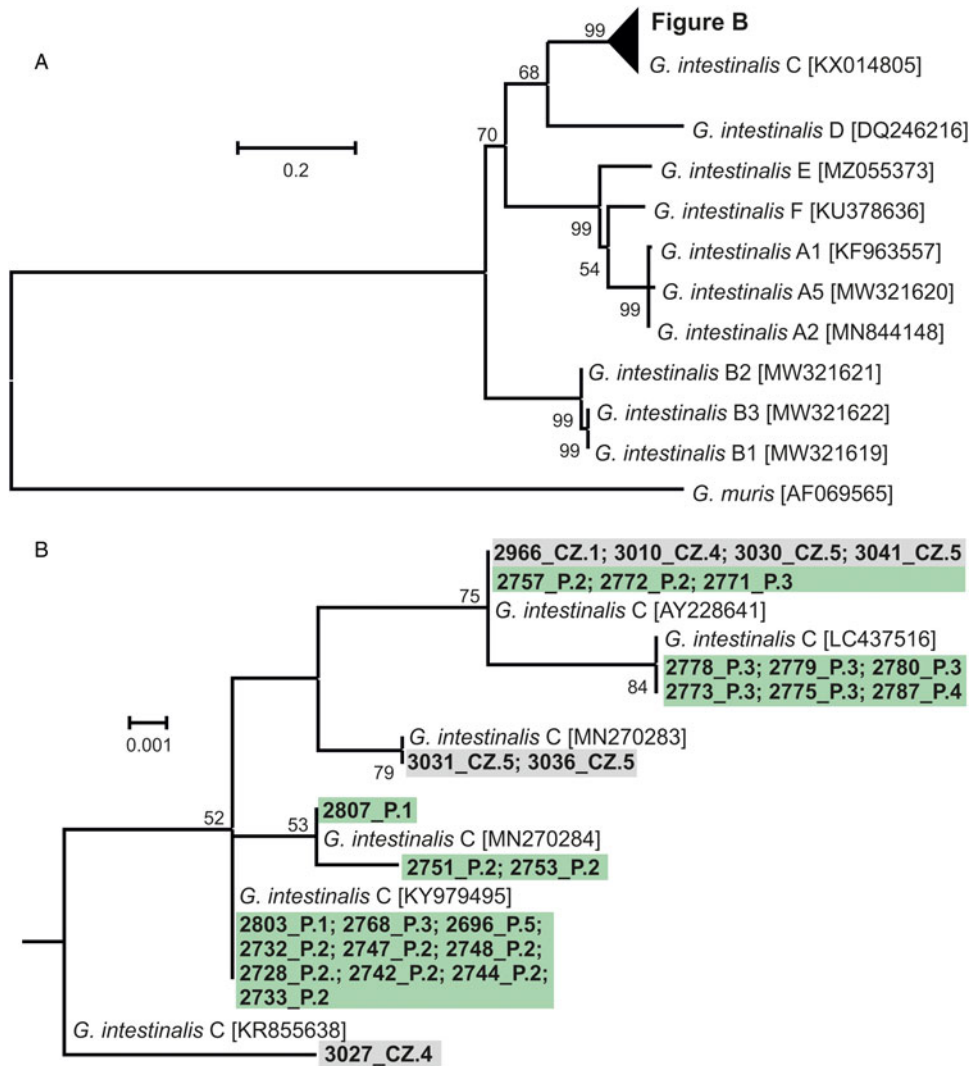
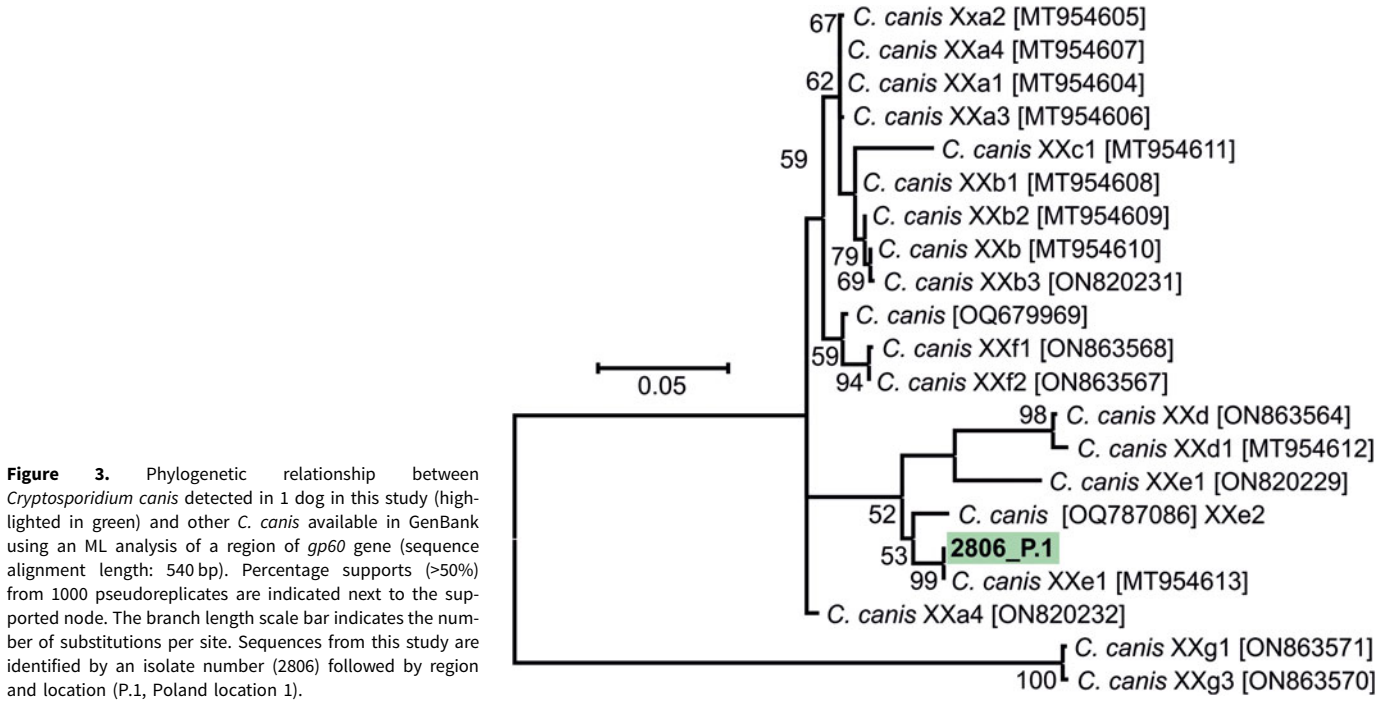


Figure 4. Phylogenetic relationships between *Giardia intestinalis* assemblages detected in dogs in this study (highlighted in green – Poland or in grey – Czech Republic) and other *G. intestinalis* assemblages available in GenBank using an ML analysis of a region of *TP1* gene (sequence alignment length: 467 bp). Percentage supports (>50%) from 1000 pseudoreplicates are indicated next to the supported node. The branch length scale bar indicates the number of substitutions per site. Sequences from this study are identified by an isolate number (e.g. 2966) followed by region and location (P.1, Poland location 1, CZ.1, Czech Republic location 1).

