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REVIEW

Cryptosporidium and *Giardia* spp. infections in humans, animals and the environment in Poland

Anna Bajer

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Abstract *Cryptosporidium* spp. and *Giardia* spp. are intestinal protozoan parasites that are prevalent and widespread pathogens of humans and many other species of mammals. The aim of this review is to summarise the last 20 years of research on the epidemiology of these parasites, with a particular emphasis on the environment and the role played by different groups of animals in Poland. The prevalence of both species has been studied in different groups of humans, in wildlife, pets and farm animals and in environmental samples. Additionally, current knowledge on the distribution of zoonotic and non-zoonotic species/genotypes has been reviewed. The usefulness of different methods for the detection and identification of the parasites in different types of samples has been evaluated. Finally, because of the wide distribution and high prevalence of both species in a range of hosts and possible vectors involved in mechanical transmission, the overall risk of outbreaks of cryptosporidiosis and giardiosis in Poland has been assessed as relatively high.

Introduction

Cryptosporidium spp. and *Giardia* spp. are intestinal protozoan parasites that are recognised as prevalent and widespread pathogens of humans and many other species of mammals. *Cryptosporidium* and *Giardia* infections are common causes of gastroenteritis (cryptosporidiosis/giardiosis), which manifests as a diarrhoea in humans. The diarrhoea may become profuse and chronic and in consequence life-threatening, particularly in immunocompromised or immunosuppressed persons (Griffiths 1998). Both parasites share a broad host range, and both cryptosporidiosis and giardiosis are believed to be zoonoses (Monis and Thompson 2003; Smith et al. 2006). Despite our knowledge of the distribution of these species among more than 100 mammalian species and numerous reports from human communities, the routes of environmental transmission are still not well defined (Appelbee et al. 2005; Smith et al. 2006).

Additional problems are generated by the fact that each genus is believed to comprise of complexes of species, and moreover genotypes within species, some of which are pathogenic, some specific to particular hosts and some zoonotic, and hence of public health significance (Xiao et al. 2004; Caccio et al. 2005; Smith et al. 2006). Epidemiological surveys have indicated that the most important sources for human infection are contaminated drinking and recreational water, food, household animals and infected people (Dillingham et al. 2002). Sexual contacts also constitute a route for direct transmission of *Cryptosporidium* spp. Sources of contamination of water and food may be diverse, but a particularly important, albeit varying, role is played by different host groups that act as reservoirs of infection (Fig. 1). Farm animals are believed to play the most significant role in this context, contributing parasite cysts/oocysts in large proportion because of their high abundance on farms. The use of faecal material as fertiliser for arable fields and pastures is another important contributory factor. Household animals, such as dogs, cats, rodents, reptiles and birds, may also contribute to the transmission of intestinal parasites because of their close association with their owners. Furthermore, cats and dogs have many opportunities to contact free-living animals, whether commensal, feral or truly wild, e.g. rodents, and to

A. Bajer (✉)
Department of Parasitology, Institute of Zoology,
University of Warsaw,
Miecznikowa 1, 02-096 Warsaw, Poland
e-mail: anabena@biol.uw.edu.pl

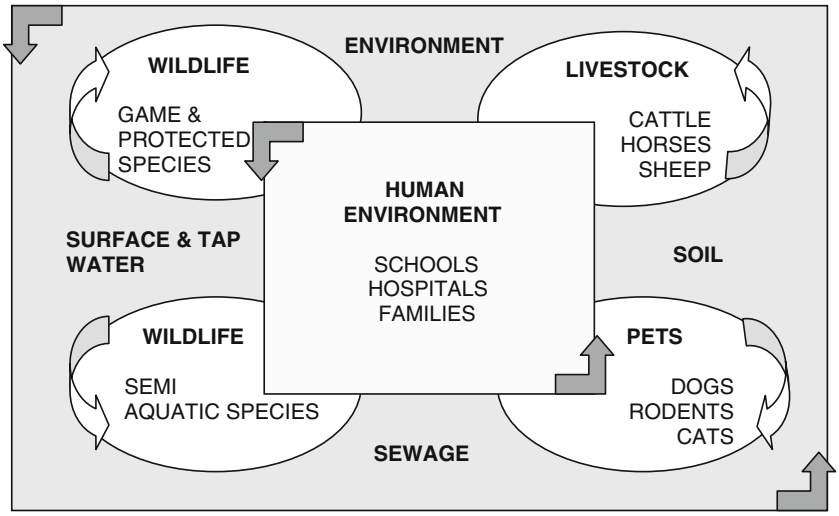

Fig. 1 Different host groups that act as reservoirs of infection

contract infections from them. Free-living animals may constitute a reservoir of pathogens in nature but also contribute to surface water, soil and food contamination. For example, semi-aquatic species, such as beavers, deposit their faeces directly in water, and commensal rodents (house mice, rats) contribute to food contamination, both for humans and domestic animals. Some species, e.g. birds or insects (e.g. species of Diptera including flies), may also act as vectors facilitating mechanical transmission of cysts/oocysts and hence contributing to their dispersal over long distances as well as directly to our tables (Graczyk et al. 2004, 2008).

Recently, a range of studies on the distribution of *Cryptosporidium* and *Giardia* have been conducted in Poland, focussing on different groups of animals, on humans and including assessment of environmental samples. The Republic of Poland covers an area of 311,888 km^2 in Central Europe and currently has 38.1 million inhabitants. Almost 60% of families own a dog or a cat. Forty percent of Poland's inhabitants work in agriculture, and an estimated 51% of available land is devoted to this industry. Around 29% of the area of Poland is covered by forests, and 32% of the country is under different forms of legal protection, including 23 National Parks where conservation of the environment and wildlife is strictly enforced. The number of tourists visiting National Parks in 2006 exceeded 11.5 million (Główny Urząd Statystyczny [GUS]—Central Statistical Office data for 2006). Despite the fact that human cryptosporidiosis and giardiosis are registered diseases in Poland, reliable information on the distribution of these infections is available mostly from scientific studies, rather than official government statistics. The aim of this review is to summarise these studies and to assess the epidemiological status of the opportunistic pathogens that cause cryptosporidiosis and giardiosis in Poland.

