International Journal for Parasitology 30 (2000) 1305–1322

INTERNATIONAL Journal for PARASITOLOGY
www.parasitology-online.com

Epidemiology of *Cryptosporidium*: transmission, detection and identification

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Received 31 May 2000; received in revised form 28 August 2000; accepted 1 September 2000

Abstract

There are 10 valid species of *Cryptosporidium* and perhaps other cryptic species hidden under the umbrella of *Cryptosporidium parvum*. The oocyst stage is of primary importance for the dispersal, survival, and infectivity of the parasite and is of major importance for detection and identification. Because most oocysts measure 4–6 μm, appear nearly spherical, and have obscure internal structures, there are few or no morphometric features to differentiate species and in vitro cultivation does not provide differential data as for bacteria. Consequently, we rely on a combination of data from three tools: morphometrics, molecular techniques, and host specificity. Of 152 species of mammals reported to be infected with *C. parvum* or an indistinguishable organism, very few oocysts have ever been examined using more than one of these tools. This paper reviews the valid species of *Cryptosporidium*, their hosts and morphometrics; the reported hosts for the human pathogen, *C. parvum*; the mechanisms of transmission; the drinking water, recreational water, and food-borne outbreaks resulting from infection with *C. parvum*; and the microscopic, immunological, and molecular methods used to detect and identify species and genotypes. © 2000 Published by Elsevier Science Ltd. on behalf of the Australian Society for Parasitology Inc.

Keywords: *Cryptosporidium*; Transmission; Detection; Epidemiology; Genotype; Species

1. Introduction

The genus *Cryptosporidium* is classified as a eukaryote in the phylum Apicomplexa. All species of *Cryptosporidium* are obligate, intracellular, protozoan parasites that undergo endogenous development culminating in the production of an encysted stage discharged in the faeces of the host. For the majority of species in the phylum the oocyst stage is of primary importance for the dispersal, survival, and infectivity of the parasite. It is also the stage of major importance for detection and identification of the parasite. For genera like *Caryospora*, *Cyclospora*, *Eimeria*, *Isospora*, *Sarcocystis* and *Toxoplasma* biological characteristics (including host specificity) combined with the unique size and shape of the oocyst and its internal structure consisting of sporocysts and sporozoites often enable specialists to identify most species. Oocysts of most of these species range from 10 to 40 μm. Differences in shape or internal structure can be seen with the aid of a high resolution microscope. Although morphometrics are often a good tool, the difficulty in species identification comes when the size, shape or internal structures of oocysts of one species cannot be distinguished from those of another. Such is the case with the relatively small oocysts of *Cryptosporidium* species.

The first difficulty in proper identification of *Cryptosporidium* spp. is to distinguish oocysts from other small particles in faecal and environmental specimens such as yeasts, moulds, algae, and plant debris. Then, because most oocysts measure 4–6 μm, appear nearly spherical, and have obscure internal structures, there are few or no morphometric differences on which to differentiate species (Table 1). Although the wall of the oocyst contains antigens that may stimulate an antibody response in immunised animals and such antibodies can be labelled to aid identification of oocysts, many oocyst wall antigens are conserved within the genus *Cryptosporidium* and appear in several species. Consequently, there are no antibodies to reliably differentiate species. Comparison of enzymes and nucleic acids from sporozoites within oocysts have provided other tools to identify species and subspecies of *Cryptosporidium* (Table 2). However, classifying organisms based on subtle molecular differences

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PII: S0020-7519(00)00135-1

Table 1
Valid named species of *Cryptosporidium*

Species name	Type host	Primary location[a]	Size of viable oocysts (μm)
C. andersoni	*Bos taurus* (cattle)	A	7.4 × 5.5 (6.0–8.1 × 5.0–6.5)
C. baileyi	*Gallus gallus* (chicken)	BF, CL	6.2 × 4.6 (5.6–6.3 × 4.5–4.8)
C. felis	*Felis catis* (cat)	SI	4.6 × 4.0 (3.2–5.1 × 3.0–4.0)
C. meleagridis	*Meleagris gallopavo* (turkey)	SI	5.2 × 4.6 (4.5–6.0 × 4.2–5.3)
C. muris	*Mus musculus* (mouse)	ST	8.4 × 6.3 (7.5–9.8 × 5.5–7.0)
C. nasorum	*Naso lituratus* (fish)	ST, SI	4.3 × 3.3 (3.5–4.6 × 2.5–4.0)
C. parvum	*Mus musculus* (mouse)	SI	5.0 × 4.5 (4.5–5.4 × 4.2–5.0)
C. saurophilum	*Eumeces schneideri* (skink)	ST, SI	5.0 × 4.7 (4.4–5.6 × 4.2–5.2)
C. serpentis	Many species of reptiles	ST	6.2 × 5.3 (5.6–6.6 × 4.8–5.6)
C. wrairi	*Cavia porcellus* (guinea-pig)	SI	5.4 × 4.6 (4.8–5.6 × 4.0–5.0)

[a] A, abomasum; BF, bursa of Fabricius; CL, cloaca; ST, stomach; SI, small intestine. Based on electron microscopy.

has not been without complications. Is the difference in an enzyme structure or in one or a few base pairs in a single gene containing over a 1000 base pairs sufficient to differentiate species? Classical biological characteristics such as host specificity, used to aid in identifying other species of Apicomplexa, has been helpful in the genus *Cryptosporidium* but such determinations are expensive and time consuming. Furthermore, they require both a significant quantity of oocysts and a variety of potentially susceptible host species with appropriate facilities to maintain them, and both must be available at the same time. The present dilemma associated with detecting, identifying and naming species of *Cryptosporidium* is that we must rely on a combination of data from all three tools: morphometrics, molecular techniques, and host specificity. Of the 152 species of mammals reported to be infected with *Cryptosporidium parvum* or a *C. parvum*-like organism very few oocysts have ever been examined using more than one of these tools (Table 3). Until we can clearly identify and confirm species or subspecies, the epidemiology and host range of an isolate will remain presumptive, imprecise, or inaccurate. Within the aforementioned limits, the goal of this paper is to identify species of *Cryptosporidium*, including genotypes of *C. parvum*, and review the hosts, outbreaks affecting humans, patterns of transmission, and methods of identification of *C. parvum*.

