Review Article

Global Distribution, Public Health and Clinical Impact of the Protozoan Pathogen Cryptosporidium

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Cryptosporidium spp. are coccidians, oocysts-forming apicomplexan protozoa, which complete their life cycle both in humans and animals, through zoonotic and anthropotonic transmission, causing cryptosporidiosis. The global burden of this disease is still underascertained, due to a conundrum transmission modality, only partially unveiled, and on a plethora of detection systems still inadequate or only partially applied for worldwide surveillance. In children, cryptosporidiosis encumber is even less recorded and often misidentified due to physiological reasons such as early-age unpaired immunological response. Furthermore, malnutrition in underdeveloped countries or clinical underestimation of protozoan etiology in developed countries contribute to the underestimation of the worldwide burden. Principal key indicators of the parasite distribution were associated to environmental (e.g., geographic and temporal clusters, etc.) and host determinants of the infection (e.g., age, immunological status, travels, community behaviours). The distribution was geographically mapped to provide an updated picture of the global parasite ecosystems. The present paper aims to provide, by a critical analysis of existing literature, a link between observational epidemiological records and new insights on public health, and diagnostic and clinical impact of cryptosporidiosis.

1. Introduction

1.1. The Cryptosporidium Parasite: General Description.

Infections of the human gastrointestinal tract with enteric pathogens are among the leading causes of disease, suffering, and death worldwide. Enteric pathogens are ingested with contaminated water and food and pass through the entire gastrointestinal tract. After establishment in a host, the infection spread to new hosts by a subsequent shedding. The most important and prevalent infections of the small intestine are caused by diarrheagenic Escherichia coli, particularly enterotoxigenic and enteropathogenic E. coli, Rotavirus, Giardia lamblia, and Cryptosporidium parvum [1–3]. Particularly, more than 58 million cases of diarrhea detected per year in children are associated to intestinal protozoa infections with high morbidity and mortality infection rates [4]. Cryptosporidium spp. are oocysts-forming apicomplexan protozoa. Following ingestion, the oocyst excystation, releases sporozoites which invade enterocytes. The excysted parasites undergo asexual (merogony) and sexual multiplication (gametogony) producing macrogametocytes and microgametocytes. Upon fertilization of the macrogametocytes by microgametes a zygotes is developed which sporulates (sporogony), generating thin-walled oocysts, involved in autoinfection and thick-walled oocysts excreted from the host (Figure 1). Once released in the environment, the parasite may cause enteric infection (cryptosporidiosis) both in humans and animals, mainly transmitted via the fecal-oral route through a zoonotic or anthropotonic modality or via contaminated water or food (Figure 2). In humans the disease results in sickness and severe diarrhea and can be life threatening in the very young, elderly and in immunosuppressed individuals, particularly those with HIV infection [5]. Contamination of drinking water by Cryptosporidium can result in major waterborne outbreaks of cryptosporidiosis [6]; additionally the Cryptosporidium is now increasingly considered an important foodborne pathogen [7, 8] causing a disease of socioeconomic significance worldwide. Three features of Cryptosporidium spp. ensure a high level of environmental contamination and
increase the likelihood of waterborne transmission. Firstly, they are responsible for disease in a broad range of hosts including man [9, 10], have a low-infectious dose (10–30 oocysts) enhancing the possibility of infection also in healthy immunocompetent people [11, 12], which may shed $10^8$–$10^9$ oocysts in a single bowel movement and excrete oocysts for up to 50 days after cessation of diarrhea [13, 14]; secondly, their transmissive stages (oocysts) are small in size and environmentally robust [15, 16] and thirdly, they are insensitive to the normal disinfectants commonly used in the water industry [17, 18].

1.2. Cryptosporidium Species and Human Infection. Since the genus Cryptosporidium was established for Cryptosporidium muris by Tyzzer in 1907, 37 species names have been introduced. However, after redescription and confirmation, currently 21 names are associated with individual species [19] and 16 species are actually regarded as valid on the basis of different oocyst morphology, site of infection, vertebrate class specificity, and genetic differences: C. muris in rodents; Cryptosporidium andersoni and Cryptosporidium bovis in cattle and sheep; Cryptosporidium suis in pigs; C. parvum in cattle, humans, and other mammals; Cryptosporidium meleagris in birds and humans; Cryptosporidium hominis in humans; Cryptosporidium baileyi and Cryptosporidium galli in birds; Cryptosporidium serpentis and Cryptosporidium saurophilum in snakes and lizards; Cryptosporidium molnari and Cryptosporidium scophthalmi in fish; Cryptosporidium wrairi in guinea pigs; Cryptosporidium felis in cats; Cryptosporidium canis in dogs [20]. The majority of these have a dominant host, but they are accidentally found in possibly unusual hosts. Remarkably, Cryptosporidium parasites are not related to other coccidians and the major recognised species in Cryptosporidium separate into two broad groups, with C. muris and C. serpentis forming one group and C. parvum, C. felis, C. wrairi, C. meleagridis, and C. baileyi forming a second broad group [21]. The accurate identification and characterisation of Cryptosporidium species and population variants are now central in the new taxonomic classification of Cryptosporidium species and in the categorization of genotypes or subtypes [20]. The picture that is emerging as a result of molecular studies clearly indicates that the species level taxonomy of the genus does not reflects the current molecular phylogenetic analyses or epidemiological data, which show high inter- and
Figure 2: Description of transmission modes of Cryptosporidium. Following ingestion (and possibly inhalation) by a suitable host (e.g., human host), excystation occurs (infective stage, (1)). The released sporozoites invade epithelial cells of the gastrointestinal tract or other tissues, complete their cycle producing oocysts which exit host (diagnostic stage, (2)) and are released in the environment (3). Transmission of Cryptosporidium mainly occurs by ingestion of contaminated water (e.g., surface, drinking or recreational water), food sources (e.g., chicken salad, fruits, vegetables) or by person-to-person contact (community and hospital infections) (4). Zoonotic transmission of *C. parvum* occurs through exposure to infected animals (person-to-animal contact) or exposure to water (reservoir) contaminated by feces of infected animals (4). Putignani and Menchella, 2010.

Intraspecific variation, and warrants reappraisal [21]. The vast majority of human cases of cryptosporidiosis worldwide are mainly caused by two species, *C. parvum* and *C. hominis* [21]. However other species, including *C. felis* [22, 23], *C. meleagridis* [23, 24], *C. canis* [23, 25], *C. suis* [23], *C. muris* [26], and more rarely *C. baileyi* [27] can infect humans too, especially children under the age of 5 years and immunocompromised individuals [28]. All *Cryptosporidium* species are transmitted in the various hosts by ingestion and inhalation of oocysts, irrespective of the species types. However, the clinical and epidemiological significance of various *Cryptosporidium* species and subtypes in humans is not yet clear. Results of recent genotyping studies nevertheless support the theory that *C. hominis* and *C. parvum* behave differently in humans especially with reference to the specificity of the clinical presentation. In *C. hominis* cases, nongastrointestinal symptoms (e.g., joint pain, eye pain, headache, dizziness and fatigue) are seen more often than in cases of *C. parvum*. Furthermore in young children, infections with *C. hominis* and, if symptomatic, *C. parvum*, are often heavy associated with fecal lactoferrin and growth shortfalls. *C. hominis* appears to stimulate inflammation irrespective of age; this raises important questions regarding how it may specifically induce greater proinflammatory response [29].

1.3. Transmission Modes and Risk Factors

1.3.1. Impact of Water Livestock on Transmission. Waterborne contamination is a growing concern causing widespread disease outbreaks. Factors that have contributed to the emergence of cryptosporidiosis in animals include increased environmental contamination and trends in livestock production. In humans the zoonotic nature of infection, along with increased numbers of at-risk population have contributed to the rate intensification of the disease [30]. Risk factors for
### Table 1: Factors that affect prevalence and adequate surveillance of cryptosporidiosis.

| Epidemiological indexes | Human activities | Environmental and social affecting factors |
|-------------------------|------------------|-------------------------------------------|
| Population age          | Hygienic and diet practices | Animal pollution |
| Gender                  | Rural and urban settings     | Famine, malnutrition, dehydration |
| Individual immunological status | Human waste contamination | Geography, international adoptions |
| Geographical distribution and ethnic group | Livestock pollution | Calamities (typhoons, local wars, floods, etc) |
|                         | Water treatment systems; food preparation styles and procedures | Climate variation, pollution, deforestation and seasonal rains |
|                         | Travels, immigration       |                           |

**Under-ascertainment factors in surveillance**
- Improper sampling of contaminated water systems and food
- Difficulty to identify the likely source of infection
- Misidentification of outbreak sources
- Multiple protozoan coinfections

**Under-ascertainment factors in clinics and diagnosis**
- Poor or diversified symptom presentations and low clinician’s sensitivity to consider protozoa as agents of gastrointestinal infections
- Limited inclusion of protozoan searching in operational diagnostic workflows
- Self-limiting infection course in immunocompetent adults and children
- Low inclusion of advanced molecular tools for routine diagnosis

Waterborne infections are deduced primarily from outbreak surveillance data. However, in the USA, only a fraction of the estimated water-related outbreaks are reported through passive surveillance [31]. While the outbreak epidemiology due to cryptosporidiosis is still a matter of concern, despite objective definition and identification of outbreak [32], the epidemiology of sporadic (non-outbreak-related) cases is largely unknown. Few papers have reported studies using the Geographical Information System (GIS) methods to map the locations of residences of sporadic cases or to assess ecosystems of cryptosporidiosis [33–36]. In the last few years a plethora of literature has been focusing on the description of advanced molecular markers and technologies [37–41], population structures [42–44], genetic variation of the parasite [45, 46], and linkage to its complex epidemiology [10, 47–50].

1.3.2. Impact of Climate and Weather on Transmission. A seasonal incidence of infection is sometimes present, possibly corresponding to rainfall peaks, increased pollution from farm waste, or calving and lambing activities [51, 52] (Table 1). Pivotal works have thoroughly investigated the seasonality of cryptosporidiosis also for children, showing highest prevalence from October to March. Such pattern may suggest a possible relationship with child care centre attendance in Europe [53], or provide correlation between seasonality and endemcity in Africa [54]. Recently, a meta-analysis has examined the seasonal patterns of cryptosporidiosis, with relation to precipitation and temperature fluctuations worldwide [55], according to the geographical Köppen Climate Classification [56]. Outcome data were linked to monthly ambient temperature and precipitation for each location and, for the Sub-Saharan Africa, to the Normalized Difference Vegetation Index, a remote sensing measure for the combined effects of temperature and precipitation on vegetation and cryptosporidiosis. Strong seasonal drivers for cryptosporidiosis showed precipitation in moist tropical locations and temperature in mid-latitude and temperate climates [55]. While climatic conditions typically define a pathogen habitat area, meteorological factors affect timing and intensity of seasonal outbreaks. Therefore, seasonality and meteorological forecasts can represent a key indicator and tool, respectively, to plan prevention programs for waterborne cryptosporidiosis (Table 1).
### 1.4. Cryptosporidiosis a “Neglected Disease” and a Poverty Index.

In developing countries the impact of protozoan pathogens represents a major cause of gastrointestinal illnesses and is becoming of growing impact, also because new epidemiological markers and indicators of infection are allowing researchers and clinicians to strengthen surveillance programs and diagnostic procedures [36, 44, 57]. However, a large proportion of diarrhoeal illnesses in these countries, especially in children, are still ascribed to an unknown etiology, often because the only available detection methods, such as microscopy and culture used in many areas, have low sensitivity. Particularly, *Cryptosporidium* and *Giardia* are still major causes of diarrhoeal diseases of humans worldwide, and are included in the World Health Organisation’s Neglected Diseases Initiative [58, 59]. The neglected tropical diseases are often indicators of poverty and disadvantage. Those most affected are the poorest populations often living in remote, rural areas, urban slums, conflict and natural disaster zones, where aggravate conditions are conducive to the spread of these diseases (Table 1). *Cryptosporidium* accounts for up to 20% of all cases of childhood diarrhea in developing countries, and is a potentially fatal complication of AIDS [30] and often, in early childhood, is often associated with poor cognitive function and failure to thrive [60].

### 1.5. Clinical Symptoms As Patognomic Evidence.

Variation in symptoms may represent and additional key indicator to set up specific diagnostic workflows for *Cryptosporidium* detection and to infer correlation between infecting species/subtypes and epidemiology (Table 1) (Figure 3). In the last few years, in low-income countries, an enhanced attention has been directed to the observation of symptom variation, which may provide a successful index for wholesale effective surveillance programs [50, 61]. A recent extensive study was performed in Bangladesh on 3646 case patients, who presented with diarrhea [61]. The study assessed the proportion of diarrhea cases attributable to *C. hominis*, *C. parvum*, *Entamoeba histolytica*, and *G. lamblia*. *Cryptosporidium* species and *E. histolytica* were more prevalent in patients with acute diarrhea, all ages and, specifically, those from 0 to 12 months of age. Remarkably, patients with diarrhea and cryptosporidiosis were less likely to have abdominal pain; patients with amebiasis were more likely to have visible blood in stool; patients with giardiasis were more likely to be dehydrated, compared with control subjects [61].

Recently, clinical symptoms such as abdominal pain and/or diarrhoea were selected as key indicators of *Cryptosporidium* and *Giardia* infections in patients in Belgium (Table 2) [62].

![Venn Diagram of factors leading to Cryptosporidium infection. Parasite, host and environmental indexes acting as key factors for the global burden of cryptosporidiosis. For details see Table 1. Putignani and Menchella, 2010.](image-url)
| Samples (surveillance study or sporadic cases) | Country | Age (human cases) | Technique/genotyping tool | Species/genotypes/subgenotypes | Reference |
|-----------------------------------------------|---------|-------------------|---------------------------|---------------------------------|-----------|
| Human stools                                  | India   | Children          | 18S rRNA, SSU, COWP, Cpgp40/15, TRAP-C1-based PCR | C. hominis (Ia, Id, le, Ib), C. parvum (lc), C. felis | [36]      |
| Environmental (water)                         | China   | —                 | 18S rRNA PCR-RFLP and sequence analyses; GP60 | C. hominis (IbA19G2, IbA20G2, and IbA21G2), Id, le (leA12G3T3), If (IfA22G1) C. meleagridis, C. baileyi, C. parvum, C. suis, C. muris, rat genotype, avian genotype 3 | [41]      |
| Human stools                                  | Perù    | Children          | GP60                       | C. hominis (Ia, Ib, Id, le, Id), C. parvum (Ilc), C. meleagridis, C. canis, C. felis | [50]      |
| Human stools                                  | Ireland | Adults and children | 18S rRNA and COWP PCR-RFLP; GP60 | C. hominis (IbA10G2), C. parvum (IaA18G3R1) | [51]      |
| Human stools                                  | Belgium | Adults and children | 70-kDa heat shock protein, 60-kDa glycoprotein (GP60) | C. hominis (IbA10G2, IaA9G3) C. parvum (IaA15G2R1, IlaA5G3a, IldA16G1 IlaA15G2R1) | [62]      |
| Human stools                                  | UK      | Adults and children | COWP and small sub-unit (SSU) rRNA gene PCR-RFLP | C. parvum, C. hominis, C. meleagridis, C. felis, C. canis, Cryptosporidium cervine, horse, skunk genotypes | [63]      |
| Human stools                                  | Haiti   | Adults and children | 18S rRNA PCR-RFLP | C. hominis, C. parvum, C. felis | [69]      |
| Human stools                                  | Perù    | Adults            | GP60                       | C. hominis (Ia, Ib, Id, le) C. canis, C. felis, C. parvum, C. meleagridis | [74]      |
| Human and animal stools                       | Portugal| Adults and children | GP60                       | C. hominis (Ib, If), C. parvum (Ila, Iib, Iic and Id) | [78]      |
| Environmental (water)                        | France  | —                 | IMS-IFA\(^2\), 18S rRNA PCR-RFLP | C. hominis, C. parvum | [96]      |
| Animal stools                                 | Ireland | Neonatal calves   | GP60                       | C. parvum (IlaA18G3R1), C. bovis, Cryptosporidium deer-like genotype | [101]     |
| Environmental (water)                        | Portugal| —                 | IMS-IFA\(^2\), PCR        | C. hominis (IaA15G2R1, IlaA16G2R1, IldA17G1) | [103]     |
| Animal and human stools                       | Portugal| Adults and children | GP60                       | C. hominis, C. parvum, C. felis, C. meleagridis | [104]     |
| Human stools                                  | MI (USA)| Adults and children | 18S rRNA and COWPPCR-RFLP; GP60 | C. hominis, C. parvum (cervine genotype, cervine genotype variant, human genotype W17) | [148]     |
| Animal and human stools                       | Iran    | Children and one adult | 18S rRNA PCR-RFLP          | C. parvum, C. hominis (anthropootic and zoonotic genotype) | [164]     |
| Animal stools                                 | China   | Neonatal calves   | 70-kDa heat shock protein; 18S rRNA, actin-based PCR | C. andersoni, C. ryanae | [165]     |
| Animal stools                                 | India   | Neonatal calves   | 18S rRNA PCR-RFLP          | C. parvum, C. hominis | [206]     |
| Human stools                                  | UK      | Adults and children | SSCP-based analysis of the 18S rRNA SSU and ITS-2 spacer | C. parvum (types 1 and 2) | [208]     |
| Human stools                                  | UK      | Adults            | GP60                       | C. hominis (IbA10G2) | [209]     |
| Human stools                                  | Kenya   | Adults and children | 18S rRNA PCR-RFLP          | C. parvum (human genotype), C. parvum (bovine genotype), C. meleagridis, C. muris | [222]     |
under one year and in females aged 15 to 44 years. Spring peaks were due to *C. parvum*, while *C. hominis* was more prevalent during the late Summer and early Autumn as well as in patients reporting recent travel abroad [63] (Table 1) (Figure 3) (Table 2). *C. parvum* and *C. hominis* are two species responsible for most human cases of cryptosporidiosis. The relationship between the global population structure of these species and the host population arrangement was thoroughly investigated by the study of Tanriverdi et al., 2008 [66], in which a series of worldwide *C. parvum* and *C. hominis* isolates were genotyped. Geographical partitions or patterns for both parasite species were observed among the countries (Uganda, Serbia, Turkey, Israel, UK, USA, and New Zealand), possibly because of different prevailing ecological determinants of transmission [66]. Rather than conforming to a strict paradigm of either a clonal or a panmictic population structure [43], these data seem to suggest a flexible reproductive strategy characterized by the cooccurrence of both propagation patterns.

A predominance of *C. hominis* was observed in persons in developing countries, such as pediatric populations from Perú [26, 50], Malawi [67], Kenya [68], India [36], Haiti [69], and Brazil [70], children and elderly persons from South Africa [71], and hospitalized HIV-infected children from South Africa [72] and Uganda [73]. A comparatively large proportion of participants infected with *C. meleagridis* was observed in a wide community in Haiti [69], a finding that was also reported at a high frequency in HIV-infected adults in Perú [74]. This species has been reported, even if rarely, by other studies regarding either children or adults with or without HIV infection from other geographical places as Portugal [75], India [36, 57], Taiwan [76], or Iran [77].