Farm animals

Poultry constitute the largest population of farm animals in Poland (111.7 million), followed by pigs (18.8 million), cattle (5.3 million), sheep and horses (about 300,000 each; data for year 2006, GUS). The great majority of studies on intestinal protozoa in farm animals have been conducted in cattle (Table 1). The earliest reports of *Cryptosporidium* infections in cattle were in the 1980s, and these indicated a high prevalence of parasites in young animals (Kozakiewicz and Maszewska 1988a, b). In the Wielkopolska region, *Cryptosporidium* has been found in cattle in the majority of farms that have been surveyed (>65%; Bednarska et al. 1998; Pilarczyk and Balicka-Ramisz 2001). The highest infection rate (>80%) and the highest intensity of infection have been observed in calves up to 3 weeks of age on dairy cattle farms (Bednarska et al. 1998; Pilarczyk et al. 2002, 2003). Infections were less common in small private farms. The dimensions and the shape of the oocysts isolated from these animals suggested that *Cryptosporidium parvum* was responsible, and this has been confirmed by molecular studies (Bednarska et al. 1998; Bajer et al. 2005; Solarczyk and Majewska 2007b). However, *Cryptosporidium* infections were generally less prevalent in adult cattle (Table 1) with an increasing frequency of species, such as *Cryptosporidium andersoni* or *Cryptosporidium felis* (Bornay-Llinares et al. 1999; Majewska et al. 2001e, 2004a), which are not considered to be highly infective to and are only very rarely found in humans. Nevertheless, in view of these studies, cattle clearly constitute an important reservoir of *C. parvum* in Poland.

In contrast, *Giardia* spp. infections appear to be rare in cattle in Poland (2.2–14%; in two of five studied farms), and there are only a few reports on isolated findings of this parasite in cattle in the country (Table 1; Bednarska et al. 1998, Majewska et al. 1998b, 2001a).

Table 1 Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in livestock and other farm animals

Host species/subgroups		*Cryptosporidium* spp.		*Giardia* spp.	
		% infected (method)	Reference	% infected (method)	Reference
Cattle	Calves	30.8% in 26 calves (*C. parvum*)	Majewska et al. (2004a)	2.2% cattle	Majewska et al. (2001a, 1998b)
	Dairy cows	4.3% in 141 cows (*C. pavum.)*			
Wielkopolska region		10% cows (*Cryptosporidium andersoni*; microscopy, EIA, PCR)			
		26–54% in cattle in 1-year study, including 0.7% *C. parvum* and 37.2% *Cryptosporidium andersoni* (microscopy, EIA)	Majewska et al. (2001a)	Single cases of *Giardia* infections in cattle (microscopy, EIA)	Majewska et al. (2001a)
		20–88% calves (51%; microscopy, IFA)	Bednarska et al. (1998)	14% calves (microscopy, IFA)	Bednarska et al. (1998)
Western Pomerania region		14.3–60% calves (microscopy, EIA)	Pilarczyk and Balicka-Ramisz (2001)		
		73.3% in calves from imported heifers (microscopy, EIA)	Pilarczyk and Balicka-Ramisz (2001)		
		6.7% calves (microscopy)	Pilarczyk and Balicka-Ramisz (2004)		
Gdańsk district		42.7% *Cryptosporidium* infection in cows, including 3 cases of *Cryptosporidium felis* infections (microscopy, PCR)	Bornay-Llinares et al. (1999)		
Sheep		10.1% sheep (microscopy, EIA, PCR)	Majewska et al. (2001a, 2000)	1.3% sheep (microscopy)	Majewska et al. (2001a, 1998b)
		11.8% lambs (microscopy, EIA)	Pilarczyk and Balicka-Ramisz (2001)		
Goats		0% in 46 adult goats (microscopy, EIA)	Majewska et al. (2000)		
Poultry		0% in 210 samples from turkeys and chickens (microscopy, EIA or FISH, PCR)	Majewska et al. (2004d)	1 domestic goose (microscopy, FISH)	Słodkowicz-Kowalska et al. (2007)
				0% in 210 samples from turkeys and chickens (microscopy, EIA or FISH, PCR)	Majewska et al. (2004d)
Pigs		9.5% pigs	Majewska et al. (2001a)		
Horses		9.4% horses (microscopy, EIA)	Majewska et al. (2001a, 1999b)		
		0–11.5% of horses (overall=3.5%), 50% stables (microscopy, EIA, FISH)	Majewska et al. (2004e)		
		14.3% colts (microscopy, EIA)	Pilarczyk and Balicka-Ramisz (2001)		
Polish Konik (*Equus caballus*)		0% of 10 samples (microscopy, IFA)	Paziewska et al. (2006, 2007)	0% of 10 samples (microscopy, IFA)	Paziewska et al. (2007)

Among the very few studies of *Cryptosporidium* spp in horses, it has been reported that 50% of stables tested positive for this species (Table 1; Majewska et al. 1999b, 2004e). Colts were more often infected than adult horses (14.3% versus 3.5%; Pilarczyk and Balicka-Ramisz 2001). Oocysts and cysts were not found in the indigenous breed of Polish wild horses known as Polish Konik (Paziewska et al. 2007). Currently, there are no other published studies on *Giardia* infections in horses in Poland (Gundłach et al. 2006).

Both parasites have been found in sheep in Poland (Table 1). Although only 1.3% of sheep excreted *Giardia* spp. cysts (Majewska et al. 2001a), *C. parvum* infections were found in 10–12% of animals and were more common among lambs than adults (Majewska et al. 2000, Pilarczyk and Balicka-Ramisz 2001). *Cryptosporidium* spp. oocysts have been detected in 9.5% of pigs and in none of 46 adult goats examined (Majewska et al. 1998b, 2000, 2001a).

Poultry have hardly been studied in Poland. Neither parasite was found in 210 faecal samples from turkeys and chickens (Majewska et al. 2004d). More recently, a single positive sample of *Giardia* spp. has been found in a domestic goose, and this diagnosis was based on sensitive molecular techniques, which are known to be more reliable than methods based on the recovery of cysts (Słodkowicz-Kowalska et al. 2007).

Wildlife/free-living animals

The largest populations of wild mammals in Poland are probably wild and commensal rodents, although no estimates of their total population sizes have been carried out across the whole country. Three hundred and ninety five species of birds inhabit Poland. Game species of mammals are distributed throughout the country, the largest population being represented by roe deer (706,500 ind.), followed by wild boars (177,100 ind.) and red deer (147,400 ind.). Growing populations of red foxes (218,800 ind.), which have become synanthropic animals in some cases, have been noted also in Poland (data for year 2006, GUS). Among protected species, stable populations of grey wolves (715 ind.), European beavers (over 49,000 ind.) and European bison (965 ind.) are established in Eastern parts of Poland (data for year 2006, GUS). A wide range of studies have been carried out on *Cryptosporidium* spp. and *Giardia* spp. in wildlife in Poland (Table 2). Both parasites have been detected in a range of host species. Both are common in rodents in Poland and were noted in four species of semi-aquatic rodents (Table 2). Infection rates for *Giardia* and *Cryptosporidium* were higher in muskrats than in European beavers; a similar picture was observed in Germany (Karanis et al. 1996). Although the morphometry of oocysts isolated from beavers suggested *C. parvum* infections, the genotyping has not yet been completed (Paziewska et al. 2006, 2007).