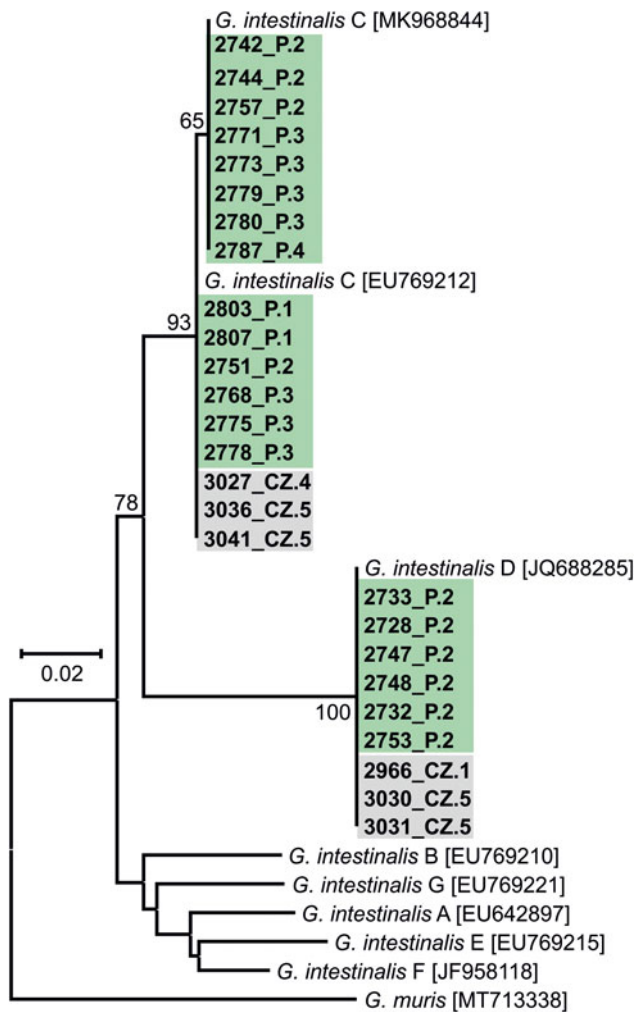


Figure 5. Phylogenetic relationships between *G. intestinalis* assemblages detected in dogs in this study (highlighted in green – Poland or in grey – Czech Republic) and other *G. intestinalis* assemblages available in GenBank using an ML analysis of a region of *BG* gene (sequence alignment length: 820 bp). Percentage supports (>50%) from 1000 pseudoreplicates are indicated next to the supported node. The branch length scale bar indicates the number of substitutions per site. Sequences from this study are identified by an isolate number (e.g. 2966) followed by region and location (P.1, Poland location 1, CZ.1, Czech Republic location 1).

Polish than in Czech animals (32.6 vs 24.4%, $\chi^2 = 1.0254$; $P = 0.3112$; Table 1). Most of the detected infections were monoinfections; only 2 dogs (ID 2966_CZ.1 and 3031_CZ.5) had a coinfection, *E. bieneusi* and *G. intestinalis*. *Giardia intestinalis* (29 dogs, 15.5%) followed by *E. bieneusi* (19 dogs, 10.2%) were the most frequently detected parasites, whereas each *Encephalitozoon* spp. and *Cryptosporidium* spp. were found in 2 dogs (Table 1). Overall, there was a significant trend towards more frequent occurrence of *G. intestinalis* in Polish (21.8%) vs Czech animals (8.1%, $\chi^2 = 6.5979$; $P = 0.0102$), while *E. bieneusi* was seen more often in dogs from Czech shelters (16.3%) than in Polish ones (4.9%, $\chi^2 = 6.5305$; $P = 0.0106$). Since detailed demographic data were obtained only from a small number of individuals, they were not subjected to statistical analysis.