Environmental isolates of *Cryptosporidium* from China were thoroughly investigated in the study of Feng et al., 2009 (Table 2) [41]. Interestingly, the predominant species was *C. hominis* followed by *C. meleagridis*. The other *Cryptosporidium* species/genotypes identified included *C. baileyi, C. parvum, C. suis, C. muris*, rat genotype, avian genotype 3, and a novel genotype. The *Ib* identified subtypes (Table 2) [41], were very different from the subtypes *IbA9G3* and *IbA10G2* commonly found in other areas of the world. The *IbA9G3* is usually observed in humans in Kenya, India, and Australia, and *IbA10G2* is commonly seen in South Africa, Perú, USA, Canada, Australia, and European countries, as France, UK, Portugal, Spain and Ireland [50, 68, 72, 78–84]; *IbA10G2* is responsible for more than half of the waterborne outbreaks in USA, and Canada [85, 86]. Likewise, the *IeA12G3T3* was different from the most common *IeA11G3T3* subtype, although observed also in Louisiana, Australia, and Jamaica [79, 87, 88] (Table 2). Also the *Ifa20G1* and *Ifa22G1* subtypes were detected, as previously in children in South Africa [72], occasionally in HIV-positive adults in Portugal [78] and in India [89], but not in most other studies, supporting the presence of unique transmission of *C. hominis* in China (Table 2).

In spite of the availability of substantial sequence data obtained by reliable typing tools (e.g., COWP, TRAP C-1, GP60) there is still no comprehensive analysis of the genetic richness, and diversity within *C. hominis* and *C. parvum* [90]. The worldwide literature produced so far highlights the need to pursue on detailed molecular epidemiological studies (e.g., GP60), especially in “neglected” geographical regions and from a wide range of hosts species. Indeed researchers have to address the key question if the low diversity associated to the substantial richness of the GP60 locus is due to the genetics of the organism or to a lack of data from countries with potential endemic transmissions (Africa, South east, China, and India subcontinents) [90]. High-throughput technologies (“genome sequence surveys”) and advanced bioinformatic platforms

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### Table 2: Continued.

| Samples (surveillance study or sporadic cases) | Country | Age (human cases) | Technique/genotyping tool | Species/genotypes/subgenotypes¹ | Reference |
|-----------------------------------------------|---------|-------------------|----------------------------|----------------------------------|-----------|
| Human stools, Switzerland, Kenya, USA         | Adults and children | 18S rRNA; HSP-70; acetyl coenzyme A synthetase | *C. parvum* (“human” genotype, “cattle” genotype), *C. felis, C. meleagridis* | [223] |
| Human stools, Spain                           | Adults and children | 18S rRNA-, COWP-based PCR-RFLP | *C. hominis, C. parvum, C. meleagridis, C. felis* | [224] |
| Human stools, Equatorial Guinea               | Adults and children | COWP-based PCR-RFLP | *C. parvum, C. hominis, C. meleagridis* | [232] |
| Human and animal stools, Thailand             | Adults | 18S rRNA PCR | *C. parvum* | [239] |
| Human stools, Perú                            | Adults and children | 18S rRNA-based PCR-RFLP | *C. hominis, C. meleagridis, C. parvum, C. canis, C. felis, Cryptosporidium (pig genotype)* | [240] |
| Human stools, Poland                          | Adults and children | COWP and β-tubulin-based PCR | *C. hominis, C. parvum, C. meleagridis* | [254] |
| Human stools, Madagascar                      | Children | GP60 | *C. hominis* (Ia, Id, Ie), *C. parvum* (Ic) | [275] |

¹When available, reported subgenotypes are the most common detected.
²IMS-IFA, immunomagnetic separation followed by immunofluorescence assay: Method 1623 of the USA Environmental Protection Agency (USEPA).
(http://cryptodb.org/cryptodb/) may allow unprecedented comparative studies of Cryptosporidium isolates and overcome the limits of incomplete global epidemiological data.

1.7. Diagnostic Pitfalls: The Case of a Developed Country. An interesting case-control study [91], performed in Lower Saxony (Germany), during 2001 to 2005, reported 744 cases of cryptosporidiosis detected by the Governmental Institute of Public Health. The study demonstrated that a broad and improved diagnostic activity in reference laboratories was able to better describe cryptosporidiosis, reflecting the real occurrence of this infection, often underestimated. The yearly incidence rate of 1.9 notified cases per 100,000 population within Lower Saxony exceeded the German mean incidence rate of 1.5. In several neighboring districts there was a striking heterogeneity of regional incidences. However, highest rates of notification were associated with one particular laboratory where all stool samples, submitted for routine microbiological diagnosis, were screened for C. parvum. Diagnostic work was done by valid, specific, CE-certified procedures. The inferred conclusion was that the increased regional incidence rate was caused by the extensive diagnostic activity of this laboratory, presuming an underestimation in other regions [91]. These data seem to suggest that, even in high-income countries, routine diagnostic protocols should be thoroughly integrated by highly advanced identification workflows.

1.8. Aims of the Review. This review aims to discuss the updated global distribution of cryptosporidiosis, focusing on the main records reported for sporadic cases and outbreaks in the last decade and exploiting spatial and temporal determinants of infection particularly for low-income countries. With this intent, key indicators were critically considered to describe dynamics of transmission linked to the principal reservoirs of environmental infection (e.g., water, food) but also to the main host factors (e.g., age, travel, immunostatus) (Table 1) (Figure 3).

The principal cryptosporidiosis ecosystems, linked to outbreak and sporadic human cases, were geographically mapped worldwide (Figure 4) and origins of infection were categorised as Waterborne (Section 3), Foodborne (Section 4), Travelers’ (Section 5), and HIV-related disease (Section 6). Cryptosporidiosis in children was separately approached, according to multivariate and specific exposure factors acting in this life age (Section 7). The set of reviewed data (outbreak and sporadic human cases, environmental and veterinary surveillance reports) was correlated, in each Section, to the geographical setting (continent) and subsetting (country) (Figure 4, Figure 5).

2. Study Approach

International surveillance networks and suites of free and open-source for epidemiology control or open-access peer-reviewed journals about infectious diseases surveillance prevention and control in Europe, Canada, USA, and Australia were exploited for our analysis (Table 3).
surveillance programs with periodical reports on etiologic agents, failures of water-treatment systems, and deficiencies associated with outbreak management [93] (Table 3). In developed countries, the detection of Cryptosporidium and Giardia should be an integral part of the quality system in the water industry and multidisciplinary approaches among public health professionals (epidemiologists, clinicians and parasitologists) should be routinely included to establish priorities in public health prevention programs and to design appropriate operational workflows for both detection and diagnosis. In developing countries the potential of infection is enhanced by the absence of sanitary and parasitological drinking water monitoring. Moreover the burden of the infection is surely underestimated for the small number of appropriate surveillance programs and for the absence of suitable diagnostic algorithms.

3.2. Waterborne Cryptosporidiosis in Europe. Recently, in Europe, the circulation of Cryptosporidium spp. populations has been thoroughly investigated because of the improved surveillance and diagnosis of both sporadic cases and outbreaks of cryptosporidiosis (Table 4). From a waterborne outbreak of diarrhea in France, the 91% of the isolates of the parasite were characterised as C. hominis type Ib [86], consistently with the current idea that Ib is the predominant allele associated with waterborne cryptosporidiosis world-wide [79, 81].

Earlier evidence had suggested that accidental ingestion of natural waters while bathing carries a risk of infection by waterborne protozoa both in UK and USA [94, 95]. In order to evaluate this risk in France, a one-year prospective study on recreational lakes and river sites located near Paris, chosen for frequent bathing and boating, was undertaken (Table 2) [96]. Giardia cysts and Cryptosporidium oocysts were detected in the recreational lakes with occasional peaks and in the river sites throughout all the year. Genetic characterization of Cryptosporidium revealed the presence of both C. hominis and C. parvum species (Table 2). Based on
a model for quantitative microbial risk assessment (QMRA), the study confirmed that bathing in surface waters was actually associated with a significant risk of infection by *Cryptosporidium* or *Giardia*. This was especially the case for rivers not protected from human or animal fecal contamination. Surface waters, especially in rural areas, may be soiled by contaminated farmyard manure or slurry used as fertiliser for crop cultivation. Pasturing of infected livestock near crops or defaecation of infected undomesticated hosts onto them is an important factor for zoonotic contamination [7]. Also sludges, night soil, and raw waters may contribute to worldwide water pollution [97]. Recently, as inferred by outbreak surveillance data, recreational, drinking and fountain waters have been identified as important source of community infections worldwide (Table 4) [6].

In Ireland, as several recent waterborne outbreaks have shown (Table 4) [80, 98, 99] and as thoroughly discussed by the latest paper of Cheng et al., 2009 on the presence of *Cryptosporidium* and *Giardia* in wastewater treatment plants [100], cryptosporidiosis poses a significant threat to public health. In a recent study [51], performed on human stool samples collected in diversified geographical areas of Ireland, *Cryptosporidium* spp. were genetically characterised (Table 2). Overall, *C. parvum* was identified in 80% and *C. hominis* in 20% of cases, with an higher proportion in older age groups. *C. parvum* was the most common species in the rural, more sparsely populated West of Ireland and exhibited a pronounced Spring peak coincident with the peak of the national cryptosporidiosis incidence rate. The most common *C. parvum* subgenotype (Table 2) was the same detected in Irish cattle [101] confirming the prevalence of zoonotic *Cryptosporidium* transmission in Ireland.

In Holland, water in canals and recreational lakes are contaminated through the discharge of raw sewage from houseboats, sewage effluent, dog and bird feces. During two successive one-year study periods, the water quality in canals and recreational lakes was tested in Amsterdam with regard to the presence of fecal indicators and waterborne pathogens [102]. *Cryptosporidium* oocysts and *Giardia* cysts were detected both in canals and recreational lakes, despite conformity with the European bathing water legislation, indicating these parasites as health risk pathogens for situations of exposure to surface waters [102].

A significant study was undertaken to monitor the presence of *Cryptosporidium* and *Giardia* in water samples, including raw and treated waters from both surface and

Figure 5: Geographical distribution of Italian studies on Cryptosporidium surveillance. A map of the principal surveillance studies performed on environmental and human samples is here reported by using the following color codes: red for environmental samples (water, animal); green for human samples associated to HIV in adults; pink for children samples. Symbols refer to different Italian regions: Ab, Abruzzo; Ap, Apulia; Ca, Campania; ER, Emilia Romagna; FVG, Friuli Venezia Giulia; La, Latium; Lo, Lombardia; Ma, Marche; Pi, Piedmont; Sa, Sardinia; Si, Sicily; Tu, Tuscany; Ve, Veneto. Putignani and Menchella, 2010.
Table 3: Public data sources exploited in the current study.

| Site name                                           | Link                                                                 | Reference |
|-----------------------------------------------------|----------------------------------------------------------------------|-----------|
| *International surveillance networks*              |                                                                      |           |
| Neglected Diseases Initiative of the World Health Organization | http://www.who.int/neglected_diseases/en/                             | [59]      |
| Center for Disease Control and Prevention           | http://emergency.cdc.gov/agent/agentlist-category.asp                 | [93]      |
| San Francisco Bay Area Cryptosporidiosis Surveillance Project | http://www.sfphes.org/water/index_crypto.htm                         | [136]     |
| C-EnterNet (Canadian Integrated Enteric Disease Surveillance System) | http://www.phac-aspc.gc.ca/c-enternet/index-eng.php                   | [153]     |
| Public Health Agency of Canada                     | http://www.phac-aspc.gc.ca/index-eng.php                             | [154]     |
| FoodNet                                             | http://www.cdc.gov/FoodNet/                                          | [183]     |
| *Suites of free and open-epidemiology data source* |                                                                      |           |
| Tri-County Health Department                       | http://www.tchd.org/                                                 | [150]     |
| AIDS site                                           | http://data.unaids.org/en/default/asp                                | [161]     |
| Communicable Diseases Branch                        | http://www.health.qld.gov.au/ph/cdb/default.asp                       | [245]     |
| NetEpi                                              | http://code.google.com/p/netepi                                       | [273]     |
| Epi Info software                                   | http://www.who.int/chp/steps/resources/EpiInfo/en/index.html          | [274]     |
| *Open-access peer-reviewed journals*                |                                                                      |           |
| Eurosurveillance Europe’s Journal on infectious disease epidemiology, prevention and control | http://www.eurosurveillance.org/                                     | [63, 81, 98, 99, 178, 246–248, 276, 291, 292] |
| Center for Disease Control and Prevention: Morbidity and Mortality Weekly Report (MMWR) | http://www.cdc.gov/mmwr/                                             | [92, 131, 132, 146, 156, 185–191, 249, 258, 260, 285, 293–295] |
| Public Health Agency of Canada                     | http://www.phac-aspc.gc.ca/surveillance-eng.php                       | [86, 296, 297] |

ground sources in Portugal [103]. *C. parvum* was the most common detected species, followed by *C. hominis*, *C. andersoni*, and *C. muris*. These results are clearly suggestive of a wide distribution of *Cryptosporidium* spp. in source and treated waters in Portugal, with high occurrence of human-pathogenic *Cryptosporidium* genotypes (Table 2) [103, 104].

An important survey of sewage influent samples from 40 Sewage Treatment Works (STWs) throughout Norway were examined for *Cryptosporidium* oocysts and *G. duodenalis* cysts [105]. The data propose giardiasis as more widespread, and occurring with greater infection intensity than cryptosporidiosis: for *Cryptosporidium*, the highest estimate was up to 5 per 100 000 individuals in Eastern Norway while for *Giardia* 40 per 100 000 persons in Western Norway. Removal efficiencies at two STW with secondary treatment processes were estimated to be approximately 50% for *Cryptosporidium* and >80% for *Giardia*. A STW with minimal treatment had negligible removal of both parasites. Because many STW in Norway have indeed minimal treatment and discharge effluent into rivers and lakes [105], thus, risk of contamination of water courses by *Cryptosporidium* and *Giardia* represents a considerable risk in this country. Contamination from sewage discharges and wild or domestic animals are also important sources for untreated waters [97]. Both *Cryptosporidium* and *Giardia* are frequently found in the stool of domestic ruminants, especially young animals. Wild ruminants may serve as reservoirs for these zoonotic parasites, as inferred from an important cross-sectional survey conducted in Belgium to estimate the occurrence of *Cryptosporidium* and *Giardia* in captive wild young ruminants. The *Cryptosporidium* prevalence was 7.5% in the zoo animals and 3.7% in the bison from a commercial breeding farm [106].

In the Russian Federation, *Cryptosporidium* has been recently included in the *Index of the Epidemic Safety of Drinking Water* as new emerging pathogen, suggesting a growing attention to the parasite control also in geographical areas with no previous surveillance programs and dedicated studies [107].

3.2.1. Waterborne Cryptosporidiosis in Italy. In Italy, an intense debate on the epidemiological and public health aspects of *Cryptosporidium* and *Giardia* infections has involved many researchers and has lead to investigate infection prevalence data especially on environmental and animal samples but less on human samples because cryptosporidiosis is not a notifiable disease in Italy [108–110] (Figure 5). Protocols recommended by the *National Institute of Health* are mainly used for detection of these protozoa...
Table 4: Worldwide distribution of principal *waterborne*, *foodborne*, *travel-related*, and community outbreaks reported in the last decade (1998–2008): case characteristics.