Intestinal protozoa are known to be widely distributed in forest and fallow rodents in the Mazury Lake District (Table 2). The epidemiology of these infections was studied during an 8-year period in naturally infected populations. Changes in the prevalence of both parasites followed changes in relative densities of rodent populations. In the case of *Cryptosporidium*, fewer older animals (especially *Myodes glareolus* and *Microtus arvalis*) carried infections, and infections in adult voles were relatively milder in comparison to mice. In contrast, in yellow-necked mice, *Giardia* infections were more common among older age classes. Although seasonal differences were significant, no consistent pattern of seasonal changes was apparent for *Cryptosporidium*, but *Giardia* infections had two peaks—in spring and the autumn. The prevalence and abundance of *Cryptosporidium* did not differ significantly between the sexes. The two protozoan species showed significant co-occurrence, and in rodents carrying both species, there was a strong significant positive correlation between intensity (abundance) of infection with each (Bajer 2008). Comparison of the dimensions of oocysts revealed that infection was entirely with the *C. parvum*-like species, and no evidence of *Cryptosporidium muris* infections was found (Bednarska et al. 2003). Preliminary molecular studies have revealed that the zoonotic species and genotypes are present in these rodent populations (Bajer et al. 2003), i.e. *C. parvum* mouse genotype, recently reported from an Indian child (Ajjampur et al. 2007), and *Giardia intestinalis* Assemblage A (Bajer et al. unpublished). Interestingly, *C. muris* infections were detected in two rodent species elsewhere in Poland, in the Wielkopolska region (Majewska et al. 2001d). Generally, *Giardia* prevalence has been reported to be higher than that of *Cryptosporidium* in rodents in Poland (Table 2).

A contrasting picture was observed in game mammals. *Cryptosporidium* spp. infections were more common than *Giardia* spp. infections in red and roe deer and wild boar. The highest prevalence of *Cryptosporidium* spp. was noted in red deer (14–17%), followed by roe deer (7–9%) and wild boar (0–2%; Pilarczyk and Balicka-Ramisz 2001; Paziewska et al. 2006, 2007). In the case of *Giardia*, infections were detected both in roe deer and red deer (<5%; Table 2). One isolate of *Giardia* from red deer was genotyped as *G. intestinalis* Assemblage A (zoonotic; Solarczyk and Majewska 2007a). Red foxes have not been studied for these intestinal protozoan infections, probably because of the high risk of *Echinococcus* spp. infection, since this tapeworm is known to be endemic in the fox populations throughout Poland (Gawor et al. 2004).

Cryptosporidium spp. and *Giardia* spp. infections have both been reported to be common intestinal parasites of grey wolves in NE Poland (Table 2). *Cryptosporidium* spp. was found in 20–55% of faecal samples, depending on the

method employed for detection (Kloch et al. 2005; Bednarska et al. 2007; Paziewska et al. 2006, 2007). The dimensions of oocysts from wolves suggested infections represented *C. parvum*-like species, and this has been confirmed by the genotyping of the COWP gene fragment. Five sequenced isolates showed full homology with a zoonotic *C. parvum* genotype (Paziewska et al. 2007). *Giardia* spp. infections were identified in 20–46% of samples from wolves, depending on the method of detection (Table 2). The high prevalence of the opportunistic protozoa in wolves suggests that young individuals dominate in Polish populations of wolves, since in most species, the prevalence of infection is higher in young compared with old individuals (Donskow et al. 2005).

Intestinal protozoa have been reported for the first time in European bison in Poland (Table 2). The prevalence of *Cryptosporidium* spp. in bison was similar to that found in cattle (20–31%). Infection rates for *Giardia* spp. were lower (8–13%), but still higher than those found in other Artiodactyls in Poland (Table 2). The dimension of the oocysts and the results of fluorescent in situ hybridisation (FISH) analysis both indicated *C. parvum* and *G. intestinalis* infections in these hosts (Bednarska et al. 2007; Paziewska et al. 2007).

Filth flies and wild birds, including aquatic species, were studied as possible mechanical vectors for intestinal protozoa (Graczyk et al. 2004). *Cryptosporidium* oocysts were found in 0.6–19% filth flies and *Giardia* cysts were detected in 1.4–7.3% flies, supporting their role in transmission of intestinal parasites (Table 2). A range of samples from various species of birds have been studied in western Poland (Majewska et al. 2004d; Słodkowicz-Kowalska et al. 2007), and the results varied between the various potential avian hosts (Table 2). Infection rates ranged from 0% to 12.5% for *Cryptosporidium* and from 0% to 7.5% for *Giardia* spp.

Domestic and captive animals

High populations of domestic dogs (about 7–8 million) and cats (5–6 million) exist in Poland because almost 60% of families own a dog or a cat. There are 19 zoological gardens in Poland, including ten that are formal members of the European Association of Zoos and Aquaria (EAZA).

Cryptosporidium oocysts have been found in 1.2–12.5% of dogs, but coproantigen detection assay indicated a higher prevalence of 27.4% (Table 3). *Giardia* cysts were detected in 6–36% of dogs, but again coproantigens were found in 53.5% of dogs. No genotyping data are available for *Cryptosporidium* isolates from dogs in Poland, but three *Giardia* isolates derived from sled dogs were genotyped as *G. intestinalis* Assemblage C, genotype specific for dogs (Bajer, unpublished). Three *Giardia* genotypes were found in dogs in Warsaw—zoonotic *G. intestinalis* Assemblage A–I in 1.7% of 350 dogs and two specific canine genotypes—*G. intestinalis* Assemblage C (1.14%) and Assemblage D (6.3%; Zygner et al. 2006).

Few studies have been conducted on the intestinal protozoa of cats in Poland, but those which have been reported have revealed only a few cases of *Cryptosporidium* and *Giardia* in these hosts (Table 3). *C. felis* was identified in 5% of cats in Poznań. Additionally, both parasites have been found in reptiles from pet shops in Poznań (Majewska et al. 2001b).

Studies on the distribution of intestinal protozoa in exotic animals from the zoological gardens in Poznań have identified three new host species for *Cryptosporidium* and six new host species for *Giardia* spp. (Table 3). One isolate of *Giardia* from Thomson's gazelle was genotyped as *G. intestinalis* Assemblage B (zoonotic; Solarczyk and Majewska 2007a).

