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Invited Review

Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission

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Abstract

The molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* is essential for accurately identifying organisms and assessing zoonotic transmission. Results of recent molecular epidemiological studies strongly suggest that zoonotic transmission plays an important role in cryptosporidiosis epidemiology. In such cases the most prevalent zoonotic species is *Cryptosporidium parvum*. Genotyping and subtyping data suggest that zoonotic transmission is not as prevalent in the epidemiology of giardiasis. Molecular characterisation of *Cryptosporidium* and *Giardia* is a relatively recent application that is evolving as new genes are found that increase the accuracy of identification while discovering a greater diversity of species and yet unnamed taxa within these two important genera. As molecular data accumulate, our understanding of the role of zoonotic transmission in epidemiology and clinical manifestations is becoming clearer.
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1. Introduction

Within the genera *Cryptosporidium* and *Giardia* are multiple species and genotypes that infect humans, domesticated livestock, companion animals and wildlife worldwide. These species and genotypes exhibit a wide range of biological diversity, each differing in their ability to infect one or multiple host species and each varying in prevalence of infection between and within countries. Hosts of all ages are affected, but generally the young are infected more frequently than adults. Age-specific preferences for some species of *Cryptosporidium* have been observed in animals. Clinical signs vary depending on the age and health of the infected host and the genetic background, condition and infective dose of the parasite. Much of the scientific literature describing host range, prevalence and clinical signs has identified *Cryptosporidium* oocysts and *Giardia* cysts based on microscopic observations that rely on very limited morphological differences. To protect public health and animal well-being, recent efforts have focused on more precisely identifying each organism associated with infections in humans and animals based on genes found in those organisms. As genetic data are accumulating in scientific literature and digital media, we are greatly improving our understanding of the complex relationship between humans and animals as hosts and reservoirs for these parasites.

Cryptosporidium, an apicomplexan protist, is reported to infect persons in 106 countries (Fayer, 2008). *Giardia*, a binucleate, flagellated, facultative anaerobic protist, similarly widespread, is the most common intestinal parasite of persons in developed countries and approximately 200 million people in Asia, Africa and Latin America have symptomatic infections with about 50,000 cases reported

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each year (Yason and Rivera, 2007). Estimates of prevalence for both of these parasites vary greatly because reporting is not universally required, diagnostic methods vary greatly, and many persons have no access to medical care or do not seek it. For cryptosporidiosis, in the USA 3,505 cases were reported in 2003, 3,911 in 2004 and 8,269 in 2005 (Yoder and Beach, 2007). The large increase in reporting in 2005 was primarily attributed to a single recreational water-associated outbreak. For giardiasis in the USA, voluntary reporting to the Centers for Disease Control and Prevention (CDC) surveillance systems from 1998 through 2003 indicated that the total number of cases varied between 24,226 and 20,075 annually (Hlavsa et al., 2005; Yoder and Beach, 2007). The greatest number of cases of both cryptosporidiosis and giardiasis was reported for children 1–9 years of age and for adults 30–39 years of age (Hlavsa et al., 2005; Yoder and Beach, 2007). Also, for both parasites a seasonal peak in age-related cases coincided with the summer recreational water season, possibly reflecting increased use of rivers, lakes, swimming pools and water parks (Hlavsa et al., 2005; Yoder and Beach, 2007).

2. Life cycles

Cryptosporidium completes its life cycle in a single host with stages similar to those of the coccidian genera *Eimeria* and *Isospora*. Oocysts containing four infectious sporozoites are excreted in faeces. Oocysts of some species such as *Cryptosporidium parvum* can remain infectious in cool, wet conditions for 6 months or longer (Fayer, 2008). After oocysts are ingested in contaminated water or food, from fomites, or from direct contact with infected persons or animals, sporozoites are released in the small intestine and invade epithelial cells. All subsequent endogenous stages are intracellular but extracytoplasmic, appearing to rest on the surface of villar epithelial cells. Two asexual cycles each produce four to eight merozoites. Second stage merozoites develop into male or female sexual stages and fertilisation results in oocyst formation. Some oocysts might auto-infect but most, if not all, oocysts are excreted in faeces.

A report of the in vitro cultivation of *Cryptosporidium andersoni* through its entire life cycle included the description of a previously unrecognised extracellular stage (Hijjawi et al., 2002). This stage, also isolated from faeces of cattle, appeared to undergo syzygy, and the ssrRNA gene from this stage confirmed it to be *C. andersoni* (Hijjawi et al., 2002). Similar extracellular stages, observed in in vitro cultures infected with *C. parvum* from cattle and from mice, led to the conclusion that these novel life cycle stages confirmed the relationship of *Cryptosporidium* to gregarines (Hijjawi et al., 2002). Hijjawi et al. (2004) later reported continuous development of *C. parvum* in cell-free medium through all life cycle stages. Attempts to obtain the developmental stages reported by Hijjawi et al. (2004) using a similar serum-free culture system were unsuccessful (Girouard et al., 2006). However, Rosales et al. (2005) reported finding similar stages in cell cultures. More recently, Woods and Upton (2007) indicated that several of the photomicrographs of developmental stages appeared to be budding yeasts (as in Hijjawi et al., 2004, Figs. 1a–c and 2d; Rosales et al., 2005, Fig. 1), host cells or other contaminating debris (as in Hijjawi et al., 2004, Figs. 2a–c, 3a–j and 4a, b, d), and fungal conidia (as in Hijjawi et al., 2004, Fig. 4c). Therefore, until an independent laboratory confirms that *Cryptosporidium* can develop extracellularly and that the forms reported as gregarine-like stages are truly stages of *Cryptosporidium*, members of this genus should still be considered obligate intracellular parasites.

Giardia has a simple and direct life cycle. Cysts excreted in faeces contain a mitotically arrested trophozoite that can remain infectious for months in a wet, cool environment. After cysts are ingested, trophozoites emerge in the duodenum and complete mitotic division. Infection in new hosts is established by ingestion of cysts and repeated divisions of trophozoites that attach to the surface of the intestinal microvilli beneath the mucus layer via a ventral adhesive disc. Cysts form in response to intestinal conditions such as the presence of major bile salts. They pass through the intestine in the faeces and are spread by contaminated water and food, and by physical contact.

3. Taxonomy and nomenclature

Cryptosporidium and *Giardia* are genera, each consisting of multiple species. The taxonomy of currently recognised species of both genera is summarised in Tables 1 and 2. Assemblages of *Giardia* and genotypes of *Cryptosporidium* have no taxonomic status but are helpful in identifying genetically unique organisms for which limited biological information is available. Some genotypes have been named as species when sufficient information regarding their morphology, biology and genetics became available.

