Iqbal et al., 1:9
http://dx.doi.org/10.4172/scientificreports.431

Open Access Scientific Reports

Review Article Open Access

Epidemiology of Cryptosporidiosis in HIV-Infected Individuals: A Global Perspective

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Abstract

Cryptosporidiosis, resulting from infection with the protozoan parasite *Cryptosporidium*, is a significant opportunistic disease among HIV-infected individuals. With multiple routes of infection due to the recalcitrant nature of its infectious stage in the environment, the formulation of effective and practical control strategies for cryptosporidiosis must be based on a firm understanding of its epidemiology. Prevalence data and molecular characterization of *Cryptosporidium* in HIV-infected individuals is currently available from numerous countries in Africa, Asia, Europe, North America and South America, and it is clear that significant differences exist between developing and developed regions. This review highlights the current global status of *Cryptosporidium* infections among HIV-infected individuals, and puts forth a contextual framework for the development of integrated surveillance and control programs for cryptosporidiosis in immune compromised patients. Given that there are few specific chemotherapeutic agents available for cryptosporidiosis, and therapy is largely based on improving the patient's immune status, the focus should be on patient compliance and non-compliance obstacles to the effective delivery of health care, as well as educating HIV-infected individuals in the prevention of infection. This would require close collaborations amongst public health professionals, governmental officials, researchers, epidemiologists and clinicians, as well as the targeted HIV-infected individuals themselves.

Keywords: HIV/AIDS; *Cryptosporidium*; Africa; Asia; Europe; North America; South America; Epidemiology; Molecular characterization; Genotyping

Introduction and Historical Background

More than 30 years have lapsed since human immunodeficiency virus type I (HIV-I) was first identified as the cause of acquired immune deficiency disease syndrome (AIDS) [1]. Currently, the world is experiencing an HIV pandemic, and the estimated number of persons living with HIV is 34 million. From its discovery in 1981 until 2010, AIDS has killed more than 25 million people. In 2010 alone, AIDS claimed an estimated 2.4–2.9 million lives, of which more than 390,000 were children. A third of these deaths occurred in sub-Saharan Africa (the hardest-hit region), which is home to approximately 15% of the world's population [2].

The enteric protozoan parasite, *Cryptosporidium*, was first recognized as a human parasite in 1976, when it was reported as a causative agent of diarrhea in a three-year-old child with self-limiting enterocolitis [3]. It was not until the emergence of the HIV pandemic in the 1980's that *Cryptosporidium* became widely recognized as an important human pathogen. The first case of cryptosporidiosis in a homosexual man with AIDS was reported in 1982 [4]. Since then, numerous reports worldwide have identified cryptosporidiosis as a significant pathogen in HIV/AIDS. There are multiple routes of transmission of cryptosporidiosis in humans, including person-to-person, waterborne, foodborne and zoonotic. In 1993, *Cryptosporidium* sparked great public health interest after a very large waterborne outbreak in Milwaukee, Wisconsin, which resulted in 403,000 people being affected, with 5,000 confirmed cases of cryptosporidiosis and 100 fatalities, mostly involving immunocompromised individuals [5].

Currently, cryptosporidiosis is commonly reported in HIV-infected individuals and is listed as an AIDS-defining illness (Clinical Category C) by the US Centers for Disease Control and Prevention [6]. The infection in individuals with HIV/AIDS is persistent and life-threatening and often involves infections of the entire gastrointestinal tract in addition to hepatobiliary and respiratory tract infections [7]. The most common presentation of cryptosporidiosis in patients is chronic or watery diarrhea, resulting in severe dehydration, abdominal pain, vomiting, nausea, low-grade fever, malnutrition and significant weight loss. Prolonged infections contribute to severe morbidity and mortality in HIV/AIDS patients [8].

Although data on prevalence and molecular characterization are currently available from various countries in regions such as Africa, Asia, Europe, North America and South America, there has been little effort in collating these data and placing information into perspective for future work, and the actual global status of the species, genotypes and sub genotypes of *Cryptosporidium* in HIV-infected individuals is currently difficult to assess. Therefore, the aims of the present article were to review the current global knowledge in terms of prevalence and molecular characterization of cryptosporidiosis in HIV-infected individuals and to offer recommendations for an improved understanding of *Cryptosporidium* infections and its global epidemiology, and control measures in HIV-infected individuals.

Cryptosporidium species and genotypes

Currently, there are more than 27 species of *Cryptosporidium* that infect humans and a wide variety of animals [9-12]. Of these, at least

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Received May 14, 2012; **Published** October 26, 2012

Citation: Iqbal A, Lim YAL, Mahdy MAK, Dixon BR, Surin J (2012) Epidemiology of Cryptosporidiosis in HIV-Infected Individuals: A Global Perspective. 1:431. doi:10.4172/scientificreports.431

seven have been found to infect HIV-infected individuals (Table 1). Due to the weakened immunological status of immunocompromized individuals, infections with *Cryptosporidium* are not only caused by the predominant human species (i.e., *C. hominis* and *C. parvum*) but these individuals are also more susceptible to infections by other minor human species, especially *C. meleagridis*, *C. felis*, *C. muris*, *C. canis* and *C. suis*.

In recent years, there has been considerable interest in the high prevalence of *Cryptosporidium* infections in livestock, and the possible role that these animals may play in the zoonotic transmission of this parasite [13]. Zoonotic transmission, however, may also occur by indirect means through contaminated water or food. For example, fruits and vegetables may become contaminated through the use of manure application to crop lands, or through irrigation or processing with contaminated water. There is also some evidence for the presence of *Cryptosporidium* oocysts in products of animal origin, such as milk and meat [14]. Livestock have also been implicated as the source of waterborne outbreaks in Canada [15] and England [16]. In addition, cattle living in close proximity to rivers should be considered potential causes of water contamination [17]. A recent study showed an increased risk of infection in humans while camping on contaminated land grazed by cattle [18]. This study suggested that an increase in time between grazing and camping was the most important control strategy, but increasing hand-washing frequency and the removal of cattle feces before camping were also beneficial.

Species	Hosts
*Cryptosporidium parvum**	Human, Mouse, Cattle, Pig, Sheep, Horse, Goat
*Cryptosporidium hominis**	Human
*Cryptosporidium meleagridis**	Human, Turkey
*Cryptosporidium felis**	Human, Cat
*Cryptosporidium muris**	Human, Mouse
*Cryptosporidium canis**	Human, Dog
*Cryptosporidium suis**	Pig, Human
Cryptosporidium andersoni	Cattle, Human
Cryptosporidium baileyi	Chicken, Human
Cryptosporidium bovis	Cattle
Cryptosporidium xiaoi	Sheep
Cryptosporidium ducismarci	Tortoises
Cryptosporidium fayeri	Kangaroo, Human
Cryptosporidium fragile	Black-spined toad
Cryptosporidium galli	Chicken
Cryptosporidium macropodum	Kangaroo
Cryptosporidium molnari	Fish
Cryptosporidium ryanae	Cattle
Cryptosporidium saurophilum	Lizard
Cryptosporidium scopthalmi	Fish
Cryptosporidium serpentis	Snake, Lizard
Cryptosporidium varanii	Lizard
Cryptosporidium wrairi	Pig
Cryptosporidium ubiquitum	Ruminants, Human
Cryptosporidium cuniculus	Rabbit, Human
Cryptosporidium tyzzeri	Mice
Cryptosporidium viatorum	Human

* Species detected in HIV/AIDS individuals. Taxonomic nomenclature adapted from [9-12]

Table 1: Current valid taxonomic nomenclature of *Cryptosporidium* species and their host ranges.