Environment—water and food

Surface waters cover 5,572 km^2 (1.8%) of the total area of the country and are often the source of drinking water for cities in Poland, i.e. the Vistula river supplies the capital of Poland—the city of Warsaw with its 1.7 million inhabitants. The annual water intake of Poland was 11,253.8 hm^3, and the annual output of untreated sewage was 167.4 hm^3 in 2006 (GUS data). Numerous lake districts are attractive areas for thousands of tourists and amateur sailors. However, drinking (tap), raw and reclaimed waters are not routinely monitored for the presence of parasite oocysts and/or cysts in Poland. Neither is the potential contamination of raw food products, such as fruits and vegetables, being monitored for risk of infection to consumers. Export of fresh berries is one of the major branches of Polish agriculture, and in 2006, Poland exported 488,000 t of fresh fruits (GUS data).

A limited number of studies monitoring the distribution of intestinal protozoa in water and food using standardised methods have been completed recently in several regions of the country (Table 4). *Cryptosporidium* oocysts were detected in a great majority of water samples taken from the surface waters in the Poznań area (Sulima et al. 2000; Nowosad et al. 2007). Additionally, rotifers have been used for monitoring contamination in recreational surface water, and parasites were found in these filter feeding organisms (Majewska et al. 2003; Kuczyńska-Kippen et al. 2004). *Giardia* cysts were less prevalent in surface water in Poznan (Table 4) but were detected in rotifers. A similar situation was reported from Tri-city area (Table 4). Again, both oocysts and cysts were found in surface water (Szostakowska et al. 2005). However, contamination of tap water with *Cryptosporidium* spp. was

Table 2 Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in wildlife

Host species/region	*Cryptosporidium* spp.		*Giardia* spp.	
	% infected (method)	Reference	% infected (method)	Reference
Rodents				
Semi-aquatic rodents	19.2% European beavers (microscopy, IFA, PCR)	Paziewska et al. (2006, 2007)	7.7% European beavers (microscopy, IFA)	Paziewska et al. (2007)
	4.5% European beavers (*Castor fiber*) 58% muskrats (*Ondatra zibethicus*) 2 of 3 (*Arvicola terrestris*) 1 of 3 rats (*Rattus norvegicus*; microscopy, IFA)	Bajer and Siński (2002)	0% European beavers (*Castor fiber*) 87% muskrats (*O. zibethicus*) 2 of 3 *A. terrestris* 2 of 3 rats (*R. norvegicus*; microscopy, IFA)	Bajer and Siński (2002)
Rodents in Mazury Lake Disrtict	54–71% bank voles (*Myodes*=*Clethrionomys glareolus*)	Bajer et al. (2001, 2002)	58–94% bank voles (*Myodes*=*C. glareolus*)	Bajer et al. (2001, 2002)
	62–73% common voles (*Microtus arvalis*) 28% yellow-necked mice (*Apodemus flavicollis*; microscopy, IFA)	Bajer (2008)	74–96% common voles (*M. arvalis*) 24–48% yellow-necked mice (*A. flavicollis*; microscopy, IFA)	Bajer (2008)
Rodents in Wielkopolska region	*Cryptosporidium muris* identified in *Apodemus agrarius* and *M. arvalis* (microscopy, EIA, PCR)	Majewska et al. (2001d)	5.4% in *A. agrarius* 10.3% in *Myodes glareolus* (microscopy, EIA, PCR)	Majewska et al. (2001d)
Artiodactyls	16.7% red deer (*Cervus elaphus*)	Pilarczyk and Balicka-Ramisz (2001)	1 pos. red deer of 22 samples from wild cervids	Solarczyk and Majewska (2007a)
Western Pomerania	6.9% roe deer (*Capreolus capreolus*; microscopy, EIA)			
Mazury Lake Disrtict	14.4% red deer (*C. elaphus*) 9.1% roe deer (*C. capreolus*; microscopy, IFA, PCR)	Paziewska et al. (2006, 2007)	1.7% red deer (*C. elaphus*) 4.5% roe deer (*C. capreolus*; microscopy, IFA, PCR)	Paziewska et al. (2007)
Wild boar (*Sus scrofa*)	2.2% boars (microscopy, EIA)	Pilarczyk and Balicka-Ramisz (2001)	0% of 5 boars (microscopy, IFA, PCR)	Paziewska et al. (2007)
	0% of 5 boars (microscopy, IFA, PCR)	Paziewska et al. (2007)		
Protected species				
Wolf (*Canis lupus*)	55% wolves of 5 packs in Mazury Lake District (microscopy, IFA)	Kloch et al. (2005)	45.5% wolves of 5 packs in Mazury Lake District (microscopy, IFA)	Kloch et al. (2005)
	37.5% wolves in Białowieża Primeval Forest (PCR)	Paziewska et al. (2006, 2007)		
	20% wolves in Białowieża Primeval Forest (FISH)	Bednarska et al. (2007)	20% wolves in Białowieża Primeval Forest (FISH)	Bednarska et al. (2007)
European bison (*Bison bonasus*)	29.1% bisons in Białowieża Primeval Forest (microscopy, IFA, PCR)	Paziewska et al. (2006, 2007)	7.5% bisons in Białowieża Primeval Forest (microscopy, IFA, PCR)	Paziewska et al. (2007)
	31% bisons in Białowieża Primeval Forest (FISH)	Bednarska et al. (2007)	13% bisons in Białowieża Primeval Forest (FISH)	Bednarska et al. (2007)

Flies (Diptera: Muscidae, Calliphoridae Sarcophagidae)	0.6% of 830 flies (83 pools; PCR)	Racewicz et al. (2002)	1.4% of 1017 flies (112 pools; IFA)	Racewicz (2007)
	2.4% of 1,017 flies (112 pools; IFA)	Racewicz (2007)		
	19.4% of filth flies (FISH)	Szostakowska et al. (2004)	7.3% of filth flies (FISH)	Szostakowska et al. (2004)
Aquatic birds in Western Poland	5.8% of wild birds (mute swan, ducks goosander, white stork, carrion crow, rook; microscopy, FISH) pos.	Słodkowicz-Kowalska et al. (2007)	7.5% of wild birds (greyleg goose, mallard duck, mute swan, ducks goosander, carrion crow)	Słodkowicz-Kowalska et al. (2007)
	1 pos. mute swan (*Cygnus olor*; microscopy, EIA)	Majewska et al. (2004d)	2.2% captive birds (white stork, black-crowned crane; microscopy, FISH)	
	Out of 84 samples from 10 species of wild birds (microscopy, EIA or FISH, PCR)		0% in 84 samples from 10 species of wild birds (microscopy, EIA or FISH, PCR)	Majewska et al. (2004d)
Nature Reserve "Ujście Warty"	1 of 51 samples from dun crow (*Corvus corone cornix*; microscopy, EIA)	Jędrzejewski et al. (2004)	1 of 51 samples from dun crow (*C. corone cornix*; FISH)	Jędrzejewski et al. (2004)
Aquatic birds in Wielkopolska				
Anser fabalis	5/192 (2.6%)	Majewska et al. (2001a)		
Anas platyrhynchos	13/200 (6.5%)			
Fulica atra	1/47 (2.1%)			
C. olor	3/19 (15.8%; IFA, PCR, microscopy)			

confirmed only in one case through a single report from the city of Poznan (Table 4; Sulima et al. 2001). In contrast, no evidence of *Giardia* spp. has been found in the same set of 12 tap water samples.

