Cryptosporidiosis: an update in molecular epidemiology

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Purpose of review

Molecular tools have been developed to detect and differentiate *Cryptosporidium* at the species/genotype and subtype levels. These tools have been increasingly used in the characterization of the transmission of *Cryptosporidium* spp. This review addresses the most recent developments in molecular epidemiology of cryptosporidiosis.

Recent findings

The recent development of subtyping tools has led to better understanding of the population genetics and transmission of *Cryptosporidium* in humans. The population structure of *C. parvum* and *C. hominis* is apparently more complicated than previously suggested, with the likely existence of both clonal and panmictic populations. Thus, the transmission of *C. parvum* (genotype II) in humans is shown to be different in different areas, with zoonotic transmission important in certain places and anthroponotic transmission in others. The use of molecular tools has also led to the identification of geographic and temporal differences in the transmission of *C. parvum* and *C. hominis*, and better appreciation of the public health importance of other *Cryptosporidium* species/genotypes and the frequency of infections with mixed genotypes or subtypes.

Summary

Factors involved in the transmission of human cryptosporidiosis are difficult to examine using conventional methods. The use of molecular tools has been helpful in the assessment of the zoonotic potential of various *Cryptosporidium* spp. and sources of human infections, and has started to play a significant role in the characterization of transmission dynamic in endemic and epidemic areas.

Keywords

Cryptosporidium, molecular epidemiology, diagnosis, zoonosis, genotyping, subtyping

Curr Opin Infect Dis 17:483–490. © 2004 Lippincott Williams & Wilkins.

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Current Opinion in Infectious Diseases 2004, 17:483–490

Abbreviations

GP60	60 kDa glycoprotein
HSP70	70 kDa heat shock protein 70
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
SSCP	single strand conformation polymorphism
rRNA	small subunit ribosomal RNA

0951-7375

Introduction

Cryptosporidiosis is a frequent cause of diarrheal diseases in humans. Several groups of humans are particularly susceptible to cryptosporidiosis. In developing countries, *Cryptosporidium* infections occur mostly in children younger than 5 years, with peak occurrence of infections and diarrhea in children under 2 years old [1••,2]. In industrialized countries, epidemic cryptosporidiosis can occur in adults via foodborne or waterborne outbreaks [3]. In immunocompromised persons, the incidence of cryptosporidiosis increases as CD4+ lymphocyte cell counts fall, especially below 200 cells/μl [4•].

Clinical manifestations of cryptosporidiosis vary with age and immunological status. In children residing in endemic areas, the most prominent symptom is diarrhea, which nevertheless occurs only in a proportion of infected persons [1••]. In outbreak settings, immunocompetent adults may have voluminous but self-limiting diarrhea, with or without abdominal cramps, fatigue, vomiting and other symptoms [5]. However, in immunodeficient humans, cryptosporidiosis can be associated with chronic, potentially life-threatening diarrhea [4•].

Because of the ability of *Cryptosporidium* to infect humans and a wide variety of animals, and because of the ubiquitous presence of *Cryptosporidium* oocysts in the environment, humans can acquire *Cryptosporidium* infections through several transmission routes, such as direct contact with infected persons (person-to-person transmission) or animals (zoonotic transmission), and ingestion of contaminated food (foodborne transmission) and water (waterborne transmission). The relative importance of these transmission routes in the epidemiology of cryptosporidiosis is not entirely clear, largely due to the fact that traditional diagnostic tools do not have the ability to differentiate sources of parasites [6]. In the last decade, however, numerous molecular biological techniques have been developed to detect and differentiate *Cryptosporidium* spp. at species/genotype and subtype levels. These tools are now increasingly used in epidemiological studies of cryptosporidiosis in endemic and epidemic areas, which has helped greatly our understanding of the transmission of cryptosporidiosis in humans and animals [7••].

Recent developments in molecular tools

A variety of tools for the detection and characterization of *Cryptosporidium* have been described recently, in addition to many previously used in epidemiological

studies. These include polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) analysis of the gene coding for small subunit ribosomal RNA (SSU rRNA) [8,9], *Cryptosporidium* oocyst wall protein [10•] and 60 kDa glycoprotein (GP60) [11], PCR single strand conformation polymorphism (SSCP) analysis of the SSU rRNA [12], internal transcribed spacer [12], 70 kDa heat shock protein 70 (HSP70) [13] and GP60 [11] genes, DNA sequence analysis of the p23, GP60 and GP900 genes [14], and heteroduplex analysis of the double-stranded RNA [15,16]. Many of the PCR-RFLP, PCR-SSCP, and PCR-heteroduplex analysis tools have incorporated a DNA sequencing step when unusual patterns are detected. A recent study suggests that direct sequencing of multiple PCR products may be better than sequencing of PCR clones, as the latter can introduce sequence artifacts when mixed *Cryptosporidium* genotypes are present in samples [17]. A biosensor technique for the detection of viable *C. parvum* oocysts has also been described [18•], which does not have a genotyping or subtyping component.