| Outbreak¹ type | Country          | Ill²          | Age         | Likely causes for outbreak occurring                                      | Species / genotype     | Reference |
|----------------|------------------|---------------|-------------|---------------------------------------------------------------------------|------------------------|-----------|
| **2008**       |                  |               |             |                                                                           |                        |           |
| Foodborne      | Finland          | 72 personnel of the Public Works Department in Helsinki | Adults      | Salad mixture suspected                                                   | *C. parvum*            | [178]     |
| Foodborne      | Sweden           | 21 guests and staff at a wedding reception             | Adults      | Sauce containing chopped fresh parsley                                     | *C. parvum*            | [179]     |
| Waterborne     | Norway           | 89 hotel guests                                       | Adults      | In-house water contamination                                              | *C. parvum*            | [298]     |
| Community      | Scotland (UK)    | 6 veterinary students                                 | Young adults| Lapse in hygiene, especially handwashing                                   | *C. parvum* (subgenotype IIaA19G2R1) | [299]     |
| Waterborne     | England (UK)     | 57 swimming pool visitors                              | Children and adults | Swimming pool contamination                                                | *C. parvum* *C. hominis* | [246]     |
| Waterborne     | Germany          | 201 soldiers                                           | Young adults| Tap water/food contamination in a military field exercise                  | *C. parvum* (genotype 2) | [300]     |
| Waterborne     | Ireland (UK)     | 182                                                    | Adults      | Contamination of treated water                                            | *C. hominis* *C. parvum* | [98]      |
| Waterborne     | Sweden           | 800–1000                                              | Children and adults | Contamination of an outdoor swimming-pool                                 | *C. parvum*            | [250]     |
| Waterborne     | ID (USA)         | 50 park visitors                                       | Children and adults | Exposure to water from a splash feature                                   | *C. hominis* (subgenotype IaA28R4) | [258]     |
| **2006**       |                  |               |             |                                                                           |                        |           |
| Waterborne     | England (UK)     | 35 school people                                      | Children and adults | Surface water contamination during a farm visit                           | *C. hominis*           | [251]     |
| Waterborne     | CO (USA)         | 21 attendes to a pool party                           | Children and adults | Swimming, pool contamination                                               | *C. hominis* (subgenotype IbA10G2) | [85]      |
| Travelers’ Infection | FL (USA)    | 29 retired people                                      | Elderly     | Environmental contamination with animal feces                             | *C. parvum* (subgenotype IIaA16G1R1b) | [207]     |
| Foodborne      | ME (USA)         | 14 people                                             | Not reported | Unknown                                                                    | *C. parvum* (genotype IIa) | [301]     |
| Foodborne      | PA (USA)         | 2 people                                              | Not reported | Unknown                                                                    | *C. parvum* (genotype IIa) | [301]     |
| Waterborne     | FL (USA)         | 9 children                                            | 4 years³   | Water fountain contamination                                               | *C. parvum* (genotype IIa) | [261]     |
| Foodborne      | Japan            | 4 company members                                     | Adults      | Contamination of raw meat dish                                            | *C. parvum* (genotype IIa) | [301]     |
| **2005**       |                  |               |             |                                                                           |                        |           |
| Foodborne      | Denmark          | 99 company employees                                   | Adults      | Buffet salad eating                                                       | *C. hominis*           | [180]     |
| Community      | Scotland (UK)    | 62 people                                             | Adults and children | Outbreak linked to a wildlife centre visit                                | *C. parvum*            | [247]     |
| Waterborne     | Wales (UK)       | 100                                                    | Mostly young adults | Contamination of raw and treated water                                    | *C. hominis*           | [291]     |
| Waterborne     | Turkey           | 191 inhabitants³                                      | Children and adults | Contamination of water tank                                               | *C. parvum*            | [276]     |
| Outbreak Type | Country | Country Detail | Ill | Age | Likely causes for outbreak occurring | Species/genotype | Reference |
|--------------|---------|----------------|-----|-----|-------------------------------------|-----------------|-----------|
| Community    | Spain   | 24 day-care children | Children | Children diaper use | C. hominis | [252] |
| Waterborne   | Norway  | 115 adults | Adults | Water supply contamination | C. parvum (bovine genotype, bovine genotype 2) | [277] |
| Community    | Croatia | One family members | Elderly and adults | Nosocomial and person-to-person contamination | C. hominis | [302] |
| Waterborne   | CA (USA) | 273 park attendants | Children and adults | Contamination of a water park | C. parvum (genotype IIc) | [127] |
| Foodborne    | NY (USA) | 212 people | Not reported | Contamination of unpasteurized apple cider | Cryptosporidium spp. | http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm |
| Waterborne   | Yorkshire and The Humber (UK) | 66 people attending at the pool | Children and adults | Contamination of water at a public pool | Cryptosporidium spp. | [293] |
| Waterborne   | South West (UK) | 21 children attending at the water park | Children | Leisure facility of a 'water splash zone' | Cryptosporidium spp. | [249] |
| Waterborne   | South East of England (UK) | 17 people attending at the pool | Children and adults | Contamination of water at a public pool | Cryptosporidium spp. | [249] |
| Waterborne   | Midlands (UK) | 122 people attending at the park | Children and adults | Contamination of a fountain water in a public park | Cryptosporidium spp. | [249] |
| Waterborne   | South West of England (UK) | 63 people attending at the animal centre | Children | Interactive water feature at an animal attraction centre | C. parvum (genotype 2) | [248] |
| Community    | Wales (UK) | 17 people attending at the school visit | Children and adults | Open farm, school visit | C. parvum (subgenotype IlaA15G2R1) | [79] |
| Community    | Wales (UK) | 36 people attending at the visit | Children and adults | Residential farm centre, school visit | C. parvum | C. hominis, C. meleagridis | [63] |
| Waterborne   | Majorca (Spain) | 179 travellers | Children and adults | Hotel pool water contamination | Cryptosporidium spp. | [292] |
| Foodborne    | OH (USA) | 144 inhabitants | Children and adults | Contamination of unpasteurized apple cider | C. parvum subgenotype IlaA15G2R1, IlaA17G2R1 | [182] |
| Community    | MN (USA) | 31 middle-/high-school students | Young people | Contact with calves | C. parvum | [303] |
| Community    | MN (USA) | 37 middle-/high-school students | Young people | Manure on hands | C. parvum | [303] |
| Foodborne    | MN (USA) | 9 people | Not reported | Contamination of food in a hotel banquet room | Cryptosporidium spp. | http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm |
| Outbreak type | Country                  | III | Age                      | Likely causes for outbreak occurring                                                                 | Species / genotype | Reference |
|---------------|--------------------------|-----|--------------------------|--------------------------------------------------------------------------------------------------------|--------------------|-----------|
| Community     | Yorkshire and The Humber (UK) | 47 people | Children and adults | Contamination at a day care nursery                                                                    | *C. parvum*, *C. hominis*, *C. meleagridis* | [63]       |
| Multiple       | Wales (UK)               | 4 people | 3 children and 1 adult  | Contamination at a day care nursery                                                                    | *C. parvum* (subgenotype IIaA17G1R1) | [79]       |
| Waterborne     | South East of England (UK) | 21 people | Not reported | Contamination of a private drinking water supply                                                       | *Cryptosporidium* spp. | [293]     |
| Waterborne     | South East of England (UK) | 31 people | Not reported | Contamination of a private drinking water supply                                                       | *Cryptosporidium* spp. | [293]     |
| Waterborne     | North West of England (UK) | 50 school people | Adults and children | Contamination of a private drinking water supply at a college                                           | *Cryptosporidium* spp. | [293, 294]|
| Community      | Northern Ireland (UK)    | 29 people | Adults                   | Contamination of raw and treated water, and land surrounding the lake watershed                        | *Cryptosporidium* spp. | [99]      |
| Community      | Netherlands              | Not reported | Children               | Not reported, during a pet farm visit                                                                  | *C. parvum* (genotype C1) | [http://www.cryptosporidium.it/](http://www.cryptosporidium.it/) |
| Community      | NY (USA)                 | 13 veterinary students | Young people   | Hands contamination by calves contacts                                                                | *Cryptosporidium* spp. | [304]     |
| Foodborne      | FL (USA)                 | 37 people | Not reported             | Contamination of food in a hotel banquet room                                                         | *Cryptosporidium* spp. | [http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm](http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm) |
| Foodborne      | GA (USA)                 | 6 people | Not reported             | Contamination of food in a private home                                                                | *Cryptosporidium* spp. | [http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm](http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm) |
| Waterborne     | France                   | 291 county inhabitants | Adults and children | Public water supply contamination                                                                    | *C. hominis* (genotype Ib,Id), *C. parvum* (genotype IIa) | [84]       |
| Waterborne     | France                   | 573 | Adults                   | Contamination of tap water                                                                            | *C. parvum* (genotype I) | [305]     |
| Waterborne     | South West of England (UK) | 14 | Adults and children      | Contact with a stream at a beach                                                                      | *Cryptosporidium* spp. | [294]     |
| Community      | South East of England (UK) | 30 | Adults and children      | Contamination at a day care nursery                                                                    | *Cryptosporidium* spp. | Unpublished data |
| Waterborne     | South East of England (UK) | 152 people | Adults and children | Contamination of outdoor school pool water                                                            | *Cryptosporidium* spp. | [294, 295]|
| Waterborne     | Canada (USA)             | 1039 people | Young adults            | Contamination of drinking water                                                                      | *C. parvum* | [296]     |
| Waterborne     | Canada (USA)             | 59 people | Attending an Ukrainian dance festival | Contamination of a swimming pool in a hotel                                                          | *C. parvum* | [297]     |
| Waterborne     | (IL) USA                 | 358 waterpark attendants | Adults and children | Contamination of waterpark and person-to-person contact                                               | *C. hominis* (genotype Ia) | [259]     |
### Table 4: Continued.

| Outbreak Type | Country | Ill | Age | Likely causes for outbreak occurring | Species/genotype | Reference |
|---------------|---------|-----|-----|--------------------------------------|-----------------|-----------|
| Foodborne     | Queensland (Australia) | 8 inhabitants | Children | Contamination of drinking unpasteurised milk | Cryptosporidium spp. | [262] |
| Community     | New Zealand | 20 farm inhabitants | Children | Hand contamination by calve contact | Cryptosporidium spp. | [264] |
| Community     | Tasmania (Australia) | 36 participants at the agricultural show | Adults | Contamination associated with an animal nursery | Cryptosporidium spp. | [306] |
| Community     | Brazil | 224 day care attendants | Children | Person-to-person contact | C. hominis (genotype 1) | [307] |
| | | | | | | |
| Waterborne    | Northern Ireland (UK) | 347 | Adults | Contamination of drinking water | C. parvum (bovine genotype; human genotype) | [80] |
| Waterborne    | Haiti | 93 patients | Adults and children | Contaminated water and overcrowded conditions of urban slums | C. hominis, C. parvum, C. felis | [69] |
| Waterborne    | England and Wales (UK) | 58 | Adults | Contamination of drinking water | C. parvum (genotype 2) | [308] |
| Waterborne    | England and Wales (UK) | 207 | Not reported | Contamination of drinking water | C. hominis and C. parvum alleles | [309] |
| Waterborne    | Yorkshire and The Humber (UK) | 41 people attending a public pool | Adults and children | Contamination of pool water | C. parvum (subgenotype IlaA17G1R1) | [79, 295] |
| Waterborne    | Majorca (Spain) | >250 | Adults and children | Contamination of a hotel pool water | Cryptosporidium spp. | [81] |
| Community     | Netherlands | Not reported | Children | School children visiting a pet farm | Cryptosporidium spp. | http://www.cryptosporidium.it/ |
| Foodborne     | IL (USA) | 8 | Not reported | Contamination of coleslaw in a private home | C. parvum | http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htm |
| Waterborne    | Russia | 50 | Adults | Contamination of drinking water | Cryptosporidium spp. | [310] |
| Waterborne    | FL (USA) | 38 park visitors | Adults and children | Contamination of a water fountain | Cryptosporidium spp. | [260] |
| Waterborne    | Spain | 21 | Children | Contamination of tap water | Cryptosporidium spp. | [253] |
| Foodborne     | DC (USA) | 88 students and employees | Young adults and adults | Contamination of food by a food handler in a cafeteria | Cryptosporidium spp. | [311] |

1. In presence of two or more cases of similar infection, with a common exposure in the community not related to waterborne or foodborne disease, the term of community disease was used.
2. Number and category of people with symptom referable to cryptosporidiosis.
3. This outbreak was characterised by a Cryptosporidium infection in 9 of the 11 children, a coinfection of Giardia and Cryptosporidium in 2 of the 11 children and a concomitant infection of other 38 additional children by only Giardia oocysts triggered by the same likely source.
4. Median age.
5. Outbreak characterised by a concomitant waterborne Cyclospora outbreak.
6. Outbreak characterised by a concomitant waterborne Giardia outbreak.
7. Outbreak characterised by a concomitant foodborne E. coli O111 outbreak.
8. Outbreak characterised by a concomitant waterborne Norovirus outbreak.
in raw and drinking water while other no well-standardised procedures are available for wastewater [109]. However, raw, reclaimed and drinking water are not subjected to routine monitoring. In surface waters (rivers, watersheds, watercourses and lakes), several studies have reported high contaminations by (oo)cysts of *Giardia* and *Cryptosporidium* all over the peninsula with the highest prevalence for *Giardia* [111–114]. Presence of both *Cryptosporidium* and *Giardia* has been monitored in sewage, surface waters, drinking water, and swimming pools by simultaneous tracing of bacterial indicators. In the Latium and Apulia regions, cysts and oocysts were detected in sewage and surface water, with *Giardia* numbers always prevailing over *Cryptosporidium*, but not in drinking waters. However, remarkably, *Cryptosporidium* was detected in 9% of samples collected from swimming pools in the Latium [112]. The paper by Di Benedetto et al., 2005 [113] described the occurrence of *Cryptosporidium* and *Giardia* (oo)cysts in water samples of two municipal treatment plants, and in surface and ground water wells in Sicily. The wastewater samples taken before and after treatment process were assayed over the course of one year: *Giardia* cysts were detected in all samples thought the year at higher concentration levels than *Cryptosporidium* oocysts, subjected to a peak during Spring. Cysts were detected in one lake at very low concentration; on the contrary, both parasites were found at high-concentration levels in all samples collected through one year from the river waters. The pattern of occurrence of both parasites showed temporal-related relationship to rainfall trend (Table 1) (Figure 3). In the Tuscan area, five drinking water treatment plants, differing for the employed handling, were monitored for the presence of *Cryptosporidium* and *Giardia* (oo)cysts, to estimate the removal capacity of each plant [114]. Water samples (from inflow raw water and outflow drinking water) were analysed during a one-year survey and both protozoa were detected. The occurrence of (oo)cysts was not associated with seasonality, turbidity or *C. perfringens*, but however low performance of plants was correlated with presence of protozoa in outflow drinking waters. *Giardia* cyst and *Cryptosporidium* oocyst removal efficiency was also evaluated in a wastewater tertiary treatment system based on membrane ultrafiltration and fed with secondary-treated municipal wastewater in Apulia: *G. duodenalis* and *C. parvum* were identified in feed water but were found in filtered water only during occasional failure of the filtration system [115]. Also in raw sewage and primary effluent more *Giardia* cysts than *Cryptosporidium* oocysts were detected in many monitoring studies [112, 113, 116, 117].

Annual rainfall reduction in some regions and increased human consumption have caused a shortage of water resources at global level. The recycling of treated wastewater has been therefore suggested for domestic, industrial, and agricultural activities. *Giardia* and *Cryptosporidium* are known to be highly resistant to water treatment procedures and to cause outbreaks through contaminated raw or treated water. The study by Caccio et al., 2003 [117] performed an investigation in four wastewater treatment plants in Lombardia, Campania, Sardinia, Sicily by sampling wastewater at each stage of the treatment process over the course of one year, and testing the presence of both parasites. While *Cryptosporidium* oocysts were rarely observed, *Giardia* cysts were detected in all samples throughout the year, with peaks observed in Autumn and Winter. The massive amounts of feces from humans and animals are discharged, dumped, or carried in runoff, bringing encysted zoonotic protozoan parasites to estuaries and coastal waters, where they contaminate bathing beaches, and are finally filtered and concentrated by shellfish eaten by humans and marine mammals, and infect a wide range of marine animal hosts, resulting in morbidity and mortality to some populations [118]. Therefore, nearshore marine sites may be considered at higher risk for exposure to livestock runoff, human sewage, or both fecal sources. Bivalves filter large volumes of water and can concentrate organisms which are pathogenic for humans and animals. In a recent paper [119] the presence of *Cryptosporidium* spp. in clams (*Chamelea gallina*) from the Adriatic coast (Abruzzo) was reported for the first time. The temporal occurrence of *Cryptosporidium* (*C. hominis* and *C. parvum*) oocysts in *Ruditapes philippinarum* were evaluated in two farms located in Veneto and in Friuli Venezia Giulia [120]. The paper of Giangaspero et al., 2009 [121], has simultaneously investigated the presence of *Giardia* and *Cryptosporidium* in inflowing water and harvested shellfish (*Ruditapes decussatus* and *Mytilus galloprovincialis*) in geographically closed environment (Varano Lagoon, Apulia). Higher concentrations of *Giardia* cysts than *Cryptosporidium* oocysts were registered in almost all wastewater and water samples, but testing of shellfish gave negative results for both protozoa. However, *Cryptosporidium* (*C. parvum*, *C. felis*, *C. andersoni*, and two novel genotypes) was detected in haemolymph samples from mussels in California [122]. In this paper, factors significantly associated with detection of *Cryptosporidium* spp. in mussel batches were exposure to freshwater outflow and collection within a week following a precipitation event, while no correlation was found with exposure to livestock feces or human sewage sources. Remarkably, mussels were proposed as tracer to monitor water quality, suggesting that humans and animals ingesting shellfish may be exposed to both host-specific and anthropozoonotic *Cryptosporidium* genotypes of public health significance [122].

In the last decade, a major concern for the scientific community has been whether infected animals can serve as reservoirs of *Giardia* and *Cryptosporidium* infection for humans. The paper by Giangaspero et al., 2007 [109], presented data on prevalence and molecular genotyping from several sample types (companion animals, sheep, cattle, goats, wastewaters, surface water, shellfish, and humans) collected in the Italian territory. Several species/genotypes of *Cryptosporidium* have a relevant zoonotic potential and ruminants may be important sources of infection for human beings [106, 108]. Cryptosporidiosis causes important economic losses to animal husbandry and livestock production. To obtain information on the occurrence of cryptosporidiosis in lambs and the potential zoonotic role of the *Cryptosporidium* isolates, faecal samples collected from lambs in Central Italy (Abruzzo) were examined for the presence of *Cryptosporidium* and discussed in the paper
by Paoletti et al., 2009 [123]. All positive samples were characterised as zoonotic *C. parvum* genotype suggesting a potential public health hazard in Italy. Also the risk related to bovine zoonotic contribution was studied by Duranti et al., 2009 [124] by considering 248 farms in Central Italy (Latium and Marche). In all positive samples, the etiological agent was identified as *C. parvum* with a large subtype genetic variability. The prevalence of farm infection ranged from 3.4% to 35.6% and appeared related to putative risk factors such as farm type, calve stalling, late supply of colostrum, number of heads and contact between calves and adults. However, the highest risk was associated with housing calves separately from their dams, whereas dam nursing resulted as a protective factor. This important evidence consistently agrees with the role of maternal milk as protective factor against cryptosporidiosis onset both in animals [125] and in humans [126].

### 3.3. Waterborne Cryptosporidiosis in USA and Canada

Besides zoonotic transmission, an important identified waterborne infection route is linked to recreational waters [6]. A cryptosporidiosis outbreak from August to September 2004 in California, affected more than 250 people visiting a waterpark [127] (Table 4). Occurring more than a decade after the first reported outbreak of cryptosporidiosis in a swimming pool [128], this outbreak demonstrates that recreational waters may represent a highly potential infection vehicle especially in childhood. Recently in USA *Cryptosporidium* species have emerged as a major cause of outbreaks of diarrhoea [6] and have been associated with consumption of contaminated recreational and drinking waters and with the attendance to child-care programmes [129]. Principal risk factors for infection seem to coincide with swallowing untreated water from a lake, river, or after exposure to recreational water or, remarkably, after contact with a child in a child-care programme or with diapers [129] (see Section 7). Although exposure to recreational water is commonly implicated in summertime cryptosporidiosis outbreaks, this evidence demonstrates that investigations of increased incidence of cases in Summer should also examine other potential risk factors, addressing the multiple transmission routes for *Cryptosporidium*. The extended review of Craun et al. 2005 [130], on outbreaks associated with recreational water during 1971–2000 in USA, provided evidence that bacterial or protozoan etiology was identified in three-quarters of the outbreaks. Outbreaks caused by *Cryptosporidium* and *Giardia* were primarily associated with treated water in swimming and wading pools. Contamination from sewage discharges and wild or domestic animals were also recognized as important sources for untreated waters. Contributing factors in swimming-pool outbreaks were inadequate attention to maintenance, operation, disinfection, and filtration [131]. For the 764 waterborne outbreaks registered from 1971 to 2002 by the USA National Surveillance of Outbreaks and associated with drinking water, 575,457 cases of illness and 79 deaths were reported [132, 133]. If properly applied, current protocols in municipal water treatment are effective at eliminating pathogens from water. However, inadequate, interrupted, or intermittent treatment has repeatedly been associated with incidence of waterborne disease outbreaks. Contamination is affected by the number of pathogens in the source water, the age of the distribution system, the quality of the delivered water, and climatic events that can tax treatment plant operations. Furthermore, private water supplies are not regulated by the USEPA and are generally not treated or monitored [134].

A case-control study [135] was conducted in the San Francisco Bay Area as part of a national study sponsored by the CDC [136] (Table 3) to ascertain the major routes of transmission for endemic cryptosporidiosis, with an emphasis on evaluating risk from drinking water. Drinking and recreational water, food items, travel, animal contact, person-to-person fecal contact, and (for adults) sexual practices were evaluated as major exposures. The study showed no significant association between cryptosporidiosis and drinking water among the immunocompetent population in the San Francisco Bay, and therefore, the key risk factor for cryptosporidiosis in this area was identified in travelling to another country [135].