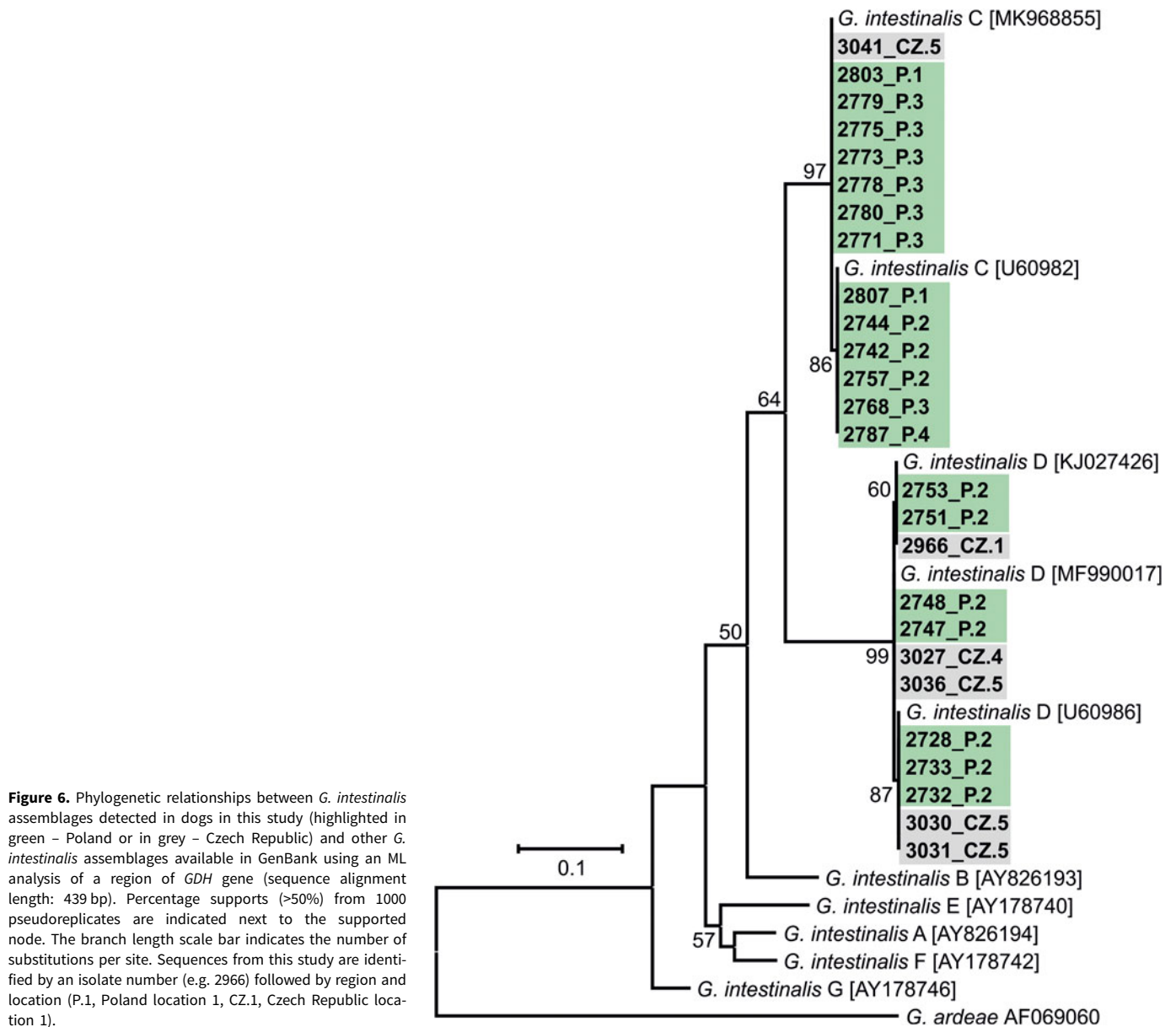
The results of genotyping of all pathogens performed in this study are presented in Table 2. Phylogeny analysis of partial sequences of 18S rRNA of *Cryptosporidium* showed the presence of *C. canis* identical to *C. canis* dog genotype (GenBank Acc. No. AB120909) in isolate 2806_P.1 and in a *Cryptosporidium* sp. isolate 2735_P.2, phylogenetically clustered near the gastric *Cryptosporidium* spp. (Fig. 2). *Cryptosporidium* sp. isolate 2735_P.2 differed from *Cryptosporidium proliferans* in 3 single-

nucleotide polymorphisms (SNPs) with 99.6% sequence identity. Based on *C. canis gp60* locus subtyping, isolate 2806_P.1 was assigned to the XXe family (Fig. 3). Genotype II, determined at ITS sequences, was detected in both dogs positive for *E. cucullii* (Table 2; phylogeny is not shown). Phylogenetic analysis of *Giardia* showed different results in 12 isolates, depending on the marker used (Table 2). Subtyping based on *TPI* gene revealed the presence of only assemblage C in all samples examined (Fig. 4), while for *BG* (Fig. 5) and *GDH* (Fig. 6) loci the presence of both C and D assemblages has been shown. Subtyping of *BG* and *GDH* failed in 2 and 3 isolates, respectively. With the exception of genotype D, which was detected in dog 2699_P.5, all other *E. bieneusi* sequences were identical to the PtEbIX genotype (Fig. 7).

Discussion

In the present study, the screening of fecal samples collected from shelter dogs in central Europe was performed in order to analyse the prevalence of zoonotic unicellular pathogens (*Cryptosporidium* spp., *G. intestinalis*, *E. bieneusi* and *Encephalitozoon* spp.) at the molecular level. Over a quarter of the tested dogs were carriers of at least one of the studied pathogens, among which the most often observed was *G. intestinalis* – one of the most common intestinal parasites infecting humans and animals (Kváč *et al.*, 2017). Nevertheless, its prevalence in other European canine populations, including shelter dogs, has shown to be even higher [27–36.5% in various regions of Spain (Gil *et al.*, 2017; Adell-Aledón *et al.*, 2018; Remesar *et al.*, 2022), 33.8% in Portugal (Pereira *et al.*, 2021), over 45% in Serbia (Sommer *et al.*, 2017), while in central Italy this value ranged from about 7% (Scaramozzino *et al.*, 2018) to 41% (Agresti *et al.*, 2022)]. Generally, such a high prevalence is most likely due to the simple and direct life cycle of *Giardia*, with easily spread dispersive forms excreted in feces, which facilitates transmission in highly dense populations, such as those found in animal shelters. *Enterocytozoon bieneusi* was also found to be a common pathogen in dogs in the present study, with an overall prevalence of 10.2%, which agrees with previous reports considering European dogs, with infection rates ranging between 4.9 and 11.7% (Mathis *et al.*, 2005; Santin and Fayer, 2011; Piekarska *et al.*, 2017). In turn, considerably low prevalences were observed for *Encephalitozoon* spp. and *Cryptosporidium* spp. (~1% for both), comparably to previous reports regarding canine populations: 0–2.4% for *Encephalitozoon* spp. (Piekarska *et al.*, 2017; Delrobaei *et al.*, 2019) and 0.6–4.9% for *Cryptosporidium* spp. (Giangaspero *et al.*, 2006; Simonato *et al.*, 2017; Yu *et al.*, 2018; Piekara-Stepińska *et al.*, 2021a), although in 1 German study the *Cryptosporidium* prevalence was as high as 10% (Murnik *et al.*, 2022). Notwithstanding, the true prevalence of these pathogens among healthy hosts may, in fact, be higher as their forms are excreted periodically and irregularly, which may be overlooked with a single sampling (Sak *et al.*, 2010). It would therefore be recommended to collect samples several times from the same animals, which may prove difficult due to the conditions specific to the shelters, such as the rotation of animals or irregular hours of cleaning the excrement. Nevertheless, the fact that the demonstrated prevalence of pathogens such as *G. intestinalis* was high even with only 1 sampling underscores the importance of their likely distribution in the population and the wide reservoir of the pathogen. Differences in the frequencies of the studied species between Polish and Czech dogs, as well as the higher prevalence of specific pathogens in individual facilities, may be related to some specific conditions typical of a particular location.