2. Distribution and prevalence of infections in humans

Human infection with *Cryptosporidium*, first reported in two cases in 1976 and a further 11 cases over the next 6 years has now been reported from over 90 countries on six continents [1]. Most data come from outbreaks or individual cases reported in scientific or medical journals. Except for outbreaks, most specimens in developed countries were submitted to diagnostic laboratories from persons with gastrointestinal illness. Estimates from United States public health records suggest that ~2% of all stools tested by health care providers are positive for *Cryptosporidium* [2]. Estimating ~15 million annual visits for diarrhoea, infection with *Cryptosporidium* might be expected in 300 000 persons annually; a figure 45 times higher than estimates based on FoodNet surveillance [2]. Indeed, CDC surveillance summaries for water-borne and food-borne disease outbreaks reported only one outbreak of cryptosporidiosis over 5 and 3 years, respectively [3,4]. The authors state that data in their reports should be interpreted with caution because the number of cases reported represent only a fraction of the total that occur. Surveys in developing countries find a higher prevalence of infection than in industrialised countries [1]. Better sanitation and cleaner drinking water in the more industrialised countries probably account most for this difference. Within these large populations are specific groups at greater risk of infection including children, malnourished

Table 2
Genotypes/cryptic species of *C. parvum*

Genotype	Loci examined	Immunocompetent host range
Cattle	18S rRNA, AcetylCoA, β-tubulin, COWP, Cp15, Cp 11, dhfr, hsp70, ITS1, 5.8S, ITS2 rRNA, poly(T), RNR, TRAPC1, TRAPC2, microsatellite loci	Artiodactyls, domestic animals, human
Human/monkey	18S rRNA, AcetylCoA, β-tubulin, COWP, Cp15, Cp 11, dhfr, hsp70, ITS1, 5.8S, ITS2 rRNA, poly(T), RNR, TRAPC1, TRAPC2, microsatellite loci	Human, dugong
Mouse	18S rRNA, AcetylCoA, COWP, dhfr, hsp70, ITS1, 5.8S, ITS2 rRNA/monkey	Mouse, large-footed mouse-eared bat
Pig	18S rRNA, COWP, dhfr, hsp70, ITS1, 5.8S, ITS2, rRNA	Pig
Marsupial	18S rRNA, dhfr, hsp70, ITS1, 5.8S, ITS2 rRNA	Koala, kangaroo
Dog	18S rRNA, hsp70	Dog
Ferret	18S rRNA, hsp70	Ferret

Table 3
Cryptosporidium parvum (and *C. parvum*-like) checklist of 152 mammalian hosts with citations for their first report

Order: Artiodactyla	
Addax nasomaculatus (addax)	[167]
Aepyceros melampus (impala)	[168]
Ammotragus lervia (Barbary sheep)	[169]
Antidorcas marsupialis (springbok)	[168]
Antilope cervicapra (blackbuck)	[167]
Axis axis (axis deer)	[168]
Bison bison (American bison)	[170]
Bison bonasus(European bison)	[171]
Bos indicus (zebu)	[172]
Bos taurus (ox)	[173]
Boselaphus tragocamelus (nilgai)	[168]
Bubalus bubalis (water buffalo)	[174]
Bubalus depressicornis (lowland anoa)	[170]
Camelus bactrianus(bactrian camel)	[175]
Capra falconeri (turkomen markhor)	[168]
Capra hircus (goat)	[176]
Capreolus capreolus (roe deer)	[177]
Cervus albirostris (Thorold's deer)	[178]
Cervus duvauceli (Barasingha deer)	[168]
Cervus elaphus (red deer/elk/wapiti)	[179]
Cervus eldi (Eld's deer)	[168]
Cervus nippon (Sika deer)	[168]
Cervus unicolor (sambar)	[170]
Connochaetes gnou (wildebeest)	[180]
Connochaetes taurinus (blue-beard gnu)	[181]
Dama dama (fallow deer)	[168]
Elaphus davidianus(Pere David's deer)	[171]
Gazella dama (Addra gazelle)	[168]
Gazella dorcas (Dorca's gazelle)	[181]
Gazella leptoceros (slender-horned gazelle)	[168]
Gazella subgutterosa (Persian gazelle)	[182]
Gazella thomsoni (Thomson's gazelle)	[183]
Giraffa camelopardalis (giraffe)	[181]
Hexaprotodom liberiensis (pygmy hippopotamus)	[170]
Hippotragus niger (sable antelope)	[167]
Kobus ellipsiprymmus (ellipsen waterbuck)	[181]
Lama glama (llama)	[184]
Lama guanicoae (guanaco)	[170]
Lama pacos (alpaca)	[185]
Muntiacus reevesi (muntjac deer)	[186]
Odocoileus hemionus (mule deer)	[168]
Odocoileus virginianus (white-tailed deer)	[187]
Oryx gazella callotys (fringe-eared oryx)	[167]
Oryx gazella dammah (scimitar-horned oryx)	[167]
Ovis aries (sheep)	[188]
Ovis musimon (mouflon)	[170]
Ovis orientalis (urial)	[189]
Sus scrofa (pig)	[190]
Syncerus caffer (African buffalo)	[181]
Taurotragus oryx (eland)	[168]
Tayassu tajacu (collared peccary)	[170]
Tragelaphus euryceros (bongo)	[170]
Order: Carnivora	
Acironyx jubatus (cheetah)	[191]
Canis familiaris (dog)	[192]
Canis latrans (coyote)	[187]
Felis catus (cat)	[193]
Helarctos malayanus (Malayan bear)	[194]
Martes foina (beech marten)	[195]
Meles meles (badger)	[186]
Mephitis mephitis (striped skunk)	[187]
Order: Artiodactyla	
Mustela putorius (ferret)	[196]
Panthera pardus (leopard)	[194]
Procyon lotor (raccoon)	[197]
Urocyon cinereoargenteus (grey fox)	[187]
Ursus americanus (black bear)	[187]
Ursus arctos(brown bear)	[191]
Ursus (Thalarctos) maritimus(polar bear)	[191]
Vulpes vulpes (red fox)	[187]
Zalophus californianus (California sea lion)	[292]
Order: Chiroptera	
Eptesicus fuscus (big brown bat)	[198]
Myotis adversus (large-footed mouse-eared bat)	[158]
Order: Insectivora	
Ateletrix albiventris (African hedgehog)	[199]
Erinaceus europaeus (European hedgehog)	[171]
Sorex araneus (long-tailed shrew)	[200]
Sorex minutus (pygmy shrew)	[186]
Order: Lagomorpha	
Oryctolagus cuniculus (rabbit)	[201]
Sylvilagus floridanus (cottontail)	[202]
Order: Marsupialia	
Antechinus stuartii (brown antechinus)	[203]
Didelphis virginiana (Opossum)	[204]
Isodon obesulus (southern brown bandicoot)	[161]
Macropus giganteus(eastern grey kangaroo)	[171]
Macropus rufogriseus (red neck wallaby)	[171]
Macropus rufus (red kangaroo)	[161]
Phascolarctos cinereus (koala)	[161]
Thylogale billardierii (pademelon)	[161]
Trichosurus vulpecula (brushtail possum)	[205]
Order: Monotremata	
Tacyglossus aculeatus (echidna)	[161]
Order: Perissodactyla	
Ceratotherium simum (southern white rhinoceros)	[181]
Equus caballus (horse)	[206]
Equus przewalski (miniature horse)	[194]
Equus zebra (zebra)	[180]
Rhinoceros unicornis (rhinoceros)	[194]
Tapirus terrestris (Brazilian tapir)	[170]
Order: Primates	
Ateles belzebuth (Marimonda spider monkey)	[207]
Calithrix jacchus (common marmoset)	[208]
Cercocebus albigena (mangabey)	[207]
Cercocebus torquatus(white-collared monkey)	[207]
Cercopithecus aethiops (velvet monkey)	[207]
Cercopithecus campbelli (Campbell's mona)	[207]
Cercopithecus talapoin (Talapoin monkey)	[207]
Erythrocebus patas (Patas monkey)	[207]
Eulemur macaco (black lemur)	[170]
Gorilla gorilla (gorilla)	[209]
Homo sapiens (human)	[210]
Hylobates syndactylus syndactylus (siamang)	[170]
Lemur catta (ring-tailed lemur)	[170]
Lemur macacomayottensis (brown lemur)	[207]
Lemur variegatus (ruffed lemur)	[170]
Macaca fascicularis (long-tailed macaque)	[211]
Macaca fuscata (Japanese macaque)	[212]
Macaca mulatta (rhesus monkey)	[213]