The recent paper by Reynolds et al., 2008 [137] has provided estimates of waterborne infection and illness risks in the USA by considering the correlation between the total number of water systems, source water type, total populations exposed, and microbial infection. The results indicated $10.7 \times 10^6$ and $2.2 \times 10^6$ infections/year in populations served by community and noncommunity groundwater systems, respectively, and $26.6 \times 10^6$ infections/year in populations with municipal surface water systems services. Water purification technologies applied at the point-of-use (POU) could be effective for limiting the effects of source water contamination, treatment plant inadequacies, minor intrusions in the distribution system, or deliberate posttreatment acts as bioterrorism (i.e. *Cryptosporidium* is a Category B bioterrorist threat) [93]. However, epidemiological studies are conflicting on the benefits of POU water treatment compared to untreated tap water [138–141]. Nevertheless, for immunocompromised and other populations, including those experiencing physiological life stages such as pregnancy, or those very young or very old, POU devices may represent water treatment options for reducing risks of *Cryptosporidium* and other types of infectious agents transmitted by drinking water. A study [142], aimed to estimate the urban contribution to the total *Cryptosporidium* and *Giardia* receiving-water loads in USA, was focused on combined sewer overflows (CSO), discharges of mixed untreated sewage and stormwaters. Interestingly, CSO from urban areas was not found to be a significant contributor of *Cryptosporidium*, but a significative source of *Giardia* [142]. Most cryptosporidiosis outbreaks in the USA are caused by *C. hominis*, and this species is often reported as the primary cause of cryptosporidiosis in this country [143, 144]. However, outbreaks account for only 10% of the overall cryptosporidiosis cases, and there are still few data on the species causing sporadic cases [145] (Table 2). The highest incidence of cryptosporidiosis in the USA has been found in the upper Midwest States [146]. In particular, Wisconsin was reported as having the highest...
incidence of cryptosporidiosis every year from 1999 to 2002 [146]. The Wisconsin city of Milwaukee also had the largest cryptosporidiosis outbreak in 1993, where more than 400,000 people were infected following contamination of the municipal water supply [147]. The pivotal study by Feltus et al., 2006 [148], identified for 49 cases of sporadic cryptosporidiosis in Wisconsin, during the period from 2003 to 2005, *C. parvum, C. hominis*, a cervine genotype [64], a cervine genotype variant, and a new W17 human genotypes [149] (Table 2). However, the study showed that most cases were linked to zoonotic *Cryptosporidium* genotypes. The Tri-County Health Department of Colorado [150] (Table 3) investigated an outbreak of cryptosporidiosis occurred in 2006, linked to a community swimming pool treated by chlorination and UV light irradiation. Risk factors appeared through swimming, getting water in mouth, and swallowing water. Important studies on waterborne-transmitted infections have been also conducted in Canada. In South Western Ontario, from July 2002 to December 2003, water samples were collected from 36 locations within the Grand River Watershed, and were analyzed for total coliforms, fecal coliforms, *E. coli*, *E. coli* O157:H7, thermophilic *Campylobacter* spp., culturable human enteric viruses, *C. perfringens*, *Cryptosporidium* spp., and *Giardia* spp. [151]. Peaks in pathogen numbers frequently preceded the peaks in numbers and turbidity of indicator organisms suggesting important implications (e.g., pathogen transport model) for designing monitoring programs in source water risk assessment [151]. In Southern Ontario, to identify management practices associated with an increased within-herd prevalence of *C. parvum* shedding on dairy farms, a large study was conducted on fecal samples collected from 1089 calves in 119 herds [125]. Overall, 30% of the calves were shedding *C. parvum* oocysts, with a prevalence ranged from 0 to 80% within herds and at least one positive calf detected in 77% of herds. Predictors significantly associated with an increased prevalence of shedding were the use of calf rub prophylaxis in cows and the feeding by milk replacer in the first week of life. In contrast, the presence of concrete flooring in calf housing areas and the use of soap or detergent when washing calf feeding utensils appeared to be protective [125].

The QMRA model was recently applied to assess the relative risks of infection associated with the presence of *Cryptosporidium* and *Giardia* in drinking water in Canada [152]. The assessment of the final risk in the contamination of the water plants resulted considerably affected by the selection of treatment performance model (filtration and ozonation). Recently, data from a sentinel site (Waterloo Region, Ontario) of the C-EnterNet (Table 3) [153, 154] were used to assess exposure factors on laboratory-confirmed *Cryptosporidium* infections [155]. Of 1204 cases of enteric illness in the sentinel area between April 2005 and December 2007, 36 cases were selected after excluding outbreak and international travel-related cases. Cryptosporidiosis was associated with swimming in a lake or river, drinking municipal water, and having a family member with a diarrhoeal illness. Since 1971, the Waterborne Disease and Outbreak Surveillance System has reported on waterborne disease and outbreak (WBDO)-related data. In 1978, WBDOs associated with recreational waters (natural and treated waters) were added [156]. During 2003-2004, a total of 62 WBDOs associated with recreational water were reported by 27 states, with typical illness occurred in 2,698 persons, resulting in 58 hospitalizations and one death [156]. Of the 62 WBDOs, 30 were outbreaks of gastroenteritis and *Cryptosporidium* was confirmed as the causal agent in 11, and all except one of these outbreaks occurred in treated water venues [156] (Table 4). Lastly, approximately 90% of waterborne outbreaks occur in treated recreational waters (swimming pools, spas and recreational parks), while the remaining 10% arise from natural waters used for leisure (e.g., bathing in rivers, beaches, etc) [6].

### 3.4. Waterborne Cryptosporidiosis in New Zealand and Australia

New Zealand has a higher incidence of cryptosporidiosis compared to other developed countries. A recent study in [157] aimed to thoroughly describe the epidemiology of this disease and to identify specific potential risk factors by analysing anonymous cryptosporidiosis notification and hospitalisation data. Human cases were designated as “urban” or “rural” and an association between disease rates and animal density was studied. Over the 10-year period from 1997 to 2006, the average annual rate of notified cryptosporidiosis was 22 cases per 100,000 population. The number of hospitalisations amounted to 3.6% of the notified cases. The annual incidence of infection appeared fairly stable, but showed marked seasonality with a peak rate in Spring. The highest rates were among Europeans, children 0–9 years of age, and those living in low-deprivation areas (Table 1) (Figure 3). Notification rates showed large geographic variations, with rates in rural areas 2.8 times higher than in urban areas, and with rural areas also experiencing the most pronounced Spring peak, correlated with farm animal density. Therefore, most transmission of *Cryptosporidium* in New Zealand appears to be zoonotic in rural settings [157]. These data seem to corroborate the evidence that the proportion of *C. parvum* sporadic cases in humans is higher in rural than in urban areas [158] and confirm the variation of the geographical distribution of *Cryptosporidium* infections within countries [159]. In New South Wales (NSW, Australia) the subtypes have global distributions and indicate both anthropootic and zoonotic transmission routes in sporadic cryptosporidiosis [160].

### 3.5. Waterborne Cryptosporidiosis in African Developing Countries

The burden of disease from cryptosporidiosis in developing countries is in the Sub-Saharan Africa, because of the disseminated status of malnutrition in children and the highest world prevalence of HIV infection in this region, with peaks reaching the 15%–28% of the adult population [161] (Table 2). However, in this vast geographical area, *Cryptosporidium* and *Giardia* infections are rampant also in adult and immunocompetent populations, due to the unhygienic and improper disposal of wastewater and to the use of surface waters as major source of potable water. The important research by Gideon et al. 2007 [162], represents
3.6. Waterborne Cryptosporidiosis in Asia. In Asia, the emerging need to facilitate the characterization of the endemic transmission of cryptosporidiosis has recently provided a large study on genotype distributions of Cryptosporidium in domestic wastewater in China (Table 2). Raw domestic wastewater samples were collected from four wastewater treatment plants in Shanghai, from December 2006 to April 2007. Interestingly, diverse Cryptosporidium species/genotypes were identified and C. hominis subtyping revealed a high complexity of Cryptosporidium populations often unique (Table 2) (see Introduction, Section 1.6).

The specific contribution of the zoonotic transmission of Cryptosporidium in China was discussed in the recent paper by Liu et al., 2009 [165], in which a total of 507 fecal specimens from six dairy farms were examined for Cryptosporidium spp. Interestingly, were identified C. andersoni and Cryptosporidium ryanae [166], a new species described from cattle and previously identified as the Cryptosporidium deer-like genotype [167], with C. andersoni as the dominant species. This interesting distribution of Cryptosporidium spp. may support the idea of unique species and transmission in these areas. In Taiwan, cryptosporidia were detected in most of the surface water specimens [168]. Water samples collected from potable water treatment plants were investigated for the presence of Giardia cysts and Cryptosporidium oocysts. The frequency of occurrence of (oo)cysts was 78% for Giardia and 72% for Cryptosporidium in 18 raw water samples. Ten out of 13 samples collected from treated water samples showed the presence of cysts, while oocysts were detected in five out of 13 treated water samples. The risk assessment for the presence of cysts and oocysts, indicates the possibility of waterborne transmission of Giardia and Cryptosporidium infection in Taiwan where adequate water treatment is almost absent. In India, an important work reported on the correlation between infections in livestock and seasonal rainfall by considering the monsoon period impact on cryptosporidiosis. The survey revealed a 30% infection with C. parvum, out of 457 fecal samples collected from neonatal bovine calves (0–3 months of age) from dairy farms for one year, across three different geographical and agro-climatic areas of India (Northern subtemperate, Eastern subtropical, and Southern subtropical region), and through the premonsoon, monsoon and post-monsoon periods. The infection was more prevalent in the Northern parts of the country than in the Eastern or Southern areas, and C. parvum was detected as the only species [169]. Highest prevalence was recorded during monsoon months. A more recent study, however, performed on 350 fecal samples collected from juvenile and mature cattle (6–24 months of age), across the three representative agro-climatic regions of the country, showed the only presence of C. andersoni with the highest occurrence in the Northern states [170]. The animals between age group of 6–12 months were mostly affected and the seasonal prevalence was higher during the hot and humid monsoon season, followed by the premonsoon season when the climate is hot and humid. However, consistently with Paul et al., 2008 [169], in the post-monsoon season the prevalence dropped, providing evidence for transmission related to survivability of the infective stages of the parasite [46]. The results seemed to suggest that C. andersoni is the major Cryptosporidium species affecting cattle with the increase in age, despite differences in species may also be associated to geography differences in sampling. However, unlike previous studies [169, 171], no cases of C. parvum, C. bovis and Cryptosporidium deer-like genotype [167] were found in the report of Paul et al., 2009 [170], evidence which may be ascribed to specific prevalence pattern of C. andersoni in the areas and season undertaken for study, corroborating the idea of space and time frames for transmission [36]. The animal age undoubtedly remains the most effective risk index in investigating veterinary transmission of Cryptosporidium [46]. Livestock fecal pollution of water sources appears to be the leading cause for both outbreaks and sporadic cases of cryptosporidiosis in developing countries, as already reported in high-income countries [159, 172, 173].

3.7. Waterborne Cryptosporidiosis in South and Central America. To study the prevalence of Cryptosporidium infection, levels of anti-Cryptosporidium IgG antibodies were measured among people inhabiting a periurban area in the Northeast of Brazil [174]. The study aimed to investigate the effects of environmental sanitation measures, hygienic habits, and household water supply, storage and handling on the frequency of these antibodies in the population sera, providing a model for low-income countries. Cryptosporidium interhousehold transmission was studied by comparing the frequency of anti-Cryptosporidium IgG antibodies among people inhabiting areas with or without different environmental sanitation measures and intrahousehold transmission by comparing the presence of these
antibodies in families with or without cases of diarrhoea, associated with the presence of *Cryptosporidium* oocysts in stools. A statistically significant difference was detected in the prevalence of *Cryptosporidium* infection between areas without and with environmental sanitation measures. Positive associations were found between poor household water supply, drinking unboiled/unfiltered water and high levels of anti-*Cryptosporidium* antibodies in sera, suggesting uncorrected household water supply, storage and handling as an important factor on *Cryptosporidium* transmission in developing countries cities [174].

In Central Mexico, the economy of the country is strongly based on sheep and bovine farm management and preventive veterinary medicine represents a useful approach to identify risk factors for zoonotic transmission. To establish the relationship between sheep farm management practices and cryptosporidiosis in this country, 37 farms were mapped to highlight facility characteristics, cleaning measures, water use and animal management practices [175]. Five indexes showed statistical significance: (i) watering frequency; (ii) bottle cleaning frequency; (iii) forage storage; (iv) place of parturition; (v) grazing place. The latter index provided the most relevant association between management practices and cryptosporidiosis. Grazing place may represent a crucial risk factor for cryptosporidiosis in Mexico, contributing to understanding how domestic animals and wildlife cycles interact, resulting in human infections and endemic locations.

3.8. Conclusions. Advanced molecular tools and improvement of international surveillance networks (Table 3) are now beginning to answer epidemiological questions related to waterborne transmission which is still difficult to address by traditional methods. Indeed environmental sampling surveys are often hampered by the absence of proper technologies able to provide reliable water sampling collections. Furthermore, many geographical gaps need to be filled to evaluate worldwide waterborne infection distributions and to assess the relationship between animal, human fecal wastes, and water transmission both in undeveloped and developed countries.

4. Cryptosporidiosis a “Foodborne Disease”

4.1. Food-Related Routes. In our analysis of *Cryptosporidium*-linked outbreaks, 15 out of 71 (21.1%) appear to be correlated to foodborne transmission, with a higher number of outbreak episodes in 2006 and 2008. Geographically, the outbreaks seem to be concentrated in the USA, Canada, and Australia and in North Europe, especially Finland and Sweden (Table 4) (Figure 4). Many infection routes have been identified, as consuming salad vegetables washed by contaminated water, eating raw meals, using contaminated water for making ice and frozen/chilled foods, or making products which receive minimum heat or preservative treatment (Table 4). However, contact with contaminated feces transmitted by coprophagoustransport hosts (e.g., birds and insects), worker aerosols (from sneezes), and exposed hand lesions have also been associated with outbreaks [176]. Transfer of pathogens has been documented through contaminated fabrics and carpets, rings, currency, skin surfaces, dust, and aerosols and through person-to-person transmission [176].

4.2. Foodborne Cryptosporidiosis in Europe. In the 27 member states of the European Union, zoonotic parasites transmitted by food are circulating with different prevalence according to the country, the environmental conditions, the human behaviour, and the socioeconomic level. Foodborne parasites can be divided into two main groups according to human transmission. They reach the human beings through the consumption of raw infected food such as muscle tissues of different animal species (*T. gondii*, *Sarcocystis hominis*, *Sarcocystis suishominis*, *Diphyllobothrium latum*, *Taenia solium*, *Taenia saginata*, *Opisthorchis felineus*, *Anisakis spp.*, *Pseudoterranova spp.*, *Trichinella spp.*), or vegetables (*Fasciola hepatica*), and contaminated food and water resources (*G. duodenalis*, *Cryptosporidium spp.*, *T. gondii*, *Echinococcus granulosus sensu latu*, *Echinococcus multilocularis*, *T. solium*, *Taenia multiceps*) [177]. Remarkably, foodborne outbreaks of cryptosporidiosis are considerably increasing in Northern Europe, as shown by the two important outbreaks registered in Finland and Sweden during 2008 [178, 179] (Table 4) (Figure 4).

In 2005 an outbreak of diarrhoea, affecting a group of 99 company employees, was described near Copenhagen [180] (Table 4). All people were ill and 13 positive for *C. hominis* infection. Disease was associated with eating from the canteen salad bar on one, possibly two, specific weekdays. Three separate salad bar ingredients were found to be likely sources: peeled whole carrots served in a bowl of water, grated carrots, and red peppers. An anthroponotic route of infection was speculated, triggered by a person excreting the parasite which may have had contaminated the buffet [180].

In Norway a searching for parasites in fruits and vegetables was undertaken in the period from 1999 to 2001 [16]. Of the 475 samples, 29 were found to be positive for *Cryptosporidium* oocysts and *Giardia* cysts, while 19 only for *Cryptosporidium* (lettuce and mung bean sprout samples). Mung bean sprouts were significantly more likely to be contaminated with *Cryptosporidium* oocysts or *Giardia* cysts than the other fruits and vegetables, despite concentrations were generally low (approximately 3 (oo)cysts per 100 g product). There was no association between imported produce and detection of parasites. *Cryptosporidium* oocysts and *Giardia* cysts were also detected in water samples concerned with field irrigation and production of bean sprouts [16]. This was the first report on detection of parasites in vegetables and fruit obtained in a highly developed wealthy country, without there being an outbreak situation.

4.3. Foodborne Cryptosporidiosis in USA and Canada. Generally, viruses and encysted parasites are more resistant than enteric bacteria to adverse environmental conditions, but all pathogens can survive long enough for transfer from a contaminated worker to food and food contact surfaces.
Also outbreaks associated with consumption of fruit juice have been growing as an emergent public health problem since the early 1990s, when the first outbreak associated with apple cider was described [181]. However, in the period from September to November 2003, 12 local residents in Northern Ohio were diagnosed with cryptosporidiosis for having drunk ozonated apple cider [182] (Table 4). In response to epidemiologic investigations of outbreaks in which juice is implicated, the USA Food and Drug Administration (FDA) has implemented process control measures to regulate the production of fruit juice, according with the Hazard Analysis Critical Control Point (HACCP) plan. However juice operations that are exempt from processing requirements or do not comply with the regulation continue to be implicated in outbreaks of illness. The CDC receives reports of food-associated outbreaks of illness (Table 3) [183] and its Foodborne Outbreak Reporting System has reviewed, from 1995 through 2005, ten implicating apple juice or cider, eight linked to orange juice, and three involving other types of fruit juice-associated outbreaks. Among the 13 outbreaks of known etiology, two were caused by Cryptosporidium and one by Shiga toxin-producing E. coli O111 and Cryptosporidium [184] (Table 4). The incidence of foodborne disease outbreaks caused by contaminated low-pH fruit juices is highly increasing [185]. The association of Cryptosporidium with fruit juice is raising a safety concern in food industries. In 1998, CDC implemented enhanced surveillance for foodborne-diseases outbreaks (FBDOs) by increasing communication with state, local, and territorial health departments and revising the outbreak report form. Since 2001, reports of FBDOs are submitted through a web internet application called electronic Foodborne Outbreak Reporting System (eFORS) (Table 3) [183, 185–191].