The genus *Cryptosporidium* consists of 18 species (Table 1) and over 40 genotypes (Xiao and Ryan, 2008). As biological and molecular data increase, many of the genotypes are expected to be named as valid species. More than 150 mammalian hosts were reported to be infected with *C. parvum*, a *C. parvum*-like parasite or simply *Cryptosporidium* (Fayer, 2008) based on microscopic observations of oocysts in faecal specimens. Most will have to be re-examined using molecular methods to validate the species or determine the genotype. Although oocysts of *Cryptosporidium* from animals in one class of vertebrates generally have not been infectious for those in another class, there are exceptions. *Cryptosporidium meleagridis*, first reported to infect turkeys, has been detected in faeces from immunocompromised and healthy humans (Pedraza-Diaz et al., 2000; Guyot et al., 2001; Xiao et al., 2001; Gatei et al., 2003) and has been transmitted from an infected patient with diarrhoea to chickens, mice, piglets and calves (Akiyoshi et al., 2003). *Cryptosporidium muris* and *C. andersoni*, originally recognised as parasites of the stomach of mammals, have been detected in avian faeces from the wood partridge

Table 1
Species of *Cryptosporidium* (modified from Fayer, 2008)

Species	Author	Type host
Cryptosporidium andersoni	Lindsay et al. (2000)	*Bos taurus* (domestic cattle)
Cryptosporidium baileyi	Current et al. (1986)	*Gallus gallus* (chicken)
Cryptosporidium bovis	Fayer et al. (2005)	*Bos taurus* (domestic cattle)
Cryptosporidium canis	Fayer et al. (2001)	*Canis familiaris* (domestic dog)
Cryptosporidium fayeri	Ryan et al. (2008)	*Macropus rufus* (red kangaroo)
Cryptosporidium felis	Iseki (1979)	*Felis catis* (domestic cat)
Cryptosporidium galli	Pavlasek (1999)	*Gallus gallus* (chicken)
ryptosporidium. hominis	Morgan-Ryan et al. (2002)	*Homo sapiens* (human)
Cryptosporidium macropodum	Power and Ryan (2008)	*Macropus giganteus* (grey kangaroo)
Cryptosporidium meleagridis	Slavin (1955)	*Meleagris gallopavo* (turkey)
Cryptosporidium molnari	Alvarez-Pellitero and Sitja-Bobadilla (2002)	*Sparus aurata* (gilthead seabream) *Dicentrarchus labrax* (European seabass)
Cryptosporidium muris	Tyzzer (1910)	*Mus musculus* (house mouse)
Cryptosporidium parvum	Tyzzer (1912)	*Mus musculus* (house mouse)
Cryptosporidium scophthalmi	Alvarez-Pellitero et al. (2004)	*Scophthalmi maximus* (turbot)
Cryptosporidium serpentis	Levine (1980) (Brownstein et al., 1977)	*Elaphe guttata* (corn snake) *Elaphe subocularis* (rat snake) *Sanzinia madagascarensus* (Madagascar boa)
Cryptosporidium suis	Ryan et al. (2004)	*Sus scrofa* (domestic pig)
Cryptosporidium varanii	Pavlasek et al. (1995)	*Varanus prasinus* (Emerald monitor)
Cryptosporidium wrairi	Vetterling et al. (1971)	*Cavia porcellus* (guinea pig)

Table 2
Cryptosporidium spp. and genotypes that infect humans and other hosts (modified from Fayer, 2008)

Species	Hosts
Cryptosporidium andersoni	Cattle, sheep, Bactrian camel, gerbil, multimammate mouse, wood partridge
Cryptosporidium baileyi	Chicken, duck, Bobwhite quail
Cryptosporidium canis	Dog, fox, coyote
Cryptosporidium felis	Cat, cattle
Cryptosporidium hominis	Primates, cattle, sheep, pig, dugong
Cryptosporidium meleagridis	Turkey, chicken, Bobwhite quail, dog, deer mouse
Cryptosporidium muris	Mouse, hamster, squirrel, Siberian chipmunk, wood mouse, bank vole, rock hyrax, Bactrian camel, mountain goat, cat, coyote, ringed seal, bilby, cynomolgus monkey, tawny frogmouth
Cryptosporidium parvum	Calf, lamb, horse, alpaca, dog, mouse, raccoon dog, eastern squirrel
Cryptosporidium suis	Pig, cattle
Cervine genotype	Cattle, sheep, ibex grey squirrel, eastern chipmunk, beaver, red squirrel, woodchuck, deer mouse, raccoon deer, mouflon sheep, blesbok, nyala, lemur
Skunk genotype	Skunk, raccoon, eastern squirrel, opossum, river otter

and tawny frogmouth, respectively (Ng et al., 2006), based on PCR. Whether the oocysts in the faeces were due to mechanical transport or an actual infection remains to be determined. *Cryptosporidium parvum*, the most studied species, was once thought to infect many, if not all, species of mammals. Under experimental conditions one study concluded that it infected chickens (Palkovic and Marousek, 1989), another found it did not infect chickens (Darabus and Olariu, 2003) and still another found it did not infect ducks or geese (Graczyk et al., 1996a, 1997). Other conflicting reports add to the confusion regarding the range of host species for *C. parvum*. One study indicated that *C. parvum* oocysts from a human infected fish, amphibians, reptiles, birds and mammals (Arcay et al., 1995) whereas another found that *C. parvum* oocysts from a bovine did not infect fish, amphibians or reptiles but simply passed through the digestive tracts (Graczyk et al., 1996b). Some species of *Cryptosporidium* appear restricted to one host species: *Cryptosporidium wrairi* apparently infects only guinea pigs (Gibson and Wagner, 1986). Other species of *Cryptosporidium* have been found to infect a predominant host species and to a much lesser extent other hosts including humans (Table 2).

The nomenclature for *Giardia* is confusing and needs clarity. The species *Giardia agilis*, *Giardia ardeae*, *Giardia muris*, *Giardia microti* and *Giardia psittaci* have been found to infect various animals but not humans (Table 3). The species names *Giardia duodenalis*, *Giardia intestinalis* and *Giardia lamblia* are all used interchangeably in current literature referring to the same organism. For purposes of consistency *G. duodenalis* is used in this review. Within this species the current trend has been to identify a complex of assemblages based on host specificity. These assemblages are identified based on the analysis of conserved genetic loci (Caccio et al., 2005). Currently, there are seven well defined assemblages of *G. duodenalis*, designated A through G. Assemblages A and B have the broadest host specificity, having been found to infect humans and various other mammals, including dogs, cats, livestock and wildlife (Karanis and Ey, 1998; Caccio et al., 2005). Assemblage A consists of mostly two subgroups AI and AII, and there is no

Table 3
Species and assemblages of *Giardia* (modified from Thompson, 2004)