Table 3 (*continued*)

Order: Artiodactyla	
Macaca nemestrina (cotton-tipped/pigtail macaque)	[214]
Macaca radiata (Bonnet macaque)	[211]
Macaca thibetana (Pere David's macaque)	[170]
Mandrillus leucophaeus (drill)	[170]
Nycticebus pygmaeus (lesser slow loris)	[207]
Papio anubis (olive baboon)	[215]
Papio cynocephalus (baboon)	[212]
Pithecia pithecia (white-faced saki)	[170]
Pongo pygmaeus (orangutan)	[194]
Saguinus oedipus (cotton-topped tamarin)	[168]
Saimiri sciureus (squirrel monkey)	[216]
Varecia variegata (red-ruffed lemur)	[168]
Order: Proboscidea	
Elephas maximus (Indian elephant)	[178]
Loxodonta africana (African elephant)	[170]
Order: Rodentia	
Apodemus agrarius (field mouse)	[217]
Apodemus flavicollis (field mouse)	[218]
Apodemus sylvaticus (field mouse)	[219]
Castor canadensis (beaver)	[220]
Castor fiber (European beaver)	[217]
Cavia porcellus (guinea-pig)	[221]
Chinchilla laniger (chinchilla)	[222]
Clethrionomys glareolus (red-backed vole)	[218]
Geomys bursarius (pocket gopher)	[187]
Glaucomys volans (flying squirrel)	[187]
Hystrix indica (Indian porcupine)	[171]
Marmota monax (woodchuck)	[187]
Mesocricetus auratus (golden hamster)	[223]
Microtus agrestis (field vole)	[224]
Microtus arvalis (Orkney vole)	[225]
Mus musculus (house mouse)	[201]
Myocastor coypus (coypu)	[226]
Ondatra zibethicus (muskrat)	[187]
Rattus norvegicus (Norwegian rat)	[227]
Rattus rattus (house rat)	[228]
Sciurus carolinensis (grey squirrel)	[229]
Sciurus niger (fox squirrel)	[187]
Sigmodon hispidus (cotton rat)	[230]
Spermophilus tridecemlineatus (13-lined ground squirrel)	[187]
Tamias sibiricus (Siberian chipmunk)	[293]
Tamias striatus (chipmunk)	[187]
Order: Sirenia	
Dugong dugon (dugong)	[231]

persons, and a range of immunocompromised individuals including AIDS patients, transplant recipients, patients receiving chemotherapy for cancer, institutionalised patients, and patients with immunosuppressive infectious diseases.

3. Transmission

The oocyst is the stage transmitted from an infected host to a susceptible host by the faecal-oral route. Routes of transmission can be (1) person-to-person through direct or indirect contact, possibly including sexual activities, (2) animal-to-animal, (3) animal-to-human, (4) water-borne through drinking water or recreational water, (5) food-borne, and (6) possibly airborne. To determine how many oocysts of *C. parvum* were required for seronegative healthy persons to become infected, 29 volunteers ingested a single dose of 30 to 1 million oocysts from a calf [5]. After ingesting 30 oocysts, one of five persons became infected. After ingesting 1000 or more oocysts seven of seven became infected. The median infective dose (ID_{50}) was calculated to be 132 oocysts. With further data the ID_{50} was recalculated to be 87 oocysts and different isolates of *C. parvum* were found to have highly different ID_{50} values [6]. The ID_{50} in human volunteers ranged from 9 to 1042 oocysts for TAMU and UCP isolates, respectively [6].

3.1. Oocyst survival: Effect of temperature and desiccation

Oocysts of *C. parvum* can remain viable for many months. When held at 20°C for 6 months many oocysts were still infectious for suckling mice [7]. Higher temperatures result in more rapid loss of viability. Some oocysts held at 25 and 30°C were infectious only to 3 months. Warming oocysts from 9 to 55°C over 20 min resulted in loss of infectivity for suckling mice [8]. Oocysts held at 59.7°C for 5 min had very low infectivity [9] and others held at 71.7°C for only 5 s were killed [10].

Freezing kills oocysts. Snap freezing and programmed freezing to −70°C resulted in immediate killing of *C. parvum* oocysts even in the presence of a variety of cryoprotectants [11,12]. At higher temperatures oocysts survive longer, some oocysts held at −20°C were viable for up to 8 h, but not at 24 h [12]. Some oocysts held at −10°C were infectious for mice up to 1 week after storage, whereas those held at −5°C remained viable for up to 2 months [7,12]. These findings suggest that fluids within oocysts offer minimal cryoprotection to the sporozoites.

Desiccation is lethal to oocysts. Only 3% of oocysts were found viable after 2 h of desiccation and 100% killing was reported at 4 h [11,13].

3.2. Mechanisms of transmission

Faeces deposited on the ground is subjected to wind and water transporting oocysts across or through soil. In some cases humans and animals contribute to the movement of oocysts. To initiate infection, oocysts must be ingested with food, water, or by close personal contact with infected people, animals or contaminated surfaces.