4.4. Foodborne Cryptosporidiosis in South and Central America. In Central America there is a high attention to foodborne infections. Recently, the role of the food handlers has been investigated in Venezuela, where cryptosporidiosis is an important public health problem [192]. Despite a basic investigation approach, fourteen out of 119 fecal samples from food workers were found positive for Cryptosporidium spp. and associated with other protozoa, being most frequent Entolimax nana, followed by B. hominis, Entamoeba coli, G. lamblia, and E. histolytica/Entamoeba dispar. In the paper of Calvo et al. 2004, [193] lettuce, parsley, cilantro, strawberries and blackberries circulating in local agricultural markets of the Central Valley of Costa Rica were investigated for the presence of Cryptosporidium spp., Cyclospora spp., and Microsporidia. Fifty different samples of each product, 25 taken in the dry season and 25 in the rainy season and coming from five different local agricultural markets, were evaluated. Although all vegetables presented fecal coliforms in high concentrations, lettuce and cilantro presented a statistical difference between the rainy and the dry season, being greater during the rainy season. Fecal coliforms were not detected in strawberries and blackberries probably due to its low pH. All products presented Cryptosporidium spp., Cyclospora spp., and Microsporidia. Cryptosporidium was not present in strawberries. Microsporidia were present in all products except blackberries and Cyclospora was only isolated from lettuce during the dry season. These results show the importance of introducing good agricultural practices, especially due to the resistance of Cryptosporidium and Cyclospora to disinfecting agents [193].

4.5. Conclusions. The considerable presence of Cryptosporidium in diversified food matrices makes it imperative to develop appropriate prevention strategies for food safety and suitable molecular techniques for parasite identification. As a general role, the control strategies should be based on the education of the consumers, farmers, and shepherds, the improvement of farming conditions, the improvement or the development of more sensitive methods to detect these parasites in slaughtered animals and in foodstuff, a control of sewage sludge on pastures and of drinking water resources, and the reduction of contacts between livestock and wild animals which frequently represent the most important reservoir of these pathogens [177].

5. Cryptosporidiosis a “Travelers’ Disease”

5.1. Traveler’s Diarrhoea and Main Pathogen Agents. Traveler’s diarrhoea (TD) occurs in 20 to 60% of European or North American travelers in intertropical areas [194]. The main agents are E. coli pathovars followed by enteroinvasive bacteria, enteric viruses, and protozoa (G. intestinalis, C. parvum and E. histolytica). Several studies have shown that a large proportion of travellers and immigrants from tropical and subtropical countries are affected by gastrointestinal disorders and harbour intestinal pathogens without clear gastrointestinal problems [195–200]. Travelling represents an important risk factor for acquiring infection also with sporforming protozoa as Cyclospora, Microsporidia, and Isospora [201]. Protozoan infections with G. lamblia and C. hominis/C. parvum are the main nonviral causes of diarrhoea in industrialised countries [202] and are even more frequently seen as the cause of gastrointestinal complaints in returning travellers [196, 203, 204].

5.2. Principal World Regions Associated to Travel-Linked Transmission. An important study investigated the relationship between Cyclospora infection and seasonality in Turkey [205]. Parasites such as Cryptosporidium, G. intestinalis, E. histolytica/dispar, B. hominis, and others were also observed (Figure 6). The incidence of cyclosporiasis was higher in Summer and early Autumn and most of the Cyclospora-infected patients were without diarrhea. On the other hand, patients with a history of recent travel to a developing country in the tropics usually present persistent diarrhea. However, very mild infections may be underdiagnosed even if causing typical traveler’s diarrhea. In a patient with a history of travel and persistent diarrhea unresponsive to the usual antibiotic and antidiarrhea treatment, stool studies for the cited protozoa infections should be always routinely performed (Table 1) (Figure 3).
Trophozoites are also passed in stool but they do not survive in the environment.

Contamination of water, food, or hands/fomites with infective cysts.

(a) *Giardia* Life Cycle

Legend:
- ▲ = Infective stage
- ▲ = Diagnostic stage

(b) *Cyclospora* Life Cycle

Legend:
- ▲ = Infective stage
- ▲ = Diagnostic stage

(c) *Blastocystis* Life Cycle

Legend:
- ▲ = Infective stage
- ▲ = Diagnostic stage

**Figure 6: Continued.**
A large study analysed 1,179 North-American travelers who visited Mexico from 2005 to 2007 [206]. TD was reported by 521 participants. A long stay in Mexico was identified as a risk factor for cryptosporidiosis. The Nassau County Health Department (NCHD) in Florida identified an outbreak of gastrointestinal (GI) illness in a returning choral group who toured Ireland in 2006 [207] (Table 4). In the long-term report performed in England and Wales from 2000 to 2003 [63] (Table 2), *C. hominis* was more prevalent in patients reporting recent foreign travel with late Summer and early Autumn picks [63] (Table 2). However, samples from other UK cases, contracted during foreign travels, were entirely characterised as *C. parvum* (type 1 and 2) [208] (Table 2). In the paper of Chalmers et al., 2008 [209], 115
isolates were investigated to assess UK transmission linked to travelling for *C. hominis*. Among the identified subtypes, the predominant was IbA10G2 (Table 2) not apparently linked to recent travel outside Europe [209].

5.3. Conclusions. Person travelling abroad, especially in regions identified as having high risk of infection (e.g., Ireland, UK, Turkey, Mexico) ought to undergo routine testing for intestinal parasites (Figure 4). A large variety of parasitic infections can be expected in homecoming travellers, and diagnostic procedures play a crucial role in the detection of intestinal parasites found in patients with and without gastrointestinal complaints. Although microscopy is considered the gold standard, it is labour intensive and its diagnostic performance critically depends on well-trained microscopists. Enzyme immunoassays [210, 211] and fluorescent antibody assays [212] have been accepted as alternative diagnostic methods for the detection of *G. lamblia* and *Cryptosporidium* in stools. Currently, the introduction of real-time PCR combining several targets into one multiplex assay offers the possibility of using DNA-based detection techniques in a high-throughput diagnostic approach [213].

6. Cryptosporidiosis in HIV-Infected Individuals

6.1. Infection Pathogenesis and Symptoms in HIV Impairment. 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 use of antiretroviral therapy although it is most frequent in men affected by gay-bowel syndrome [214, 215]. *C. parvum* is primarily responsible for watery diarrhoea, but it may also trigger biliary disease, hepatitis, pancreatitis, arthritis, and possibly respiratory tract infections [214, 216]. Diarrhoea is self-limited in immunocompetent individuals or in those whose CD4 cell count >200/mm³, but may be severe, and unremitting or relapsing in severely immunodeficient patients (CD4 cell count <100/mm³). In these cases chronic infection can lead to dehydration, malnutrition, malabsorption, wasting and, frequently, death [214]. Biliary cryptosporidiosis is more frequent in patients with CD4 cell counts <50/mm³ and commonly presents with right upper quadrant pain, nausea, fever, vomiting and often with absence of diarrhea. Coinfection with cytomegalovirus or microsporidia has been frequently found in biliary cryptosporidiosis [216]. All segments of the gastrointestinal tract may be involved, but the small bowel is the main target organ followed by the colon [217]. Esophageal cryptosporidiosis, with parasites attached to the squamous mucosa and the luminal borders of submucosal glands and ducts, has been described both in adults and in children with AIDS [214]. Intestinal coinfection by *C. parvum* and *Cyclospora* species or cytomegalovirus is not rarely documented [216, 217] (Figure 6). Recent evidence suggests that epithelial apoptosis mediated by cytotoxic host T cells might play a role in the development of colonic lesions in AIDS-related cryptosporidiosis [218], suggesting a modified pathogenesis in HIV-positive patients. With the introduction of highly active antiretroviral therapy (HAART), the incidence of cryptosporidiosis has declined and chronic diarrhea and cryptosporidial infection often resolve with increases in CD4 lymphocyte count [219, 220]. In countries where HAART is available, HIV infection is generally a chronic disease strictly depending on the patient’s adherence to treatment [221].

6.2. HIV-Related Cryptosporidiosis in Europe. Recently, in Europe few studies have traced the entire spectrum of epidemiological diffusion routes in HIV-infected patients. In a large study [222], *Cryptosporidium* isolates from HIV infected and uninfected patients from UK were compared to other isolates collected in different geographical areas (Kenya, Malawi, Brazil and Vietnam). Among the *C. parvum* group, strains clustered distinctly into either human or bovine genotypes regardless of the geographical origin, age, or HIV status of the patients (Table 2). The intragenotypic variation observed in the *C. parvum* human genotype was wide-ranging compared to that within the *C. parvum* bovine genotype group. The variation within genotypes was conserved in all geographical regions regardless of the patient HIV status (Table 2). Independent widespread of genotypes was also observed in the study by Morgan et al., 2000 [223], where isolates from HIV infected patients from Switzerland were compared to other isolates from Kenya and the USA (Table 2). In Portugal, to investigate a possible zoonotic transmission in HIV-seropositive patients, isolates from patients, cattle, sheep and wild ruminants were collected from different regions and appeared largely limited to the only Portugal (Table 2) [78]. A surveillance study on *Cryptosporidium* in HIV-infected adults was carried out in Spain [224]. *C. hominis* was detected in 10 HIV-infected and *C. parvum* in six HIV-infected individuals showing a similar prevalence of the two species (Table 2).

6.2.1. HIV-Related Cryptosporidiosis in Italy. In Italy, during the previous decade, remarkable epidemiological and clinical studies have been provided [225–229] (Figure 5). An outbreak affected both HIV-positive and HIV-negative members of a drug rehabilitation community in 1995 in Northern Italy (Emilia Romagna) [226]. The 31% of the HIV-positive individuals were affected, with a severity grade according to CD4 cell count. The *Cryptosporidium* oocysts were identified in the sediment of the water tanks used to store drinking water for the community, suggesting water as the vehicle of infection [226] (Figure 5). However, following these pivotal studies, only a limited literature on AIDS-related cryptosporidiosis has been produced in the last ten years in Italy, clearly reflecting the positive impact of the HAART therapy on incidence and severity of opportunistic infections.

6.3. HIV-Related Cryptosporidiosis in USA and Canada. An unusual aspect of cryptosporidiosis onset in HIV/AIDS persons was approached by evaluating events of recreational water activities and risk of exposure to *Cryptosporidium* in
waterways of Baltimore (Maryland, USA) [230]. Interviews conducted on HIV/AIDS patients showed that approximately 48% of respondents participated in recreational water activities and had almost equally gender probability to contract waterborne pathogens.

6.4. HIV-Related Cryptosporidiosis in Africa. In Iran ten health centers were mapped for searching of Cryptosporidium in diarrheal patients. The study [231] showed that overall, 1.4% of all patients and 6.3% of diarrheal patients were infected by Cryptosporidium while AIDS patients who were suffering from diarrhea reached the 33.4%.

In Equatorial Guinea a study identified C. parvum, C. hominis and C. meleagridis in 35 cases: remarkably over 90% of the species were isolated from HIV-positive patients (Table 2) [232].

6.5. HIV-Related Cryptosporidiosis in Asia. A prevalence of intestinal parasites in HIV patients in India was determined by testing acute, chronic diarrhea, and controls without diarrhea [233]. I. belli was found in 18.6% of chronic diarrhea and 7.3% of acute diarrhea. Cryptosporidium was detected irrespective of specific clinical signs. Microsporidia and C. cayetanensis were detected only in one chronic case. Remarkably, I. belli appeared the predominant parasite associated with diarrhea among HIV patients, providing an important evidence of a low-represented but emerging parasite in gastrointestinal infections [234]. Reports on the prevalence of cryptosporidial diarrhea in HIV-infected adults from different parts of India from the mid-1990s have shown a range from 0.7 to 83% in symptomatic and from 1.4 to 57% in asymptomatic individuals, with very high rates in both groups and a strong correlation between immune status impairment and diversity of symptoms [235, 236]. In Taiwan the extremely low prevalence of intestinal cryptosporidiosis among HIV patients [76], despite detection of cryptosporidia in most of the surface waters [168], may be the result of using boiling water [5, 76]. In Malaysia, the commonness of fecal wastes from human and nonhuman hosts suggests that many environments, particularly water and soil, act as vehicles for the spreading of the disease [237]. A recent paper [238] investigated the occurrence of intestinal parasites in HIV/AIDS patients with chronic diarrhea in Indonesia. Parasites were found in 84% of samples (single species infections, 71%; polyparasitism, 13%), with protozoan pathogens occurring most commonly. Cryptosporidium, C. cayetanensis, and G. duodenalis were the most frequent single infections. Cryptosporidium and C. cayetanensis occurred in 12% and 8% of all infections. The most common coinfection was with B. hominis and Cryptosporidium (6.3%) (Figure 6). No seasonal influence was observed for Cryptosporidium, C. cayetanensis, or B. hominis. A study [239], representing the first genetic identification of Cryptosporidium species in cattle in Thailand, showed that all HIV and cattle stool samples were characterize as C. parvum, suggesting a possible zoonotic transmission for HIV individuals (Table 2) [239].

6.6. HIV-Related Cryptosporidiosis in South and Central America. In Peru, a study on the genetic diversity of Cryptosporidium spp. in HIV-positive people [240] suggested that C. hominis is the predominant species in HIV patients, while zoonotic Cryptosporidium spp. accounts for about 30% of cases (Table 2) [240]. A prospective longitudinal cohort study [241] conducted in Haiti showed that AIDS patients were infected by either human or animal genotypes. These data confirm that immunocompromised individuals are susceptible to a wide range of Cryptosporidium spp. which remains a frequent hazard especially in countries with poor hygiene and overcrowded conditions associated with urban slums [69].

6.7. Conclusions. In developing countries with no or limited access to HAART, AIDS is rapidly expanding with a high fatality ratio (Figure 4). Furthermore, new HAART baselines, where introduced, are now modifying HIV circulation modes and opportunistic infections in these geographic areas. The data on the parasite's transmission in the Sub-Saharan Africa clearly show high rates of severe or even fatal Cryptosporidium infections, massively contributing to the entire worldwide burden of sporadic cases (Figure 4). In HIV patients the impact of C. felis infection in tropical countries is becoming an emerging issue. In developed countries (Figure 4), therapeutic approaches are effective in reducing fecal output, but the eradication of the parasite is rarely obtained. Cryptosporidiosis is still a leading opportunistic infection in HIV persons and, despite HAART therapy, it should not be underestimated in epidemiological tracing and clinical followup of these patients. Immunocompromised persons should be cautioned on the potential risks from recreational water contact and their water-related practices should always be considered in the clinical monitoring of their health status.

7. Cryptosporidiosis in Children

7.1. General Notes. In the early 1980s, diarrhoeal disorders were the biggest child killers, responsible for an estimated 4.6 million deaths worldwide every year. Despite widespread use of oral rehydration therapies and an increased understanding of the pathogenesis of diarrheoa, 2.5 million children still die from these illnesses every year, almost all of them in developing countries [242]. Parasites such as Cryptosporidium and Giardia are leading agents of chronic or persistent diarrhoea worsened by specific risk factors such as malnutrition or immune deficiency [243].

7.2. Children Cryptosporidiosis in Europe. Studies on cryptosporidiosis in children have been progressively developed in the last few years in Europe [35, 244]. Survey laboratory practices in the UK have recently included screening of all fecal specimens from children aged 15 or younger, with routine reports to the Communicable Disease Surveillance Centre (CDSC) of the PHLS (Public Health Laboratory Service) (Table 3) [245]. Among the described outbreaks (see Sections 3.1 and 3.2), in many episodes the children represented the largest portion of confirmed cases of cryptosporidiosis.
The work by Chalmers et al., 2009 [63] showed that the epidemiology of human cryptosporidiosis in UK, from 2000 to 2003, importantly differed among Cryptosporidium infecting species with reference to children age groups (Table 1) (Figure 3). The mean age of C. parvum cases was lower than that of C. hominis cases. However, an opposite trend in infants under one year, independently from the gender and possibly linked to the stay of the babies in day-care nurseries, was observed. A seasonal distribution of cryptosporidiosis in children in a region of North-Eastern Spain, was determined [53] (see Introduction, Section 1.3.2). Prevalence was highest in children aged 1 to 3 years old and significantly more elevated in the Autumn–Winter period than in the Spring-Summer period [53]. Furthermore, the stay within a nursery and the improper diaper usage, for a group of 24 day-care children, were analysed as triggering factors in the community outbreak described by Ortega et al. in 2006 in Spain [252] (Table 4). In Spring 1998, an acute gastroenteritis outbreak, which mainly affected preschool children, took place in Guadarrama (Spain) (Table 4) [253]. In Spain a large surveillance study on a set of stool samples collected from Cryptosporidium-infected patients, including 92 children [224], revealed a high heterogenicity of species (Table 2). A recent study [254] performed, amongst the others, on 32 hospitalised children, revealed an impairment-dependent prevalence of Cryptosporidium species in hospitalised children [254] (Table 2). Large-scale surveys of representative population groups in the Central and North-Western regions of the Russian Federation showed a mean population incidence of 3.3%, much higher in children (3.7%) than in adults (0.4%). There were differences in the infection rates between genders (boys more affected than girls) but not between rural and urban children [255].

### 7.2.1. Children Cryptosporidiosis in Italy

A large group of 618 children with diarrhea was prospectively evaluated for viral, bacterial, and parasitic enteric pathogens in a multicenter study performed by the Italian Study Group on Gastrointestinal Infections [256] (Figure 5). The agents mainly associated with disease were Rotavirus, Salmonella, and Campylobacter. Cryptosporidium and Giardia were observed only in 10 patients [256].

Another important study [257] evaluated the prevalence of C. parvum in 368 hospitalized children with enteritis, of whom 359 were immunocompetent and 9 were HIV-infected. C. parvum oocysts were found in seven out of 368 specimens. All subjects with cryptosporidiosis were living in Apulia (South Italy) and had not travelled outside of Italy. No differences between those living in urban or rural areas was observed and no correlation was found between seasonal timing of specimen collection and positivity for C. parvum. Importantly, the areas were served by chlorinated water systems. In two out of seven children the parasite was associated also with S. typhimurium in one case and Rotavirus spp. in the other. The population study on the drug community members [226], included the subset of their 135 children. Interestingly, 28 out of 135 children, aged in the range 0–12 years, were affected by Cryptosporidium. Updated data on cryptosporidiosis prevalence in pediatric population are nowadays missing, despite the growing clinical interest on children cryptosporidiosis.

### 7.3. Children Cryptosporidiosis in USA and Canada

In 2007 the Idaho waterborne outbreak in a municipal park [258] (Table 4) affected, over 50 ill people, 36 children with a peak ranging in the 4–6 age group. In 2006, in a waterborne outbreak the 83% of primary cases occurred in children [85] (Table 4). Also in the waterborne outbreak registered in Illinois in 2001, children were predominantly involved [259]. Interestingly, C. hominis was the only etiological agent and one of the risk agents was the heavy usage of recreational water by diaper-aged children (Figure 3) (Table 4). Also in the outbreak registered in Florida in 1999 [260] (Table 4), over the 86 park visitors interviewed, the 38 which had gastroenteritis were 8 year old over an age range of 2–65 years. During an outbreak of giardiasis and cryptosporidiosis in Central Florida in September 2006, including also coinfection (Figure 6), only children were affected [261] (Table 4).