Sequence analyses of detected pathogens showed that most infections involved dog-specific genotypes or species, the transmission of which may be favoured by intensive contact among



large numbers of dogs living together. In the case of 12 isolates, assignment to the appropriate *G. intestinalis* assemblage was difficult because subtyping results varied depending on the locus used. Similar discrepancies have been reported in previous studies (Read *et al.*, 2004; Traub *et al.*, 2004), emphasizing the importance of using multilocus genotyping in the molecular analysis of *Giardia* diversity. Nevertheless, all *G. intestinalis*-positive samples harboured assemblage C or D, which have a strong host specificity for dogs and other canines (Feng and Xiao, 2011). These assemblages were also found to be highly prevalent in different dog populations in Europe (Simonato *et al.*, 2015; Adell-Aledón *et al.*, 2018; Pereira *et al.*, 2021) and although sporadically they have been reported in humans as well (Broglia *et al.*, 2013; Liu *et al.*, 2014; Villamizar *et al.*, 2019), their zoonotic relevance seems to be low and limited to individuals at risk, for instance, children or immunocompromised persons. Likewise, cases of *C. canis* colonization in humans were described in individuals at increased risk (children, HIV-infected adults) and immunocompetent people as well (Learmonth *et al.*, 2004; Gatei *et al.*, 2007; Feng *et al.*, 2012; Liao *et al.*, 2020). However, due to the relatively transient nature of these infections in humans, dogs do not seem to represent an important source of cryptosporidiosis for people

(Villamizar *et al.*, 2019; Liao *et al.*, 2020). To date, 9 families of *C. canis* subtypes (XXa–XXi) have been identified based on *gp60* locus subtyping, occurring not only in canids, but also in minks, foxes and humans (Jiang *et al.*, 2021; Murnik *et al.*, 2022; Wang *et al.*, 2022). According to the study of Jiang *et al.*, the zoonotic potential may concern XXa family, detected in both dog and human samples (Jiang *et al.*, 2021). In our study, analysis of the *C. canis* *gp60* locus in the 2806_P.1 isolate revealed that it belongs to the XXe family (Fig. 3), which was also the most prevalent among dogs in the report from Germany (Murnik *et al.*, 2022). In turn, the newly detected *Cryptosporidium* sp. isolate 2735_P.2 also does not pose a significant risk to humans and probably not to dogs as well. An incidental infection/contamination was likely caused by rodent feces. *Cryptosporidium* sp. isolate 2735_P.2 is closely related to *Cryptosporidium muris* and *C. proliferans*, whose hosts are rodents. However, the phylogenetic position is not related to host specificity, and therefore another host cannot be excluded (Kváč *et al.*, 2018). To summarize the subtyping results, as in previous studies (de Lucio *et al.*, 2017; Rehbein *et al.*, 2019), zoonotic transmission of giardiasis or cryptosporidiosis between dogs and humans is most likely a rare event.

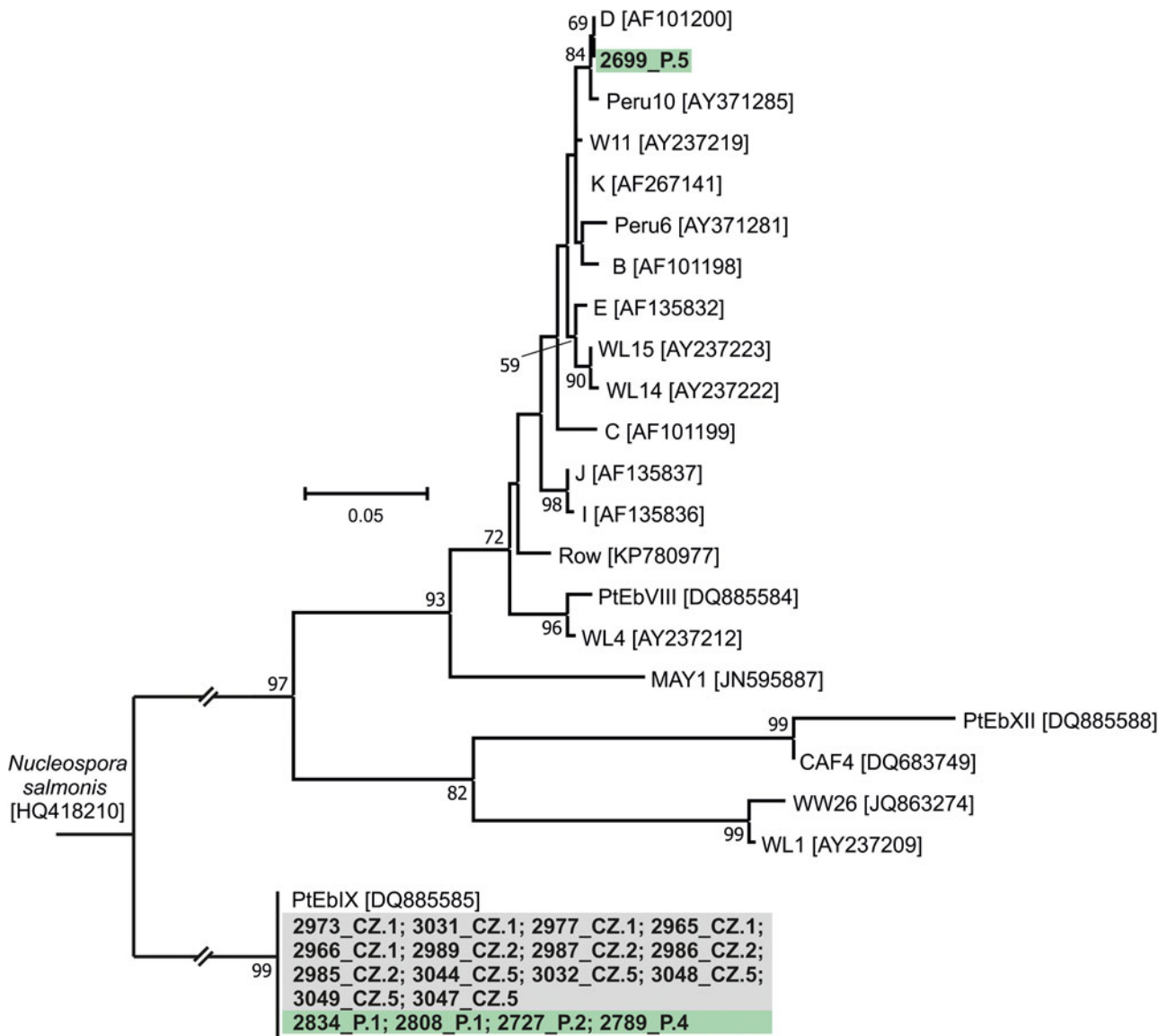


Figure 7. Phylogenetic relationships between *Enterocytozoon bieneusi* genotypes detected in dogs in this study (highlighted in green – Poland or in grey – Czech Republic) and other *E. bieneusi* genotypes available in GenBank using an ML analysis of *ITS* region of rRNA gene (sequence alignment length: 309 bp). Percentage supports (>50%) from 1000 pseudoreplicates are indicated next to the supported node. The branch length scale bar indicates the number of substitutions per site. Sequences from this study are identified by an isolate number (e.g. 2973) followed by region and location (P.1, Poland location 1, CZ.1, Czech Republic location 1).