Genetic variants of *C. parvum* isolated from humans that are rarely found in animals have been identified using subgenotyping analysis, suggesting that human infection with *C. parvum* might have originated from humans themselves [19]. *Cryptosporidium meleagridis*, a parasite originally described in turkeys [20], has been detected in humans in the UK [16], Thailand [21] and Peru [22,23], implicating domestic animals as a potential source of infection for humans. Zoonotic cryptosporidiosis from exposure to pets has been reported in both healthy adults and in HIV-infected persons [24,25]. Dogs and cats seem to be most commonly infected with the predominantly host-adapted *C. canis* and *C. felis* [26], and may represent important zoonotic reservoirs of *Cryptosporidium* infection in HIV patients. In Spain and the UK, there have been cases of *C. felis*, in which cats were identified as the source for human infections, and were found to infect both immunocompetent and immunosuppressed patients [27,28]. The first Italian case of a human *C. felis* infection occurred in an HIV-positive man who did not have a cat at home [29].

Transmission of cryptosporidiosis

Cryptosporidium has different stages which include oocysts, trophozoites, schizonts, merozoites, and sexual stages such as micro- and macrogamonts. Oocysts are the exogenous stage, consisting of four sporozoites within a tough two-layered wall, and range in size from 3.8 by 4.6 μm to 6.3 by 8.4 μm, depending on the species [30]. Oocysts are highly infectious, and only 10^1 to 10^3 oocysts are sufficient to produce infection and disease in susceptible hosts [31]. The life cycle starts with the ingestion of the infectious oocyst stage by an appropriate host. Thin-walled oocysts may also develop and can excyst in the intestinal tract causing autoinfection and heavy, persistent infection, with massive shedding of oocysts in the feces of an infected patient [32]. This phenomenon may explain the mechanism of persistent infection in AIDS patients in the absence of successive oocyst exposure [33]. Cases of human-to-human transmission have been reported between family members, sexual partners, children in day-care centres, and hospital patients and staff [33].

Transmission may occur through direct contact with oocysts in animal feces, as in the case of animal handlers, veterinarians, farmers and their families, and farm or petting zoo visitors. Waterborne cryptosporidiosis associated with water supplies results from either human or animal pollution of the environment [15]. *Cryptosporidium* has been isolated from untreated and treated drinking water supplies, swimming pools, splash pads, water parks, rivers, streams, and reservoirs in North America, South America, United Kingdom and Europe, and it may be ubiquitous in surface waters throughout the world [5,20,34]. Cryptosporidiosis has sometimes been associated with consumption of certain foods. *C. parvum* have been detected in oysters, clams, mussels, raw vegetables and green leafy vegetables [35-38]. Outbreaks associated with raw milk, fresh-pressed apple cider, and social event foods have also been reported in the UK [39] and USA [40]. Several reports strongly suggested but were unable to confirm that cryptosporidiosis may also be acquired by sexual transmission [15]. Data comparing HIV/AIDS patients, homosexual men and intravenous drug users (IVDU), showed a higher prevalence of cryptosporidiosis among homosexual men [41].

Pathophysiology and clinical symptoms

The main site of *Cryptosporidium* infection is the small intestine, although infection may spread throughout the gastrointestinal tract and extra-intestinal sites. In HIV patients, proximal small intestinal

infections generally cause more severe diarrhea and reduced survival rates, compared to heavy infection of the colon which, in the absence of small bowel infection, may result in asymptomatic infection or intermittent diarrhea [42]. Ultrasonic examination of AIDS patients with biliary cryptosporidiosis has revealed a generalized dilation of the bile duct and gall bladder [43], and commonly presents with right upper quadrant pain, nausea, fever, vomiting and often the absence of diarrhea [44]. Esophageal cryptosporidiosis, with parasites attached to the squamous mucosa and the luminal borders of sub mucosal glands and ducts has been described both in adults and in children with AIDS [45]. Infections within the bronchial tree have also been reported [44].

While cryptosporidiosis-associated diarrhea almost always resolves within a few days in otherwise healthy individuals, severe diarrhea caused by *Cryptosporidium* in HIV-infected patients is common and the effect of reduced CD4 T-cell counts on the persistence of diarrhea has been well documented [46]. *Cryptosporidium* infections in HIV-infected individuals can reduce both their quality of life and life span, especially those who are severely immunosuppressed, with a CD4 T-cell counts of <200 cells/mm^3 [46]. It is evident from previous studies that resistance to and recovery from infection with *Cryptosporidium* is dependent on T-cell activity [47]. At higher CD4 T-cell levels, spontaneous clearing of the parasite generally takes place [44].

In developing countries such as Mexico, Haiti and in some countries in Africa, diarrhea has been reported in more than 40% of AIDS patients [48]. Studies from India indicated that diarrhea is the third most common clinical presentation in AIDS patients [49]. The prevalence of cryptosporidiosis in HIV-infected patients with diarrhea has been reported to range from 3 to 16% in developed countries, depending on the population studied, degree of immunosuppression and the use of antiretroviral therapy [50]. A study in Atlanta, USA, found that lower CD4 T-cell counts were predictive of chronic diarrhea [51]. Another study in the USA identified four distinct clinical syndromes of cryptosporidiosis such as transient diarrhea, relapsing illness, chronic diarrhea, and cholera-like illness in AIDS patients with CD4 T-cell counts of <200 cells/mm^3 [52].

Treatment of cryptosporidiosis

Despite the magnitude and severity of *Cryptosporidium* infection in HIV-infected individuals, its pathogenesis is poorly understood, and there is currently no drug of choice. However, with the introduction of Highly Active Antiretroviral Therapy (HAART) for the reconstitution of immune response, the incidence of cryptosporidiosis has declined [53], and it is clear that chronic diarrhea and cryptosporidial infection often resolves with the increase in CD4 T-cell numbers [46]. In the severely affected immune compromised patient, drug treatment is of uncertain and probably limited efficacy, and the infection responds best to an improved host's immune status, for example, by means of HAART. Conversely, relapses can occur following deterioration in immune function, if infection has been suppressed and not completely cleared [44]. Usually, the aim of treatment is to reduce the duration of diarrhea, prevent complications, eliminate the organism from the host, and reduce mortality [54]. To ensure absorption of antiretroviral drugs, symptomatic treatment with loperamide and/or opium tincture, as controlled prescriptions given at the maximum doses is advised. If this is unsuccessful, treatment with other anti-diarrheal medications can be attempted. Sufficient hydration of the patient is also important, and may involve infusions in the case of severe dehydration.

The emergence of cryptosporidiosis has triggered the screening of many compounds for potential anti-cryptosporidial activity, but unfortunately the majorities have been ineffective. Currently, the drugs most commonly used to treat cryptosporidiosis include paromomycin, spiramycin, azithromycin and rifaximin. Rossignol et al. [55], reported good results in individual cases of cryptosporidiosis when treated with the antihelminthic agent, nitazoxanide. Nitazoxanide has been found to be effective in a small, randomized study and is possibly the first drug with real efficacy for treating cryptosporidiosis [55]. In the American Expanded-Access Program (EAP), almost two-thirds of the patients responded to treatment [56]. In June 2005, nitazoxanide was licensed for *Cryptosporidium*-associated diarrhea in the US. However, there is no approval yet for AIDS patients, either in the US or in Europe.