Species	Author	Hosts
Giardia agilis	Hegner (1922)	Amphibians
Giardia ardeae	Noller (1920)	Birds
Giardia microti	Benson (1908)	Muskrats and voles
Giardia muris	Benson (1908)	Rodents
Giardia psittaci	Erlandsen and Bemrick (1987)	Birds
Giardia duodenalis	Davaine (1875)	
Assemblage A		Humans, primates, dogs, cats, cattle, rodents, wild mammals
Assemblage B		Humans, primates, dogs, horses, cattle
Assemblage C		Dogs
Assemblage D		Dogs
Assemblage E		Artiodactyls
Assemblage F		Cats
Assemblage G		Rodents

clear subgrouping in assemblage B (Monis et al., 1999, 2003; Thompson, 2000; Sulaiman et al., 2003; Read et al., 2004; Wielinga and Thompson, 2007). Assemblages C and D have been found to infect only dogs (Hopkins et al., 1997; Monis et al., 1998; Leonhard et al., 2007). Assemblage E has been found to infect only cloven-hooved mammals (Ey et al., 1997). Assemblages F and G have been found to infect only cats and rodents, respectively (Monis et al., 1999).

4. Host specificity of *Cryptosporidium* and *Giardia* species and genotypes in animals

With some exceptions, most *Cryptosporidium* and *Giardia* species and genotypes are host-adapted in nature, having a narrow spectrum of natural hosts. Thus, one species or genotype usually infects only a particular species or group of related animals. Recognizing the zoonotic potential of each species is based upon knowing the host range or host specificity of that species. This can be determined in part by: (i) accurately identifying the species/genotype from the oocyst/cyst in the faeces of naturally infected hosts using molecular methods and (ii) obtaining oocysts or cysts from one host species and feeding those to putative hosts of another species. When feeding these stages results in completion of the life cycle in the putative host and de novo stages are excreted that are genetically identical to those that initiated the infection, the confirmed host range is extended. However, the inability to obtain sufficient numbers of oocysts or cysts and difficulty in obtaining and/or housing wildlife, or scarce or expensive domesticated animals, limits testing. Both (i) and (ii) rely on molecular methods to clearly identify the species because morphological methods lack the specificity required to distinguish many species and genotypes. For example, oocysts of *C. parvum*, *Cryptosporidium hominis*, *C. meleagridis* and *Cryptosporidium bovis* have no apparent internal features that are unique among them and they overlap in size, differing only at the extremes by a few tenths of a micrometre. The oocyst size ranges for each species are 4.8–6.0 × 4.8–5.4 μm, 4.4–5.9 × 4.4–5.4 μm, 4.5–6.0 × 4.2–5.3 μm and 4.8–5.4 × 4.2–4.8 μm, respectively (Fayer et al., 2005; Fayer, 2008). Likewise, cysts of the assemblages of *G. duodenalis* are difficult or impossible to distinguish from one another by microscopy (Filice, 1952).

Confirmation of infection is complicated when only one or two faecal specimens serve as the source of oocysts or cysts because it can be argued that these stages were ingested and simply passed through the gut without actually infecting the host. This has been demonstrated experimentally (Graczyk et al., 1996a). For humans, even when it appears conclusive that there is an infection with a potentially zoonotic species or genotype one might argue that the infection was acquired from a human source rather than an animal and therefore transmission is anthroponotic not zoonotic. Because the two foregoing issues cannot always be resolved by investigators we are even less able to resolve these issues by review of their publications, therefore, interpretation of some of the examples presented as zoonotic infections in this review will fall in part to the judgment of the reader.

4.1. *Cryptosporidium*

Surveys conducted in cattle, sheep, pigs, cats, dogs, kangaroos, squirrels, other wild mammals, Canada geese and reptiles have shown that most animal species are infected with only a few host-adapted *Cryptosporidium* species or genotypes (Iseki, 1979; Asahi et al., 1991; Guselle et al., 2003; Jellison et al., 2004; Power et al., 2004; Ryan et al., 2004, 2005; Xiao et al., 2004c; Zhou et al., 2004a,b; Fayer et al., 2006c; Feng et al., 2007a; Langkjaer et al., 2007). The existence of host-adapted *Cryptosporidium* species or genotypes indicates that cross transmission of *Cryptosporidium* among different groups of animals is usually limited. Cross-species transmission is possible when animals share a similar habitat and/or the parasite is biologically capable of infecting multiple host species. Two genotypes that appear to fit this description are the skunk genotype and the cervine genotype. The skunk genotype has been detected in faeces of skunks, raccoons, squirrels and opossums (Feng et al., 2007a). The cervine genotype has been detected in faeces of domestic and wild ruminants (domesticated sheep, mouflon sheep, blesbok, nyala and deer), rodents (squirrels, chipmunks, woodchucks, beavers and deer mice), carnivores (raccoons) and primates (lemurs and humans) (Perz and Le Blancq, 2001; Ong et al., 2002; da Silva et al., 2003; Ryan et al., 2003; Ryan et al., 2005; Blackburn et al., 2006; Feltus et al., 2006; Leoni et al., 2006; Nichols et al., 2006; Soba et al., 2006; Trotz-Williams et al., 2006; Feng et al., 2007a).

Cryptosporidium parvum has received the most attention with regard to cross-species transmission. Although *C. parvum* was once thought to infect all mammals, genetic characterisation of *Cryptosporidium* specimens have mostly failed to detect this species in wild mammals (Zhou et al., 2004a; Feng et al., 2007a). It is now generally accepted that *C. parvum* (previously referred to as genotype II or the bovine genotype) primarily infects ruminants and humans. Even in cattle, only calves less than 2 months of age (primarily monogastric) are frequently infected with this species. The prevalence in beef calves is often lower than in dairy calves, even when raised in similar conditions (Kvac et al., 2006). Most infections in older dairy calves are caused by *C. bovis* and the deer-like genotype, and infections in mature cattle are mostly with *C. andersoni* (Santin et al., 2004; Fayer et al., 2006c; Feng et al., 2007b; Langkjaer et al., 2007). Therefore, the major contributors of zoonotic *C. parvum* appear to be dairy calves less than 2 months of age. *Cryptosporidium parvum* has only been detected in small numbers in other farm animals. Two recent studies in Australia and the USA report that oocysts of *C. parvum* are not commonly detected in sheep faeces, which more often contain oocysts of the *Cryptosporidium* cervine genotype and other genotypes (Ryan et al., 2005; Santin et al., 2007). Although *C. parvum* has been detected in a few horses and alpacas, its prevalence is not known (Grinberg et al., 2003; Hajdusek et al., 2004; Chalmers et al., 2005b; Starkey et al., 2007; Twomey et al., 2008) and horses are known to be infected with a *Cryptosporidium* horse genotype (Ryan et al., 2003). Natural *C. parvum* infections have been found occasionally in other animals such as mice, raccoon dogs and dogs (Morgan et al., 1999; Matsubayashi et al., 2004; Giangaspero et al., 2006).