All *E. bieneusi* isolates detected in the studied dogs, except for 1 clustering to genotype D, were identical to *E. bieneusi* genotype PtEbIX. This genotype appears to be specific to dogs; to date, it has been detected almost exclusively in dogs and sporadically in wolves, cats and swans (Santín *et al.*, 2008; Abe *et al.*, 2009; Santín and Fayer, 2011; Mori *et al.*, 2013; Karim *et al.*, 2014a; Piekarska *et al.*, 2017; Kváč *et al.*, 2021). In turn, both *E. bieneusi* genotype D and *E. cuniculi* genotype II detected in the present study have been reported in a broad range of hosts so far, including humans (Li *et al.*, 2012; Kváč *et al.*, 2017; Piekarska *et al.*, 2017; Delrobai *et al.*, 2019). Observation of microsporidian genotypes with a human-infection capacity in companion animals suggests that pets may be of importance as one of the potential sources of infection. However, the presented results do not indicate that dogs in shelters in Poland and the Czech Republic represent a significant source of zoonotic species and genotypes of the studied parasites for humans.

Our study had some limitations. Firstly, due to its screening nature, a detailed analysis in the context of the demographic data of the tested dogs or the drugs used was not possible to

conduct. Moreover, the study groups differed in size – in Poland it was possible to collect material from a larger number of animals than in the Czech Republic, which may have an impact on the differences in prevalence.

This study included clinically healthy animals without signs of intestinal infection, yet dispersive forms of potentially pathogenic and infectious organisms were observed. It should also be borne in mind that asymptomatic hosts could shed cysts, oocysts or spores occasionally and irregularly, and thus their screening with multiple sampling could increase the real observed prevalence (Sak *et al.*, 2010). Nevertheless, since infections of the studied pathogens in dogs can often be asymptomatic, they may not be detected by routine veterinary examinations, and their occurrence in companion animals may be underestimated. On the contrary, the majority of species and genotypes observed in canine samples herein are not commonly associated with human infections, and aforesaid transmission routes seem to be rare. The exceptions are genotypes D (*E. bieneusi*) and II (*E. cuniculi*) observed in the present study, whose zoonotic potential should be emphasized due to their occurrence in a wide range of different

hosts (Hinney *et al.*, 2016; Li *et al.*, 2019, 2020). Despite the low likelihood of transmission of the studied pathogens and due to the fact that they mainly affect immunosuppressed individuals, in whom the consequences of opportunistic infections may be life-threatening, the awareness among new dog owners is recommended, especially those with various levels of immunosuppression, on the relevance of diagnosing and treating zoonotic diseases.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S003118202400009X>

Data availability statement. The data that support the findings of this study are available from the corresponding author, MS, upon reasonable request.

Acknowledgements. The authors acknowledge the staff of dog shelters for their assistance in sample collection and the technician staff of the Laboratory of Veterinary and Medical Protistology, Biology Centre CAS, for sample processing during this study.

Author's contribution. M. S., M. Ki. and Ž. Z. conceived and designed the study. M. S., M. Ki., Ž. Z., M. Ka., M. Kv., B. S. and N. H. conducted data gathering. M. S., M. Ki., Ž. Z., M. Ka., B. L. and A. L. prepared the samples and conducted molecular analysis. M. S. conducted statistical analyses. M. Kv. prepared phylogenetic analyses. M. S., M. Ki. and M. Kv. wrote the article.

Financial support. This work was supported with a subvention by the Polish Ministry of Health (M. S., grant number SUBK.A060.23.027 from the IT Simple system of Wrocław Medical University) and the Grant Agency of the Czech Republic (M. Kv., GACR 21-23773S and B. S. 23-06571S).

Competing interests. None.

Ethical standards. The collection of samples carried out in a non-invasive way, without interfering with the organism of the animals included in the study.