Recent monitoring of the contamination of food with parasite transmission stages revealed the presence of *Cryptosporidium* in a range of fresh food products, including vegetables, fruits and herbs (Table 4; Jędrzejewski and Majewska 2007). The presence of *Giardia* was confirmed in only 2 units of berries (10%; Jędrzejewski and Majewska 2007).

Humans

Human cases of *Cryptosporidium* spp. and *Giardia* spp. are registered in Poland, but no cases of human cryptosporidiosis can be found in reports of the National Institute of Hygiene (PZH- Państwowy Instytut Hygieny), including those of the last 2 years (Table 5). The average annual number of registered giardiosis cases is low and probably a significant underestimate (around 3,000 annually) and in terms of comparison with other notifiable diseases about half of the registered number of cases of borreliosis for 2006 and 2007. Diagnosis of giardiosis is routinely performed in clinical laboratories, but often *Cryptosporidium* infections remain undiagnosed.

More reliable data on the prevalence of these intestinal protozoa in humans in Poland are available through scientific research projects. *Cryptosporidium* spp. infection was discovered for the first time in Poland in children in 1986 (Siński et al. 1988). Using the Ziehl-Neelsen staining method, Siński et al (1988) detected *Cryptosporidium* spp. in 2.5% of children with diarrhoea. Both intestinal protozoa were identified in different groups of humans, but prevalence depended on their immunological status (Table 5). No *Cryptosporidium* infections were found in healthy immunocompetent adults (Table 5) even when studies incorporated high-risk groups of subjects (cattle farm workers, stable personnel). Interestingly, the parasite was detected in one horse rider frequenting a stable where infected horses were also identified (Majewska et al. 1999b). No *Cryptosporidium* infections have been found in healthy children (Majewska et al. 2004a). However, *Giardia* infections have been identified in 1–8.8% of healthy children and in 3.1–6.5% of healthy adults (Table 5; Okulewicz et al. 1998; Stelmaszyk and Owsikowski 2001; Stelmaszyk et al. 2001; Solarczyk and Majewska 2007b). Four isolates of *Giardia* of human origin were genotyped as *G. intestinalis* Assemblage B (zoonotic; Solarczyk and Majewska 2007b).

In a group of immunocompetent individuals with diarrhoea, the infection rates for *Cryptosporidium* infection was much higher. Parasites were identified in 5.7% of

Table 3 Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in pets and captive animals

Host species	*Cryptosporidium* spp.		*Giardia* spp.	
	% infected (method)	Reference	% infected (method)	Reference
Dogs	27.4% (EIA)	Gundłach et al. (2004)	53.5% (EIA)	Gundłach et al. (2005)
			6.5% (flotation/decantation)	Gundłach et al. (2004)
			26.2% (EIA)	
Dogs from animal shelter in Poznań and private owners	1.2% in 326 dogs (microscopy, EIA, IFA)	Majewska et al. (2001c)	9.2% in 326 dogs (microscopy, EIA, IFA)	Majewska et al. (2001c)
			6.1% of dogs	Majewska et al. (2001a)
Dogs from private owners, Warsaw			5.14% (microscopy)	Zygner et al. (2006)
			9.14% (PCR)	
Sled dogs, World Championship Lubliniec 2004	12.5% (microscopy, IFA)	Bajer and Bednarska (2007)	36% (microscopy, IFA)	Bajer and Bednarska (2007)
Cats	1/6 cats (EIA)	Gundłach et al. (2004)		
Cats from animal shelter in Poznań and private owners	5% *Cryptosporidium felis* in 100 cats (microscopy, EIA, IFA)	Majewska et al. (2001c)	1% in 100 cats (microscopy, EIA, IFA)	Majewska et al. (2001c)
			1.3% cats	Majewska et al. (2001a)
	Cryptosporidium identified in reptiles from Zoo-shops (microscopy, EIA, PCR)	Majewska et al. (2001b)	*Giardia* identified in rodents from Zoo-shops (microscopy, EIA, PCR)	Majewska et al. (2001b)
Poznań ZOO	1 giraffe	Solarczyk and Majewska (2007b)	3 cactus mice (*Peromyscus eremicus*; microscopy)	Solarczyk and Majewska (2007b)
	1 gopher (*Cittelus cittelus*; microscopy)		1 tamandua (*Tamandua tetradactyla*)	
			1 giant toad (*Bufo marinus*)	
			1 silvery marmoset *(Callihtrix argentata*)	
	1 Mandarin duck	Słodkowicz-Kowalska et al. (2007)	1 lemur katta (*Lemur catta*)	Majewska et al. (2004c)
			1 gazelle (*Gazella thomsoni*)	
	0% in 83 samples from 34 species of birds from Zoo (microscopy, EIA or FISH, PCR)	Majewska et al. (2004d)	0% in 83 samples from 34 species of birds from Zoo (microscopy, EIA or FISH, PCR)	Majewska et al. (2004d)