Currently, the number of internationally adopted children worldwide has rapidly increased during the past decade, providing an additional surveillance indicator for cryptosporidiosis acquired in their country of origin. The work of Saaiman [195] performed a retrospective cohort study on 504 children adopted from abroad in the USA from 1997 to 1998 to determine the prevalence of infectious diseases. Being born in Eastern Europe was a risk factor for the acquisition of G. lamblia. Thirty-two children had one or more organisms identified by stool microscopy: B. hominis, Dientamoeba fragilis, E. nana, Hymenolepis nana, Ascaris lumbricoides, Chilomastix mesnili, and Entamoeba hartmanni were detected. Cryptosporidium species were identified only in four out of 504 children, but probably underestimated for the low sensitivity of the direct fluorescent method.

### 7.4. Children Cryptosporidiosis in New Zealand and Australia

An outbreak affecting eight children in Australia was associated to a contamination of drinking unpasteurised milk [262] (Table 4). Despite being rarely observed, a pivotal work [263] described a previous children outbreak of cryptosporidiosis linked to drinking school milk in September 1995 in the UK. The only exposure significantly associated with illness was drinking school milk, possibly infected by a temporarily failing pasteurisation plant at the local producing farm. A children community outbreak was also reported in New Zealand in 2001 [264] (Table 4). The 19 cases aged under 7 years, were linked to a specific farm event identified as parasite hand-to-mouth transfer after touching an infected calf.

### 7.5. Children Cryptosporidiosis in Africa

C. parvum is a leading pathogen in children in African developing countries. Here, as in other low-income areas, with no or limited access to HAART, AIDS is rapidly expanding in infants [265]. The fatality rate increased due to opportunistic infections, with C. parvum being one of the leading agents.
of severe diarrhea in infants affected by HIV/AIDS [265]. However, stunting is a major burden in developing countries, affecting ~147 million children. Repeated or prolonged episodes of diarrhea during childhood increase the risk of stunting, which is believed to be associated with significant morbidity. Although the relationships between malnutrition, environmental and diarrheal illnesses are complex, studies have suggested a connection between stunting and diarrhoea causing pathogens, including *G. lamblia*, *C. parvum*, and enteric *E. coli* (EAggEC) [266]. A recent paper [267], showed that the microsporidian parasite *E. bieneusi* is associated with lower rates of weight gain in children with persistent diarrhea in Uganda. Children with microsporidiosis were predicted to weigh 1.3 kg less than children without microsporidiosis at 5 years of age [267]. The benefits of exclusive breastfeeding for health in infants have been widely described. The study by Bilenko et al., 2008 [126], considered whether partial breastfeeding has protective effects against enteric infection and associated morbidity in population where early addition of supplementation is common. In this study, 238 Bedouin infants were followed from birth to 18 months. Exclusive breastfeeding was protective against infection and morbidity at ages 0 to 3 months. In the age range of 4 to 6 months, partial versus nonbreastfeeding was associated with lower rates of infection with *Cryptosporidium* spp. and *Campylobacter* spp. In older children (10–12 month age range) partial breastfeeding as compared to none, protected against infections with *Cryptosporidium* spp. and *G. lamblia*. Short-term protection from maternal antibodies passed to infants during breastfeeding may result in a lack of cryptosporidial infection in infancy. This protection of breastfeeding children may, however, result in such children developing less anti-*Cryptosporidium* immunity of their own, so that, by school age, the children who had been breastfed are those most likely to be found infected [268].

Hospital- and community-based studies in Sub-Saharan Africa document a high prevalence of cryptosporidiosis in children aged 6–36 months, particularly among those who are malnourished or HIV-positive and during rainy seasons. Transmission appears to occur predominantly through an anthropoconotic cycle [269]. Prevalence of *Cryptosporidium* and *Giardia* infections was assessed among children using protected and unprotected water sources in Eastern Ethiopia, in November 2005 to May 2006 [270]. Of 655 children examined, 80 were infected with *Cryptosporidium* and 231 with *Giardia*. No difference was observed in the prevalence of cryptosporidiosis and giardiasis between children drinking water from protected and unprotected sources [270]. The study of Dlamini et al., 2005 [271], reports the first finding on *Cryptosporidium* spp. detection among children of the Swazi ethnic group in Zimbabwe. A study focused on the prevalence of cryptosporidiosis in pediatric hospital patients in Niger [272], where malnutrition and diarrhoea are two major public health issues. The aim of this study was to get a first evaluation on the prevalence of *Cryptosporidium* spp. in the stools of hospitalized children younger than 5 years of age. The weight/age ratio to describe malnutrition was calculated and analyzed with the Epi-Info software [273, 274], (Table 3). In the three months study 220 children were included (mean age 20 months) showing that 65% of the children were suffering from moderate and severe malnutrition. Diarrhoea was reported in 52% of the children. *Cryptosporidium* oocysts were detected in 12 out of 220 children with 10 children malnourished. A study on children (median age 13.5 months) presenting with acute diarrhea and rehydration clinics in Madagascar, was undertaken between May 2004–May 2005 [275]. Twelve cases of cryptosporidiosis were detected only in the rainy season. As 11 of the 12 cases were caused by *C. hominis* and only one by *C. parvum*, most of the cases were probably the result of anthropoconotic transmission (Table 4) [275]. A large study was performed in Kuwait to investigate the incidence of cryptosporidial infection in children presenting with gastrointestinal symptoms at the local hospitals [34]. Over a period of three years, fecal samples from 3549 children were analyzed for the presence of *Cryptosporidium* oocysts, detected in 51 children with diarrhea. Prevalence was highest in children older than two years of age. The maximum number of cases was seen during the months January to April, indicating a marked seasonal variation. Three possible modes of infection transmission were inferred: (i) drinking contaminated water stored in overhead water tanks; (ii) person to person; (iii) contact with infected animals. A common polyparasitism was mainly due to the recurrent *Cryptosporidium* co-infective *Giardia* and *Cyclospora* parasites, as also reported by recent outbreak reports [261, 276, 277] (Table 4), confirming these parasites as largely emerging pathogens [1, 201] also with *B. hominis* and *E. histolytica/dispar* [278] (Figure 6).

### 7.6. Children Cryptosporidiosis in Asia

An interesting correlation between subtypes distribution and geographical settings for children infections, was investigated to identify geographical-dependent variation [36] (Table 2). In this study, species, genotypes, and subgenotypes of *Cryptosporidium* spp. infections were identified for the first time in a well-defined cohort of children in Southern India. Only one previous report was produced on genotypes distribution of *Cryptosporidium* spp. in children in Eastern India [57]. *C. parvum*-positive samples revealed that all were subgenotype Ic, usually associated with anthropoconotic transmission (Table 2). There were no significant differences in demographic or clinical (nutritional status, vomiting, fever) characteristics between *C. hominis* and *C. parvum* or *C. felis* [87] infected children and those infected with different subgenotypes. However, *C. hominis*-infected children had a significantly greater severity of diarrhea [36]. There was also a trend toward a longer average duration of diarrhea in *C. hominis*-infected children than in those infected with other species (Table 1) (Figure 3). There were two significant time clusters of cryptosporidial diarrhea, one during February-March and the other during June–August. In the other study of Aijampur [235], fecal samples from 158 children with and 99 children without diarrhoea were tested for enteric pathogens in Southern India. Remarkably, *Cryptosporidium* spp. resulted in one of the most common causes of diarrhoea in hospitalized children [235, 236].
The epidemiology, clinical features, nutritional status, and causative agents of diarrhea were studied in 289 children in Bangladesh [279]. Compared with malnourished and/or stunted children, better-nourished children experienced significantly fewer diarrheal episodes. *G. lamblia*, *C. parvum*, and *E. histolytica* were the most common protozoan agents. A very recent study [280] performed in Bangladesh, examined whether malnutrition, may increase the risk of diarrheal illness. A study [281] was conducted to investigate the presence of intestinal parasites among 475 preschool children (aged 3 months to 5 years) in Thailand. The most frequent parasites identified were *G. lamblia* and *Cryptosporidium* spp. Highest proportion of intestinal parasites occurred during the rainy season (June–October). A 5-year hospital-based retrospective analysis was aimed to find out the intestinal protozoal parasitic profile in 1790 preschool and school-age children visiting the hospital with gastrointestinal illness in Nepal [282]. *G. lamblia* was the most prevalent pathogenic protozoan intestinal parasite, followed by *E. histolytica*. Interestingly, opportunistic pathogens like *C. cayetanensis* and *Cryptosporidium* spp. were detected in immunocompromised children below two years of age as a result of vertical transmission, which is alarming for a country like Nepal presenting a “concentrated epidemic” HIV infection period [282]. Intestinal parasites are still a major health problem in Turkey. The study of Börekçüz and Uzel [283] identified one or more parasites in 43% of the children. *G. intestinalis* was found to be the most common parasite, followed by *E. histolytica* plus *E. coli*, *E. coli*, *E. nana*, and *Cryptosporidium* spp. in Turkey, the first waterborne outbreak of cryptosporidiosis with *Cyclospora* coinfection mainly affected children aged between 0 and 14 years [276] (Table 4) (Figure 6). An interesting topic for children infections in an Asian high-income country, was discussed by Matsubayashi et al., 2005, on *Cryptosporidium* and *Giardia* transmission in a zoo in Japan [284]. *Cryptosporidium* spp. was found only in a raccoon dog, and *Giardia* spp. was detected in a mandarin duck and two ruddy shelducks. These results corroborate the idea that infected animals could serve as a direct source of contamination for children. In a recent compendium [285], recommendations for public health officials, veterinarians, animal venue staff members and exhibitors, visitors to animal venues, and physicians have been provided. Pet and wild animal transmission has been more critically revised as an important reservoir for cryptosporidiosis in children [78, 118, 284, 286].

7.8. Conclusions. Infectious diarrheal diseases remain an important cause of childhood morbidity in developed countries. The recent confirmation of an infection route of *Cryptosporidium* linked to drinking unpasteurised milk highlights the need to evaluate the entire transmission variety of the parasite, besides the already well-known and identified classical courses of disease in children. In pediatric populations, prevalence data are still underestimated, due to a poor clinical valuation of pathognomic symptoms and to the absence of advanced laboratory tools in diagnostic routine panels. Literature regarding developing countries shows that anthroponotic transmission is the principal mode of infection in children population, while less representative are the water or environmental sources contaminated with zoonotic Cryptosporidium genotypes, a direct

7.7. Children Cryptosporidiosis in South and Central America. In South America, cryptosporidiosis is often observed as a pediatric disease in areas where *Cryptosporidium* spp.
contact with animals or the presence within farms and/or associated waters. Interestingly, of the 71 Cryptosporidium-linked outbreaks (Table 4), 17 outbreaks (23.9%), defined as community-linked, appear to involve predominantly children, underlying the person-to-person contact as the prevailing transmission route. Geographically, this type of outbreaks seem to be concentrated in the USA and in the UK but have been also mapped in New Zealand and South-America (Table 3) (Figure 4). The high heterogeneity of C. hominis genotypes, notified in many developing areas for childhood, represents an indicator of endemicity for the transmission of cryptosporidiosis (Figure 4). In the poorest areas, gastrointestinal parasitism, enhanced by malnutrition, play a major role in children with severe immune impairment, with C. hominis being the leading agent of severe diarrhea. Intestinal dysfunction contributes to growth failure and further immune derangement, leading to wasting, and significantly enhancing children mortality. New areas of research on the relationship between breastfeeding and onset/progression of the cryptosporidiosis should be explored, especially in children population characterised by paucity of hygiene, nutrition and hydration, particularly in the first months of life. Lastly, the growing number of internationally adopted children requires an appropriate surveillance to ensure the long-term health of adopted children as well as their families. Thus, periodic surveys of large cohorts of internationally adopted children are now essential to monitor global changing epidemiologic trends.

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References

[1] R. W. Goodgame, “Understanding intestinal spore-forming protozoa: cryptosporidia, microsporidia, Isospora, and Cyclospora,” Annals of Internal Medicine, vol. 124, no. 4, pp. 429–441, 1996.
[2] L. Eckmann, “Small bowel infections,” Current Opinion in Gastroenterology, vol. 18, no. 2, pp. 197–202, 2002.
[3] K. K. Pierce and B. D. Kirkpatrick, “Update on human infections caused by intestinal protozoa,” Current Opinion in Gastroenterology, vol. 25, no. 1, pp. 12–17, 2009.
[4] World Health Organisation, “WHO/PAHO informal consultation on intestinal protozoal infections,” Tech. Rep. WHO/CDS/IP/92.2, World Health Organisation, Geneva, Switzerland, 1992.
[5] X.-M. Chen, J. S. Keithly, C. V. Paya, and N. F. LaRussa, “Cryptosporidiosis,” The New England Journal of Medicine, vol. 346, no. 22, pp. 1723–1731, 2002.
[6] P. Karanis, C. Kourenti, and H. Smith, “Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt,” Journal of Water and Health, vol. 5, no. 1, pp. 1–38, 2007.
[7] H. V. Smith, S. M. Cacciò, N. Cook, R. A. B. Nichols, and A. Tait, “Cryptosporidium and Giardia as foodborne zoonoses,” Veterinary Parasitology, vol. 149, no. 1-2, pp. 29–40, 2007.
[8] S. M. Cacciò and E. Pozio, “Molecular identification of food-borne and water-borne protozoa,” The Southeast Asian Journal of Tropical Medicine and Public Health, vol. 32, no. 2, pp. 156–158, 2001.
[9] S. M. Cacciò, “Molecular epidemiology of human cryptosporidiosis,” Parasitologia, vol. 47, no. 2, pp. 185–192, 2005.
[10] A. Grinberg, N. Lopez-Villalobos, W. Pomroy, G. Widmer, H. Smith, and A. Tait, “Host-shaped segregation of the Cryptosporidium parvum multilocus genotype repertoire,” Epidemiology and Infection, vol. 136, no. 2, pp. 273–278, 2008.
[11] H. L. DuPont, C. L. Chappell, C. R. Sterling, P. C. Okhuysen, J. D. Rose, and W. Jakubowski, “The infectivity of Cryptosporidium parvum in healthy volunteers,” The New England Journal of Medicine, vol. 332, no. 13, pp. 855–859, 1995.
[12] P. C. Okhuysen, C. L. Chappell, J. H. Crabb, C. R. Sterling, and H. L. Du Pont, “Virulence of three distinct Cryptosporidium parvum isolates for healthy adults,” The Journal of Infectious Diseases, vol. 180, no. 4, pp. 1275–1281, 1999.
[13] L. Jokipiï and A. M. M. Jokipiï, “Timing of symptoms and oocyst excretion in human cryptosporidiosis,” The New England Journal of Medicine, vol. 315, no. 26, pp. 1643–1647, 1986.
[14] C. L. Chappell, P. C. Okhuysen, C. R. Sterling, and H. L. Du Pont, “Cryptosporidium hominis: intensity of infection and oocyst excretion patterns in healthy volunteers,” The Journal of Infectious Diseases, vol. 173, no. 1, pp. 232–236, 1996.
[15] B.-M. Hsu, C. Huang, and J. R. Pan, “Filtration behaviors of Giardia and Cryptosporidium-ionic strength and pH effects,” Water Research, vol. 35, no. 16, pp. 3777–3782, 2001.
[16] J. L. Robertson and B. Gjerde, “Occurrence of parasites on fruits and vegetables in Norway,” Journal of Food Protection, vol. 64, no. 11, pp. 1793–1798, 2001.
[17] R. Reinoso, E. Becares, and H. V. Smith, “Effect of various environmental factors on the viability of Cryptosporidium parvum oocysts,” Journal of Applied Microbiology, vol. 104, no. 4, pp. 980–986, 2008.
[18] J. M. Shields, V. R. Hill, M. J. Arrowood, and M. J. Beach, “Inactivation of Cryptosporidium parvum under chlorinated recreational water conditions,” Journal of Water and Health, vol. 6, no. 4, pp. 513–520, 2008.
[19] J. Słapeta, “Centenary of the genus Cryptosporidium: from morphological to molecular species identification,” in Giardia and Cryptosporidium: From Molecules to Diseases, G. Ortega-Pierres, S. M. Cacciò, R. Fayer, T. G. Mank, H. V. Smith, and R. C. A. Thompson, Eds., chaper IV, pp. 31–50, CABl, Oxfordshire, UK.
[20] U. M. Ryan and L. Xiao, “Molecular epidemiology and typing of non-human isolates of Cryptosporidium,” in *Giardia and Cryptosporidium: From Molecules to Diseases*, G. Ortega-Pierres, S. M. Caccio, R. Fayer, T. G. Mank, H. V. Smith, and R. C. A. Thompson, Eds., chaper VI, pp. 65–80, CABI, Oxfordshire, UK.

[21] U. M. Morgan, L. Xiao, R. Fayer, A. A. Lal, and R. C. A. Thompson, “Variation in Cryptosporidium: towards a taxonomic revision of the genus,” *International Journal for Parasitology*, vol. 29, no. 11, pp. 1733–1751, 1999.

[22] C. P. Raccurt, “Worldwide human zoonotic cryptosporidiosis caused by *Cryptosporidium felis*,” *Parasite*, vol. 14, no. 1, pp. 15–20, 2007.

[23] F. Leoni, C. I. Gallimore, J. Green, and J. McLauchlin, “Characterisation of small double stranded RNA molecule in *Cryptosporidium hominis, Cryptosporidium felis* and *Cryptosporidium meleagridis*,” *Parasitology International*, vol. 55, no. 4, pp. 299–306, 2006.

[24] B. Wolska-Kusnierz, A. Bajer, S. M. Cacció et al., “Cryptosporidium infection in patients with primary immunodeficiencies,” *Journal of Pediatric Gastroenterology and Nutrition*, vol. 45, no. 4, pp. 458–464, 2007.

[25] L. Xiao, V. A. Cama, L. Cabrera, Y. Ortega, J. Pearson, and R. H. Gilman, “Possible transmission of *Cryptosporidium canis* among children and a dog in a household,” *Journal of Clinical Microbiology*, vol. 45, no. 6, pp. 2014–2016, 2007.

[26] L. Xiao, C. Bern, J. Limor et al., “Identification of 5 types of *Cryptosporidium* parasites in children in Lima, Peru,” *The Journal of Infectious Diseases*, vol. 183, no. 3, pp. 492–497, 2001.

[27] O. Ditrich, L. Palkovic, J. Sterba, J. Prokopik, J. Loudová, and M. Giboda, “The first finding of *Cryptosporidium baileyi* in man,” *Parasitology Research*, vol. 77, no. 1, pp. 44–47, 1991.

[28] I. Abubakar, S. H. Aliyu, C. Arumugam, P. R. Hunter, and N. K. Usman, “Prevention and treatment of cryptosporidiosis in immunocompromised patients,” *Cochrane Database of Systematic Reviews*, no. 1, Article ID CD004932, 2007.

[29] L. M. Kortbeek, “Clinical representation in Cryptosporidium-infected patients,” in *Giardia and Cryptosporidium: From Molecules to Diseases*, G. Ortega-Pierres, S. M. Cacció, R. Fayer, T. G. Mank, H. V. Smith, and R. C. A. Thompson, Eds., chaper XI, pp. 131–137, CABI, Oxfordshire, UK.