Companion animals are most often infected with host-specific *Cryptosporidium* spp. Thus, dogs are almost exclusively infected with *Cryptosporidium canis* (Morgan et al., 2000; Satoh et al., 2006; Huber et al., 2007; Rimhanen-Finne et al., 2007) and most cats are infected with *Cryptosporidium felis*, although *C. muris* was found in faeces from two cats (Fayer et al., 2006a; Pavlasek and Ryan, 2007; Santin et al., 2006; Rimhanen-Finne et al., 2007). Because *C. canis* and *C. felis* infections are infrequently reported for humans, despite close and widespread contact with dogs and cats, the role of dogs and cats in the transmission of human cryptosporidiosis appears quite limited.

4.2. Giardia

Of all the species of *Giardia* only *G. duodenalis* has been found in humans, livestock and companion animals. Most of the animals in these groups have unique *G. duodenalis* assemblages, but some have been found to harbour assemblages A and/or B, which also infect humans. Most cattle, sheep and pigs are infected with *G. duodenalis* assemblage E (Table 4). A significant number of cattle, however, are also infected with assemblage A. In contrast, assemblage B was found in a small number of cattle in a few studies, and other assemblages have never been convincingly found in cattle (Table 4). The only exception is in New Zealand, where limited studies indicate that assemblages A and B appeared to be common in cattle and assemblage E was largely absent (Hunt et al., 2000; Learmonth et al., 2003). There are no age-associated differences in the distribution of assemblages A and E in cattle. Four multi-state prevalence studies for *G. duodenalis* in the eastern USA involving nearly 2,000 cattle from birth to adulthood reported that assemblage E was found in 28% of preweaned calves, 45% of post-weaned calves, 33% of heifers and 25% of adult cows, whereas assemblage A was detected in 6% of preweaned calves, 7% of post-weaned calves, 3% of heifers and 2% of adult cows (Trout et al., 2004, 2005, 2006b, 2007).

For sheep, studies have all identified a predominance of assemblage E in both lambs and ewes, with assemblage A identified infrequently (Table 4). Assemblage B has rarely been found in sheep. Although an outbreak of giardiasis in lambs with severe weight loss and some mortality was attributed to assemblage B, this conclusion was based on PCR analysis of only two specimens (Aloisio et al., 2006). Results of a study of pigs indicated a pattern similar to that of cattle and sheep (Langkjaer et al., 2007). In the only survey conducted of horses, albeit just nine animals, only assemblages A and B were found (Traub et al., 2005).

Dogs are infected with a broader range of *G. duodenalis* assemblages. Dogs in Australia were found to be infected with assemblages A, B, C and D (Monis et al., 1998). The same assemblages have been found in dogs in other countries with a dominance of assemblages C and D (Table 5). Many studies have also shown modest to high prevalence of assemblage A (Table 5). In contrast, infection with assemblage B has rarely been found in dogs (Read et al., 2004; Traub et al., 2004).

Cats are infected with assemblages A and F, with the cat-specific assemblage F found more frequently than assemblage A (Table 5). Although one study identified almost all known assemblages (A, B, C, D and E) except F in cats (Read et al., 2004) there was a high level of discrepancy in genotyping results of animal samples between the two genotyping tools used in the study. Assemblage A was found in a ferret in a pet shop (Abe et al., 2005).

Little information is available on the prevalence of *G. duodenalis* assemblages infecting wildlife. Limited reports showed mostly the presence of assemblage A and the lack of assemblage E in wild cervids. Two cases of giardiasis in a white-tailed deer in the USA and a roe deer in the Netherlands were identified as assemblage A (Trout et al., 2003; van der Giessen et al., 2006). Likewise, only assemblage A was found in 15 moose and nine reindeer in Norway (Robertson et al., 2007), eight fallow deer in Italy (Lalle et al., 2007), one fox and three kangaroos in Australia (McCarthy et al., 2008).

Assemblages B, C and D are found in other wild mammals. Of 15 positive faecal specimens from beavers and

Table 4
Giardia duodenalis genotypes in farm animals

Animal	Location	Sample size	Assemblage				Reference
			A	B	E	Others	
Cattle	Italy	24	12	5	3	A+B (2), A+E (2)	Lalle et al. (2005b)
Cattle	Portugal	14	2	1	11		Mendonca et al. (2007)
Cattle	Denmark	145	8		133	4 unknown	Langkjaer et al. (2007)
Cattle	Belgium	101	16		54	A+E (31)	Geurden et al. (2008a)
Cattle (preweaned calves)	USA	164	25		139		Trout et al. (2004)
Cattle (weaned calves)	USA	237	31		196		Trout et al. (2005)
Cattle	USA	58	8		48	A+E (2)	Feng et al. (in press)
Cattle	USA	7			7		Sulaiman et al. (2003)
Cattle (1–2 years)	USA	204	18		186		Trout et al. (2006b)
Cattle (cows)	USA	144	9		135		Trout et al. (2007)
Cattle	Canada	42	1		41		Appelbee et al. (2003)
Cattle	Canada	14	6		14		Uehlinger et al. (2006)
Cattle	Canada	60		35	25		Coklin et al. (2007)
Cattle	Brazil	5	1		4		Souza et al. (2007)
Cattle	Taiwan	4	2		2		Hsu et al. (2007)
Cattle	Japan	5	1		4		Itagaki et al. (2005)
Cattle (preweaned calves)	Vietnam	17	1		16		Geurden et al. (in press)
Cattle	Australia	16			16		Read et al. (2002)
Cattle (preweaned calves)	Australia	31			31		Becher et al. (2004)
Cattle	New Zealand	15	11	4			
Cattle	New Zealand	48	26	22			Learmonth et al. (2003)
Italian water buffalo	Italy	8	2		6		Caccio et al. (2007)
Sheep	Italy	5	5				Giangaspero et al. (2005)
Sheep	Spain	12		1	11		Castro-Hermida et al. (2007)
Sheep	USA	14	1		13		Santin et al. (2007)
Sheep	Mexico	14			14		Di Giovanni et al. (2006)
Sheep	Australia	46	11		33	2 unknown	Ryan et al. (2005)
Goat	Spain	39			39		Ruiz et al. (in press)
Alpaca	USA	3	3				Trout et al. (in press)
Pig	Denmark	82	10		65	C (2) and unknown (1)	Langkjaer et al. (2007)
Horse	USA and Australia	9	4	6			Traub et al. (2005)