Table 4 Distribution of *Cryptosporidium* spp. and *Giardia* spp. in environment

Environmental samples	*Cryptosporidium* spp.		*Giardia* spp.	
	% infected (method)	Reference	% infected (method)	Reference
Surface waters+rotifers	20 l of water from Kierskie Lake (+), Strzeszyńskie Lake (+)	Nowosad et al. (2007) Majewska et al. (2003)	Cysts in rotifers (FISH)	Majewska et al. (2003)
	Rusałka Lake (+) (EIA, IFA, FISH)	Majewska et al. (2004b)	1 pos. of 41 water samples, Poznan (flocculation)	Sulima et al. (2000)
Surface waters				
Tri-city	11 pos. of 22 surface water samples from 17 localities	Szostakowska et al. (2005)	2 pos. of 75 water samples, Poznań	Sulima et al. (2002)
	30.6% of 72 surface water samples from 17 water bodies (microscopy, IFA, FISH, PCR)	Szostakowska et al. (2006)	6.9% of 72 surface water samples from 17 water bodies, Tri-city (microscopy, IFA, FISH, PCR)	Szostakowska et al. (2006)
Poznań	11 pos./41 water samples (6 from 4 lakes, 2 from 2 clay-pits, 2 from a river, 1 from a pond; flocculation)	Sulima et al. (2000)		
	24% water samples of 75, Poznań	Sulima et al. (2002)		
Tap water in Poznań	1 pos. of 12 samples of 250 l each (microscopy, EIA, IFA, PCR)	Sulima et al. (2001)	0c% in 12 samples of 250 l each (microscopy, EIA, IFA, PCR)	Sulima et al. (2001)
Food	3 units of sprouts of 9 studied 4 units of lettuce of 16 2 units of herbs of 7 1 unit of fruit of 20 1 unit of vegetable of 18 (EIA, IFA)	Jędrzejewski and Majewska (2007)	10%=2 units of berries (EIA, IFA)	Jędrzejewski and Majewska (2007)

Table 5 Prevalence of *Cryptosporidium* spp. and *Giardia* spp. in humans

Groups	*Cryptosporidium* spp.		*Giardia* spp.	
	% infected (method)	Reference	% infected (method)	Reference
Registered cases per country (PZH data)	0 in 2006 0 in 2007	www.pzh.gov.pl/epimeld/2007	2,944 cases in 2006 3,093 cases in 2007	www.pzh.gov.pl/epimeld/2007
Immunocompetent children	0% in 8 children from diary farm (microscopy, EIA, PCR)	Majewska et al. (2004a)	1% in 621 schoolgirls and schoolboys in Western Pomerania region (microscopy)	Stelmaszyk and Owsikowski (2001)
			7.4–8.8% in 304 children (microscopy, EIA)	Okulewicz et al. (1998)
With diarrhoea	2.5% of 201 children (microscopy)	Siński et al. (1988)		
	5.7% in 123 infants (microscopy)	Śpiewak et al. (1998)		
	42.9% of 14 children (microscopy, IFA, PCR)	Bajer et al. (2008)		
	29.5% of 122 children (microscopy, IFA)	Gołąb et al. (2007)		
	6.6% of children with chronic diarrhoea (microscopy, EIA)	Wesołowska et al. (2004)	*G. intestinalis* identified in children with chronic diarrhoea (microscopy)	Wesołowska et al. (2004)
	0% in 16 children (microscopy, EIA)	Grzeszczuk and Kalinowska (1998)		
Immunocompetent adults	0% in 17 workers from diary farm (microscopy, EIA, PCR)	Majewska et al. (2004a)	6.5% of 400 soldiers in Szczecin (microscopy)	Stelmaszyk et al. (2001)
	0% in 12 farm workers (microscopy, EIA)	Kołodziejczyk et al. (2003)	3.1% in 131 adults (PCR)	Solarczyk and Majewska (2007b)
	0% in 5 stable personnel (microscopy, EIA, FISH)	Majewska et al. (2004e)		
	1 horse rider (of 3 persons in direct contact with horses)	Majewska et al. (1999b)		
With diarrhoea	0.5% in 2,134 samples of adults and children, Poznań (microscopy, EIA)	Werner et al. (2004)	0.4% in 2,134 samples of adults and children (microscopy, EIA)	Werner et al. (2004)
	1.5% in 538 samples of adults and children, Poznań (microscopy, EIA)	Werner et al. (2001)	0.7% in 538 samples of adults and children (microscopy, EIA)	Werner et al. (2001)
	7.9% in 89 patients in Białystok (microscopy, EIA)	Grzeszczuk and Kalinowska (1998)		
Primary immunodeficiencies (PID)	4 of 6 boys with hyper-IgM syndrome (5) and primary CD4 lymphopenia (1; microscopy, IFA, PCR)	Wolska-Kuśnierz et al. (2007)		
	29.4% of 17 children (microscopy, IFA, PCR)	Bajer et al. (2008)		
Secondary immunodeficiencies	11.5% in immunodeficient children (microscopy, EIA)	Wesołowska et al. (2004)		

Patients with HIV	12.5–28.6% in patients in Poznań (microscopy, EIA, PCR)	Majewska et al. (1998a, 1999a)
	11% in patients in Wrocław (microscopy, EIA, IFA, PCR)	Wesołowska—report on-line; Wesołowska and Ditrich (2003)
	6.7% in patients in Białystok (microscopy, EIA)	Grzeszczuk and Kalinowska (1998)
	0.7% in 151 patients in Białystok	Wiercińska-Drapało et al. (1998)
Patients after transplantation	0 of 2 children after liver/kidney transplantation (microscopy, IFA, PCR)	Bajer et al. (2008)
	23.3% (7 of 30) children after allo-HPCT (microscopy)	Turkiewicz et al. (2006)
Patients with cancers	1 of 2 adults after chemotherapy (microscopy, IFA, PCR)	Bajer et al. (2008)
	36% of patients with colon cancer (EIA)	Kołodziejczyk et al. (2003)
	20.5% of children with cancer disease (microscopy, EIA, IFA, PCR)	Wesołowska—report on-line

infants and in 0% to 43% children with diarrhoea (Table 5). Only a few *Cryptosporidium* isolates were genotyped, and only *C. parvum* (previous genotype 2 or zoonotic) was identified in this group of patients (Bajer et al. 2008). *Giardia* infection has been detected also in children with chronic diarrhoea (Wesołowska et al. 2004). *Cryptosporidium* infections were less common among immunocompetent adults with diarrhoea (0.5–7.9%; Table 5). Low infection rates were found for *Giardia* in this group (0.4–0.7%; Werner et al. 2001, 2004).

Cryptosporidium infections have been reported to be common in patients with primary immunodeficiencies in Poland (Table 5; Wolska-Kuśnierz et al. 2007; Bajer et al. 2008). In some patients, disseminated infection involving the gall bladder, accompanied by sclerosing cholangitis, was observed, and the majority of these cases were long lasting despite the treatment that was administered. Three different *Cryptosporidium* species were detected in this group of patients—the most common being the zoonotic *C. parvum* genotype, a single case of *Cryptosporidium hominis* infection and a single case of *Cryptosporidium meleagridis* infection (Bajer et al. 2008). No evidence of *Giardia* invasion was found in these patients.