3.2.1. Mechanical transport across and through soil and transport hosts

Faecal contamination of soil and surface water can ultimately lead to contamination of fresh foods, drinking water, and recreational water. Although oocysts can be detected in soil [14], their movement from faeces on land surfaces to surface and ground water has received little attention. A greenhouse soil tilting table was used to detect movement of *C. parvum* oocysts in a variety of soil types [15,16]. Oocysts in livestock faeces were applied to soil blocks which were then intermittently irrigated. Oocysts moved within the soil for several weeks, and in some cases for over 70 days. Most oocysts were found within the upper 2 cm of soil, the numbers decreasing with increasing depth. Some were recovered at 30 cm but none were recovered at 70 cm.

When oocysts of *Cryptosporidium* were isolated from gulls in the United Kingdom it was not known if the oocysts were *C. parvum* or an avian species, but the investigators postulated that oocysts could be distributed by birds over wide areas [17]. Subsequently, it was shown that *C. parvum* oocysts ingested by Canada geese (*Branta canadensis*) and Peking ducks (*Anas platyrhynchos*) passed through the gastrointestinal tract, were excreted in the faeces for nearly 1 week, and were capable of infecting mice [18,19]. Later, viable oocysts of *C. parvum* were recovered from faeces of Canada geese in fields where they rested along their migration route [20].

What appeared to be oocysts of *C. parvum* were found in the intestinal tracts of cockroaches (*Periplaneta americana*) collected in the household where a child had cryptosporidiosis, suggesting that roaches had a role in disseminating the parasite [21]. House flies, exposed under laboratory conditions to bovine faeces containing oocysts of *C. parvum* and wild filth flies trapped in a barn where a calf had cryptosporidiosis, had oocysts both in their faeces and on their external surfaces [22,23]. Although most oocysts of *C. parvum* ingested by dung beetles were destroyed by digestion, some passed through the intestinal tract and appeared morphologically normal in beetle faeces [24]. Oocysts also were recovered from the external surfaces of beetles, suggesting they may be capable of disseminating oocysts in the environment. Six genera of rotifers (microscopic invertebrates found worldwide in lakes, ponds, puddles, moss, damp soil, or virtually anywhere water can accumulate) were observed ingesting oocysts of *C. parvum*; it was not determined whether oocysts were digested or rendered nonviable [25].

3.2.2. Transmission via drinking water

Positive findings of oocysts in untreated wastewater, filtered secondarily treated wastewater, activated sludge effluent, combined sewer overflows, groundwater, surface water, and treated drinking water indicate widespread faecal contamination [26,27]. Numerous reports worldwide provide strong circumstantial evidence that contaminated water is a high risk factor for cryptosporidiosis [28]. Contamination of surface source waters in North America has been reported from many studies. Representative of these are the studies [29,30] in which the same sites were revisited after a 4-year interval and it was found that 89 and 45%, respectively, of all samples were positive for oocysts of *C. parvum*. In drinking water treatment plants using conventional filtration, a summary of studies indicated that oocysts were found in finished water 3.8–33.3% of the time at concentrations from 0.1 to 48 oocysts per 100 l [27]. These levels represent daily exposure to persons using filter purified tap water in the USA. In the USA in 1988 surface water was used by over 155 million people in 6000 community water systems of which 23% provided unfiltered water to 21 million people and protection from infectious agents relied solely on disinfection [31]. Unless source water is protected, higher exposure levels might be expected at households served by systems providing unfiltered water. What is not well documented about *Cryptosporidium* is the viability, species, and source of the oocysts found in tap water. Because identification of species of oocysts in water is not routine, the public health significance of oocysts found in water is unclear. Although water-borne infections in individuals are difficult to document, outbreaks of cryptosporidiosis linked to drinking water (Table 4) clearly confirm that viable *C. parvum* oocysts enter and pass through drinking water purification processes. Nonviable oocysts of *C. parvum* and other species also may be present in source water and finished water.

The first reported water-borne outbreak of cryptosporidiosis, confirmed by stools and serologic tests, was in the summer of 1984 in Braun Station, a suburb of ~5900 persons 32 km from San Antonio, Texas [32]. Diarrhoea was the major symptom. A telephone survey of 100 homes identified an attack rate of 34%. Potable, unfiltered artesian well water supplied to all 1791 homes was contaminated with faecal coliforms. Dye introduced into the community sewage system appeared in the well water.

In 1987 an outbreak, first recognised as a dramatic increase in gastroenteritis among college students, affected ~13 000 of 64 900 residents in Carroll County, GA, USA [33]. Oocysts were identified in treated water from the water treatment plant, dead water mains, and streams above the plant. Dye added to a sewage overflow caused by a blocked sewer line above the treatment plant reached the plant within 6 h. Within the plant, failures included removal of mechanical agitators from the flocculation basins, impaired filtration, and use of filters that were not being back washed.

In 1993 ~403 000 out of ~1 610 000 people in the greater Milwaukee, WI area experienced the largest documented water-borne disease outbreak in US history [34]. An epidemiologic investigation began after the health department was notified of gastrointestinal illness causing high absenteeism of hospital employees, students, and teachers. Within 4 days, oocysts were identified in residents' stools, treated water from one of the two water treatment plants was found highly turbid, a boil water advisory was issued, and that plant was closed. Oocysts were identified in ice made before and during the outbreak. Oocysts from Lake Michigan water apparently entered the southern treatment plant. Possibly, inadequate amounts of polyaluminium chloride or alum coagulant failed to reduce the high turbidity, and recycling of filter backwash water may have increased the number of oocysts in the finished water. Heavy rains, cattle manure on fields in the watershed, abattoir waste, and sewage overflow were considered potential sources. However, after oocysts from four affected persons failed to infect animals and were identified genetically to be of human origin, the probable source was sewage overflow [35].

Cattle (and sheep) are repeatedly implicated as sources of water-borne outbreaks outside the United States but have not been conclusively identified (by genotyping) as the source of any water-borne outbreak within the United States [35]. The outbreak in Cranbrook, BC is the only water-borne outbreak in North America in which oocysts of the bovine genotype have been identified [35].

Many other water-borne outbreaks have been documented with patterns similar to those above (Table 4). Most epidemiologic investigations have detected a combination of causes including contaminated source water, high turbidity, and failures at the treatment plant.

3.2.3. Food-borne transmission

Reports of food related outbreaks are few, difficult to document, and greatly under-reported (Table 4). Individual cases and small group outbreaks are less likely to be recognised.