Table 5
Giardia duodenalis genotypes in domestic pets

Animal	Location	Sample size	Assemblage						Reference
			A	B	C	D	F	Others	
Dog	Italy	17	2		11	1		A+C (2), C+D (1)	Berrilli et al. (2004)
Dog	Italy	21	6		1	12		A+D (1)	Lalle et al. (2005b)
Dog	Germany	55	33		5	2		A+C (15)	Leonhard et al. (2007)
Dog	Germany	150	4		54	83		C+D (8), A+D (1)	Barutzki et al. (2007)
Dog	Hungary	15			5	9		C+D (1)	Szenasi et al. (2007)
Dog	India	7	5	2					Traub et al. (2004)
Dog	Japan	4				4			Abe et al. (2003)
Dog	Japan	24	14		1	6		A+D (3)	Itagaki et al. (2005)
Dog	Thailand	13	5		1	3		A+B (3), A+D (1)	Inpankaew et al. (2007)
Dog	Australia	11			10	1			Monis et al. (1998, 2003)
Dog	Australia	9	1	2	4	2			Read et al. (2002)
Dog	USA	15			15				Sulaiman et al. (2003)
Dog	Mexico	5	4					A+B (1)	Lalle et al. (2005a)
Dog	Mexico	11	7					A4 (probably C or D) (4)	Eligio-Garcia et al. (2005)
Dog	Brazil	27			7	20			Souza et al. (2007)
Dog	Brazil	7	7						Volotao et al. (2007)
Cat	Italy	10	10						Papini et al. (2007)
Cat	USA	17	6				11		Vasilopulos et al. (2007)
Cat	USA	8 from 1 colony					8		Fayer et al. (2006a)
Cat	Brazil	19	8				11		Souza et al. (2007)
Cat	Columbia	3					3		Santin et al. (2006)
Cat	Japan	3					3		Itagaki et al. (2005)
Cat	Australia	18	6	2	2	7		E (1)	Read et al. (2004)

muskrats trapped in Maryland, USA, assemblage B was found in seven beavers and five muskrats, and *G. microti* was found in three other muskrats (Sulaiman et al., 2003). Of 62 beavers trapped in Massachusetts, USA, four had assemblage B (Fayer et al., 2006b). One rabbit was found infected with assemblage B (Sulaiman et al., 2003). Among seven coyotes in the USA positive for *G. duodenalis*, one had assemblage B, three had assemblage C, and three had assemblage D (Trout et al., 2006a). In contrast, assemblage A was found in two gorillas in Uganda (Graczyk et al., 2002) and assemblage B was found in three wild Japanese monkeys (*Macaca fuscata*) (Itagaki et al., 2005).

5. *Cryptosporidium* and *Giardia* species and genotypes in humans

5.1. Cryptosporidium

Cryptosporidium parvum was once considered the only *Cryptosporidium* species to infect humans. The use of genotyping tools in the late 1990s identified two genotypes (I and II) of *C. parvum*, which eventually became *C. hominis* and *C. parvum senso stricto*, both infectious for immunocompetent and immunocompromised persons (Xiao et al., 2004a,b; Caccio, 2005). Subsequently, the use of ssrRNA-based genotyping tools revealed the presence of several other species, including *C. canis*, *C. felis* and *C. meleagridis* in human faeces (Pieniazek et al., 1999; McLauchlin et al., 2000; Alves et al., 2001; Guyot et al., 2001; Xiao et al., 2001; Caccio et al., 2002a; Gatei et al., 2002b, 2006; Tiangtip and Jongwutiwes, 2002; Cama et al., 2003; Leoni et al., 2006). Still other species found in human faeces, but in fewer cases, include *C. muris* (Katsumata et al., 2000; Guyot et al., 2001; Gatei et al., 2002a, 2006; Palmer et al., 2003; Muthusamy et al., 2006) and *Cryptosporidium suis* (Xiao et al., 2002; Leoni et al., 2006; Nichols et al., 2006). In addition, several genotypes of *Cryptosporidium* have been detected in faeces from patients experiencing cryptosporidiosis including the cervine genotype (Ong et al., 2002; Blackburn et al., 2006; Feltus et al., 2006; Leoni et al., 2006; Nichols et al., 2006; Soba et al., 2006; Trotz-Williams et al., 2006), a *C. suis*-like genotype (Ong et al., 2002), a *C. andersoni*-like genotype (Leoni et al., 2006; Morse et al., 2007), the chipmunk genotype I (W17) (Feltus et al., 2006), the skunk genotype (Nichols et al., 2006), and the *C. hominis* monkey genotype (Mallon et al., 2003a). Because these genotypes were detected in microscopically positive faeces from patients it is improbable that these persons simply had their faeces examined around 49–63 h (mean gut transit time; Probert et al., 1995) after they ingested oocysts that transited the gastrointestinal tract.

Among the human-pathogenic *Cryptosporidium* spp., *C. hominis* and *C. parvum* are responsible for most human cases of infection (Xiao and Ryan, 2004). Geographic differences have been reported in disease burdens attributed to these two species. In the UK, several earlier large scale studies all found that *C. parvum* was responsible for slightly more infections than *C. hominis* (McLauchlin et al., 2000; Hunter et al., 2003; Smerdon et al., 2003; Sopwith et al., 2005; Leoni et al., 2006). A more recent study in the UK found that of 13,112 cases of cryptosporidiosis 50.3% were associated with *C. hominis* and 45.6% were associated with*C. parvum* (Nichols et al., 2006). Likewise, although earlier studies generally showed a higher prevalence of *C. parvum* than *C. hominis* in humans in other European countries, more recent studies have shown a slightly higher prevalence of *C. hominis* than *C. parvum*. Thus, in the Netherlands, 91 human isolates consisted of 70% *C. hominis*, 19% *C. parvum*, a 10% mixture of *C. hominis* and *C. parvum*, and 1% *C. felis* (Wielinga et al., 2008). In Spain, Llorente et al. (2007) detected *C. hominis* in 59 of 92 immunocompetent children and 10 of 16 HIV-infected adults (63.8%), and *C. parvum*, in 28 children and six adults (31.5%); *C. parvum* was significantly more frequent in children from rural areas. In general *C. hominis* has been associated with more infections than *C. parvum* in the USA, Canada, Australia, Japan and developing countries where molecular methods have been used to identify specimens (Peng et al., 1997; Morgan et al., 1998; Sulaiman et al., 1998; Ong et al., 1999, 2002; Xiao et al., 2004a) although in New Zealand 46.8% of 423 cases were associated with *C. hominis* and 52.7% were associated with *C. parvum* (Learmonth et al., 2004). A much higher prevalence of *C. parvum* is seen in humans in the Middle East. In highly urbanised Kuwait City, almost all cryptosporidiosis cases in children were caused by *C. parvum* (Sulaiman et al., 2005). In Iran seven of eight HIV patients and four of seven children had cryptosporidiosis caused by *C. parvum* and the others had *C. hominis* (Meamar et al., 2007). In Turkey, all four cases of cryptosporidiosis in children had *C. parvum* (Tamer et al., 2007).