Immune reconstitution using HAART is the treatment of choice for those with HIV-related immunodeficiency, as it drastically diminishes the occurrence of life-threatening diarrhea [57]. Some studies using protease inhibitors, especially serine and cysteine protease, as a part of HAART in the treatment of HIV infection, have shown an improvement in the health status of AIDS patients suffering from cryptosporidiosis [58]. Thus, combination therapy, restoring immunity in AIDS patients along with antimicrobial treatment of *Cryptosporidium* infections, is necessary. Although HAART has been found to be effective, this treatment regime is not widely available, and is too expensive in many developing countries where cryptosporidiosis is particularly prevalent in HIV-infected patients [54,59]. This is especially true in the African and Asian regions. Hence, effective control and prevention of cryptosporidiosis is of utmost importance.

HIV-cryptosporidiosis in Africa

HIV/AIDS is a major public health concern in Africa, where there are 22.9 million HIV cases and the largest burden of AIDS cases in the world [2]. Although Africa is inhabited by an estimated 14.7% of the global population, it contributed 66.6% of the global AIDS deaths in 2010 [2]. The potent combination of HIV and extreme poverty also results in intestinal parasitic infections being widespread in Africa [60]. Among the intestinal parasitic infections, cryptosporidiosis is one of the most common, and is of particular concern among HIV-infected individuals.

Currently, 18 reports on cryptosporidiosis among HIV-infected individuals are available from 12 countries in Africa, with prevalence ranging from 3.8 % to 73.6% (Table 2). Uganda has recorded the highest rate of cryptosporidiosis in HIV-infected individuals [69], whilst Cameroon has the lowest. Rates of cryptosporidiosis do not, however, correlate with the number of HIV cases each country has. For example, Uganda contributes 5.4% of HIV people on the African continent, whilst Cameroon contributes 6.1%. Prevalence and species distribution of *Cryptosporidium* spp. vary greatly with the regions or countries studied, and even within specific demographic groups (i.e., children, adults, and women). This creates a complex picture of the epidemiology of cryptosporidiosis. Diarrhea and low CD4 T-cell counts have been found to be significant predictors of cryptosporidiosis in African countries (Table 2).

Studies in Ethiopia, which contributes 2.1% of the African HIV/AIDS patients [2], have reported high prevalence of cryptosporidiosis (11% to 25%) [60,71,77]. This was evident in a study of 52 Ethiopian HIV patients with chronic diarrhea, whereby 11% had cryptosporidiosis [71]. Similar findings were also obtained when 20.1% of 214 HIV/AIDS patients with CD4 T-cell counts of <200 cells/mm^3 had cryptosporidiosis, with diarrhea as the most frequent symptom [60]. A prospective cross-sectional study in Nairobi, Kenya, revealed *Cryptosporidium* as the most common pathogen (17% of 75 cases). Thirty-one patients died, and

detection of *Cryptosporidium* oocysts was the single most significant predictor of death ($p < 0.05$) [63]. Among Nigerian HIV-infected adult patients with chronic diarrhea, *C. parvum* was found to be the most common parasitic infection (16.8% of 101 cases) [73], whilst another study indicated a very high percentage of cryptosporidiosis (52.7%) amongst 100 HIV-infected individuals presenting with diarrhea [74]. However, in Zimbabwe, only 9% of 82 HIV-infected patients with diarrhea were found to have *C. parvum* [64]. In hospitalized children with diarrhea between the ages of 15 months and 5 years in Zambia, *Cryptosporidium* was again the most common parasite identified, being isolated from 14% of 44 HIV-seropositive children [61]. Unlike for individuals with HIV/AIDS, immune defects that increase infection

Regions	Country	Sample size (n)	Age (yrs)	CD4 Count cells/mm^3	Prevalence (%)	Diarrhea (%)	References
Africa	Cameroon	154	23 - 58	238	3.8	29.0	[70]
	Equatorial Guinea	185	-	-	18.9	-	[76]
	Ethiopia	52	15 - >45	-	11.0	51.1	[71]
	Ethiopia	214	17 - 67	<200	20.1	45.3	[60]
	Ethiopia	192	15 - 67	-	25.0	-	[77]
	Guinea-Bissau	37	24 - 60	3-153	25.0	100.0	[65]
	Kenya	75	-	-	17.0	100.0	[63]
	Nigeria	100	9 - 54	-	52.7	100.0	[74]
	Nigeria	101	15 - 24	-	16.8	100*	[73]
	South Africa	101	0.5 - 3	24-1,932	24.8	24.0	[66]
	South Africa	31	0 - >60	-	12.5	59.1	[72]
	Tanzania	89	1.1-61	<50	8.5	100.0	[68]
	Tanzania	127	18 - 65	179	17.0	48.0	[67]
	Tunisia	75	-	-	10.7	37.0	[75]
	Uganda	91	0 - 5	<25	73.6	81.0	[69]
	Zambia	44	1.25 - 5	-	14.0	100.0	[61]
	Zambia	108	0.5-2	-	26.0	100.0	[62]
	Zimbabwe	82	20 - 59	-	9.0	100.0	[64]
Asia	Cambodia	80	40 - 60	4 - 285	45.0	50.0	[81]
	India	120	-	30	10.8	44.2	[92]
	India	152	-	-	16.6	68.0	[93]
	India	150	14 - 66	-	14.4	66.6	[94]
	India	200	21 - >40	<200	56.5	38.0	[47]
	India	111	-	44 - 745	25.2	100.0	[95]
	India	423	-	113 - 686	4.7	0.0	[95]
	India	75	20 - 55	2 - 660	33.0	66.6	[96]
	India	111	36 - 64	-	25.0	100.0	[97]
	India	80	19 - 72	-	28.7	100.0	[98]
	India	206	21 - 67	2 - 583	19.9	48.1	[99]
	India	366	31 - 40	<200	39.8	100.0	[100]
	India	113	4 - 65	180 - 261	23.6	30.0	[49]
	India	100	20 - 50	-	9.0	41.3	[101]
	India	137	-	<200 - 499	12.0	100.0	[102]
	Indonesia	318	<5 - > 50	0 - 400	4.9	100.0	[88]
	Indonesia	122	-	-	52.5	100.0	[89]
	Iran	206	-	240 - 496	1.5	13.6	[104]
	Iran	781	-	50 - 137	0.9	1.1	[105]
	Iran	75	<20 - >50	127 - 351	26.7	14.6	[106]
	Iran	64	10 - 50	272 - 354	9.4	-	[107]
	Korea	67	10 - 39	10 - 200	10.5	16.4	[90]
	Malaysia	168	-	-	23.0	-	[83]
	Malaysia	66	29-56	-	3.0	9.1	[84]
	Malaysia	59	-	-	16.0	-	[86]
	Malaysia	122	>35	<200	22.1	8.3	[85]
	Malaysia	346	1 - 65	2 - 525	12.4	8.7	[87]
	Nepal	75	-	-	10.7	30.7	[103]
	Taiwan	1,044	15-83	81 - 265	0.5	68.2	[53]
	Thailand	250	-	-	8.8	100.0	[108]
	Thailand	45	24 - 65	0 - 360	20.0	100.0	[109]
	Thailand	22	-	-	9.1	100.0	[110]
	Thailand	61	20 - 39	-	10.0	16.0	[111]
	Thailand	43	<37	0 - 545	25.6	55.8	[112]
	Thailand	288	16 - 64	1 - 279	19.2	86.1	[113]