Most of the tools are genotyping in nature. Several tools, however, have been used in the differentiation of *C. parvum* and *C. hominis* subtypes, thus representing the second-generation molecular epidemiological tools and are increasingly used in the characterization of *Cryptosporidium* transmission. The latter include DNA sequence analysis of the GP60 [11,14,19•,20,21] and HSP70 [20] genes, heteroduplex analysis and nucleotide sequencing of the double-stranded RNA [15,16], and single [22] or multilocus mini and micro-satellite analysis [23••,24]. With the recent completion of *C. parvum* genomic sequencing [25•,26••], it is expected that more high-resolution subtyping tools will be developed.

Most of the molecular tools were developed using nucleotide sequences of *C. parvum*. Because of the extensive genetic diversity among the human-pathogenic *Cryptosporidium* spp., it is expected that these tools may have difficulties in detecting those species that are very divergent from *C. parvum*, such as *C. felis, C. canis, C. muis* and *C. suis*. Indeed, a recent study has compared the ability of 10 commonly used genotyping tools in detecting seven human-pathogenic *Cryptosporidium* species/genotypes. With the exception of SSU rRNA-based PCR tools, which detected all seven *Cryptosporidium* species/genotypes, most of the genotyping tools examined had only the ability to detect *C. parvum* (genotype II or the bovine genotype), *C. hominis* (genotype I or the human genotype) and *C. meleagridis* [27]. More recently, however, using an array of primers (23 primers in a nested PCR) to cover all combinations of sequence heterogeneity in the primer region, a *Cryptosporidium* oocyst wall protein based nested PCR-RFLP tool has been developed for the detection and differentiation of various *Cryptosporidium* spp. [10•].

Cryptosporidium genotypes and biological and public health significance

There is extensive genetic variation within *Cryptosporidium*. In addition to the 13 accepted species of *Cryptosporidium*, over 30 *Cryptosporidium* genotypes have been described and new genotypes are continually being discovered [7••]. Most of the species and genotypes are host-adapted in nature and have a narrow spectrum of natural hosts (Table 1). The biological and taxonomic significance of most *Cryptosporidium* genotypes has been reviewed [7••]. Recently, several genotypes are described as species and a few new genotypes have been found, such as *Cryptosporidium galli* [28•], *Cryptosporidium suis* (pig genotype I) [29•], marsupial genotype II in eastern grey kangaroos [30•], goose genotype II in Canada geese [31•,32], muskrat genotype II [33], a mongoose genotype [34•], a horse genotype and a new Eurasian woodcock genotype [35], two unnamed genotypes in Canada geese [31•], and several unnamed genotypes in reptiles [36].

Results of experimental infections with some common genotypes have shown significant differences in biology and host specificity among *Cryptosporidium* genotypes, indicating that many described genotypes may represent different species. The establishment of *C. hominis* as a separate species is supported by more recent studies in gnotobiotic and conventional piglets, which have shown significant biological differences between *C. hominis* and *C. parvum* [37•,38]. Similarly, *Cryptosporidium* pig genotype I has shown uniqueness in infectivity, prepatent period and pathogenicity from *C. parvum* in experimental infections in pigs [39•], which has led to the establishment of a new species, *C. suis* [29•]. The finch genotype has been re-described as *C. galli* on the basis of molecular and biological evidence [28•].