5.2. Giardia

Of the established *Giardia* spp. and *G. duodenalis* assemblages, only *G. duodenalis* assemblages A and B are known to infect humans. Assemblage F was reported in humans in one study, but this was likely the result of technical errors (Gelanew et al., 2007). Because both assemblages (A and B) have also been found to infect animals, zoonotic transmission has long been suspected to play a role in epidemiology of human giardiasis. The distribution of these two assemblages in humans varies among studies, sometimes within the same country (see Caccio et al., 2005, for review). The number of molecular epidemiological studies of giardiasis in humans is very small, and do not demonstrate clear geographic or socioeconomic differences in the distribution of assemblages A and B, or clearly indicate the role of zoonotic infections in human giardiasis.

6. Molecular epidemiological evidence for zoonotic transmission of cryptosporidiosis and giardiasis

6.1. Cryptosporidium

Geographic differences in the distribution of *C. parvum* and *C. hominis* infections within a country support the likelihood of zoonotic cryptosporidiosis. This situation is observed in the USA, the UK and New Zealand where *C. hominis* infection is more common in urban areas and *C. parvum* is more common in rural areas (McLauchlin et al., 1999, 2000; Learmonth et al., 2004; Feltus et al., 2006).

In the UK, *C. hominis* infection was found more often in persons with a history of foreign travel (McLauchlin et al., 2000; Goh et al., 2004; Hunter et al., 2004, 2007). In an extensive analysis of human cases of cryptosporidiosis Sopwith et al. (2005) reported that from 1996 to 2000 elevated rates of infection in the North West Region of England (13% of the population) was responsible for 31–42% of all cases reported in England and Wales and might have skewed national data for the UK, especially the spring peak caused by *C. parvum*. The coincidental timing of a foot-and-mouth epidemic in the UK in 2001 and the decrease in the number of cases in spring that year led to speculation that the enforced restriction of farm visits and culling of farm animals was responsible for the great reduction in cases associated with *C. parvum* after 2001 (Hunter et al., 2003; Smerdon et al., 2003). It appears more likely that the decrease was due to measures taken by the water supplier in the North West Region to improve treatment of drinking water by coagulation and filtration and to restrict livestock from water catchment areas (Sopwith et al., 2005; Lake et al., 2007).

The role of zoonotic infections in human cryptosporidiosis in developing countries appears much less important than in industrialised nations. Studies conducted in Brazil, India, Kenya, Malawi, Peru, Thailand, South Africa and Vietnam have shown that 70–90% of infections in humans in these developing countries result from *C. hominis* (Peng et al., 2001, 2003a; Xiao et al., 2001; Leav et al., 2002; Tiangtip and Jongwutiwes, 2002; Cama et al., 2003; Gatei et al., 2003, 2006, 2007; Tumwine et al., 2003, 2005; Das et al., 2006; Muthusamy et al., 2006; Bushen et al., 2007). Because the prevalence of *C. parvum* in human cases of cryptosporidiosis is much lower, this strongly suggests that zoonotic infection is much less common in developing countries than in industrialised countries.

The number of *C. parvum* infections in humans attributable to zoonotic transmission remains unclear because the source of *C. parvum* in humans can be of bovine or human origin. Results of sequence analyses of the 60 kDa glycoprotein (GP60) gene support the probable zoonotic transmission in industrialised nations. One major GP60 *C. parvum* subtype family, IIa, is common in humans in rural areas in the USA and in Europe (Glaberman et al., 2002; Alves et al., 2003, 2006; Stantic-Pavlinic et al., 2003; Chalmers et al., 2005a; Feltus et al., 2006). Many of the IIa subtypes found in humans have also been found in calves in the same area. For example, in Portugal one major *C. parvum* subtype in humans, IIaA15G2R1, is the predominant subtype in calves and zoo ruminants (Alves et al., 2003, 2006). Likewise, in Northern Ireland, most of the common IIa subtypes found in calves have been found in human outbreaks or sporadic cases (Glaberman et al., 2002; Thompson et al., 2007). In Wisconsin, USA, most of the *C. parvum* subtypes found in humans were also found in calves in neighbouring Michigan and Ontario (Peng et al., 2003b; Feltus et al., 2006; Trotz-Williams et al., 2006). In an apple cider-associated outbreak of cryptosporidiosis in Ohio, USA, all patients had *C. parvum* subtype IIaA15G2R1 or IIaA17G2R1, and the latter was found in the apple cider (Blackburn et al., 2006). Subtype IIaA17G2R1 is rare in the USA, having been reported only in some calves in Ohio and Vermont (Xiao et al., 2007b). Despite evidence of the presence of IIa subtypes in humans and cattle in the same areas, there is minimal molecularly based epidemiological evidence quantifying the extent of zoonotic transmission.

Another less common bovine *C. parvum* subtype family, IId, might potentially be responsible for some zoonotic infections in industrialised nations. In southern Europe (Portugal, Italy, Serbia and Hungary), although IIa subtypes were the dominant *C. parvum* in calves, IId subtypes were found occasionally (Alves et al., 2003, 2006; Wu et al., 2003; Misic and Abe, 2006; Plutzer and Karanis, 2007). Some IId subtypes have been found in HIV+ persons in Portugal (Alves et al., 2003, 2006). About half the *C. parvum* infections in children in Kuwait City are IId subtypes, but transmission appears to be anthroponotic in origin (Sulaiman et al., 2005). IId subtypes of *C. parvum* have not been found in cattle or humans in the UK, USA, Canada or Australia (Glaberman et al., 2002; Peng et al., 2003b; Chalmers et al., 2005a; Trotz-Williams et al., 2006; Thompson et al., 2007; Xiao et al., 2007b).

Multilocus typing data provide evidence of zoonotic transmission of *C. parvum* in Wales and the North West Region of England (Hunter et al., 2007). At the ML1, ML2 and GP60 loci, there were significant differences in *C. parvum* isolates from persons who had contact with farm animals and those with no animal contact before the onset of illness (Hunter et al., 2007). At the ML1 and ML2 loci, significantly more persons with *C. parvum* subtype ML1-242 and ML2-223-237 had farm animal contact than those with ML1-227 and ML2-193 or 197, respectively. Similarly, at the GP60 locus, specimens from patients with farm animal contact generated significantly greater PCR product sizes than those who had no animal contact.