[30] D. A. Mosier and R. D. Oberst, “Cryptosporidiosis: a global challenge,” *Annals of the New York Academy of Sciences*, vol. 916, pp. 102–111, 2000.

[31] J. M. Balbus and M. A. Embrey, “Risk factors for waterborne enteric infections,” *Current Opinion in Gastroenterology*, vol. 18, no. 1, pp. 46–50, 2002.

[32] A. L. Reingold, “Outbreak investigations—a perspective,” *Emerging Infectious Diseases*, vol. 4, no. 1, pp. 21–27, 1998.

[33] S. Kato, L. Ascoliolo, J. Egas et al., “Waterborne Cryptosporidium oocyst identification and genotyping: use of G3S for ecosystem studies in Kenya and Ecuador,” *The Journal of Eukaryotic Microbiology*, vol. 50, pp. 548–549, 2003.

[34] S. Hughes, Q. Syed, S. Woodhouse et al., “Using a geographical information system to investigate the relationship between reported cryptosporidiosis and water supply,” *International Journal of Health Geographics*, vol. 3, article 15, 2004.

[35] I. R. Lake, F. C. D. Harrison, R. M. Chalmers et al., “Case-control study of environmental and social factors influencing cryptosporidiosis,” *European Journal of Epidemiology*, vol. 22, no. 11, pp. 805–811, 2007.

[36] S. S. R. Ajampur, B. P. Gladstone, D. Selvaprandian, J. P. Muliyil, H. Ward, and G. Kang, “Molecular and spatial epidemiology of cryptosporidiosis in children in a semiurban community in South India,” *Journal of Clinical Microbiology*, vol. 45, no. 3, pp. 915–920, 2007.

[37] A. R. Jex and R. B. Gasser, “Analysis of the genetic diversity within *Cryptosporidium hominis* and *Cryptosporidium parvum* from imported and autochthonous cases of human cryptosporidiosis by mutation scanning,” *Electrophoresis*, vol. 29, no. 20, pp. 4119–4129, 2008.

[38] A. R. Jex, A. Pangasa, B. E. Campbell et al., “Classification of *Cryptosporidium* species from patients with sporadic cryptosporidiosis by use of sequence-based multilocus analysis following mutation scanning,” *Journal of Clinical Microbiology*, vol. 46, no. 7, pp. 2252–2262, 2008.

[39] H.-P. Beck, D. Blake, M.-L. Darde et al., “Molecular approaches to diversity of populations of apicomplexan parasites,” *International Journal for Parasitology*, vol. 39, no. 2, pp. 175–189, 2009.

[40] A. Pangasa, A. R. Jex, B. E. Campbell et al., “High resolution melting-curve (HRM) analysis for the diagnosis of cryptosporidiosis in humans,” *Molecular and Cellular Probes*, vol. 23, no. 1, pp. 10–15, 2009.

[41] Y. Feng, N. Li, L. Duan, and L. Xiao, “Cryptosporidium genotype and subtype distribution in raw wastewater in Shanghai, China: evidence for possible unique *Cryptosporidium hominis* transmission,” *Journal of Clinical Microbiology*, vol. 47, no. 1, pp. 153–157, 2009.

[42] M. E. Mallon, A. MacLeod, J. M. Wastling, H. Smith, and A. Tait, “Multilocus genotyping of *Cryptosporidium parvum* type 2: population genetics and sub-structuring,” *Infection, Genetics and Evolution*, vol. 3, no. 3, pp. 207–218, 2003.

[43] M. Mallon, A. MacLeod, J. Wastling, H. Smith, B. Reilly, and A. Tait, “Population structures and the role of genetic exchange in the zoonotic pathogen *Cryptosporidium parvum*,” *Journal of Molecular Evolution*, vol. 56, no. 4, pp. 407–417, 2003.

[44] G. Widmer, X. Feng, and S. Tanriverdi, “Genotyping of *Cryptosporidium parvum* with microsatellite markers,” *Methods in Molecular Biology*, vol. 268, pp. 177–187, 2004.

[45] S. M. Cacció, “New methods for the diagnosis of *Cryptosporidium* and *Giardia*,” *Parasitologia*, vol. 46, no. 1–2, pp. 151–155, 2004.

[46] S. Tanriverdi and G. Widmer, “Differential evolution of repetitive sequences in *Cryptosporidium parvum* and *Cryptosporidium hominis*,” *Infection, Genetics and Evolution*, vol. 6, no. 2, pp. 113–122, 2006.

[47] L. Xiao, “Molecular epidemiology of cryptosporidiosis: an update,” *Experimental Parasitology*, vol. 124, no. 1, pp. 80–89, 2010.

[48] L. Xiao and U. M. Ryan, “Cryptosporidiosis: an update in molecular epidemiology,” *Current Opinion in Infectious Diseases*, vol. 17, no. 5, pp. 483–490, 2004.

[49] S. M. Cacció, R. C. A. Thompson, J. McLauchlin, and H. V. Smith, “Unravelling *Cryptosporidium* and *Giardia* epidemiology,” *Trends in Parasitology*, vol. 21, no. 9, pp. 430–437, 2005.

[50] V. A. Cama, C. Bern, J. Roberts et al., “Cryptosporidium species and subtypes and clinical manifestations in children, Peru,” *Emerging Infectious Diseases*, vol. 14, no. 10, pp. 1567–1574, 2008.
[51] A. Zintl, A. F. Proctor, C. Read et al., “The prevalence of Cryptosporidium species and subtypes in human faecal samples in Ireland,” Epidemiology and Infection, vol. 137, no. 2, pp. 270–277, 2009.

[52] G. Wilkes, T. Edge, V. Gannon et al., “Seasonal relationships among indicator bacteria, pathogenic bacteria, Cryptosporidium oocysts, Giardia cysts, and hydrological indices for surface waters within an agricultural landscape,” Water Research, vol. 43, no. 8, pp. 2209–2223, 2009.

[53] A. Clavel, J. L. Oliwares, J. Fleta et al., “Seasonality of cryptosporidiosis in children,” European Journal of Clinical Microbiology & Infectious Diseases, vol. 15, no. 1, pp. 77–79, 1996.

[54] I. Qibal, P. R. Hira, F. Al-Ali, and R. Philip, “Cryptosporidiosis in Kuwaiti children: seasonality and endemicity,” Clinical Microbiology and Infection, vol. 7, no. 5, pp. 261–266, 2001.

[55] J. S. Jagai, D. A. Castronovo, J. Monchak, and E. N. Naumova, “Seasonality of cryptosporidiosis: a meta-analysis approach,” Environmental Research, vol. 109, no. 4, pp. 465–478, 2009.

[56] M. C. Peel, B. L. Finlayson, and T. A. McMahon, “Updated world map of the Köppen-Geiger climate classification,” Hydrology and Earth System Sciences, vol. 11, no. 5, pp. 1633–1644, 2007.

[57] P. Das, S. S. Roy, K. MitraDhar et al., “Molecular characterisation of Cryptosporidium spp. from children in Kolkata, India,” Journal of Clinical Microbiology, vol. 44, no. 11, pp. 4246–4249, 2006.

[58] L. Savioli, H. Smith, and A. Thompson, “Giardia and Cryptosporidium join the ‘neglected diseases initiative’,” Trends in Parasitology, vol. 22, no. 5, pp. 203–208, 2006.

[59] Neglected Diseases Initiative of the World Health Organization, http://www.who.int/neglected_diseases/en/.

[60] D. S. Berkman, A. G. Lescano, R. H. Gilman, S. L. Lopez, and M. M. Black, “Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study,” The Lancet, vol. 359, no. 9306, pp. 564–571, 2002.

[61] R. Haque, D. Mondal, A. Karim et al., “Prospective case-control study of the association between common enteric protozoal parasites and diarrhoea in Bangladesh,” Clinical Infectious Diseases, vol. 48, no. 9, pp. 1191–1197, 2009.

[62] T. Geurden, B. Levecke, S. M. Cacciò et al., “Multilocus genotyping of Cryptosporidium and Giardia in non-outbreak related cases of diarrhoea in human patients in Belgium,” Parasitology, vol. 136, no. 10, pp. 1161–1168, 2009.

[63] R. M. Chalmers, K. Elwin, A. L. Thomas, E. C. Guy, and B. Mason, “Long-term Cryptosporidium typing reveals the aetiology and species-specific epidemiology of human cryptosporidiosis in England and Wales, 2000 to 2003,” Eurosurveillance, vol. 14, no. 2, Article ID 19086, 2009.

[64] P. H. P. Wong and C. S. L. Ong, “Molecular characterization of the Cryptosporidium cervine genotype,” Parasitology, vol. 133, no. 6, pp. 693–700, 2006.

[65] G. Robinson, K. Elwin, and R. M. Chalmers, “Unusual Cryptosporidium genotypes in human cases of diarrhoea,” Emerging Infectious Diseases, vol. 14, no. 11, pp. 1800–1802, 2008.

[66] S. Tanriverdi, A. Grinberg, R. M. Chalmers et al., “Inferences about the global population structures of Cryptosporidium parvum and cryptosporidium hominis,” Applied and Environmental Microbiology, vol. 74, no. 23, pp. 7227–7234, 2008.

[67] M. M. Peng, S. R. Meshnick, N. A. Cunliffe et al., “Molecular epidemiology of cryptosporidiosis in children in Malawi,” The Journal of Eukaryotic Microbiology, vol. 50, supplement, pp. 557–559, 2003.

[68] W. Gatei, C. N. Wamae, C. Mbale et al., “Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya,” The American Journal of Tropical Medicine and Hygiene, vol. 75, no. 1, pp. 78–82, 2006.

[69] C. P. Raccurt, P. Brasseur, R. I. Verdier et al., “Human cryptosporidiosis and Cryptosporidium spp. in Haiti,” Tropical Medicine & International Health, vol. 11, no. 6, pp. 929–934, 2006.

[70] O. Y. Bushen, A. Kohli, R. C. Pinkerton et al., “Heavy cryptosporidial infections in children in northeast Brazil: comparison of Cryptosporidium hominis and Cryptosporidium parvum,” Transactions of the Royal Society of Tropical Medicine and Hygiene, vol. 101, no. 4, pp. 378–384, 2007.

[71] A. Samie, P. O. Bessong, C. L. Obi et al., “Cryptosporidium species: preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda region, Limpopo Province, South Africa,” Experimental Parasitology, vol. 114, no. 4, pp. 314–322, 2006.

[72] B. A. Leav, M. R. Mackay, A. Anyanwu et al., “Analysis of sequence diversity at the highly polymorphic Cpg40/15 locus among Cryptosporidium isolates from human immunodeficiency virus-infected children in South Africa,” Infection and Immunity, vol. 70, no. 7, pp. 3881–3890, 2002.

[73] J. K. Tumwine, A. Kekitiinwa, S. Bakeera-Kitaka et al., “Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhoea with and without concurrent infection with the human immunodeficiency virus,” The American Journal of Tropical Medicine and Hygiene, vol. 73, no. 5, pp. 921–925, 2005.

[74] V. A. Cama, J. M. Ross, S. Crawford et al., “Differences in clinical manifestations among Cryptosporidium species and subtypes in HIV-infected persons,” The Journal of Infectious Diseases, vol. 196, no. 5, pp. 684–691, 2007.

[75] O. Matos, M. Alves, L. Xiao, V. Cama, and F. Antunes, “Cryptosporidium felis and C. meleagris in persons with HIV, Portugal,” Emerging Infectious Diseases, vol. 10, no. 12, pp. 2256–2257, 2004.

[76] C.-C. Hung, J. C. Tsaihong, Y.-T. Lee et al., “Prevalence of intestinal infection due to Cryptosporidium species among Taiwanese patients with human immunodeficiency virus infection,” Journal of the Formosan Medical Association, vol. 106, no. 1, pp. 31–35, 2007.

[77] A. R. Meamar, K. Guyot, G. Cerdà et al., “Molecular characterization of Cryptosporidium isolates from humans and animals in Iran,” Applied and Environmental Microbiology, vol. 73, no. 3, pp. 1033–1035, 2007.

[78] M. Alves, L. Xiao, F. Antunes, and O. Matos, “Distribution of Cryptosporidium subtypes in humans and domestic and wild ruminants in Portugal,” Parasitology Research, vol. 99, no. 3, pp. 287–292, 2006.

[79] R. M. Chalmers, C. Ferguson, S. M. Cacciò et al., “Direct comparison of selected methods for genetic categorisation of Cryptosporidium parvum and cryptosporidium hominis species,” International Journal for Parasitology, vol. 35, no. 4, pp. 397–410, 2005.

[80] S. Glaberman, J. E. Moore, C. J. Lowery et al., “Three drinking-water-associated cryptosporidiosis outbreaks, Northern Ireland,” Emerging Infectious Diseases, vol. 8, no. 6, pp. 631–633, 2002.
[81] W. Smerdon, "Cryptosporidiosis outbreak associated with Majorcan hotel," Eurosurveillance, vol. 4, no. 34, Article ID 1540, 2000.

[82] L. Zhou, A. Singh, J. Jiang, and L. Xiao, "Molecular surveillance of Cryptosporidium spp. in raw wastewater in Milwaukee: implications for understanding outbreak occurrence and transmission dynamics," Journal of Clinical Microbiology, vol. 41, no. 11, pp. 5254–5257, 2003.

[83] W. Gatei, P. Das, P. Dutta et al., "Multilocus sequence typing and genetic structure of Cryptosporidium hominis from children in Kolkata, India," Infection, Genetics and Evolution, vol. 7, no. 2, pp. 197–205, 2007.

[84] S. Cohen, F. Dalle, A. Gallay, M. Di Palma, A. Bonnin, and H. D. Ward, "Identification of Cggp40/15 type Ib as the predominant allele in isolates of Cryptosporidium spp. from a waterborne outbreak of gastroenteritis in South Burgundy, France," Journal of Clinical Microbiology, vol. 44, no. 2, pp. 589–591, 2006.

[85] T. K. Boehmer, N. B. Alden, T. S. Ghosh, and R. L. Vogt, "Cryptosporidiosis from a community swimming pool: outbreak investigation and follow-up study," Epidemiology and Infection, vol. 137, no. 11, pp. 1651–1654, 2009.

[86] C. S. Ong, S. Chow, P. P. L. So, et al., "Identification of two different cryptosporidium hominis subtypes from cases in the 2001 waterborne cryptosporidiosis outbreak in North Battleford, Saskatchewan," in Proceedings of the 11th Canadian National Conference and the 2nd Policy Forum on Drinking Water, Calgary, Canada, April 2004, pp. 628–638, Canadian Water and Wastewater Association, Ottawa, Canada, 2005.

[87] L. Xiao, R. Fayer, U. Ryan, and S. J. Upton, "Cryptosporidium taxonomy: recent advances and implications for public health," Clinical Microbiology Reviews, vol. 17, no. 1, pp. 72–97, 2004.

[88] W. Gatei, D. Barrett, J. F. Lindo, D. Eldemire-Shearer, V. S. J. Robertson, and L. Hermansen, and B. K. Gjerde, "Occurrence of Cryptosporidium population in HIV-infected persons, Jamaica," Emerging Infectious Diseases, vol. 14, no. 5, pp. 841–843, 2008.

[89] D. Muthusamy, S. S. Rao, S. Ramani et al., "Multilocus genotyping of Cryptosporidium sp. isolates from human immunodeficiency-virus-infected individuals in South India," Journal of Clinical Microbiology, vol. 44, no. 2, pp. 632–634, 2006.

[90] A. R. Jex and R. B. Gasser, "Genetic richness and diversity in cryptosporidium hominis and C. parvum reveals major knowledge gaps and a need for the application of "next generation" technologies—research review," Biotechnology Advances, vol. 28, no. 1, pp. 17–26, 2010.

[91] J. Dreesman, D. C. Villarreal-Conzales, S. Cleves, H. A. Reins, and M. Pulz, "Regionally increased incidence of notified cryptosporidiosis cases due to different laboratory methods," Gesundheitswesen, vol. 69, no. 8–9, pp. 483–487, 2007.

[92] J. S. Yoder and M. J. Beach, "Cryptosporidiosis surveillance—United States, 2003–2005," Morbidity and Mortality Weekly Report, vol. 56, no. 7, pp. 1–10, 2007.

[93] Center for Disease Control and Prevention, http://emergency.cdc.gov/agent/agentlist-category.asp.

[94] J. M. Stuart, H. J. Orr, F. G. Warburton et al., "Risk factors for sporadic giardiasis: a case-control study in Southwestern England," Emerging Infectious Diseases, vol. 9, no. 2, pp. 229–233, 2003.

[95] S. L. Roy, S. M. DeLong, S. A. Stenzel et al., "Risk factors for sporadic cryptosporidiosis among immunocompetent persons in the United States from 1999 to 2001," Journal of Clinical Microbiology, vol. 42, no. 7, pp. 2944–2951, 2004.

[96] S. Couple, K. Delambre, R. Pouillot, S. Houdart, M. Santillana-Hayat, and F. Derouin, "Detection of Cryptosporidium, Giardia and Enterocytozoon bieneusi in surface water, including recreational areas: a one-year prospective study," FEMS Immunology and Medical Microbiology, vol. 47, no. 3, pp. 351–359, 2006.

[97] R. Fayer, "Cryptosporidium: a water-borne zoonotic parasite," Veterinary Parasitology, vol. 126, no. 1-2, pp. 37–56, 2004.

[98] H. Pelly, M. Cormican, D. O’Donovan et al., "A large outbreak of cryptosporidiosis in western Ireland linked to public water supply: a preliminary report," Eurosurveillance, vol. 12, no. 5, Article ID 3187, 2007.

[99] P. Jennings and A. Rhatigan, "Cryptosporidiosis outbreak in Ireland linked to public water supply," Eurosurveillance, vol. 6, no. 22, Article ID 2089, 2002.

[100] H.-W. A. Cheng, F. E. Lucy, T. K. Graczyk, M. A. Broaders, L. Tamang, and M. Connolly, "Fate of Cryptosporidium parvum and Cryptosporidium hominis oocysts and Giardia duodenalis cysts during secondary wastewater treatments," Parasitology Research, vol. 105, no. 3, pp. 689–696, 2009.

[101] H. P. Thompson, J. S. G. Dooley, J. Kenny et al., "Genotypes and subtypes of Cryptosporidium spp. in neonatal calves in Northern Ireland," Parasitology Research, vol. 100, no. 3, pp. 619–624, 2007.

[102] F. M. Schets, J. H. van Wijnen, J. F. Schijven, H. Schoon, and A. M. de Roda Husman, "Monitoring of waterborne pathogens in surface waters in Amsterdam, the Netherlands, and the potential health risk associated with exposure to Cryptosporidium and Giardia in these waters," Applied and Environmental Microbiology, vol. 74, no. 7, pp. 2069–2078, 2008.

[103] M. L. Lobo, L. Xiao, F. Antunes, and O. Matos, "Occurrence of Cryptosporidium and Giardia genotypes and subtypes in raw and treated water in Portugal," Letters in Applied Microbiology, vol. 48, no. 6, pp. 732–737, 2009.