The existence of host-adapted *Cryptosporidium* species or genotypes indicates that cross transmission of *Cryptosporidium* between humans and most animal species or among different groups of animals is probably limited. Surveys conducted in pigs, grey kangaroos, Canada geese, fur-bearing mammals, and reptiles have shown that most animals are infected with only a few host-adapted *Cryptosporidium* species/genotypes [30•,31•,32, 33,36,40]. Even though human-pathogenic species have been occasionally found in a few animals, such as *C. canis* dog genotype infection in one fox and the excretion of *C. hominis* and *C. parvum* oocysts in a few Canada geese, the role of these animals in the transmission of *Cryptosporidium* infection to humans is probably minimal [32,33]. Several animal species such as domestic and wild ruminants [21,41], horses [42], and raccoon dogs [43],

Table 1. *Cryptosporidium* species and genotypes described so far

Cryptosporidium species and genotypes	Major hosts	Locus/loci examined	GenBank accession No.[a]	References
Birds				
C. baileyi	Chickens, turkeys, other birds	SSU rRNA, HSP-70, actin, COWP	L19068, AF266276, AF316634, AF382346	[7••,35,49]
C. galli	Finches, chickens, capercaillies, grosbeaks	SSU rRNA, HSP-70, actin	AY1608847, AY168849, AY163901	[7••,28•]
C. meleagridis	Turkeys and other birds, humans	SSU rRNA, HSP-70, actin, COWP, TRAP C1, DHFR	AF112574, AF329189, AF382351, AF266266 AY391726, AY391725	[7••,49]
Goose genotype I and II	Geese	SSU rRNA, actin	AY120912, AY504512, AY504513, AY504515-AY504517	[31•,32]
Unnamed goose genotype (#3b)	Geese	SSU rRNA	AY324638	[31•]
Unnamed goose genotype (#7)	Geese	SSU rRNA	AY324641	[31•]
Duck genotype	Ducks, geese	SSU rRNA	AF316630, AY504514	[31•,32]
Woodcock genotype	Eurasian woodcock	SSU rRNA, HSP-70	AY273769, AY273773	[35]
Humans and domestic animals				
C. andersoni	Cattle, Bactrian camels, sheep	SSU rRNA, HSP-70, actin, COWP	L19069, AF221542, AF382352, AF266262	[7••,21]
C. hominis	Humans, monkeys	SSU rRNA, HSP-70, actin, COWP, etc.	L16997, AF401506, AF382337, AF266265	[7••]
C. parvum	Cattle, sheep, goats, deer, raccoon dog, horses	SSU rRNA, HSP-70, actin, COWP, etc.	L16996, AF221528, AF382338, AF266273	[7••,41–43,50]
C. canis	Dogs	SSU rRNA, HSP-70, actin, COWP	AF112576, AF221529, AF382340, AF266274	[7••]
C. felis	Cats	SSU rRNA, HSP-70, actin, COWP	AF112575, AF221538, AF382347, AF266263	[7••]
C. wrairi	Guinea pigs	SSU rRNA, HSP-70, actin	AF115378, AF221536, AF382348, AF266271	[7••]
C. suis (pig genotype I)	Pigs	SSU rRNA, HSP-70, actin	AF108861, AF221533, AF382344	[29•,39•,40]
Bovine genotype B	Cattle, sheep	SSU rRNA	AY120911	[7••]
Deer-like genotype	Cattle	SSU rRNA		[7••]
Pig genotype II	Pigs	SSU rRNA	AY271721	[51]
Horse genotype	Horses	SSU rRNA, HSP 70	AY273770, AY273774	[35]
Wildlife				
C. muris	Rodents, Bactrian camels, bilbies	SSU rRNA, HSP-70, actin	AF093498, AF221543, AF382350	[7••,52,53]
Bear genotype	Bear	SSU rRNA, HSP-70, actin	AF247535, AF247536, AF382339	[7••]
Cervine genotype	Deer, sheep, lemurs	SSU rRNA, HSP-70	AF262328, AF442484, AY273776, AY273772	[7••,35,46]
C. canis fox genotype	Foxes	SSU rRNA, actin	AY120908, AY120908, AY120926	[7••,33]
C. canis coyote genotype	Coyotes	SSU rRNA, HSP-70, actin	AY120909, AY120920, AY120927	[7••,33]
Deer genotype	Deer	SSU rRNA, actin	AY120910, AY120928	[7••]
Deer-mouse genotype	Deer-mice	SSU rRNA, HSP-70, actin	AY120905, AY120919, AY120925	[7••]
Ferret genotype	Ferrets	SSU rRNA, HSP-70, actin, COWP	AY120905, AF112572, AF221532, AF221532, AF382341, AF266267	[7••,54]
Fox genotype	Foxes	SSU rRNA	AY120907	[7••]
Muskrat genotype I and II	Muskrats	SSU rRNA	AY120904, AY545546-AY545548	[7••,33]
Marsupial genotype I and II (EGK3)	Marsupials	SSU rRNA, HSP-70, actin, COWP	AF112570, AF221531, AF382345, AF266269, AF513227, AY237630, AY237632- AY237635	[7••,30•]
Mouse genotype	Mice, rats	SSU rRNA, HSP-70, actin, COWP	AF112571, AF221530, AF382343, AF266268	[7••,47]
Mongoose genotype	Mongooses	SSU rRNA, HSP-70, COWP	AB102769, AB102771, AB102770	[34•]
C. hominis monkey genotype	Monkeys	SSU rRNA, HSP-70, actin, COWP	AF112569, AF221534, AF382342, AF266272	[7••]