Not all *C. parvum* infections in humans result from zoonotic transmission. In Portugal, the genetic diversity of *C. parvum* is much higher in HIV+ persons than in calves or other ruminants, and one of the three *C. parvum* subtype families, IIc, has never been found in animals. The anthroponotic nature of the *C. parvum* IIc subtype family is sup-

ported by subtyping studies of human and bovine cryptosporidiosis in Portugal, USA, Canada, UK and Australia. In urban areas in the USA, IIc subtypes are responsible for most human *C. parvum* infections (Xiao et al., 2004b). In European countries such as Portugal and the UK, both IIa and IIc are fairly common in humans (Alves et al., 2003, 2006).

Multilocus typing data also provide evidence of anthroponotic transmission of *C. parvum* in Europe. Analyses of three minisatellite and four microsatellite markers identified three groups of *C. parvum* in humans and cattle in Scotland, with two small groups exclusively found in humans and one large group in both humans and calves (Mallon et al., 2003a,b). Similar findings in England and Wales were based on three of the same microsatellite markers (Leoni et al., 2007). As in Portugal, based on GP60 gene findings, multilocus data confirmed that humans in Scotland were infected with significantly wider spectra of *C. parvum* subtypes than were cattle, indicating that some human *C. parvum* infections were not related to subtypes found in cattle (Grinberg et al., 2008). In contrast, lower genetic diversity of *C. parvum* was observed in humans than in animals in France, and there was no host restriction of *C. parvum* populations (Ngouanesavanh et al., 2006). It is not clear whether the omission of some more polymorphic markers such as GP60 in the French study has contributed to this different observation.

The anthroponotic IIc subtypes of *C. parvum* are responsible for most human infections in developing countries (Leav et al., 2002; Peng et al., 2003a; Xiao et al., 2004a; Xiao and Ryan, 2004; Akiyoshi et al., 2006) whereas the zoonotic IIa subtypes are more common in European countries, Australia and the USA. In some regions such as Lima, Peru, IIc subtypes are the only *C. parvum* found in humans. In developing countries such as Malawi and Kenya, the anthroponotic *C. parvum* subtype family IIe is found (Peng et al., 2003a; Xiao et al., 2004a; Xiao and Ryan, 2004; Cama et al., 2007). In Uganda, several new *C. parvum* subtype families are present in humans (Akiyoshi et al., 2006). Most of these studies were done in urban areas but a study in Malawi has shown a higher rate of human *C. parvum* infection in rural areas than in urban areas (Morse et al., 2007); unfortunately subtyping was not done. An earlier subtyping study found almost exclusive anthroponotic transmission of cryptosporidiosis in the country (Peng et al., 2003a).

Whether *C. meleagridis*, *C. canis*, *C. felis* and *C. muris* are predominantly transmitted in developing countries zoonotically remains unclear. Using *C. hominis* and *C. parvum*-specific genotyping tools, the analysis of *C. canis*- and *C. felis*-infected specimens from HIV+ persons in Lima, Peru revealed concurrent presence of *C. hominis* and *C. parvum* IIc subtype family in six of 21 patients (Cama et al., 2006). There are no multilocus subtyping studies to determine whether there is any host segregation in *C. canis* or *C. felis*, although an earlier study of a small number of human and bird specimens failed to show this in *C. meleagridis* (Glaberman et al., 2001). Transmission of *C. canis* between humans and a dog, however, has been speculated in a recent report (Xiao et al., 2007a). In one household, two diarrhoeic children and one dog were diagnosed during the same period having cryptosporidiosis by microscopic examinations of faecal smears stained with a modified acid-fast stain. *Cryptosporidium canis* was found in faeces of all three by PCR-restriction fragment length polymorphism (RFLP) and DNA sequence analysis of the ssrRNA gene. The direction of the *C. canis* transmission, however, was not clear.

6.2. Giardia

Because humans are infected only with *G. duodenalis* assemblages A and B and both genotypes have been found in animals, studies on zoonotic transmission of giardiasis have concentrated on these two genotypes. This especially applies to assemblage A, detected in faeces from many mammals (Tables 4 and 5). Some wild animals such as beavers and muskrats have a high prevalence of assemblage B, and these animals historically have been considered important contamination sources of waterborne giardiasis (see Section 7).

Few studies have compared *Giardia* isolates from humans and animals living in the same locality or household. In Aboriginal communities in Australia ssrRNA gene sequences from 13 humans and nine dogs identified human isolates in genetic groups 1 and 2, and dog isolates in groups 3 and 4 (Hopkins et al., 1997). Only one dog isolate contained groups 2 and 3, suggesting that zoonotic transmission between humans and dogs, if present, was infrequent. In Bangkok, faeces were collected from 204 humans and 229 dogs from 20 temples and surrounding communities. Of 13 *Giardia* isolates from dogs most were assemblage A, followed by D, B and C, whereas three isolates from humans were assemblages A and B. One dog and two monks in the same monastery had assemblage A (Inpankaew et al., 2007). In two households in a village in northern India the same assemblage A or B subtype was found in both humans and dogs living in the same household (Traub et al., 2003, 2004). In Brazil a child with giardiasis and her dog were both reported to be infected with subtype AI (Volotao et al., 2007).

Although assemblage A has been considered the most important *G. duodenalis* genotype involved in zoonotic giardiasis, subtyping data do not support widespread findings of zoonotic transmission. The two most common subtypes of assemblage A, AI and AII, differ significantly in host preference. Humans are mostly infected with AII, although AI is also seen in some areas or studies (Table 6). In contrast, animals are mostly infected with AI, although the AII subtype is seen occasionally (Table 7). Based on the β-giardin gene, subtype AIII has been found in a few humans (Table 6). This could be due to the higher resolution of the locus, because the β-giardin AIII subtype was linked to AII at the glutamate dehydrogenase (GDH)

Table 6
Subtypes of *Giardia duodenalis* assemblage A in humans

Location	Sample size	Subtypes				Reference
		AI	AII	AIII	Others	
Italy	17	1	7	3	6	Lalle et al. (2005b)
Italy	13		10	2	AII+AIII (1)	Caccio et al. (2002b)
England	12		12			Amar et al. (2002)
France	8		8			Bertrand et al. (2005)
Mexico	19		19			Ponce-Macotela et al. (2002)
Mexico	17	15		2		Lalle et al. (2005a)
Nicaragua	16		3	13[a]		Lebbad et al. (in press)
Brazil	62	60	2			Volotao et al. (2007)
Brazil	29		29			Souza et al. (2007)
Peru	6		6			Sulaiman et al. (2003)
Korea	3		3			Lee et al. (2006)
China	4		4			Lee et al. (2006)
China	2		2			Lu et al. (2002)
Bangladesh	29	8	20		AI+AII (1)	Haque et al. (2005)
Philippines	50	3	47			Yason and Rivera (2007)
Australia	9	4	5			Read et al. (2004)
Ethiopia	23	1	5	16	1	Gelanew et al. (2007)

[a] AII at glutamate dehydrogenase locus.