References

- Abe N, Kimata I and Iseki M (2009) Molecular evidence of *Enterocytozoon bieneusi* in Japan. *Journal of Veterinary Medical Science* **71**, 217–219.
- Adell-Aledón M, Köster PC, de Lucio A, Puente P, Hernández-de-Mingo M, Sánchez-Thevenet P, Dea-Ayuela MA and Carmena D (2018) Occurrence and molecular epidemiology of *Giardia duodenalis* infection in dog populations in eastern Spain. *BMC Veterinary Research* **14**, 1–11.
- Agresti A, Berrilli F, Maestrini M, Procesi IG, Loretti E, Vonci N and Perrucci S (2022) Prevalence, risk factors and genotypes of *Giardia duodenalis* in sheltered dogs in Tuscany (Central Italy). *Pathogens (Basel, Switzerland)* **11**, 1–9.
- Alderisio KA, Mergen K, Moessner H and Madison-Antenucci S (2023) Identification and evaluation of *Cryptosporidium* species from New York City cases of cryptosporidiosis (2015 to 2018): a watershed perspective. *Microbiology Spectrum* **11**, 1–8.
- Bajer A and Bednarska M (2007) *Cryptosporidium* spp. and *Giardia* spp. infections in sled dogs. *Medycyna Weterynaryjna* **63**, 681–687.
- Bouzid M, Halai K, Jeffreys D and Hunter PR (2015) The prevalence of *Giardia* infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. *Veterinary Parasitology* **207**, 181–202.
- Brogli A, Weitzel T, Harms G, Cacció SM and Nöckler K (2013) Molecular typing of *Giardia duodenalis* isolates from German travellers. *Parasitology Research* **112**, 3449–3456.
- Buckholt MA, Lee JH and Tzipori S (2002) Prevalence of *Enterocytozoon bieneusi* in swine: an 18-month survey at a slaughterhouse in Massachusetts. *Applied and Environmental Microbiology* **68**, 2595–2599.
- Cacció SM, De Giacomo M and Pozio E (2002) Sequence analysis of the β -giardin gene and development of a polymerase chain reaction–restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. *International Journal for Parasitology* **32**, 1023–1030.
- Cacció SM, Beck R, Lalle M, Marinculic A and Pozio E (2008) Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. *International Journal for Parasitology* **38**, 1523–1531.
- Delrobaei M, Jamshidi S, Shayan P, Ebrahimzade E, Ashrafi Tamai I, Rezaeian M and Mirjalali H (2019) Molecular detection and genotyping of intestinal microsporidia from stray dogs in Iran. *Iranian Journal of Parasitology* **14**, 159–166.
- de Lucio A, Bailo B, Aguilera M, Cardona GA, Fernández-Crespo JC and Carmena D (2017) No molecular epidemiological evidence supporting household transmission of zoonotic *Giardia duodenalis* and *Cryptosporidium* spp. from pet dogs and cats in the province of Álava, northern Spain. *Acta Tropica* **170**, 48–56.
- Dengjel B, Zahler M, Hermanns W, Heinritz K, Spillmann T, Thomschke A, Löscher T, Gothe R and Rinder H (2001) Zoonotic potential of *Enterocytozoon bieneusi*. *Journal of Clinical Microbiology* **39**, 4495–4499.
- Didier ES, Vossbrinck CR, Baker MD, Rogers LB, Bertucci DC and Shadduck JA (1995) Identification and characterization of three *Encephalitozoon cuniculi* strains. *Parasitology* **111**, 411–421.
- Didier ES, Didier PJ, Snowden KF and Shadduck JA (2000) Microsporidiosis in mammals. *Microbes and Infection* **2**, 709–720.
- Dubná S, Langrová I, Nápravník J, Jankovská I, Vadlejš J, Pekár S and Fechtner J (2007) The prevalence of intestinal parasites in dogs from Prague, rural areas, and shelters of the Czech Republic. *Veterinary Parasitology* **145**, 120–128.
- European Scientific Counsel Companion Animal Parasites (ESCCAP) (2018) Control of intestinal protozoa in dogs and cats. Available at <https://www.esccap.org/guidelines/gl6/> (accessed 13 January 2024).
- Feng Y and Xiao L (2011) Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clinical Microbiology Reviews* **24**, 110–140.
- Feng Y, Wang L, Duan L, Gomez-Puerta LA, Zhang L, Zhao X, Hu J, Zhang N and Xiao L (2012) Extended outbreak of cryptosporidiosis in a pediatric hospital, China. *Emerging Infectious Diseases* **18**, 312–314.
- Gatei W, Das P, Dutta P, Sen A, Cama V, Lal AA and Xiao L (2007) Multilocus sequence typing and genetic structure of *Cryptosporidium hominis* from children in Kolkata, India. *Infection, Genetics and Evolution* **7**, 197–205.
- General Veterinary Inspectorate (2023) Annual report on visits to animal shelters for 2022. Available at <https://www.wetgiw.gov.pl/nadzor-weterynaryjny/schroniska-dla-bezdomnych-zwierzat> (accessed 13 January 2024).
- Giagaspero A, Iorio R, Paoletti B, Traversa D and Capelli G (2006) Molecular evidence for *Cryptosporidium* infection in dogs in central Italy. *Parasitology Research* **99**, 297–299.
- Gil H, Cano L, de Lucio A, Bailo B, de Mingo MH, Cardona GA, Fernández-Basterra JA, Aramburu-Aguirre J, López-Molina N and Carmena D (2017) Detection and molecular diversity of *Giardia duodenalis* and *Cryptosporidium* spp. in sheltered dogs and cats in northern Spain. *Infection, Genetics and Evolution* **50**, 62–69.
- Hall, T A (1999) BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 95–98.
- Hinney B, Sak B, Joachim A and Kváč M (2016) More than a rabbit's tale – *Encephalitozoon* spp. in wild mammals and birds. *International Journal for Parasitology: Parasites and Wildlife* **5**, 76–87.
- Jiang W, Roellig DM, Guo Y, Li N, Feng Y and Xiao L (2021) Development of a subtyping tool for zoonotic pathogen *Cryptosporidium canis*. *Journal of Clinical Microbiology* **59**, 1–8.
- Karim MR, Dong H, Yu F, Jian F, Zhang L, Wang R, Zhang S, Rume FI, Ning C and Xiao L (2014a) Genetic diversity in *Enterocytozoon bieneusi* isolates from dogs and cats in China: host specificity and public health implications. *Journal of Clinical Microbiology* **52**, 3297–3302.
- Karim MR, Wang R, Dong H, Zhang L, Li J, Zhang S, Rume FI, Qi M, Jian F, Sun M, Yang G, Zou F, Ning C and Xiao L (2014b) Genetic polymorphism and zoonotic potential of *Enterocytozoon bieneusi* from nonhuman primates in China. *Applied and Environmental Microbiology* **80**, 1893–1898.
- Katzwinkl-Wladarsch S, Lieb M, Helse W, Löscher T and Rinder H (1996) Direct amplification and species determination of microsporidian DNA from stool specimens. *Tropical Medicine & International Health* **1**, 373–378.
- Kicia M, Wesolowska M, Jakuszko K, Kopacz Z, Sak B, Květonová D, Krajewska M and Kváč M (2014) Concurrent infection of the urinary tract with *Encephalitozoon cuniculi* and *Enterocytozoon bieneusi* in a renal transplant recipient. *Journal of Clinical Microbiology* **52**, 1780–1782.
- Kicia M, Wesolowska M, Kopacz Z, Jakuszko K, Sak B, Květonová D, Krajewska M and Kváč M (2016) Prevalence and molecular characteristics of urinary and intestinal microsporidia infections in renal transplant recipients. *Clinical Microbiology and Infection* **22**, 462.e5–462.e9.