(*continued overleaf*)

Table 1. (*continued*)

Cryptosporidium species and genotypes	Major hosts	Locus/loci examined	GenBank accession No.[a]	References
Opossum genotype I and II	Opossums	SSU rRNA, HSP-70, actin	AY120902, AY120906, AY120916, AY120918, AY120921, AY120922	[7••]
Rabbit genotype	Rabbits	SSU rRNA, HSP-70, actin	AY120901, AY273775, AY120924	[7••]
Squirrel genotype	Squirrels	SSU rRNA		[7••]
Skunk genotype	Skunks, raccoons	SSU rRNA, HSP-70, actin	AY120903, AY120917, AY120923	[33]
Reptiles/fish				
C. molnari	Fish			[7••]
C. saurophilum	Lizards	SSU rRNA, HSP-70, actin	AY382170, AF221540, AF382349	[36]
C. serpentis	Snakes, lizards	SSU rRNA, HSP-70, actin, COWP	AF093502, AF221541, AF382353, AF266275	[36]
Unnamed snake genotype (W11)	Snakes	SSU rRNA, actin	AY120913, AY120930	[36]
Unnamed snake genotype	Snakes	SSU rRNA	AY268584	[36]
Unnamed lizard genotype	Lizards	SSU rRNA, actin	AY120915, AY120932	[36]
Tortoise genotype	Tortoises	SSU rRNA, actin	AY120914, AY120931	[36]

SSU rRNA, small subunit ribosomal RNA; HSP70, 70 kDa heat shock protein 70; COWP, *Cryptosporidium* oocyst wall protein; TRAP C1, thrombospondin-related adhesive protein 1 of *Cryptosporidium*; DHFR, dihydrofolate reductase. [a]Only representative sequences are quoted.

however, are natural hosts of *C. parvum*, one of the two major human *Cryptosporidium* pathogens. These animals obviously can be a source of contamination with human pathogenic *Cryptosporidium*. The ability to infect a wide range of mammals experimentally with *C. meleagridis* [44,45] is in agreement with the suggestion that *C. meleagridis* is increasingly becoming an important human pathogen instead of merely an avian pathogen [4•]. Likewise, the finding of the cervine genotype in lemurs [46] also supports the previously demonstrated human-infective nature of the parasite. In addition, *C. hominis* monkey genotype has also been found in two persons in the UK for the first time [24]. The suggestion that *Cryptosporidium* mouse genotype is a potential human pathogen because of its close relatedness to *C. parvum* [47,48], however, needs support from finding the parasite in human patients.

Population structure of *Cryptosporidium*

The development of genotyping and subtyping tools has made it possible to examine the population genetics of *Cryptosporidium*, which is essential to the understanding of *Cryptosporidium* transmission in humans and animals, and assessing the value of multilocus subtyping in the characterization of cryptosporidiosis epidemiology. A recent multilocus study of 180 fecal specimens from humans and cattle living in a small area in Scotland using three mini and four micro-satellite markers identified 38 multilocus subtypes of *C. parvum* and *C. hominis* [23••]. Linkage disequilibrium analysis between pairs of loci combined with measures of genetic distance and similarity showed the presence of four genetically isolated populations of parasites in this area. The *C. hominis* group consisted primarily of two closely related multilocus subtypes, suggesting the population structure was essentially clonal. In contrast, *C. parvum* isolates in the study belonged to three distinct lineages, two of which were seen in only humans and one in both humans and cattle. The *C. parvum* population comprising both human and bovine isolates had a panmictic population structure and was in linkage equilibrium, suggesting that genetic exchange occurred frequently. Nevertheless, genetic exchange between *C. parvum* and *C. hominis* was never observed, which is in agreement with the separation of *C. hominis* from *C. parvum* as an individual species [23••]. The presence of human-adapted *C. parvum* subtypes is well known and they have been found in South Africa, Portugal, the USA, and Peru [7••,19•]. It is important to point out that these human-adapted *C. parvum* subtypes are not the various host-adapted *Cryptosporidium* genotypes (see Table 1) previously described based on sequence analysis of conservative genes such as SSU rRNA, HSP70 [7••], as the former would have minimal sequence variations at these loci.