Oocysts of *Cryptosporidium* were found in sea water near a sewage outfall site in Honolulu, Hawaii [26] and in estuarine waters in the Chesapeake Bay [36]. Molluscan shellfish filter large quantities of water, extract tiny particles that remain on their gills and thereby make excellent biological indicators of water-borne pathogens. Oocysts of *C. parvum* have been detected in oysters, clams, and mussels collected from the Chesapeake Bay [37,38], in mussels from the coast of Ireland [39], and in oysters from Galicia, Spain [40]. Although none of these findings were associated with outbreaks of cryptosporidiosis, repeated outbreaks of viral and bacterial illness associated with ingestion of raw shellfish should serve as a warning that cooking of shellfish will reduce the risk of illness from all these pathogens.

Oocysts have been found on the surface of raw vegetables from the market place. Cool, moist vegetables provide an optimal environment for survival. In Costa Rica oocysts were found on cilantro leaves and roots, lettuce, radishes, tomatoes, cucumbers, and carrots but not cabbage [41]. In a suburban slum of Lima, Peru, basil, cabbage, celery, cilantro, green onions, ground green chilli, leeks, lettuce, parsley, and yerba buena from several markets were contaminated with oocysts of *C. parvum* [42]. Vegetables can be contaminated from fertiliser of animal or human faeces; by contaminated water used to irrigate or moisten produce; by soiled hands of farm workers, produce handlers, or food workers; and from contaminated surfaces where vegetables are packed, stored, sold or prepared. Detecting oocysts washed from foods is difficult. Although only 1% of oocysts experimentally added to fruit and vegetables were recovered [43] molecular methods to detect and identify small numbers of oocysts are becoming more important with increasing international trade in fresh produce.

A cryptosporidiosis outbreak involving 50 school children was associated with milk from a local, small-scale producer in the United Kingdom using an on-farm pasteuriser [44]. Environmental health officers responding to a complaint of dirt in the milk found the pasteuriser was not working properly at the time of the outbreak. Outbreaks were associated with drinking fresh-pressed apple juice (non-alcoholic cider). In Maine, USA, apples from the ground near a cattle pasture were used for cider at an agricultural fair; 160 attendees developed cryptosporidiosis [45]. Oocysts from the attendees had genotype characteristics implicating a bovine source [35]. In New York, apples for cider may have been washed with well water contaminated with faeces. In both outbreaks cider was not pasteurised.

In Minnesota, chicken salad was associated with an outbreak among 50 people attending a social event [46]. The caterer changed a baby's diaper in her home day-care facility and later prepared chicken salad for that social event. In Spokane, Washington, 54 of 62 persons who attended a catered banquet became ill 3–9 days later [47]. The buffet of 18 foods and beverages contained seven uncooked produce items. Food eaten by 51 affected persons contained uncooked green onions. Between 3–4 weeks after the banquet 2 of 14 food preparers were positive for *Cryptosporidium*; one was symptomatic at the time of the banquet. Similarly, 88 students and four cafeteria employees were diagnosed with cryptosporidiosis at a university in Washington, DC [48]. A prep cook who cut up vegetables and fruit to be eaten raw was ill for 10 days beginning ~3 days before the implicated meal and may have acquired infection from a child with diarrhoea in his family. Restriction fragment length polymorphism (RFLP) analysis of polymerase chain reaction (PCR) products and DNA sequencing showed that all positive specimens were the human genotype, all were identical, and were linked to the food handler.

These outbreaks highlight important issues. Food handlers should thoroughly wash their hands before handling food items and utensils. Raw fruits and vegetables as well as previously cooked items should not be handled with bare hands. Uncooked produce should be thoroughly washed before being placed on kitchen surfaces. Food preparation surfaces should be washed between preparations. Food workers should not work when experiencing gastrointestinal illness.

3.2.4. Transmission via recreational water

Swimming is a very popular recreational activity worldwide. There are over 350 million person-events annually in the United States alone [49]. In the past 12 years reported outbreaks of cryptosporidiosis related to recreational waters affected over 10 000 people (Table 5). Frequent faecal contamination coupled with oocyst resistance to chlorine

Table 4
Outbreaks related to food and drinking water

Year	Locality	Estimated no. of cases	Suspected cause	Key references
1984	Braun Station, TX	2006	Sewage contaminated artesian well	[32]
1986	Great Yarmouth, UK	36	Unknown	[232]
1987	Carrollton, GA	12960	Treatment deficiencies of river water	[33]
1988	Ayrshire, UK	27	Treatment deficiencies of spring water	[233]
1989	Swindon/Oxfordshire, UK	516	Treatment deficiencies of river water	[234]
1990	Loch Lomond, UK	442	Treatment deficiencies of loch water	[235]
1990–91	Isle of Thanet, UK	47	Treatment deficiencies of river water	[236]
1991	South London, UK	44	Treatment deficiencies of tap water	[237]
1991	Berks County, PA	551	Treatment deficiencies of well water	[238]
1992	South Devon, UK	?	Contaminated drinking water	[239]
1992	North-west UK	42	Contaminated drinking water	[240]
1992	North-west UK	63	Contaminated drinking water	[240]
1992	South-west UK	108	Contaminated drinking water	[240]
1992	Jackson County, OR	15000	Treatment deficiencies of spring/river	[238,241,242]
1992	Yorkshire, UK	125	Contaminated tap water	[240]
1992	Mersey, UK	47	Contaminated tap water	[240]
1992	Bradford, UK	125	Contaminated tap water	[243]
1992–93	Warrington, UK	47	Contaminated tap water	[244]
1993	Milwaukee, WI	403000	Treatment deficiencies of lake water	[34,35,245–254]
1993	Waterloo, Canada	>1000	Contaminated tap water	[27]
1993	Las Vegas, NV	103	Unknown; perhaps tap water	[255]
1993	Wessex, UK	40	Contaminated tap water	[240]
1993	Northern UK	5	Contaminated water at university	[240]
1993	Yorkshire, UK	97	Contaminated tap water	[240]
1993	Wessex, UK	27	Contaminated tap water	[240]
1993	Central Maine	>150	Contaminated apple cider	[45]
1994	Kanagawa, Japan	461	Contaminated drinking water	[256]
1994	Walla Walla, WA	104	Sewage contaminated well	[257]
1994	SW Thames, Wessex, Oxford, UK	224	Contaminated tap water	[240]
1994	Trent, UK	33	Contaminated tap water (?)	[240]
1995	Gainesville, FL	77	Contaminated tap water at day camp	[258,259]
1995	Torbay, Devon, UK	575	Non flocculated river water	[156,239,260]
1995	Northern Italy	294	Community water tanks	[261,262]
1995	South-west UK	575	Contaminated tap water	[240]
1995	Ireland	13	Playing in sand during farm visit	[263,264]
1995	Minnesota	50	Contaminated chicken salad	[44]
1996	Eagle Harbor, FL	16	Unknown	[265,266]
1996	Kelowna, BC	~ 14500	Unfiltered water from lake	[267–269]
1996	Cranbrook, BC	~ 2097	Unfiltered water from reservoir	[270]
1996	Ogose, Japan	>9000	Unfiltered spring and ground water	[271,272]
1996	Northern England, UK	~ 126	Contaminated drinking water	[273]
1996	Yorkshire, UK	20	Contaminated drinking water	[273]
1996	North-western England, UK	?	Contaminated drinking water	[273]
1996	New York	>30	Contaminated apple cider	[274–276]
1996	Collingwood, Ontario	~ 182	Unfiltered municipal water	[277]
1997	Shoal Lake, Ontario	~ 100	Unfiltered lake water	[278]
1997	North Thames, UK	345	Filtered borehole water	[135,279–281]
1997	England and Wales, UK	>4321	Multiple outbreaks and causes	[282,283]
1998	Chilliwack, BC	25–30	Unknown	[284]
1998	Brushy Creek, TX	32	Sewage contamination of creek/wells	[285]
1998	Spokane, WA	~ 54	Unknown banquet food	[286,287]
1999	Hawke's Bay, New Zealand	20	Unknown	[288]
1999	North Island, New Zealand		Unknown	[289]
1999	North-west England, UK	~ 360	Unfiltered surface water	[290,291]