Cryptosporidium infections appear to be common also among patients with secondary immunodeficiencies (Table 5). The parasite has been found in immunodeficient children, including 23.3% of children after allogeneic haematopoietic progenitor cell transplantation and 20.5% of children with various types of cancer (Wesołowska et al. 2004, report on-line 2006; Turkiewicz et al. 2006). Also, in this group of children, disseminated infections were noted, because jaundice was common among these patients (Turkiewicz et al. 2006). *Cryptosporidium* infections have been found in 0.7% to 28.6% of HIV-infected patients (Grzeszczuk and Kalinowska 1998; Wiercińska-Drapało et al. 1998; Wesołowska et al. 2006, on-line; Majewska et al. 1998a, 1999a) and in a large proportion of patients with cancer (Table 5; Kołodziejczyk et al. 2003; Bajer et al. 2008). To date, few *Cryptosporidium* isolates from this group of patients have been genotyped, but from those that have been examined closely, so far, only *C. parvum* has been identified (Bajer et al. 2008).

Methods of detection of infection

The method for detection that still dominates in the diagnosis of intestinal protozoa in Poland is conventional microscopy and diagnosis by recognition of the morphological features of cysts/oocysts, especially in samples of human or animal origin (Tables 1, 2, 3, 4 and 5). The most common methods are based on Ziehl-Neelsen staining for the confirmation of *Cryptosporidium* and Giemsa stain for identification of

Giardia cysts. However, recently commercial enzyme-linked immunoassays (EIA) have been used more frequently for the detection of *Cryptosporidium*/*Giardia* coproantigens in human and animal faecal samples, in both cases with increased sensitivity of detection. Other sensitive immunoassay have been employed but mostly confined to scientific projects rather than routine testing, e.g. the commercial immunofluorescent assay (IFA), i.e. MeriFluor Cryptosporidium/Giardia (Meridian Diagnostics, Cincinnati, OH, USA). Additionally, in recent years, an interesting trend, evident in increasing numbers of reports, has been the concurrent use of more than one sensitive method for the identification of parasites (Tables 1, 2, 3, 4 and 5). Polymerase chain reaction (PCR) is usually used in order to define parasite species/genotypes, but not many data are available up to date in Poland. The common problems in using this techniques are connected with DNA extraction from faecal and environmental samples, probably due to low intensity of infection in naturally infected hosts/environmental samples (Paziewska et al. 2006, 2007).

For the detection of viable (infective) protozoan cysts/oocysts in environmental samples, such as water, one method has been applied predominantly—FISH (Table 4). FISH, using small-subunit (SSU) ribosomal RNA (rRNA) probes, was developed to mark the presence of rRNA in cysts or oocysts, assuming that rRNA is degraded upon cell death and will not be found in dead cysts/oocysts (Vesey et al. 1998). However, Smith et al. (2004) have recently stated that during the transitional state when oocysts no longer transcribe SSU rRNA but previously transcribed copies had not yet degraded below the threshold of detection, FISH was not capable of determining *Cryptosporidium* oocysts viability accurately. However, neither PCR nor FISH method can be routinely applied in every diagnostic laboratory because of the costs involved and the need for skilled, qualified personnel and relevant technical equipment.

Very interesting results have been published recently, comparing the efficiency of standardised Method 1623 (recommended by the U.S. Environmental Protection Agency [USEPA]) applied to water samples with the FISH technique for *Cryptosporidium*/*Giardia* in rotifers (Nowosad et al. 2007). The FISH technique enabled easier and more sensitive detection of *C. parvum* oocysts in planktonic rotifers than Method 1623 in surface waters. Thus, rotifers provide very good bio-indicators of water contamination with cysts/oocysts.

The genotyping of isolates of parasites is still only sporadically applied in Poland, but the number of research reports in this field is increasing gradually. Recognition of parasite species/genotypes is crucial for a better understanding of transmission routes and the risk hazards to public health.

Discussion and conclusions

This review summarises studies carried out in Poland on the epidemiology of two opportunistic parasites that have the potential to cause severe disease in humans. The prevalence of each parasite has been compared among different groups of humans, species of wildlife, pets and farm animals and in environmental samples, since both species have transmission stages (oocysts and cysts) that once shed with host faeces and are freely distributed in environment and may be ingested by mammals to continue the infection cycle. The distribution of *Giardia* spp. infections in humans in Poland (1–9% in children, 3–7% in adults) was similar to that noted in other European countries (Giangaspero et al. 2007), but *Cryptosporidium* infections were found in surprisingly high numbers of patients among the high-risk groups. Although rare in immunocompetent individuals, this parasite was common in patients with diarrhoea (up to 43%) or with primary or secondary immunodeficiencies (23–36%; Bajer et al. 2008; Wolska-Kuśnierz et al. 2007; Turkiewicz et al. 2006; Kołodziejczyk et al. 2003; Majewska et al. 1998a). However, in comparison to other European surveys (Leoni et al. 2006; Semenza and Nichols 2007), only limited numbers of patients were involved in the Polish studies (around 3,000 individuals). This limited sample warrants some caution but emphasises also the need for further investigations on the local prevalence of this parasite in human communities and among people who are at special risk of infection.

Comparable high rates of *Cryptosporidium* infection in immunocompetent children with chronic diarrhoea were confirmed recently in other countries in various parts of the world (Ajjampur et al. 2007; Llorente et al. 2007; ten Hove et al. 2007; Sanad and Al-Malki 2007). High prevalence of *Cryptosporidium* infection in individuals with primary and secondary immunodeficiencies has been recorded elsewhere, exceeding 82% in some cases, notably among patients with AIDS and subjects with chronic diarrhoea (Winkelstein et al. 2003; Sanad and Al-Malki 2007).

However, to date, only a few *Cryptosporidium* isolates of human origin have been genotyped. Amplification and sequencing of the COWP and beta-tubulin gene fragments have revealed the presence of three *Cryptosporidium* species in humans in Poland (Bajer et al. 2008). The most common is *C. parvum*, which was detected in diarrhoeic children and adults. In contrast, other studies in immunocompetent children have shown the most common species (>80%) to be *C. hominis* (Gatel et al. 2006; Ajjampur et al. 2007). In children with different immunodeficiencies, *C. parvum* has been detected in two cases, and *C. hominis* and *C. meleagridis* were identified in the remaining two cases (Bajer et al. 2008; Wolska-Kuśnierz et al. 2007). In studies in other parts of the world, up to eight different *Crypto-*

sporidium species/genotypes have been identified in immunocompetent and immunodeficient children, including the mouse and cervine genotypes of *C. parvum* as well as *C. meleagridis*, *C. felis*, *Cryptosporidium canis* and *C. muris* (Pedraza-Diaz et al. 2001b; Xiao et al. 2001; Soba et al. 2006; Ajjampur et al. 2007; Gatel et al. 2006; Llorente et al. 2007). Clearly, the range of species/genotypes isolated so far from subjects in Poland may not yet represent the full range, and, therefore, there is a need for more extensive monitoring and genotyping to achieve a better understanding of the transmission routes for *Cryptosporidium* in Poland. Thus far, no outbreaks of waterborne or foodborne cryptosporidiosis have been recorded in Poland, but this may simply be due to the poor monitoring at a national level.