	Thailand	156	0.08 - 65	-	12.8	-	[114]
	Thailand	90	-	2 - 563	34.4	78.9	[118]
	Thailand	78	25 - 39	<100	11.5	32.0	[115]
	Thailand	143	20 - 65	621 - 1,337	16.1	100.0	[116]
	Thailand	46	24 - 62	-	28.7	28.2	[117]
Europe	Denmark	60	8 - 40	7 - 304	33.3	26.6	[126]
	France	81	22 - 62	0 - 973	37.3	70.6	[127]
	Italy	154	-	90 - 387	11.1	43.08	[132]
	Portugal	465	-	3 - 600	8.0	100.0	[131]
	Spain	275	14 - 38	<200	15.6	72.1	[129]
	Spain	505	-	<200	8.5	59.4	[130]
	Spain	16	-	-	14.8	-	[134]
	Turkey	38	-	<200	39.0	60.5	[133]
	United Kingdom	234	-	-	11.0	26.0	[124]
	United Kingdom	44	-	20 - 250	25.0	100.0	[125]
	United Kingdom	38			29.0		[128]
North America	Cuba	67	18 - 43	-	11.9	-	[148]
	USA	16,953	6 - 44	-	3.8	-	[147]
	USA	3,564	30 - 39	19 - 209	5.4	-	[146]
	USA	137	-	-	3.6	-	[145]
	USA	602	23 - 63	0 - 1,470	8.5	48.9	[51]
	USA	6,913	13 - >44	<100	3.5	7.2	[144]
South America	Brazil	131	-	-	19.1	-	[153]
	Brazil	200	-	-	7.0	40.0	[155]
	Brazil	75	20 - 50	-	9.3	100.0	[158]
	Brazil	100	-	1 - 958	4.0	27.0	[159]
	Brazil	359	2 - 72	50 - 800	8.6	70.2	[8]
	Brazil	105	18 - 64	<200	10.5	-	[162]
	Brazil	482	18 - 70	<200 - >350	8.1	-	[163]
	Chile	27	30 - 40	-	11.1	100.0	[161]
	Colombia	111	1 - 69	-	3.6	52.3	[157]
	Peru	2,672	18 - 67	<200	13.3	43.0	[22]
	Peru	2,490	>17	-	9.2	26.5	[23]
	Venezuela	515	0.08 - 86	-	13.0	-	[154]
	Venezuela	35	14 - 34	-	22.8	-	[156]
	Venezuela	397	17 - 63	4 - 412	15.0	34.0	[160]

[a]Data not available

Table 2: Prevalence of cryptosporidiosis and occurrence of diarrhea in HIV-infected individuals by region.

risk in malnourished children are not well defined. There is, however, a clear and significantly higher association between cryptosporidiosis and malnourished diarrheic children (26% of 108 cases) in Zambia [62]. Although malnutrition may impair cell-mediated immunity, thus predisposing to infection, it is highly plausible that pathology associated with infection actually impairs nutrient absorption and therefore causes weight loss and growth. Houpt et al. [67] reported from Tanzania, the percentage of *Cryptosporidium* positive patients with diarrhea was not significantly different from the number of *Cryptosporidium*-positive patients without diarrhea (17% of 127 cases) in HIV infected individuals ($p > 0.05$). One of the pediatric study from Tanzania showed that cryptosporidiosis (8.5% of 89 cases) with chronic diarrhea in particular have an adverse and sustained impact on child growth [68]. In 2001, patients from Guinea-Bissau, the country with the highest prevalence of HIV-2 in the world [2] were evaluated and results showed that 25% of 37 HIV-2-positive patients were infected with *C. parvum* [65].

Recently, advances in molecular techniques have increased the sensitivity of *Cryptosporidium* detection, as well as allowing for a simultaneous genetic characterization of the isolates obtained from African HIV/AIDS patients. The most common PCR target gene utilised in molecular studies on *Cryptosporidium* in Africa is 18s rRNA, although some studies have also targeted hsp70, acetyl-CoA, TRAP, COWP and gp60 genes (Table 3). *Cryptosporidium hominis* was found to be the most common species reported in HIV-infected individuals in most of the African countries, followed by *C. parvum* (Table 3). One exception was in Equatorial Guinea, where 18.9% of 185 HIV-infected patients were identified as positive for *Cryptosporidium*, and PCR-restriction fragment length polymorphism (RFLP) analysis revealed that *C. parvum* was the most common species found at 52.9%, while *C. hominis* was reported at 44.1% [76]. This study also provided some evidence for zoonotic transmission as *C. meleagridis* was reported in 2.9% of these samples. In addition to Equatorial Guinea, *C. meleagridis* has also been reported in Kenya [78,79], Tunisia [75] and Uganda [69], whereas *C. muris* has been reported only in Kenya [79, 80]. However, *C. felis*, a species which has been reported in humans in other areas of the world, has not been reported in Africa.

South Africa has the highest rate of reported HIV/AIDS cases in

Regions	Country	Total No. of Isolates	No. of Isolates							PCR target	References
			C. parvum	*C. hominis*	*C. meleagridis*	*C. felis*	*C. canis*	*C. muris*	*C. suis*		
Africa	Equatorial Guinea	31	17	13	1					18s rRNA, COWP	[79]
	Kenya	6	1	4	1					18s rRNA, hsp70, acetyl-CoA	[81]
	Kenya	24	8	14	1			1		18s rRNA	[82]
	Malawi	2	1	1						18s rRNA	[82]
	South Africa	21	5	16						18s rRNA, TRAP, COWP,GP60	[70]
	South Africa	31	1	3						18s rRNA	[75]
	Tunisia	8	3	4	1					18s rRNA	[78]
	Uganda	76	14	56	3					18s rRNA	[72]
Asia	Cambodia	9	1	8						18s rRNA	[81]
	India	48[a]	8	33	1	5		1		18s rRNA, COWP, TRAP-C, Cpgp40/15	[95,97]
	Iran	17	13	4						18s rRNA	[120]
	Iran	15	11	4						18s rRNA	[121]
	Malaysia	9	9							18s rRNA	[86]
	Malaysia	26	16	6	2	2				18srRNA, GP60	[85]
	Malaysia	32	27	2	2	1				18s rRNA, GP60	[122]
	Taiwan	4		2	1	1				18s rRNA	[53]
	Thailand	29		24	3	1		1		18s rRNA	[119]
	Thailand	34	5	17	7	3	2			18s rRNA	[21]
	Thailand	2	2							18s rRNA	[117]
	Vietnam	3		3						18s rRNA	[79]
Europe	France	13	7	6						18s rRNA	[138]
	France	46	22	14	3	6		1		18s rRNA	[139]
	Italy	9	8			1				COWP, MS	[29,136]
	Italy	9	8	1						GP60	[137]
	Portugal	29	16	7	3	3				18s rRNA, GP60	[140]
	Portugal	40	22	11	3	4				18s rRNA	[141]
	Portugal	22	9	14						GP60	[142]
	Spain	16	6	10						18s rRNA, COWP	[134]
	Switzerland	13	7	2	1	3				18s rRNA, hsp70, acetyl-CoA	[78]
	Switzerland	5	2	3						18s rRNA	[143]
	UK	6	2		2	2				18s rRNA	[46]
Central America	Guatemala	4		4						TRAP-C2	[151]
North America	Jamaica	35[b]	7	25		1	1			GP60, 18s rRNA, MLST, hsp 70	[152]
	USA	17	2	15						TRAP-C2	[151]
	USA	13	3	10						18s rRNA	[149]
	USA	10	1	5		3	1			18s rRNA	[150]
	USA	3				3				18s rRNA, hsp70	[78]
	USA	29	8	18		3				18s rRNA	[168]
South America	Brazil	5[c]	3	1						TRAP-C1, COWP	[164]
	Brazil	5	1	2	2					COWP	[165]
	Brazil	27	4	17		5	1			18s rRNA, COWP	[166]
	Chile	3	1	2						18s rRNA	[163]
	Peru	300[d]	34	204	38	10	12		1	18s rRNA	[22]
	Peru	118	20	76	10	4	9			18s rRNA	[25]
	Peru	21	1	4	2	7	7			18s rRNA, COWP, DHFR	[167]
	Peru	193	22	141	17	6	6			GP 60	[23]

a: one isolate of *C. parvum* mouse genotype
b: one isolate of mixed infection with *C. parvum* and *C. hominis*
c: one isolate of undetermined species
d: one isolate of *C. pig* genotype

Table 3: Molecular characterisation of *Cryptosporidium* detected in HIV-infected individuals by region.