[50], low infectious dose, and high bather densities have facilitated transmission. Even optimal conditions of pool design, water quality, filtration, and disinfection cannot prevent faecal accidents. However, routine use of recreational waters by incontinent persons, including diapered children and toddlers, increases the potential for water-

Table 5
Outbreaks of cryptosporidiosis related to use of recreational water facilities modified from Ref. [31]

Recreational facility	Location	Disinfectant	No. of cases (estimated/confirmed)	Date (year)
Lake	Albuquerque, NM	None	56/[b]	1986
Pool	Doncaster, UK	Chlorine	[b]/79	1988
Pool	Los Angeles county	Chlorine	44/5	1988
Pool	British Columbia, Canada	Chlorine	66/23	1990
Pool	Gloucestershire, UK	Ozone/chlorine	[b]/13	1992
Water slide	Idaho	Chlorine	500/[b]	1992
Pool (wave)	Oregon	Chlorine	[b]/52	1992
Pool (motel)	Wisconsin	Chlorine	51/22	1993
Pool (motel)	Wisconsin	Chlorine	64[b]	1993
Pool	Wisconsin	Chlorine	5[b]	1993
Pool	Wisconsin	Chlorine	54[b]	1993
Pool (motel)	Missouri	Chlorine	101/26	1994
Lake	New Jersey	None	2070/46	1994
Pool	Sutherland, Australia	Chlorine	[b]/70	1994
Pool	Kansas	[a]	101/2	1995
Water park	Georgia	Chlorine	2470/6	1995
Water park	Nebraska	[a]	[b]/14	1995
Pool	Florida	[a]	22/16	1996
Water park	California	Chlorine	3000/29	1996
Pool	Andover, UK	Chlorine	8/[b]	1996
Lake	Indiana	None	3/[b]	1996
River	North-west England and Wales, UK	None	27/7	1997
Pool	South-west England and Wales, UK	Ozone and chlorine	[b]/9	1997
Fountain	Minnesota	Sand filter	369/73	1997
3 Pools	Canberra, Australia	[a]	[b]/210	1998
Pool	Oregon	[a]	51/8	1998
Pools	Queensland, Australia	[a]	129/[b]	1997
Pools	New South Wales, Australia	[a]	370/[b]	1998
Pools	Hutt Valley, New Zealand	[a]	[b]/171	1998

[a] No data available.
[b] Reference did not identify cases as estimated or confirmed.

borne transmission. Recognition of cryptosporidiosis as a major cause of recreational water-borne disease necessitates public health officials, pool operators, and users to collaborate in developing plans to reduce the risk of water-borne transmission. Plans should include engineering changes such as improved filtration and turnover rates, separation of plumbing/ filtration for high risk 'kiddie' pools. Pool policies should: establish specific response actions to faecal accidents, test effectiveness of barrier garments such as swim diapers, and educate both patrons and staff. Education should stress water-borne disease transmission and suggest simple prevention measures such as refraining from water related recreational activities during a current or recent diarrhoeal episode, refraining from swallowing recreational water, using good diaper changing and hand washing practices, frequent bathroom breaks for young children, and promoting showers to remove faecal residue before pool use.

3.2.5. Sexual transmission

A series of reports convincingly suggested but were unable to confirm cryptosporidiosis acquired by sexual transmission. Data comparing HIV/AIDS patients, homosexual men and intravenous drug users, showed a higher prevalence of cryptosporidiosis in homosexual men [51]. However, the possibility of transmission related to other behaviours could not be ruled out.

3.2.6. Airborne transmission

Although there have been no proven cases of airborne transmission in humans the concept was theorised by investigators in 1987 [52]. There are, however, numerous reports of high rates of cough or other pulmonary symptoms in children and immune compromised persons with cryptosporidiosis [51]. Although lethal respiratory cryptosporidiosis has been reported for persons with AIDS, malignant lymphoma, and bone marrow transplantation, the occurrence of respiratory cryptosporidiosis rarely reported. A summary of the anatomical distribution of *Cryptosporidium* in naturally infected birds [53] suggests that chickens, turkeys, quail, ducks, pheasant, peafowl and budgerigars apparently acquire respiratory infections with species of avian *Cryptosporidium* more frequently than mammals acquire such infections.

4. Detection and identification

4.1. Detection methods

4.1.1. Microscopic staining methods

Conventional detection methods include concentration and staining of faecal smears [54–69]. Differential staining methods including safranin-methylene blue stain [70], Kinyoun [71], Ziehl-Neelsen [55] and DMSO-carbol fuchsin [60] stain oocysts red and counterstain the background. Differential staining, however, is time consuming and varies in sensitivity and specificity [67,70,72]. Fluorochrome stains [73,74], although sensitive, are complex and oocyst-like structures in faecal debris often take up the stain. Negative staining techniques with nigrosin [59], light green, merbromide [66] and malachite green [75] stain background yeasts and bacteria but not oocysts. Many of these stains require an experienced microscopist, however, and are labour-intensive.

4.1.2. Immunological-based detection methods

Immunological-based techniques including polyclonal fluorescent antibody tests [76], latex agglutination reactions [77] immunofluorescence (IF) with monoclonal antibodies (mAbs) [78–83], enzyme-linked immunosorbent assays (ELISA) [84–90], reverse passive haemagglutination (RPH) [91] immunoserology using IF detection [92] and ELISA [28,93,94], and solid-phase qualitative immunochromatographic assays [95] have been developed for the detection of cryptosporidiosis. Non-specificity of antibody-based methods due to cross-reactivity with other microorganisms can be problematic. For example, in the study of gill washings and haemolymph from oysters that harboured oocysts of *Cryptosporidium* [37] a variety of organisms and particulate material of many sizes and shapes were observed that fluoresced as brightly as the oocysts.