Whether this difference in population genetic structure between *C. parvum* and *C. hominis* is valid in other areas is still uncertain [55•,56]. Even though the three populations of *C. parvum* were also seen in a subsequent study with more samples from several areas in Scotland [24], linkage disequilibrium in subtyping results between the GP60 and HSP70 loci was observed in *C. hominis* in Malawi, suggesting that *C. hominis* in some areas may also have a panmictic population structure [20,56]. Indeed, it is uncertain whether the observed clonal population structure of *C. hominis* in Scotland is valid, because in the Scotland study, the two

Table 2. Distribution of *Cryptosporidium* spp. in humans in recent studies

Location	Type of patients	No. of patients	*C. hominis*	*C. parvum*	*C. hominis* + *C. parvum*	*C. meleagridis*	Other	Reference
Portugal	AIDS	29	7	16	0	3	3 *C. felis*	[19•]
Switzerland	Adults	9	0	9	0	0	0	[60]
Switzerland	Children with diarrhea	14	11	3	0	0	0	[61]
UK	Adults	151	78	73	0	0	0	[13]
UK	Adults	184	108	76	0	0	0	[12]
UK	Immunodeficient children	15	2	5	4	3	1 *C. hominis* + *C. parvum* + *C. meleagridis*	[53]
New Zealand	Adults	66	22	44	0	0	0	[41]
Uganda	Children with diarrhea	444	326	85	19	5	9 with unknown genotype	[1••]
Kenya	HIV+ children and adults	33[a]	23	8	0	1	1 *C. muris*	[51]
Malawi	Children	43	41	2	0	0	0	[20]
Peru	HIV+ adults	300	204	34	0	38	12 *C. canis*, 10 *C. felis*, 1 *C. suis*, 2 *C. parvum* + *C. canis*, 1 *C. parvum* + *C. meleagridis*	[4•]

[a]Including samples from nine HIV– adults.

major multilocus subtypes (89% of the isolates) differed from each other only at one locus (MS5), which made it impossible to calculate linkage disequilibrium. The same region of the HSP70 gene was used in both the Malawi and Scotland studies. However, the Scotland investigators relied on length polymorphism of the gene to determine subtypes, whereas the Malawi study showed that even though there was no length polymorphism in the HSP70 gene among *C. hominis* isolates examined, there were six subtypes which differed from each other at seven previously identified polymorphic sites [20]. Thus, if DNA sequence analysis were used in the Scotland study, the conclusion could be different. In any case, more extensive studies in different epidemiological settings using more polymorphic loci are needed before firm conclusions on the population structure of *C. parvum* and *C. hominis* can be made [55•,56].

Recent developments in molecular epidemiology of human cryptosporidiosis

The development of molecular tools for the species differentiation, genotyping, and subtyping of *Cryptosporidium* has been useful in studies aimed at understanding host specificity of *Cryptosporidium* spp. and the transmission of human cryptosporidiosis. They have been used in the establishment of the identity of *Cryptosporidium* in humans, the identification of infection or contamination sources, and the characterization of transmission dynamics of cryptosporidiosis in communities.

Thus far, eight *Cryptosporidium* species/genotypes have been identified in humans, including *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, *C. suis* and *Cryptosporidium* cervine genotype [4•,7••,19•,57–59]. Among them, *C. hominis* and *C. parvum* are responsible for most human infections (Table 2), even though in some areas *C. meleagridis* infection rate is as high as *C. parvum* [4•]. The distribution of *C. parvum* and *C. hominis* in humans differs in geographic regions, probably as the result of differences in transmission routes. In European countries, *C. parvum* is generally found in more human cases than *C. hominis* (Table 2), although a more recent study in the UK has shown a comparable rate of both pathogens in autochtonous, sporadic cases [13]. In the rest of the world, *C. hominis* is usually the predominant species in humans (Table 2). A shift in human infection from predominantly *C. parvum* in the spring to *C. hominis* in the autumn has been reported in New Zealand [41]. In studies conducted in Peru, there was no significant difference in the distribution of *Cryptosporidium* species or genotypes between children and HIV+ persons, indicating that there is no preferential infection with zoonotic species/genotype in immunocompromised persons [4•].