Africa [2]. Currently, the only available subgenotyping information is based on 24.8% of 101 HIV-infected South African children found positive for *Cryptosporidium*. The study analyzed nucleic acid and amino acid sequence polymorphisms at the Cpgp40/15 locus of 20 *C. parvum* isolates from these children. Fifteen isolates exhibited one of four previously identified genotype I (now known as *C. hominis*) alleles at the Cpgp40/15 locus (Ia, Ib, Ic, and Id), while five isolates displayed a novel set of polymorphisms that defined a new Cpgp40/15 genotype I allele, designated Ie. Only 15 of these isolates exhibited concordant *C. hominis* at the TRAP and COWP loci, while five isolates (all of which displayed Cpgp40/15 genotype Ic alleles) displayed *C. parvum* at these loci. Children infected with isolates having genotype Ic alleles were significantly older than those infected with isolates displaying other *C. hominis* genotypes [66].

The occurrence of these species and genotypes has revealed substantial variation amongst the different geographical locations studied, and amongst the populations selected for these studies. The generally high prevalence of *C. hominis* infections indicated that humans are a major source of infection and that person-to-person transmission has played a major role in the spread of *Cryptosporidium* infection in Africa, especially among HIV-infected patients. In addition, with the presence of zoonotic species such as *C. parvum*, *C. meleagridis* and *C. muris*, the role of infected animals in transmitting *Cryptosporidium* to humans must not be underestimated. This was evident in Tunisia, where *C. meleagridis* was mainly identified in children living on farms and having close contact with animals [75].

HIV-cryptosporidiosis in Asia

Asia is the largest continent in the world and is home to 60% of the global population. In terms of the number of people living with HIV, Asia has the second highest number of cases of HIV and AIDS (~4.7 million HIV cases) in the world after Africa [2]. Regionally however, the number of cases has decreased since 2000 due to the advent of HAART.

Currently, Asia has the greatest number of published studies on cryptosporidiosis in HIV-infected individuals (40 studies to date), with data available from nine countries (Table 2). These studies have shown a wide range of *Cryptosporidium* infection rates (i.e., 0.5% to 56.5%) in HIV-infected individuals with or without diarrhea (Table 2). The highest prevalence was recorded in a study from India, where 56.5% of 200 HIV-infected individuals had cryptosporidiosis [47], whereas a study in Taiwan reported only 0.9% of 1,044 HIV-infected patients harbouring *Cryptosporidium* [53].

Among the southeastern Asian countries, Thailand has a relatively high number (1.4% of global prevalence) of HIV cases [2]. Research on cryptosporidiosis in this country has been quite substantial, with 11 reports on HIV-infected individuals documenting prevalence ranging from 8.8 % to 34.4%, with mean CD4 T-cell counts of <100 cells/ mm^3 (Table 2). Another country with high rates of HIV infection is Cambodia, with 0.8% of the global numbers [2]. However, except for one case-control study, very little is known about the epidemiology of HIV infection or its co-infections in this country. This case-control study involving 80 HIV-infected individuals found that 45% of these patients harboured *Cryptosporidium*. Using PCR-RFLP, this study showed the presence of *C. hominis* in chronic diarrheic patients, and both *C. hominis* and *C. parvum* in asymptomatic patients [81]. In Malaysia, the first cryptosporidiosis case was reported in 1984 [82]. Subsequently, there were 5 reports with prevalence ranging from 3% to 23% [83-87]. Given that 70% of HIV-infected individuals in Malaysia are intravenous drug users, a study was carried out to determine the prevalence of *Cryptosporidium* amongst asymptomatic intravenous drug users, and it was found that 23% of 168 patients were positive [83]. Among hospitalized HIV patients, 3% to 16% were positive with cryptosporidiosis [84-87]. Two studies indicated that HIV patients with CD4 T-cell counts of <200 cells/mm^3 had cryptosporidiosis [85,87], and these patients were more likely to have diarrheal symptoms. A study in Indonesia associated the occurrence of cryptosporidiosis (4.9% of 318 cases) in HIV-infected individuals with chronic diarrhea and CD4 T-cell counts < 50 cells/mm^3 [88]. A recent study in Indonesia indicated a very high percentage of cryptosporidiosis (52.5%) amongst 100 HIV-infected individuals presenting with diarrhea [89].

Although limited, information on cryptosporidiosis in HIV-infected individuals is available from eastern Asia, namely Korea and Taiwan, both of which have HIV/AIDS cases of less than 0.5% [2]. Guk et al. [90] reported *C. parvum* infections in 10.5% of 67 HIV-infected individuals. Interestingly, another study in Korea has suggested that naturally infected pigs may be a significant reservoir for *C. parvum* in humans, and that pigs should be viewed as being more important than calves, because the number of pigs overwhelms the number of calves in Korea [91].

India accounts for approximately 50% of the HIV infections in Asia [2]. There are 14 reports available on the prevalence of *Cryptosporidium* infections from different parts of India, with prevalence ranging from 4.7% to 56.5% in HIV-infected individuals (Table 2). As observed in other parts of Asia, there is a paucity of data on the correlation of CD4 T-cell counts and the etiology of diarrhea among HIV-infected individuals. However, in one study, diarrhea was determined to be the most common symptom in HIV/AIDS patients with CD4 T-cell counts of <200 cells/ mm^3, and *Cryptosporidium* infection was the highest (56.5% of 200 cases) and statistically significant as compared with the other intestinal parasites [47]. A study in India analysed the correlation between CD4 T-cell counts of HIV patients and the occurrence of intestinal protozoan infections in different seasons, and the results showed that the highest incidence of *Cryptosporidium* infection (39.8% of 366 cases) occurred during the rainy season [100]. In Nepal, cryptosporidiosis was reported in 10.7% of 75 HIV-infected individuals, associated with chronic watery diarrhea of more than one month's duration [103].

In Iran, there have been four reports on cryptosporidiosis in HIV-positive individuals. Using Ziehl-Neelsen staining and conventional microscopy method, the prevalence was determined to be between 0.9% and 26.7% [104-107]. Zali et al. [104] highlighted the significance of intestinal parasites among HIV-infected individuals, especially those with low immunity presenting with diarrhea. In another study, it was found that 26.7% of 75 HIV-infected individuals were infected with *Cryptosporidium*, and the probability of infection with opportunistic parasites increased as the number of CD4 T-cells decreased in the patients [106].