4.1.3. Concentration techniques for detection of oocysts in water

Before oocysts can be detected in water they must be concentrated using methods such as continuous flow centrifugation, membrane filtration, calcium carbonate flocculation, Envirochek (Gelman) cartridge filters and polycarbonate track etch membrane systems (Corning Costar) [96]. Concentrated oocysts can then be separated from accompanying debris by density gradient centrifugation or immunomagnetic bead separation (IMS). Recovery rates are affected by many factors including turbidity and other physical-chemical properties of the water, antibody reactivity with other micro-organisms, removal from filters, and loss during centrifugation [96–99]. Following concentration from water, most conventional detection methods have relied on microscopy of chemically or immunologically stained specimens. The difficulties in these methods comes from the inability to distinguish *C. parvum* from *Cryptosporidium* species not of public health significance and to distinguish live from dead oocysts.

4.1.4. Molecular techniques

A variety of PCR tests offer alternatives to conventional diagnosis of *Cryptosporidium* for both clinical and environmental specimens [97,100–105]. Although PCR is rapid, highly sensitive, and accurate, it has several limitations. False positives can result from detection of naked nucleic acids, non-viable microorganisms, and laboratory contamination. Some environmental contaminants interfere with qualitative and/or quantitative assays [106]. For routine acceptance of PCR as a diagnostic tool, interference must be overcome, and a standardised, reliable method of recovering oocysts from water supplies must be developed.

4.1.5. Techniques to determine oocyst infectivity and viability

A reliable indicator of oocyst infectivity is needed to differentiate potentially infectious from non-infectious oocysts and for valid disinfection studies [96]. Vital dyes such as propidium iodide (PI: not membrane permeant) and 4, 6, diamidino-2′-phenylindole (DAPI, membrane permeant), as indicators of viability, once reported to correlate well with in vitro excystation [107], have been reported to significantly overestimate oocyst viability [108]. In vitro excystation is not an accurate measure of viability or infectious potential [109]. Oocysts that failed to excyst in vitro were found infectious in vivo [109]. Furthermore, sporozoites can excyst from oocysts and appear viable but are not infectious. Sporozoites depleted of amylopectin (polysaccharide required for energy) lacked infectivity in vivo [110]. Reverse transcriptase (RT)–PCR demonstrated that the quantity of amyloglucosidase correlated with infectivity [111]. Other molecular tests for viability include fluorescent in situ hybridisation (FISH) [112] and cell culture followed by RT–PCR [113,114].

4.2. Identification: molecular epidemiology of *Cryptosporidium parvum*

Isolates of *C. parvum* possess different antigens [115–118], virulence, infectivity, and drug sensitivity [119–121]. An important advantage of molecular techniques is that they allow not only for accurate and sensitive detection of *Cryptosporidium* but also provide information on genetic variability of isolates of *Cryptosporidium*. Recent molecular evidence has demonstrated that *C. parvum* is not a uniform species, but consists of several distinct genotypes or cryptic species.

4.2.1. *Cryptosporidium* in humans and domestic livestock - 'human' and 'cattle' genotypes

Genetic and biological studies indicate at least two genotypes of *Cryptosporidium* infecting humans: a human genotype found only in humans, and a zoonotic cattle genotype found in animals such as cattle, sheep, goats etc. as well as humans [35,42,104,122–149]. The latter is infectious for other animals such as laboratory rodents also [35,151].

Genetic diversity in human and animal isolates of *C. parvum* was clearly identified by isoenzyme analysis; zoonotically transmitted isolates from humans exhibited animal profiles [122,123,151]. Differences between these isolates were confirmed by random amplified polymorphic DNA (RAPD) analysis [125,126,152,153]. Because few oocysts are usually recovered from environmental and faecal specimens and these contain contaminants, most genetic studies use parasite-specific PCR primers to overcome these problems. Sequence analysis examines all bases at a particular locus and is the 'gold standard' of genotyping studies. RFLP analysis examines only those bases at particular restriction sites within the locus. Sequence analysis provides the most complete and reliable data but is more costly and time-consuming whereas RFLP analysis allows a larger data set to be examined. Both techniques have yielded valuable information on genetic variation within this genus.

Sequence and/or PCR–RFLP analysis of the 18S rDNA gene [125,127,128,143–145] and the more variable internal transcribed rDNA spacers (ITS1 and ITS2) [126,129] the acetyl-CoA synthetase gene [108] the COWP gene [134], the dihydrofolate reductase-thymidylate synthase (dhfr-ts) gene [104,138,141] the 70 kDa hsp70 [139] the thrombospondin-related adhesion protein (TRAP-C1 and TRAP-C2) genes [35,135–137] and an unidentified genomic fragment [124] have all confirmed the genetic distinctness of the human and cattle genotypes.

A recent multilocus approach analysed 28 isolates of *Cryptosporidium* originating from Europe, North and South America and Australia [136]. PCR–RFLP analysis of the polythreonine [poly(T)] and COWP gene, TRAP-C1 gene and ribonucleotide reductase gene (RNR), and genotype specific PCR analysis of the rDNA ITS1 region, clustered all the isolates into two groups, one comprising both human and animal isolates and the other comprising isolates only of human origin [136]. PCR–RFLP analysis of the poly(T) and COWP gene, RNR and PCR analysis of the 18S rDNA gene was also conducted on *C. parvum* isolates from AIDS patients [150]. Five of the patients exhibited the human genotype and two exhibited the cattle genotype. In both studies, neither recombinant genotypes nor mixed infections were detected [150]. Another study reported that sequence and PCR–RFLP analysis of the β-tubulin intron revealed polymorphism within the human genotype and evidence of recombination between the human and cattle genotypes [142]. Others have analysed the same region and have not found recombination [138,154,155]. A study that analysed 211 faecal specimens 'positive' for *Cryptosporidium* by microscopy used PCR–RFLP analysis of 18S rRNA, COWP, and TRAP-C1 gene fragments and found 38% human genotype and 62% cattle genotype [147]. The human genotype was found in significantly more samples with larger numbers of oocysts and the cattle genotype in significantly more samples with small numbers of oocysts, suggesting differences in fecundity between the two genotypes in humans. The distribution of the genotypes however, was significantly different in patients with a history of foreign travel and in those from different regions in England [147].