The finding of different species/genotypes has frequently been used as an indication of infection sources because of differences in host specificity of *Cryptosporidium* spp. Thus, the predominance of *C. parvum* in humans in European countries suggests that contamination from farm animals plays a significant role in the

transmission in areas with extensive animal husbandry [62]. Indeed, during the 2001 outbreaks of food and mouth disease in England and Wales, due to the extensive culling of animals and strict restriction on access to the countryside, there was a dramatic reduction in the incidence of cryptosporidiosis and increase in the proportion of human infection caused by *C. hominis* [63••,64•], supporting the role of zoonotic transmission in the cryptosporidiosis epidemiology in the UK. In contrast, the dominance of *C. hominis* in other parts of the world indicates that the anthroponotic transmission cycle is important in epidemiology in these areas [1••,20,57].

Nevertheless, results of recent subtyping studies have shown the presence of human-adapted *C. parvum* subtypes, even in areas with intensive transmission of *C. parvum* between humans and farm animals [19•,23••,24]. Thus, not all *C. parvum* infections in humans are the result of zoonotic transmission. For example, a study conducted in Portugal has shown substantial disparity in the distribution of *C. parvum* subtypes between humans and cattle, even though zoo ruminants had a *C. parvum* subtype distribution similar to cattle [19•]. Indeed, a whole *C. parvum* GP60 subtype allelic family, Ic, has been widely found in humans in South Africa, Portugal, the US and Peru, but has never been found in animals [7••,19•,21]. Human infections of other 'zoonotic' species or genotypes, such as *C. felis* and *C. suis* (pig genotype I), have sometimes been seen as mixed infections together with *C. hominis* [27]. Thus, anthroponotic transmission of *C. parvum* and other *Cryptosporidium* species/genotypes traditionally associated with animals is probably not rare. One study has even shown the presence of a low level of *C. hominis* in a few *C. parvum* laboratory isolates maintained through long-term passage in calves, arguing that animals may play a role in the transmission of *C. hominis* in humans [65•]. It is not clear how the low-grade *C. hominis* infection was maintained in calves over time in the presence of overwhelming *C. parvum* infection, as another study in gnotobiotic pigs, which are more susceptible to *C. hominis* than calves, has shown a rapid displacement of *C. hominis* by *C. parvum* in mixed infections [37•].

Genotyping and subtyping tools have also been used in the investigation of waterborne outbreaks of human cryptosporidiosis. A drinking water-associated outbreak of cryptosporidiosis in France was shown to be caused by *C. hominis*, which led to the conclusion that contamination of finished water by human sewage was the cause of the outbreak [3]. In a study conducted in Milwaukee, the genotypes and subtypes of *Cryptosporidium* in raw wastewater were monitored for 1 year. It was demonstrated that the subtype in the *C. hominis* GP60 allelic family Ib, which was found in the 1993 cryptosporidiosis outbreak, was still the predominant *Cryptosporidium* spp. in humans in Milwaukee during 2001 and 2002, indicating this parasite is quite infectious [66•]. Oocysts of *C. hominis* have been found in finished water in the UK by PCR-RFLP [9], and viable *C. parvum* and *C. hominis* oocysts have also been detected in finished water in the US by cell culture PCR [67•] and in river water in Japan by animal inoculation and genotyping [68].

Conclusion

Molecular epidemiological studies of cryptosporidiosis are still in their infancy, but significant progress has been made towards a better understanding of the transmission of cryptosporidiosis in humans and the public health significance of *Cryptosporidium* spp. from animals. Gone are the days when *C. parvum* was considered a homogeneous species and the only species infecting humans. We now have a much better appreciation of the complexity of *Cryptosporidium* infection in humans. We are also beginning to use the second-generation molecular tools to answer some epidemiological questions that are difficult to address by traditional methods, such as the role of zoonotic infections, frequency of mixed infections, maintenance of immunity and cross protection, transmission dynamics in different settings, temporal and geographic variations in *Cryptosporidium* transmission, and the role of parasite factors in transmission and the clinical spectrum of cryptosporidiosis. With the development of new subtyping tools and better characterization of the population structure of *Cryptosporidium*, we should soon have a more in-depth understanding of the epidemiology of cryptosporidiosis in humans and animals.

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