Determining the true prevalence of cryptosporidiosis in any region is important for epidemiological data; hence, a more sensitive and reliable technique is required to improve the detection rate [86]. Differences in prevalence's reported worldwide may therefore be partially accounted for by the different detection methods used. The use of microscopic diagnostic techniques (Ziehl-Neelsen staining) in combination with nested-PCR, offers one of the most sensitive methods for detecting *Cryptosporidium* (Table 3). Based on genotyping studies,

C. hominis was more prevalent than *C. parvum* in Cambodia [85], India [95,97], Taiwan [53], Thailand [119] and Vietnam [79]. However, in Iran [120,121] and Malaysia [85,86], *C. parvum* was more common.

Lim et al. [85] published the first report of *C. hominis*, *C. meleagridis* and *C. felis* from Malaysian HIV patients. The sequencing of amplicons derived from small subunit (SSU) rRNA revealed that *C. parvum* was the most commonly detected species at 64% of 25 cases (16 isolates), followed by *C. hominis* (six isolates), *C. meleagridis* (two isolates) and *C. felis* (one isolate). Sequencing of the gp60 gene identified *C. parvum* subgenotype IId and *C. hominis* subgenotype Ia, Ib, Id, Ie and If in HIV patients [85]. Further subgenotype analysis demonstrated *C. parvum* subgenotype IIdA15G2R1 and *C. hominis* subgenotypes IaA14R1, IbA10G2R2, IdA15R2, IeA11G2T3R1 and IfA11G1R2. Another study in Malaysia reported that of 346 fecal samples from HIV-positive patients, microscopically *Cryptosporidium* positive samples (i.e., 43 samples) tested via PCR targeting the small subunit of nuclear ribosomal RNA (SSU rRNA), 32 (74.4% of 43) were found positive. Subsequent sequence analysis identified four distinct *Cryptosporidium* species which included *C. parvum* (84.3%) as the most frequently detected species, followed by *C. hominis* (6.3%), *C. meleagridis* (6.3%) and *C. felis* (3.2%) [122]. Subgenotype analysis targeting partial 60kDa glycoprotein (pgp60) gene identified 18 (5.2% of 346 cases) isolates as *Cryptosporidium*-positive, with 72.2% of the 18 identified as *C. parvum* and 27.7% as *C. hominis*. Gp60 analysis revealed that the *C. parvum* isolates included subgenotypes IIaA13G1R1 (two isolates), IIaA13G2R1 (two isolates), IIaA14G2R1 (three isolates), IIaA15G2R1 (five isolates) and IIdA15G1R1 (one isolate). *C. hominis* was represented by subgenotypes IaA14R1 (two isolates), IaA18R1 (one isolate) and IbA10G2R2 (two isolates) [123]. These data demonstrated the potential significance of zoonotic transmission of *C. parvum* as it was the predominant species in HIV individuals in Malaysia. The advantage of using subgenotyping is in clarifying the epidemiology of *Cryptosporidium* and raising interesting questions with regards to its population genetics. Besides the two most common human species (*C. parvum* and *C. hominis*), *C. meleagridis* and *C. felis* are also relatively common in India [95,97], Taiwan [53] and Thailand [21]. Other less common species included *C. canis*, which has only been reported in Thailand [29], and *C. muris* in India and Thailand [95,97,119].

Furthermore, in a study carried out in India, 48 isolates of *Cryptosporidium* were identified, with 33 being identified as *C. hominis*. Of those patients who have potentially acquired infection via zoonotic transmission, molecular characterization identified eight as *C. parvum*, five as *C. felis*, one as *C. meleagridis*, one as *C. muris* and one as *C. parvum* (mouse genotype). Twenty one of these patients confirmed contact with animals such as cows, goats and dogs in both urban and rural households [95]. Based on the analysis of the *Cryptosporidium* pgp40/15 locus, the subgenotypes found among 31 *C. hominis* isolates from Indian HIV patients included Ia, Ib, Ic, Id and If, with one mixed infection with subgenotypes Ic and Id, whereas among five *C. parvum* isolates, four were IIa or IIb, which have similar-sized fragments, and one was IIc [95].

In Thailand, molecular characterisation studies showed that only *C. meleagridis* and *C. muris* were present in HIV-infected children, whereas *C. hominis* predominated in HIV-infected adults [119]. During this investigation, 34 *Cryptosporidium* isolates were obtained from the patients who had symptomatic cryptosporidiosis. Genotyping of these isolates, by RFLP analysis and DNA sequencing of the 18S rRNA gene, indicated that 17 were *C. hominis*, with the rest being *C. meleagridis* (seven isolates), *C. parvum* (five isolates), *C. felis* (three isolates) and *C. canis* (two isolates). This was the first report of *C. canis* and *C. parvum* in HIV-infected Thai patients [21].

The Asian and African regions show an obvious difference in genetic characterization of *Cryptosporidium* species. As with the African regions, direct or indirect transmission of oocysts through human-to-human or waterborne routes appears to be most common in Asia, where infections with *C. parvum* and *C. hominis* predominate. However, the presence of zoonotic species such as *C. canis*, *C. felis*, *C. muris*, and *C. meleagridis* in humans indicated that animal reservoirs may also be important. Therefore, continued molecular characterization of *Cryptosporidium* species and genotypes, combined with field epidemiology, is likely to lead to more rational approaches to disease control in this region and elsewhere.

HIV-cryptosporidiosis in Europe

The number of AIDS cases has declined slightly in western and central Europe due to widespread availability of antiretroviral drugs, especially HAART, in this region [169]. The prevalence of *Cryptosporidium* infection among HIV-infected individuals in Europe ranges from 8% to 39% (Table 2). In the mid 1990's, the epidemiology of cryptosporidiosis among European patients with AIDS was described based on a cohort of 6,548 AIDS patients seen at 52 centres across 17 European countries. Overall, cryptosporidiosis was diagnosed in 6.6% of these patients. Waterborne transmission associated with drinking water and swimming pool contact is now one of the most common causes of human cryptosporidiosis in Europe and the UK [135].

In addition to the routes of transmission mentioned above, nosocomial cases were identified at a hospital in Copenhagen, Denmark. Thirty three percent of 60 HIV-infected patients were affected during this outbreak. The source of the outbreak was identified from contaminated ice dispensed from an ice machine in the ward, which was contaminated by an incontinent, psychotic patient with cryptosporidiosis [126]. In Lisbon, Portugal, a study assessed the prevalence and factors associated with *Cryptosporidium* infection among 465 HIV-infected individuals. Cryptosporidiosis was reported in 36 (8% of 465) patients. Prevalence of cryptosporidial infection was higher among HIV-infected individuals whose exposure category was through sexual contact compared to patients in other HIV exposure categories, with few cases occurring in intravenous drug users [131]. The first Italian case of human *C. felis* infection occurred in a homosexual HIV-positive man who, in spite of a very low CD4 T-cell count, successfully recovered with paromomycin treatment. While this individual did not have a cat at home, the city where he lives (Rome) is home to a plethora of stray and domestic cats (approximately 0.1 cats per inhabitant). Infection may have occurred upon accidental contact with oocysts in the environment [29]. Also in Italy, a study reported nine cases of cryptosporidiosis in AIDS patients. Formalin-fixed faecal specimens were treated to obtain high quality genomic DNA for amplification and sequencing of the 60-kDa glycoprotein (gp60) gene. Sequence analysis revealed that one of these patients was infected with *C. hominis* whereas the remaining eight patients were infected with *C. parvum*. Interestingly, the patients showing severe cryptosporidiosis harboured two subgenotypes within the *C. parvum* family IIc (IIcA5G3 and IIcA5G3R2), whereas patients with moderate or mild infections showed various subgenotypes of the *C. parvum* family IIa (IIaA14G2R1, IIaA15G2R1, IIaA17G3R1 and IIaA18G3R1). DNA extraction and genotyping of *Cryptosporidium* species is a challenging task on formalin-fixed stool samples, whose diagnostic outcome is dependent on the age of the sample. This method represented a step

forward in routine diagnosis, and improved the epidemiology of HIV-related clinical cases [129].