In food-borne, water-borne, and day-care centre outbreaks of cryptosporidiosis, oocysts of both human and bovine genotypes have been identified, the former identified more frequently [35,137,139,143,148,156]. Outbreaks caused by the bovine genotype have been linked to contamination from or direct contact with animals, such as the Maine apple cider outbreak in 1995, the British Colombia outbreak in 1996, the Pennsylvania rural family outbreak in 1997 and the Minnesota Zoo outbreak in 1997 [139]. Results of these studies were also very useful in clarifying the source of contamination in outbreaks, such as the massive outbreak in Milwaukee in 1993, which was probably caused by *Cryptosporidium* of human origin contaminating the water supply [35,137].

Despite substantial genetic differences between the human and cattle genotypes, little variation is found within these genotypes. Within the human genotype minor differences have been found in the 18S rRNA [143], TRAP-C2 [35,137] and poly(T) genes [150]. Preliminary analysis of *Cryptosporidium* databases has indicated that most microsatellite sequences are AT-rich and of low complexity [149]. Microsatellite analysis of 94 *C. parvum* human and animal isolates differentiated the human genotype into two subgenotypes and the cattle genotype into four subgenotypes [149]. Some subgenotypes showed a wide geographical distribution, whereas others were restricted to specific regions. Another study characterised nine microsatellite loci and identified two subgenotypes within the human genotype and two subgenotypes within the cattle genotype [157]. A number of subgenotypes have also been identified within the human and cattle genotypes using sequence analysis of the hsp70 locus (Xiao et al., unpublished observations). Additional loci need to be characterised in order to obtain greater intragenotype variation.

4.2.2. Additional *C. parvum*-like genotypes/cryptic species

A number of additional genetically distinct genotypes/cryptic species have been identified. Recent research, genetically characterising isolates of *C. parvum* from mice (*Mus musculus*) in Australia, the United Kingdom, Spain and the United States using sequence analysis of the 18S rRNA, ITS, dhfr, AcetylCo A and hsp70 loci as well as RAPD analysis has revealed that these isolates carry a distinct genotype referred to as the 'mouse' genotype ([128,129,132,144,158]; Xiao, et al., unpublished). Interestingly, some of the mice were also infected with the cattle genotype indicating that they might serve as reservoirs of infection for humans and other animals. Oocysts of the mouse genotype were also identified from a large-footed mouse-eared bat *(Myotus adversus)*, extending the host range of this genotype [158]. Pigs have also been shown to be infected with a genetically distinct host-adapted form of *Cryptosporidium* [128,129,132,144,159,160]. Little is known about the prevalence of *Cryptosporidium* in marsupials. *Cryptosporidium* infections have been reported in southern brown bandicoots (*Isoodon obesulus)*, a hand-reared juvenile red kangaroo *(Macropus rufus)* from South Australia and a Tasmanian wallaby (*Thylogale billardierii*) [161]. Genetic analysis of marsupial isolates at the 18S rDNA, ITS, dhfr and hsp70 loci have all confirmed their genetic identity, and distinctness from other all other genotypes of *C. parvum* [132,145].

Genetic analysis of *C. parvum*-like isolates from dog (*Canis familiaris*) isolates from the United States and Australia and from ferret (*Mustela furo*) isolates at the 18S rDNA and hsp70 loci have also revealed distinct genotypes [145,162]. Recently, a monkey genotype has also been identified based on the analysis of the 18S rRNA, hsp70 and COWP genes ([145]; Xiao et al., unpublished). As expected, this genotype is most related to the human genotype. As more isolates of *Cryptosporidium* from other animal species are analysed genetically, it is likely that new additional genotypes will be identified. The species status of these genotypes is currently under review [132,133,144] as there is both biological and genetic evidence to support their separation into discrete species.

4.2.3. Infectivity of other Cryptosporidium species and genotypes for humans

Few genotyping studies have been conducted on isolates of *Cryptosporidium* from immunocompromised patients [124,137,150,163,164]. In a study of 10 *Cryptosporidium* isolates from HIV-infected individuals at the 18S rDNA locus, one isolate exhibited the cattle genotype, five isolates exhibited the human genotype, three were infected with *C. felis* and one exhibited the newly identified ‘dog’ genotype [163]. For some patients, multiple specimens collected over 12 months were available and in these cases the same *Cryptosporidium* genotype persisted throughout the infection [163]. In another study of *Cryptosporidium* isolates from HIV-infected individuals from Switzerland, Kenya and the United States in which the 18S rDNA, hsp70 and Acetyl-CoA synthethase genes were analysed, the majority of patients (64%) were infected with the human and cattle *C. parvum* genotypes [164]. However, several patients were infected with *C. felis* (27%) and *C. meleagridis* (9%) [164]. These results indicate that immunocompromised individuals are susceptible to a wide range of *Cryptosporidium* species and genotypes and host-factors must play a role in controlling susceptibility to these divergent parasites. Two healthy, asymptomatic 4- and 5-year-old girls in Indonesia passed oocysts resembling those of *C. muris* for 5 and 6 days [165]. PCR products identified the oocysts as those of *Cryptosporidium* but not *C. parvum*. The recent finding of the *C. parvum* human genotype in a dugong (*Dugong dugon*) [166], complicates our understanding of the epidemiology and transmission dynamics of this ubiquitous parasite. Future studies on a larger number of isolates with more extensive clinical information is required in order to understand the transmission dynamics and full public health significance of *Cryptosporidium* species and genotypes in both immunocompetent and immunocompromised hosts.

5. Conclusion

Cryptosporidiosis is a worldwide disease in humans. Of 10 valid species of *Cryptosporidium* only *C. parvum* is widespread in humans and other mammals. Faecal–oral transmission of the oocyst stage has resulted in outbreaks through contamination of drinking water, food, and recreational water. Detection and identification of oocysts, including microscopy, immunological and molecular methods are constantly improving. We now recognise human and bovine genotypes of *C. parvum*, identified by isoenzyme analysis and confirmed by RAPD, RFLP, and sequence analysis, both of which are infectious for immunocompetent persons. Despite substantial genetic differences between these genotypes, little variation has been found within each genotype. Because few reports of *C. parvum* in mammals have been characterised by methods other than microscopy there may be other species hidden under the *C. parvum* umbrella. Of the few genotyping studies conducted on *Cryptosporidium* isolated from immunocompromised persons, most have been found infected with the human and cattle genotypes, some with *C. felis* and *C. meleagridis*, and a few with the dog genotype. Furthermore, two healthy persons have passed oocysts resembling *C. muris* and the human genotype has been found in a dugong (sea mammal). These findings suggest a greater host range for species and genotypes of *Cryptosporidium* than has been documented. These findings also indicate the need for further research on molecular characterisation and speciation of this genus so that the epidemiology can be better understood.

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