- Kicia M, Szydłowicz M, Cebulski K, Jakuszko K, Piesiak P, Kowal A, Sak B, Krajewska M, Hendrich AB, Kváč M and Kopacz Ż (2019) Symptomatic respiratory *Encephalitozoon cuniculi* infection in renal transplant recipients. *International Journal of Infectious Diseases* **79**, 21–25.
- Kozisek F, Craun GF, Cerovska L, Pumann P, Frost F and Muller T (2008) Serological responses to *Cryptosporidium*-specific antigens in Czech populations with different water sources. *Epidemiology and Infection* **136**, 279–286.
- Kumar S, Stecher G and Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.
- Kváč M, Hofmannová L, Ortega Y, Holubová N, Horčíčková M, Kicia M, Hlášková L, Květoňová D, Sak B and McEvoy J (2017) Stray cats are more frequently infected with zoonotic protists than pet cats. *Folia Parasitologica* **64**, 034. doi: 10.14411/fp.2017.034
- Kváč M, Vlnatá G, Ježková J, Horčíčková M, Konečný R, Hlášková L, McEvoy J and Sak B (2018) *Cryptosporidium occultus* sp. n. (Apicomplexa: Cryptosporidiidae) in rats. *European Journal of Protistology* **63**, 96–104.
- Kváč M, Myšková E, Holubová N, Kellnerová K, Kicia M, Rajský D, McEvoy J, Feng Y, Hanzal V and Sak B (2021) Occurrence and genetic diversity of *Cryptosporidium* spp. in wild foxes, wolves, jackals, and bears in central Europe. *Folia Parasitologica* **68**, 1–8.
- Lalle M, Jimenez-Cardosa E, Cacciò SM and Pozio E (2005) Genotyping of *Giardia duodenalis* from humans and dogs from Mexico using a β -giardin nested polymerase chain reaction assay. *Journal of Parasitology* **91**, 203–205.
- Learmonth JJ, Ionas G, Ebbett KA and Kwan ES (2004) Genetic characterization and transmission cycles of *Cryptosporidium* species isolated from humans in New Zealand. *Applied and Environmental Microbiology* **70**, 3973–3978.
- Li N, Xiao L, Wang L, Zhao S, Zhao X, Duan L, Guo M, Liu L and Feng Y (2012) Molecular surveillance of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* by genotyping and subtyping parasites in wastewater. *PLoS Neglected Tropical Diseases* **6**, e1809.
- Li W, Feng Y and Santin M (2019) Host specificity of *Enterocytozoon bieneusi* and public health implications. *Trends in Parasitology* **35**, 436–451.
- Li W, Feng Y and Xiao L (2020) Diagnosis and molecular typing of *Enterocytozoon bieneusi*: the significant role of domestic animals in transmission of human microsporidiosis. *Research in Veterinary Science* **133**, 251–261.
- Li J, Ryan U, Guo Y, Feng Y and Xiao L (2021) Advances in molecular epidemiology of cryptosporidiosis in dogs and cats. *International Journal for Parasitology* **51**, 787–795.
- Liao S, Lin X, Sun Y, Qi N, Lv M, Wu C, Li J, Hu J, Yu L, Cai H, Xiao W, Sun M and Li G (2020) Occurrence and genotypes of *Cryptosporidium* spp., *Giardia duodenalis*, and *Blastocystis* sp. in household, shelter, breeding, and pet market dogs in Guangzhou, southern China. *Scientific Reports* **10**, 1–11.
- Liu H, Shen Y, Yin J, Yuan Z, Jiang Y, Xu Y, Pan W, Hu Y and Cao J (2014) Prevalence and genetic characterization of *Cryptosporidium*, *Enterocytozoon*, *Giardia* and *Cyclospora* in diarrheal outpatients in China. *BMC Infectious Diseases* **14**, 1–6.
- Mathis A, Weber R and Deplazes P (2005) Zoonotic potential of the microsporidia. *Clinical Microbiology Reviews* **18**, 423–445.
- Mori H, Mahittikorn A, Thammasonthijarern N, Chaisiri K, Rojekkittikhun W and Sukthana Y (2013) Presence of zoonotic *Enterocytozoon bieneusi* in cats in a temple in central Thailand. *Veterinary Parasitology* **197**, 696–701.
- Murnik L-C, Dauschies A and Delling C (2022) *Cryptosporidium* infection in young dogs from Germany. *Parasitology Research* **121**, 2985–2993.
- Pereira A, Teixeira J, Sousa S, Parreira R, Campino L, Meireles J and Maia C (2021) *Giardia duodenalis* infection in dogs from the metropolitan area of Lisbon, Portugal: prevalence, genotyping and associated risk factors. *Journal of Parasitic Diseases* **45**, 372–379.
- Piekara-Stepińska A, Piekarska J and Gorczykowski M (2021a) *Cryptosporidium* spp. in dogs and cats in Poland. *Annals of Agricultural and Environmental Medicine* **28**, 345–347.
- Piekara-Stepińska A, Piekarska J, Gorczykowski M and Bania J (2021b) Genotypes of *Giardia duodenalis* in household dogs and cats from Poland. *Acta Parasitologica* **66**, 428–435.
- Piekarska J, Bajzert J, Gorczykowski M, Kantyka M and Podkowik M (2016) Molecular identification of *Giardia duodenalis* isolates from domestic dogs and cats in Wrocław, Poland. *Annals of Agricultural and Environmental Medicine* **23**, 410–415.
- Piekarska J, Kicia M, Wesolowska M, Kopacz Ż, Gorczykowski M, Szczepankiewicz B, Kváč M and Sak B (2017) Zoonotic microsporidia in dogs and cats in Poland. *Veterinary Parasitology* **246**, 108–111.
- Plutzer J, Lassen B, Jokelainen P, Djurković-Djaković O, Kucsera I, Dorbek-Kolin E, Šoba B, Sréter T, Imre K, Omeragić J, Nikolić A, Bobić B, Živičnjak T, Lučinger S, Stefanović LL, Kučinar J, Sroka J, Deksne G, Keidāne D, Kváč M, Hůzová Z and Karanis P (2018) Review of *Cryptosporidium* and *Giardia* in the eastern part of Europe, 2016. *Eurosurveillance* **23**, 1–23.
- Raza A, Rand J, Qamar AG, Jabbar A and Kopp S (2018) Gastrointestinal parasites in shelter dogs: occurrence, pathology, treatment and risk to shelter workers. *Animals* **8**, 108. doi: 10.3390/ani8070108
- Read CM, Monis PT and Andrew Thompson RC (2004) Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. *Infection, Genetics and Evolution* **4**, 125–130.
- Rehbein S, Klotz C, Ignatius R, Müller E, Aebischer A and Kohn B (2019) *Giardia duodenalis* in small animals and their owners in Germany: a pilot study. *Zoonoses and Public Health* **66**, 117–124.
- Remesar S, García-Dios D, Calabuig N, Prieto A, Díaz-Cao JM, López-Lorenzo G, López C, Fernández G, Morrondo P, Panadero R and Díaz P (2022) Cardiorespiratory nematodes and co-infections with gastrointestinal parasites in new arrivals at dog and cat shelters in northwestern Spain. *Transboundary and Emerging Diseases* **69**, e3141–e3153.
- Ryan U and Zahedi A (2019) Molecular epidemiology of giardiasis from a veterinary perspective. *Advances in Parasitology* **106**, 209–254.
- Sak B, Kašičková D, Kváč M, Květoňová D and Ditrich O (2010) Microsporidia in exotic birds: intermittent spore excretion of *Encephalitozoon* spp. in naturally infected budgerigars (*Melospittacus undulatus*). *Veterinary Parasitology* **168**, 196–200.
- Sak B, Brady D, Pelikánová M, Květoňová D, Rost M, Kostka M, Tolarová V, Hůzová Z and Kváč M (2011) Unapparent microsporidial infection among immunocompetent humans in the Czech Republic. *Journal of Clinical Microbiology* **49**, 1064–1070.
- Santín M and Fayer R (2011) Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. *Research in Veterinary Science* **90**, 363–371.
- Santín M, Vecino JAC and Fayer R (2008) *Enterocytozoon bieneusi* genotypes in dogs in Bogota, Colombia. *The American Journal of Tropical Medicine and Hygiene* **79**, 215–217.
- Scaramozzino P, Carvelli A, Iacoponi F and De Liberato C (2018) Endoparasites in household and shelter dogs from central Italy. *International Journal of Veterinary Science and Medicine* **6**, 45–47.
- Simonato G, Frangipane di Regalbono A, Cassini R, Traversa D, Beraldo P, Tessarin C and Pietrobelli M (2015) Copromicroscopic and molecular investigations on intestinal parasites in kennel dogs. *Parasitology Research* **114**, 1963–1970.
- Simonato G, Frangipane di Regalbono A, Cassini R, Traversa D, Tessarin C, Di Cesare A and Pietrobelli M (2017) Molecular detection of *Giardia duodenalis* and *Cryptosporidium* spp. in canine faecal samples contaminating public areas in northern Italy. *Parasitology Research* **116**, 3411–3418.
- Soliman RH, Fuentes I and Rubio JM (2011) Identification of a novel assemblage B subgenotype and a zoonotic assemblage C in human isolates of *Giardia intestinalis* in Egypt. *Parasitology International* **60**, 507–511.
- Sommer MF, Zdravković N, Vasić A, Grimm F and Silaghi C (2017) Gastrointestinal parasites in shelter dogs from Belgrade, Serbia. *Veterinary Parasitology: Regional Studies and Reports* **7**, 54–57.
- State Veterinary Administration (2024) Registered animal shelters. Available at <https://www.svscr.cz/registrovane-subjekty-svs/registrovane-utulky-pro-zvirata/> (accessed 13 January 2024).
- Sulaiman IM, Fayer R, Bern C, Gilman RH, Trout JM, Schantz PM, Das P, Lal AA and Xiao L (2003) Triosephosphate isomerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. *Emerging Infectious Diseases* **9**, 1444–1452.
- Traub RJ, Monis PT, Robertson I, Irwin P, Mencke N and Thompson RCA (2004) Epidemiological and molecular evidence supports the zoonotic transmission of *Giardia* among humans and dogs living in the same community. *Parasitology* **128**, 253–262.
- Tůmová L, Ježková J, Prediger J, Holubová N, Sak B, Konečný R, Květoňová D, Hlášková L, Rost M, McEvoy J, Xiao L, Santín M and Kváč M (2023) *Cryptosporidium mortiferum* n. sp. (Apicomplexa: Cryptosporidiidae), the species causing lethal cryptosporidiosis in Eurasian red squirrels (*Sciurus vulgaris*). *Parasites & Vectors* **16**, 1–21.

