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 anthropogenic 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

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], 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. suis 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. meleagris [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],
| Cryptosporidium species and genotypes | Major hosts | Locus/loci examined | GenBank accession No.* | 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, capercaille, 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 | [7***, 49] |
| Goose genotype I and II              | Geese       | SSU rRNA, actin     | AY120912, AY504512, AY504513-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        | 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 | AY112576, AY221529, AF382340, AF266274 | [7**] |
| *C. felis*                           | Cats        | SSU rRNA, HSP-70, actin, COWP | AY112575, AY221538, AF382347, AF266263 | [7**] |
| *C. wrairi*                          | Guinea pigs | SSU rRNA, HSP-70, actin | AY115378, AY221536, AF382348, AF266271 | [7**] |
| *C. suis* (pig genotype I)           | Pigs        | SSU rRNA, HSP-70, actin | AY108861, AF221533, AF382344 | [29*, 39*, 40] |
| Bovine genotype B                    | Cattle, sheep | SSU rRNA            | AF382344               |            |
| Deer-like genotype                   | Cattle      | SSU rRNA            | AY120911               | [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, AF382341, AF266267 | [7**, 54] |
| Fox genotype                         | Foxes       | SSU rRNA            | AY120907               | [7**]       |
| Muskrat genotype I and II (EGK3)     | 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, AF237630, AF373632, AF373635 | [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, actin, COWP | AB102769, AB102771, AB1031270 | [34*] |
| *C. hominis monkey genotype*         | Monkeys     | SSU rRNA, HSP-70, actin, COWP | AF112569, AF221534, AF382342, AF266272 | [7**] |

(continued overleaf)
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* is increasingly becoming an important human pathogen instead of merely an avian pathogen [4,45]. 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. hominis* [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* [23,45]. 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 [23,45]. 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 [7,47], as the former would have minimal sequence variations at these loci.

| Table 1. (continued) |
|----------------------|
| **Cryptosporidium species and genotypes** | **Major hosts** | **Locus/loci examined** | **GenBank accession No.*** | **References** |
| **Opossum genotype I and II** | Opossums | SSU rRNA, HSP-70, actin | AY120902, AY120906, AY120916, AY120918, AY120921, AY120922, AY120924 | [7••] |
| **Rabbit genotype** | Rabbits | SSU rRNA, HSP-70, actin | AY120901, AY273775, AY120924 | [7••] |
| **Squirrel genotype** | Squirrels | SSU rRNA | AY120903, AY120917, AY120923 | [7••] |
| **Skunk genotype** | Skunks, raccoons | SSU rRNA, HSP-70, actin | AY120902, AY120906, AY120908, AY120910, AY120912, AY120914, AY120916, AY120918, AY120920, AY120922 | [7••] |
| **Reptiles/fish** | | | | |
| C. molnari | Fish | SSU rRNA, HSP-70, actin | AY382170, AF221540, AF392349 | [7••] |
| C. saurophilum | Lizards | SSU rRNA, HSP-70, actin | AF093502, AF221541, AF823539, AF266275 | [36] |
| C. serpentis | Snakes, lizards | SSU rRNA, HSP-70, actin | AF823539, 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; HSP-70, 70 kDa heat shock protein 70; COWP, *Cryptosporidium* oocyst wall protein; TRAP C1, thrombospondin-related adhesive protein 1 of *Cryptosporidium*; DHFR, dihydrofolate reductase. *Only representative sequences are quoted.*
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 autochthonous, 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

| 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              | 0                      | 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*        | 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*]            |

*Including samples from nine HIV– adults.
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 anthropogenic 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 zoonotic transmission 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, anthropogenic 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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- of outstanding interest

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