In Europe, studies on cryptosporidiosis have employed molecular techniques much earlier than other areas of the world. Generally, *C. parvum* is more common in Europe, followed by *C. hominis*, *C. meleagridis*, *C. felis* and *C. muris* (Table 3). No reports of *C. canis* nor *C. suis* have been documented thus far. In the UK, *C. parvum* was detected in both animals and humans, and *C. parvum* bovine genotype was found to be dominant.

In France, 13 *C. parvum* isolates typed using PCR-RFLP at the 18S rRNA gene locus indicated possible zoonotic transmission, while six *C. hominis* isolates were also reported [138]. The prominence of zoonotic species was also reported by Guyot et al. [139] who found that the majority of HIV-infected patients were infected with cattle (22 out of 46 cases) or human (14 out of 46 cases) genotypes of *C. parvum*. In addition, three patients were infected with *C. meleagridis*, six with *C. felis*, and one with *C. muris*. This was the first report of these three zoonotic species in humans reported in France, and the findings indicated that immunocompromised individuals were susceptible to a wider range of *Cryptosporidium* species and genotypes [139]. The same observation was made in a study in Switzerland [78].

Similarly, in Portugal, molecular analysis showed that 22 HIV-infected patients were infected with *C. parvum*, 11 with *C. hominis*, four with *C. felis*, and three with *C. meleagridis* [141]. Of those infected with *C. meleagridis* and *C. felis*, three were heterosexual, two were homosexual persons, one was an intravenous drug user and one acquired HIV infection through vertical transmission [141]. Another study in Portugal characterized 16 *C. parvum*, seven *C. hominis*, three *C. felis* and three *C. meleagridis* isolates using PCR-RFLP, and subsequent DNA sequencing analysis of 18s rRNA and 60-kDa glycoprotein genes showed extensive genetic diversity in both *C. parvum* and *C. hominis* isolates. Seven alleles were identified, three corresponding to *C. hominis* and four corresponding to *C. parvum* [140]. A further subgenotyping study characterized *Cryptosporidium* spp. from HIV-seropositive patients, as well as cattle, sheep and wild ruminants, from different regions of Portugal using a gp60-based PCR sequencing protocol. Results showed 14 *C. hominis* and nine *C. parvum* isolates, with five different subgenotypes belonging to five subgenotype families in *C. hominis*, and eight subgenotypes belonging to four subgenotype families in *C. parvum*. Seven of the eight *C. parvum* subgenotypes were observed in human parasites, while only four subgenotypes were observed in animals. All animal parasites in family IIa were of the IIaA15G2R1 subgenotype, with the exception of those from seven calves that exhibited the subgenotype IIaA16G2R1. Human parasites in the IIa family were identified as subgenotype IIaA15G2R1, but not IIaA16G2R1. In humans, four subgenotypes within the subgenotype family IId were identified (i.e., IIdA17G1, IIdA19G1, IIdA21G1, and IIdA22G1), two of which, IIdA17G1 and IIdA21G1, were also found in calves and sheep, respectively. All wild ruminants had the same subgenotype (IIa), which was also the predominant subgenotype in cattle all over Portugal, and was found in nine HIV-infected individuals [142]. These findings highlighted the significance of zoonotic transmission. In Switzerland, HIV-infected individuals were found to harbour *C. hominis* Ib and Id subgenotypes and *C. parvum* IIa and IId subgenotypes [143].

HIV-cryptosporidiosis in North America

As in Europe, the incidence of AIDS-opportunistic illness in the United States (US) has also decreased following the introduction of HAART [144]. However, there are some reports of *Cryptosporidium* infection among HIV-positive children, with acute diarrheal disease, as well as among asymptomatic HIV-positive individuals in the US with prevalence rates ranging from 3.5% to 8.5% (Table 2).

Data from the AIDS surveillance registry for the 10-year period 1983-1992 found an overall rate of 3.8% of individuals to be positive for cryptosporidiosis during the study period. Significant risk factors for cryptosporidiosis included sexual contact (3.9%), immigrants from Mexico (5.2%) and Latinos (4.2%) [147]. Seasonal variation was also reported, in New Orleans, Louisiana, where a greater number of cryptosporidiosis cases were observed among HIV-infected individuals in the spring compared to other seasons [144]. A prospective cohort study in Atlanta, Georgia, determined the role of enteric parasites in acute and chronic diarrhea in patients infected with HIV. This report described the incidence of diarrhea and its association with CD4+ T-lymphocyte counts, and cryptosporidiosis was found in 10.8% of 602 HIV-infected individuals [51]. Other than the US, the only report of cryptosporidiosis in HIV-infected individuals in North America was from Cuba, where 11.9% of 67 AIDS patients with cryptosporidiosis had continuous diarrhea [150].

Regarding the species of *Cryptosporidium* reported in HIV-infected individuals in the US, the most prevalent was *C. hominis*, followed by *C. parvum*, *C. canis*, and finally *C. muris* (Table 3). A study in the US characterized 13 *Cryptosporidium* isolates from people with AIDS and found that 10 were *C. hominis* and three were *C. parvum* [149]. A subsequent study in the US found that five of 10 AIDS patients with cryptosporidiosis were infected with *C. hominis*, one had *C. parvum*, three had *C. felis* and one had *C. canis* [150].

Characterization of isolates from outbreaks and sporadic cases of human cryptosporidiosis using PCR-RFLP and DNA sequencing confirmed that *C. parvum* is highly conserved at the TRAP-C2 locus. Of 17 *Cryptosporidium* isolates from HIV-infected patients in New Orleans, Louisiana, two were *C. parvum*, while the rest were *C. hominis* [151]. More recently genotyping and multilocus sequence typing techniques employed to determine the transmission patterns of cryptosporidiosis among HIV-infected individuals in Jamaica, demonstrated that 25 individuals had *C. hominis*, seven had *C. parvum*, one had *C. canis*, one had *C. felis*, and one had both *C. hominis* and *C. felis*. Subgenotyping of *C. hominis* and *C. parvum* using sequence analysis of the gp60 gene demonstrated that 22 *C. hominis* isolates were of the subgenotype IbA10G2, and three were subgenotype IeA12G3T3. All seven *C. parvum* specimens belonged to subgenotype IIcA5G3d. This finding suggested that the anthroponotic route of transmission predominated [152].

HIV-cryptosporidiosis in South America

A significant number of cases of cryptosporidiosis have been reported in HIV-infected individuals from various countries in South America with prevalence ranging from 4% to 22.8% (Table 2). In addition to geographic variability, there also appears to be seasonal differences in *Cryptosporidium* infection, with more infections occurring in warmer or more humid months [8].

The greatest numbers of studies on the prevalence of *Cryptosporidium* infection were reported from Brazil, with seven studies conducted in different regions on both symptomatic and asymptomatic HIV-infected adults and children. Prevalence in these studies ranged from 4% to 19.1% (Table 2) Using molecular tools, six species of *Cryptosporidium* have been identified among HIV-infected individuals

in South America. These include *C. hominis*, *C. parvum*, *C. meleagridis*, *C. muris*, *C. canis* and *C. suis* (Table 3). Currently, the occurrence of *C. suis* in HIV-infected individuals is only reported in Peru [22]. Recently, the predominance of *C. hominis* over *C. parvum* was reported [166]. Seventeen isolates were identified as *C. hominis*, five as *C. felis*, four as *C. parvum*, and one as *C. canis*. These findings suggested that while human-to-human transmission predominates in urban environments of Brazil, the cat species *C. felis* may play a potential role in the zoonotic transmission of cryptosporidiosis [166].