Table 7
Subtypes of *Giardia duodenalis* assemblage A in animals

Animal	Location	Sample size	Subtypes			Reference
			AI	AII	AIII	
Cattle	Portugal	2		2		Mendonca et al. (2007)
Cattle	Denmark	8	8			Langkjaer et al. (2007)
Cattle	Brazil	1	1			Souza et al. (2007)
Cattle	USA	10	3	6[a]	1	Feng et al. (in press)
Water buffalo	Italy	2	2			Caccio et al. (2007)
Sheep	Italy	5	5			Giangaspero et al. (2005)
Pig	Denmark	10	10			Langkjaer et al. (2007)
Horse	USA and Australia	4	3	1		Traub et al. (2005)
Dog	Germany	33	33			Leonhard et al. (2007)
Dog	Mexico	5	4		1	Lalle et al. (2005a)
Dog	Brazil	7	7			Volotao et al. (2007)
Dog	India	5	2	3		Traub et al. (2004)
Cat	USA	6	6			Vasilopulos et al. (2007)
Cat	Brazil	8	8			Souza et al. (2007)
Cat	Australia	6	6			Read et al. (2004)

[a] Appeared on one farm briefly.

locus in a study that used both genotyping tools (Lebbad et al., in press). There are no clear geographic differences in the distribution of the two common assemblage A subtypes in humans. Although AI was detected in humans in high frequency in two studies in South America, two other studies conducted in the same countries found only AII in humans (Table 6). Increasing the typing resolution and further epidemiological studies are needed to determine whether the occasional human AI infections are results of anthroponotic or zoonotic transmission.

7. Epidemiological evidence for zoonotic cryptosporidiosis and giardiasis

The strongest epidemiological evidence for zoonotic transmission of cryptosporidiosis is from investigations associating cattle with outbreaks in veterinary students in contact with infected young calves, animal researchers in contact with infected young calves, and children attending agricultural camps and fairs (Preiser et al., 2003; Smith et al., 2004; Kiang et al., 2006). In case-control studies in industrialised nations, contact with cattle was implicated as a risk factor for human cryptosporidiosis (Robertson et al., 2002; Goh et al., 2004; Hunter et al., 2004; Roy et al., 2004; Hunter and Thompson, 2005). In the UK, cryptosporidiosis case numbers are higher in areas with a high estimate of *Cryptosporidium* oocysts applied to land from manure (Lake et al., 2007). Thus cattle, especially dairy calves, are likely the major reservoir for *C. parvum* in some areas. In contrast, few epidemiological studies have implicated sheep and companion animals as a source of human cryptosporidiosis (Molbak et al., 1994; Duke

et al., 1996; Glaser et al., 1998; Katsumata et al., 1998; Robinson and Pugh, 2002; Goh et al., 2004).

Little epidemiological evidence exists that strongly supports the importance of zoonotic transmission of giardiasis to humans. In New Zealand, case-control studies of giardiasis did not identify contact with pets as a risk factor for children or adults, although contact with farm animals was associated with an increased risk of infection for adults (Hoque et al., 2002, 2003), Also in New Zealand, the prevalence of giardiasis was higher in rural than urban areas (Mitchell et al., 1993). In the UK, farm visitation was frequent among case patients but specified exposure to dogs, cats, horses, cattle and sheep was not significant (Stuart et al., 2003). One case-control study in eastern England found associations with exposure to farm animals and pets, particularly pigs, dogs and cats (Warburton et al., 1994). Other studies in the USA, Canada and UK have not found such associations (Mathias et al., 1992; Dennis et al., 1993; Gray et al., 1994). Although giardiasis affects persons in all age groups, the number of reported cases in the USA was highest among children aged 1–9 years and adults aged 30–39 years (Hlavsa et al., 2005; Yoder and Beach, 2007). A marked onset of illness was reported in early summer through early autumn, with a doubling in giardiasis during summer, coinciding with increased outdoor activities such as swimming (essentially communal bathing venues) and camping (Hlavsa et al., 2005).

Beavers are probably the most accused animal source of giardiasis for humans in the USA. often resulting from investigations of waterborne outbreaks and reports by hikers and campers who became infected and recalled drinking water from streams or lakes. The first accusation began in 1976 with an outbreak in Camas, Washington, USA when 128 townspeople had laboratory-confirmed giardiasis. A questionnaire survey revealed that 3.8% of residents had clinical giardiasis, whereas none of 318 residents in a control town were ill. *Giardia* cysts were recovered from water entering the city water treatment system and also from two storage reservoirs containing chlorinated and filtered stream water. Trapping in the watershed yielded three beavers infected with *Giardia* that was infective for beagle pups (Dykes et al., 1980). Similar conclusions were made from investigations of waterborne giardiasis outbreaks in New Hampshire and Nevada, USA, largely based on finding *Giardia* cysts in beavers caught in source watersheds (Lopez et al., 1980; Navin et al., 1985). These reports lack hard evidence of involvement in human infections and merely report circumstantial evidence based on beavers found at contaminated sites; in some cases the beavers were infected. The roles of other animals and humans are virtually omitted from such reports and the possible role of humans as a source of infection for beavers is rarely addressed. In case-control studies, the evidence for an association between drinking wilderness water and acquiring giardiasis is also minimal (Welch, 2000).

8. Perspective

The use of molecular epidemiological tools has significantly changed our understanding of zoonotic transmission of *Cryptosporidium* spp. and *Giardia* spp. Genotyping and subtyping data have clearly demonstrated the presence of anthroponotic as well as zoonotic *Cryptosporidium* types in humans in industrialised nations. The latter is especially true in rural areas of Europe, New Zealand and North America. In contrast, transmission of cryptosporidiosis appears largely anthroponotic in developing countries. These molecular epidemiological data have already shown that zoonotic transmission of *Cryptosporidium* is less common than once perceived. Nevertheless, more usage of subtyping analysis in case-control studies is needed to accurately assess the extent of zoonotic transmission of cryptosporidiosis in industrialised nations.

The story is clearly different with giardiasis, where epidemiological evidence indicates high rates of infection from anthroponotic transmission whereas genotyping and subtyping data point only to the potential role for zoonotic transmission with little or no epidemiological support. Further molecular epidemiological studies of giardiasis in humans, utilising case-control study design and subtyping analysis of specimens from both humans and animals, are needed before more definitive conclusions can be made and possible geographic differences in the occurrence of zoonotic giardiasis can be detected. Systematic comparisons of assemblage A subtypes and assemblage B at various genetic loci and elucidation of parasite population genetics are also needed to better understand zoonotic transmission of *G. duodenalis*.

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