- Villamizar X, Higuera A, Herrera G, Vasquez-A LR, Buitron L, Muñoz LM, Gonzalez-C FE, Lopez MC, Giraldo JC and Ramirez JD (2019) Molecular and descriptive epidemiology of intestinal protozoan parasites of children and their pets in Cauca, Colombia: a cross-sectional study. *BMC Infectious Diseases* **19**, 1–11.
- Wang W, Wei Y, Cao S, Wu W, Zhao W, Guo Y, Xiao L, Feng Y and Li N (2022) Divergent *Cryptosporidium* species and host-adapted *Cryptosporidium canis* subtypes in farmed minks, raccoon dogs and foxes in Shandong, China. *Frontiers in Cellular and Infection Microbiology* **12**, 1–9.
- Xiao L (2010) Molecular epidemiology of cryptosporidiosis: an update. *Experimental Parasitology* **124**, 80–89.
- Xiao L, Morgan UM, Limor J, Escalante A, Arrowood M, Shulaw W, Thompson RC, Fayer R and Lal AA (1999) Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. *Applied and Environmental Microbiology* **65**, 3386–3391.
- Xiao L, Cama VA, Cabrera L, Ortega Y, Pearson J and Gilman RH (2007) Possible transmission of *Cryptosporidium canis* among children and a dog in a household. *Journal of Clinical Microbiology* **45**, 2014–2016.
- Xu H, Jin Y, Wu W, Li P, Wang L, Li N, Feng Y and Xiao L (2016) Genotypes of *Cryptosporidium* spp., *Enterocytozoon bieneusi* and *Giardia duodenalis* in dogs and cats in Shanghai, China. *Parasites & Vectors* **9**, 1–9.
- Yu Z, Ruan Y, Zhou M, Chen S, Zhang Y, Wang L, Zhu G and Yu Y (2018) Prevalence of intestinal parasites in companion dogs with diarrhea in Beijing, China, and genetic characteristics of *Giardia* and *Cryptosporidium* species. *Parasitology Research* **117**, 35–43.
- Zemanová I, Husník R and Svobodová V (2005) *Giardia intestinalis* u psů – výskyt, zoonotický potenciál a využití endoskopické diagnostiky. [*Giardia intestinalis* in dogs – occurrence, zoonotic potential and use of endoscopic diagnostic]. *Veterinarství* **55**, 319–325.