Similar findings on the diversity of *Cryptosporidium* spp. were also reported in Peru. *Cryptosporidium* oocysts were detected in 13.3% of 2,672 HIV-infected patients. Using PCR-RFLP, these authors identified six species; *C. hominis* (204 isolates) was the most frequently detected, followed by 38 isolates of *C. meleagridis*, 34 of *C. parvum*, 12 of *C. canis*, 10 of *C. felis*, and one of *C. suis*. These findings indicated that *C. hominis* is the predominant species in Peruvian HIV-positive persons, and that zoonotic *Cryptosporidium* spp. accounted for about 30% of cryptosporidiosis in these patients [22]. In addition, mixed *Cryptosporidium* infection was found to be more frequent and persist longer in HIV-infected individuals than in the general population [167]. Distribution of the five most common *Cryptosporidium* spp., namely *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, and *C. canis*, were found to be the same in HIV-infected adults and children [25].

Another genotyping and subgenotyping study in Peru, determined that 230 (9.2% of 2,490) HIV-infected individuals were infected with *Cryptosporidium*. Of these, there were 141 *C. hominis* isolates, 22 *C. parvum*, 17 *C. meleagridis*, six each of *C. canis* and *C. felis*, and one *C. suis*. All *C. parvum* isolates belonged to subgenotype family IIc, which is considered anthroponotic in origin. Although *Cryptosporidium* infections were associated with diarrhea, only those infections with *C. canis*, *C. felis*, and subgenotype family Id of *C. hominis* were associated with diarrhea without vomiting, while infections with *C. parvum* were associated with both chronic diarrhea and vomiting. These results demonstrated that different *Cryptosporidium* genotypes and sub genotype families are linked to different clinical manifestations [23].

Conclusion

This review confirms that HIV-positive individuals worldwide are at high risk of acquiring *Cryptosporidium* infection. The prevalence of cryptosporidiosis in these individuals, however, varies considerably amongst studies, depending on where the study was conducted, the season, the age of the populations studied, the stage of the disease, and the laboratory methods used. Cryptosporidiosis in HIV-infected people may result in severe and prolonged diarrhea, especially in patients with low CD4 T-cell counts, while at higher CD4 T-cell levels, spontaneous clearance of the parasite generally occurs. Due to the availability of HAART in developed nations (i.e., countries in Europe and North America), there has been a reduction in the prevalence of *Cryptosporidium* infection in HIV/AIDS patients. However, in the resource poor settings of developing countries (i.e., countries in Africa, Asia and South America), patients usually go undiagnosed for long periods and present late in the course of the disease [46]. With lack of access to HAART in some of these countries, infections with *Cryptosporidium* are usually prolonged and, in some instances, fatal. With limited effective treatment available for cryptosporidiosis, an intact immune system is crucial in resolving the infection [160].

In the last decade, numerous molecular tools have been employed to enhance the molecular epidemiology of *Cryptosporidium* by detecting and differentiating *Cryptosporidium* spp. at the species, genotype and subgenotype levels, which significantly improved our understanding of the transmission of cryptosporidiosis in humans and animals, and provided a better understanding of population genetics of *Cryptosporidium* transmission in humans [19]. The high prevalence of *C. hominis* reported in many of these studies indicated that humans are a major source of infection, and that person-to-person transmission probably played a major role in the spread of *Cryptosporidium* infection in HIV-infected patients. However, the presence of *C. parvum*, *C. meleagridis*, *C. muris*, *C. canis* and *C. suis*, especially in African, Asian, European and South American countries, is suggestive of zoonotic transmission, with infected individuals having direct or indirect (e.g., contaminated water or foods) contact with animals. These findings also demonstrated that immune compromised individuals are susceptible to a wide range of *Cryptosporidium* species and genotypes.

Future Priorities

Data is currently limited in a few high-priority areas with respect to cryptosporidiosis in HIV-infected people. For example, further information is required on the relative risks of acquiring cryptosporidiosis from drinking water, poor hygiene, unsafe sexual practices, direct or indirect contact with animals, household or nosocomial infections, oocyst-contaminated foods, and other sources. Another area that requires further study is in the association amongst clinical manifestations, CD4 T-cell counts, and the molecular type of *Cryptosporidium* in HIV/AIDS patients. Clinical studies will be needed to clearly define the asymptomatic carrier rate for different species, genotypes and subgenotypes of *Cryptosporidium* in HIV-infected patients who recover from a clinical episode of cryptosporidiosis and who have CD4 T-cell counts of >200 cells/mm^3. It will also be important to determine if such carriers are likely to develop severe cryptosporidiosis if their CD4 T-cell count drops below this level. Finally, as there are currently few viable options for drug treatment, especially in developing countries, continued screening of compounds for their efficacy in treating cryptosporidiosis is imperative. In particular, accessible, affordable, and efficacious drugs for the treatment of diarrhea caused by infection with *Cryptosporidium* spp., or for the outright clearance of infection, will be of considerable importance in minimizing the impact of this global opportunistic pathogen on HIV/AIDS patients.

A number of laboratory methodologies will need to be addressed in order to fill some of these research gaps. For example, sensitive, specific, and validated standardized methods for the routine detection and molecular characterization of human *Cryptosporidium* isolates identified in surveillance studies worldwide, as well as isolates from animals and environmental sources (e.g., water and foods), are required to facilitate epidemiological studies of cryptosporidiosis, and more firmly identify the possible sources of contamination. The use of second generation molecular diagnostic tools in conjunction with traditional epidemiological methods such as gp60-based subgenotyping, and more recently multi locus subgenotyping, can be used to determine the species, genotypes and subgenotypes present and will be very useful in identifying the source(s) of infections, whether anthroponotic or zoonotic. Development and incorporation of these methods, as well as those for viability determination of oocysts, will improve the efficiency and accuracy of surveillance studies and outbreak investigations, and could also facilitate screening of potential therapeutic agents for infections due to *Cryptosporidium* species.

Continued efforts in this area will provide the scientific data needed to better advice public health professionals, care givers and

the general public, on the sources of infection with *Cryptosporidium* and the means to reduce the likelihood of transmission. Regional and national reporting systems for collecting data on the numbers of cases of cryptosporidiosis are also needed to better quantify the public health impact of this disease, and to identify illness outbreaks. In light of the evidence supporting zoonotic transmission of cryptosporidiosis in HIV-infected individuals worldwide, prevention and control measures against cryptosporidiosis also need to be adopted and regulated at the farm level. The role of the veterinarian in diagnoses, treatment and guidance concerning cryptosporidiosis is significant in the management and prevention of this disease in farm animals as well as in companion animals. Since these animals have been implicated as a major source of *Cryptosporidium* transmission to humans, effective management of the disease in animals will likely translate into fewer human infections.

Finally, in order to develop effective disease reduction strategies related to *Cryptosporidium*, smart partnerships need to be built amongst governments, agencies, and health care workers responsible for the care of HIV-infected patients worldwide, as well as the targeted HIV-infected population itself. By addressing the data gaps mentioned above, the prevention and management of clinical disease in HIV-infected patients, and other immunocompromised individuals, could be significantly improved. Communication of the findings of such studies would also serve to focus the immunocompromised patients' attention on avoidance of exposures that would put them at greater risk.

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