The unexpectedly high prevalence rates of *Cryptosporidium* infections in humans have been complemented by numerous records of infections detected in a range of reservoir hosts in Poland. The significant role of livestock (i.e. cattle, sheep and horses) was confirmed through the high prevalence of *Cryptosporidium* in these hosts, especially in calves. *C. parvum* was identified in these hosts (Solarczyk and Majewska 2007b; Bajer et al. 2005) and was also the most common species found in humans in Poland (Bajer et al. 2008). The high prevalence in calves was not unexpected, since comparable high prevalence rates have been reported in these hosts in many countries worldwide (de Graaf et al. 1999; Anderson 1998).

Cryptosporidium spp. infections have also been studied in a range of wildlife. Oocysts were detected in 12 of 13 mammalian species, and in three dipteran families and in many different species of birds (Table 2). In some of these species, the prevalence and abundance of *Cryptosporidium* spp. were high (e.g. rodents, wolves, European bison); in others, low (roe and red deer); but nevertheless, the potential risk of environment contamination with oocysts by dispersal of these stages is considered to be high in Poland. The role of birds and dipteran flies as vectors of viable oocysts has been reliably confirmed using the FISH technique (Graczyk et al. 2004, 2008; Szostakowska et al. 2004). *Cryptosporidium* infections were identified more often in domestic dogs than in cats (27 versus 5%), but the risk of human infections from pets cannot be evaluated from the currently published studies because the species/genotypes were not analysed. Although *Cryptosporidium* isolates from wild animals have been genotyped in few studies only, genotypes of public health significance have been identified in wolves (e.g. zoonotic *C. parvum*; previous genotype 2) and rodents (*C. parvum* mouse genotype in rodents; Paziewska et al. 2006, 2007; Bajer et al. 2003).

Complementing the studies in people and wildlife, few studies have been carried out on the distribution of *Cryptosporidium* spp. in the environment in Poland. These have revealed widespread contamination of surface waters with parasite oocysts (Nowosad et al. 2007; Szostakowska et al. 2005; Sulima et al. 2000). However, to date, only one positive sample of tap water has been reported, and no waterborne cryptosporidiosis cases have been identified in Poland.

Summing up, the wide distribution of *Cryptosporidium* among reservoir hosts, surface waters and among humans from risk groups indicates that risk of infection with this parasite in Poland has to be assumed as high. It is highly probable that the number of registered cases of cryptosporidiosis is grossly underestimated probably because of insufficient knowledge and awareness among physicians about the clinical manifestations of cryptosporidiosis and/or inappropriate diagnostic methods applied in clinical diagnosis.

In contrast to *Cryptosporidium*, *Giardia* infections were not common in diarrhoeic patients and were only sporadically recognised in individuals with primary or secondary immunodeficiencies. Likewise, the parasite was not common in cattle, sheep and poultry (1–2.2%), although there is a single report of 14% prevalence in calves (Bednarska et al. 1998). However, *Giardia* spp. infections were common in wildlife, parasites being found in 12 of 13 mammalian species that have been studied, in three dipteran families (1.4–7%) and in many species of birds (2–8%). In several species of mammals, prevalence and abundance were very high (rodents, wolves, European bisons); in others, much lower (game species). However, as with *Cryptosporidium*, the available data clearly indicate that the potential risk of environment contamination with the dispersal stages (cysts) has to be considered as high in Poland. The role of birds and flies as vectors of viable cysts was confirmed using FISH technique (Graczyk et al. 2008; Szostakowska et al. 2004). *Giardia* spp. infections were more common in domestic dogs than cats (5–54% versus 1%). Evidence for environmental contamination comes from studies reporting *Giardia* spp. cysts in surface waters and also on fresh fruits (Szostakowska et al. 2005; Jędrzejewski and Majewska 2007) but not yet in tap water, and so far, water- or foodborne outbreaks of giardiosis are not notified in Poland.

Nevertheless, the true importance of these infections in the context of public health cannot be evaluated reliably because very few samples have been subjected to molecular analysis, and hence, data on parasite species/genotypes involved in animal infections and in environmental samples are still extremely limited. Interestingly, despite the limited numbers of *Giardia* isolates from mammals that have been genotyped so far, genotypes of public health significance have already been identified (i.e. *G. intestinalis* Assemblages A or B in: dogs, *Artiodactyla* [red deer, Thomson's gazelle, sheep] and rodents; Solarczyk and Majewska 2007a, b; Zygner et al. 2006; Bajer unpublished). *G. intestinalis* Assemblage B has been identified also in four isolates of human origin in Poland (Solarczyk and

Majewska 2007b). A number of non-zoonotic species/genotypes of *Giardia* have been reported also, particularly in dogs (*G. intestinalis* Assemblages C and D) and rodents (*Giardia microti*, *G. intestinalis* Assemblage G; Caccio unpublished; Zygner et al. 2006).

Summing up, the high prevalence of *Giardia* spp. in reservoir hosts, surface waters and fresh fruits indicates that there is a risk of infection for humans in Poland. As with cryptosporidiosis, the number of registered giardiosis cases (around 3,000 clinical cases per year per 38 million population) is probably highly underestimated because screening studies have shown that infection rates of 1–9% are not uncommon in healthy individuals in the country (Okulewicz et al. 1998; Stelmaszyk et al. 2001; Stelmaszyk and Owsikowski 2001).

As elsewhere, there is an extensive range of genotypes that comprise the *Giardia* species complex circulating in the urban, rural and wild environments in Poland, and sorting out which pose the greatest threat to public health and analysis of their respective epidemiologies are tasks that have only just begun to be tackled. There is still a long road ahead and clearly a major need for more extensive monitoring, surveying and genotyping of the different parasite isolates.

Finally, from the evidence reviewed above covering infections in people, domestic and wild animals and samples from the environment, it is evident that *Cryptosporidium* or *Giardia* infection is currently widely distributed in Poland, but there is still a lot to do, and it will only be possible to evaluate the risk of infection comprehensively when far more samples are genotyped. This is a prerequisite for better understanding of the role of the different host species and parasites genotypes in the context of transmission of disease to human communities and among domestic livestock.

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