[104] M. Alves, L. Xiao, I. Sulaiman, A. A. Lal, O. Matos, and F. Antunes, "Subgenotype analysis of Cryptosporidium isolates from humans, cattle, and zoo ruminants in Portugal," Journal of Clinical Microbiology, vol. 41, no. 6, pp. 2744–2747, 2003.

[105] L. J. Robertson, L. Hermansen, and B. K. Gjerde, "Occurrence of Cryptosporidium oocysts and Giardia cysts in sewage in Norway," Applied and Environmental Microbiology, vol. 72, no. 8, pp. 5297–5303, 2006.

[106] T. Geurden, E. Goossens, B. Levecke, F. Vercammen, J. Vercruyssse, and E. Claerebout, "Occurrence and molecular characterization of Cryptosporidium and Giardia in captive wild ruminants in Belgium," Journal of Zoo and Wildlife Medicine, vol. 40, no. 1, pp. 126–130, 2009.

[107] N. A. Romanenko, V. P. Sergiev, and I. A. Rakhmanin, "Cryptosporidium oocysts and epidemic safety of drinking water in the Russian Federation," Meditsinskaya Parazitologiya i Parazitarnye Bolezni, no. 2, pp. 11–13, 2001.

[108] A. Giangaspero, "Giardia, Cryptosporidium and the spectre of zoonosis: the Italian experience from land to sea," Parasitologia, vol. 48, no. 1-2, pp. 95–100, 2006.

[109] A. Giangaspero, F. Berrilli, and O. Brandonisio, "Giardia and Cryptosporidium and public health: the epidemiological scenario from the Italian perspective," Parasitology Research, vol. 101, no. 5, pp. 1169–1182, 2007.

[110] O. Brandonisio, "Waterborne transmission of Giardia and Cryptosporidium," Parasitologiå, vol. 48, no. 1-2, pp. 91–94, 2006.
[111] O. Brandonisio, L. Fumarola, R. Spinelli, F. Donadio, P. Montemurro, and F. Portincasa, “Giardia e Cryptosporidium spp: rassegna critica e monitoraggio in acque superficiali e reflue,” L’Igiene Moderna, vol. 122, pp. 137–160, 2004.

[112] R. Briancesco and L. Bonadonna, “An Italian study on Cryptosporidium and Giardia in wastewater, fresh water and treated water,” Environmental Monitoring and Assessment, vol. 104, no. 1–3, pp. 445–457, 2005.

[113] M. A. Di Benedetto, F. Di Piazza, C. M. Maida, A. Firenze, and R. Oliveri, “Occurrence of Giardia and Cryptosporidium in wastewater, surface water and ground water samples in Palermo (Sicily),” Annali di Igiene, vol. 17, no. 5, pp. 367–375, 2005.

[114] C. Sacco, M. Bianchi, C. Lorini, D. Burrini, S. Berchielli, and E. Lanciotti, “Removal of Cryptosporidium and Giardia in drinking water treatment in a Tuscan area,” Annali di Igiene, vol. 18, no. 2, pp. 117–126, 2006.

[115] A. Lonigro, A. Pollice, R. Spinelli et al., “Giardia cysts and Cryptosporidium oocysts in membrane-filtered municipal wastewater used for irrigation,” Applied and Environmental Microbiology, vol. 72, no. 12, pp. 7916–7918, 2006.

[116] E. Carraro, E. Fea, S. Salva, and G. Gilli, “Impact of a wastewater treatment plant on Cryptosporidium oocysts and Giardia cysts occurring in a surface water,” Water Science and Technology, vol. 41, no. 7, pp. 31–37, 2000.

[117] S. M. Cacciò, M. De Giacomo, F. A. Aulicino, and E. Pozio, “Giardia cysts in wastewater treatment plants in Italy,” Applied and Environmental Microbiology, vol. 69, no. 6, pp. 3393–3398, 2003.

[118] R. Fayer, J. P. Dubey, and D. S. Lindsay, “Zoonotic protozoa: from land to sea,” Trends in Parasitology, vol. 20, no. 11, pp. 531–536, 2004.

[119] A. Giangaspero, U. Molini, R. Iorio, D. Traversa, B. Paoletti, and C. Gianstone, “Cryptosporidium parvum oocysts in seawater clams (Chamelea gallina) in Italy,” Preventive Veterinary Medicine, vol. 69, no. 3–4, pp. 203–212, 2007.

[120] U. Molini, D. Traversa, G. Ceschia et al., “Temporal occurrence of Cryptosporidium in the Manila clam Ruditapes philippinarum in Northern Adriatic Italian Lagoons,” Journal of Food Protection, vol. 70, no. 2, pp. 494–499, 2007.

[121] A. Giangaspero, R. Cirillo, V. Lacasella et al., “Giardia and Cryptosporidium in inflowing water and harvested shellfish in a Lagoon in Southern Italy,” Parasitology International, vol. 58, no. 1, pp. 12–17, 2009.

[122] W. A. Miller, M. A. Miller, I. A. Gardner et al., “New genotypes and factors associated with Cryptosporidium detection in mussels (Mytilus spp.) along the California coast,” International Journal for Parasitology, vol. 35, no. 10, pp. 1103–1113, 2005.

[123] B. Paoletti, A. Giangaspero, A. Gatti et al., “Immunoenzymatic analysis and genetic detection of Cryptosporidium parvum in lambs from Italy,” Experimental Parasitology, vol. 122, no. 4, pp. 349–352, 2009.

[124] A. Duranti, S. M. Cacciò, E. Pozio et al., “Risk factors associated with Cryptosporidium parvum infection in cattle,” Zoonoses and Public Health, vol. 56, no. 4, pp. 176–182, 2009.

[125] L. A. Trotz-Williams, S. W. Martin, K. E. Leslie, T. Duffield, D. V. Nydam, and A. S. Peregrine, “Association between management practices and within-herd prevalence of Cryptosporidium parvum shedding on dairy farms in southern Ontario,” Preventive Veterinary Medicine, vol. 83, no. 1, pp. 11–23, 2008.

[126] N. Bilenko, R. Ghosh, A. Levy, R. J. Deckelbaum, and O. Fraser, “Partial breastfeeding protects Bedouin infants from infection and morbidity: prospective cohort study,” Asia Pacific Journal of Clinical Nutrition, vol. 17, no. 2, pp. 243–249, 2008.

[127] C. Wheeler, D. J. Vugia, G. Thomas et al., “Outbreak of cryptosporidiosis at a California waterpark: employee and patron roles and the long road towards prevention,” Epidemiology and Infection, vol. 135, no. 2, pp. 302–310, 2007.

[128] F. J. Sorvillo, K. Fujioka, B. Nahlen, M. P. Tormey, R. Kebabian, and L. Mascola, “Swimming-associated cryptosporidiosis,” American Journal of Public Health, vol. 82, no. 5, pp. 742–744, 1992.

[129] A. L. Valderrama, M. C. Hlavsa, A. Cronquist et al., “Multiple risk factors associated with a large statewide increase in cryptosporidiosis,” Epidemiology and Infection, vol. 137, no. 12, pp. 1781–1788, 2009.

[130] G. F. Craun, R. L. Calderon, and M. F. Craun, “Outbreaks associated with recreational water in the United States,” International Journal of Environmental Health Research, vol. 15, no. 4, pp. 243–262, 2005.

[131] J. S. Yoder, M. C. Hlavsa, G. F. Craun et al., “Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events—United States, 2005–2006,” Morbidity and Mortality Weekly Report, vol. 57, no. 9, pp. 1–29, 2008.

[132] B. G. Blackburn, G. F. Craun, J. S. Yoder et al., “Surveillance for waterborne-disease outbreaks associated with drinking water-United States, 2001–2002,” Morbidity and Mortality Weekly Report, vol. 53, no. 8, pp. 23–45, 2004.

[133] R. L. Calderon and G. F. Craun, “Estimates of endemic waterborne risks from community-intervention studies,” Journal of Water and Health, vol. 4, no. 2, pp. 89–100, 2006.

[134] G. F. Craun, N. Nwachuku, R. L. Calderon, and M. F. Craun, “Outbreaks in drinking-water systems, 1991–1998,” Journal of Environmental Health, vol. 65, no. 1, pp. 16–23, 2002.

[135] A. Khalakdina, D. J. Vugia, I. Nadle, G. A. Rothrock, and I. M. Colford Jr., “Is drinking water a risk factor for endemic cryptosporidiosis? A case-control study in the immunocompetent general population of the San Francisco Bay Area,” BMC Public Health, vol. 3, no. 1, article 11, 2003.

[136] San Francisco Bay Area Cryptosporidiosis Surveillance Project, http://www.sfphes.org/water/index_crypto.htm.

[137] K. A. Reynolds, K. D. Mena, and C. P. Gerba, “Risk of waterborne illness via drinking water in the United States,” Reviews of Environmental Contamination and Toxicology, vol. 192, pp. 117–158, 2008.

[138] P. Payment, E. Franco, L. Richardson, and J. Siemiatycki, “Gastrointestinal health effects associated with the consumption of drinking water produced by point-of-use domestic reverse-osmosis filtration units,” Applied and Environmental Microbiology, vol. 57, no. 4, pp. 945–948, 1991.

[139] P. Payment, A. Berte, and C. Fleury, “Sources of variation in isolation rate of Giardia lamblia cysts and their homogeneous distribution in river water entering a water treatment plant,” Canadian Journal of Microbiology, vol. 43, no. 7, pp. 687–689, 1997.

[140] M. E. Hellard, M. I. Sinclair, A. B. Forbes, and C. K. Fairley, “A randomized, blinded, controlled trial investigating the gastrointestinal health effects of drinking water quality,” Environmental Health Perspectives, vol. 109, no. 8, pp. 773–778, 2001.
[173] S. Goh, M. Reacher, D. P. Casemore et al., “Sporadic cryptosporidiosis, North Cumbria, England, 1996–2000,” Emerging Infectious Diseases, vol. 10, no. 6, pp. 1007–1015, 2004.

[174] M. C. A. Teixeira, M. L. Barreto, C. Melo, L. R. Silva, L. R. S. Moraes, and N. M. Alcântara-Neves, "A serological study of Cryptosporidium transmission in a periurban area of a Brazilian Northeastern city," Tropical Medicine & International Health, vol. 12, no. 9, pp. 1096–1104, 2007.

[175] M. U. Alonso-Fresán, J. C. Vázquez-Chagoyán, V. Velázquez-Ordoñez, N. Pescador-Salas, and J. Saltijeral-Oaxaca, "Sheep management and cryptosporidiosis in central Mexico," Tropical Animal Health and Production, vol. 41, no. 4, pp. 431–436, 2009.

[176] E. C.D. Todd, J. D. Greig, C. A. Bartleson, and B. S. Michaels, "Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment," Journal of Food Protection, vol. 72, no. 1, pp. 202–219, 2009.

[177] E. Pozio, "Epidemiology and control prospects of foodborne parasitic zoonoses in the European Union," Parassitologia, vol. 50, no. 1-2, pp. 17–24, 2008.

[178] A. Pönka, P. Kotilainen, R. Rimhanen-Finne et al., "A foodborne outbreak due to Cryptosporidium parvum in Helsinki, November 2008," Eurosurveillance, vol. 14, no. 28, Article ID 19269, 2009.

[179] M. Insulander, B. de Jong, and B. Svenungsson, "A foodborne outbreak of Cryptosporidium hominis infection," Epidemiology and Infection, vol. 137, no. 3, pp. 348–356, 2009.

[180] S. Ethelberg, M. Lisby, L. S. Vestergaard et al., "A foodborne outbreak of Cryptosporidium hominis infection," Epidemiology and Infection, vol. 137, no. 3, pp. 348–356, 2009.

[181] P. S. Millard, K. F. Gensheimer, D. G. Addiss et al., "An outbreak of cryptosporidiosis from fresh-pressed apple cider," Journal of the American Medical Association, vol. 272, no. 20, pp. 1592–1596, 1994.

[182] B. G. Blackburn, J. M. Mazurek, M. Hlavsa et al., "Cryptosporidiosis associated with ozonated apple cider," Emerging Infectious Diseases, vol. 12, no. 4, pp. 684–686, 2006.

[183] FoodNet, http://www.cdc.gov/FoodNet/.

[184] J. D. Vojdani, L. R. Beuchat, and R. V. Tauxe, "Juice-associated outbreaks of human illness in the United States, 1995 through 2005," Journal of Food Protection, vol. 71, no. 2, pp. 356–364, 2008.

[185] M. Lynch, J. Painter, R. Woodruff, and C. Braden, "Surveillance for foodborne-disease outbreaks—United States, 1998–2002," Morbidity and Mortality Weekly Report, vol. 55, no. 10, pp. 1–42, 2006.

[186] Centers for Disease Control and Prevention (CDC), “Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food-10 States, United States 2003,” Morbidity and Mortality Weekly Report, vol. 53, no. 16, pp. 338–343, 2004.

[187] Centers for Disease Control and Prevention (CDC), “Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food-10 States, United States 2004,” Morbidity and Mortality Weekly Report, vol. 54, no. 14, pp. 352–356, 2005.

[188] D. Vugia, A. Cronquist, J. Hadler et al., “Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 States, United States, 2005,” Morbidity and Mortality Weekly Report, vol. 55, no. 14, pp. 392–393, 2006.

[189] D. Vugia, A. Cronquist, J. Hadler et al., “Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 States, 2006,” Morbidity and Mortality Weekly Report, vol. 56, no. 14, pp. 336–339, 2007.

[190] D. Vugia, A. Cronquist, J. Hadler et al., “Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 States, 2007,” Morbidity and Mortality Weekly Report, vol. 57, no. 14, pp. 366–370, 2008.

[191] Centers for Disease Control and Prevention (CDC), “Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food-10 States, United States 2008,” Morbidity and Mortality Weekly Report, vol. 58, no. 13, pp. 333–337, 2009.

[192] A. D. Freites-Martínez, D. Colmenares, M. Pérez, M. García, and O. Díaz de Suárez, “Cryptosporidium sp infections and other intestinal parasites in food handlers from Zulia state, Venezuela,” Investigacion Clinica, vol. 50, no. 1, pp. 13–21, 2009.

[193] M. Calvo, M. Carazo, M. L. Arias, C. Chaves, R. Monge, and M. Chinchilla, “Prevalence of Cyclospora sp., Cryptosporidium sp, microsporidia and fecal coliform determination in fresh fruit and vegetables consumed in Costa Rica,” Archivos Latinoamericanos de Nutrición, vol. 54, no. 4, pp. 428–432, 2004.

[194] J.-D. Cavallo and E. Garrabé, “Infectious aetiologies of travelers’ diarrhoea,” Médecine et Maladies Infectieuses, vol. 37, no. 11, pp. 722–727, 2007.

[195] L. Saiman, J. Aronson, J. Zhou et al., “Prevalence of infectious diseases among internationally adopted children,” Pediatrics, vol. 108, no. 3, pp. 608–612, 2001.

[196] D. O. Freedman, L. H. Weld, P. E. Kozarsky et al., “Spectrum of disease and relation to place of exposure among ill returned travelers,” The New England Journal of Medicine, vol. 354, no. 2, pp. 119–130, 2006.

[197] S. Ansart, L. Perez, O. Vergely, M. Danis, F. Bricaire, and E. Caumes, “Illnesses in travelers returning from the tropics: a prospective study of 622 patients,” Journal of Travel Medicine, vol. 12, no. 6, pp. 312–318, 2005.

[198] S. R. Caruana, H. A. Kelly, J. Y. Y. Ngeow et al., “Undiagnosed and potentially lethal parasitic infections among immigrants and refugees in Australia,” Journal of Travel Medicine, vol. 13, no. 4, pp. 233–239, 2006.

[199] C. J. M. Whitty, B. Carroll, M. Armstrong et al., “Utility of history, examination and laboratory tests in screening those returning to Europe from the tropics for parasitic infection,” Tropical Medicine & International Health, vol. 5, no. 11, pp. 818–823, 2000.

[200] R. Fotedar, D. Stark, N. Beebe, D. Marriott, J. Ellis, and J. Harkness, “Laboratory diagnostic techniques for Entamoeba species,” Clinical Microbiology Reviews, vol. 20, no. 3, pp. 511–532, 2007.

[201] R. Goodgame, “Emerging causes of traveler’s diarrheaa: Cryptosporidium, Cyclospora, Isospora, and Microsporidia,” Current Infectious Disease Reports, vol. 5, no. 1, pp. 66–73, 2003.

[202] M. A. S. de Wit, M. P. G. Koopmans, L. M. Kortbeek et al., “Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology,” American Journal of Epidemiology, vol. 154, no. 7, pp. 666–674, 2001.
[203] N. M. Thielman and R. L. Guerrant, “Persistent diarrhea in the returned traveler,” *Infectious Disease Clinics of North America*, vol. 12, no. 2, pp. 489–501, 1998.

[204] P. C. Okhuysen, “Traveler’s diarrhea due to intestinal protozoa,” *Clinical Infectious Diseases*, vol. 33, no. 1, pp. 110–114, 2001.

[205] N. Turgay, A. Yolaisigmez, D. D. Erdogân, F. Y. Zeyrek, and A. Uner, “Incidence of cyclosporiasis in patients with gastrointestinal symptoms in western Turkey,” *Medical Science Monitor*, vol. 13, no. 1, pp. CR34–CR39, 2007.

[206] P. Nair, J. A. Mohamed, H. L. DuPont et al., “Epidemiology of cryptosporidiosis in northern American travelers to Mexico,” *The American Journal of Tropical Medicine and Hygiene*, vol. 79, no. 2, pp. 210–214, 2008.

[207] R. Lazensky, R. M. Hammond, K. Van Zile, and K. Geib, “Cryptosporidiosis outbreak in a Nassau County, Florida, return travel group from Ireland, may 24, 2006–june 4, 2006,” *Journal of Environmental Health*, vol. 71, no. 2, pp. 20–24, 2008.

[208] R. B. Gasser, Y. G. Abs EL-Osta, and R. M. Chalmers, “Electrophoretic analysis of genetic variability within *Cryptosporidium parvum* from imported and autochthonous cases of human cryptosporidiosis in the United Kingdom,” *Applied and Environmental Microbiology*, vol. 69, no. 5, pp. 2719–2730, 2003.

[209] R. M. Chalmers, S. J. Hadfield, C. J. Jackson, K. Elwin, L. Xiao, and P. Hunter, “Geographic linkage and variation in *Cryptosporidium hominis*,” *Emerging Infectious Diseases*, vol. 14, no. 3, pp. 496–498, 2008.

[210] T. Weitzen, S. Dittrich, I. Möhl, E. Adusu, and T. Jelinek, “Evaluation of seven commercial antigen detection tests for *Giardia* and *Cryptosporidium* in stool samples,” *Clinical Microbiology and Infection*, vol. 12, no. 7, pp. 656–659, 2006.

[211] M. T. Katanik, S. K. Schneider, J. E. Rosenblatt, G. S. Hall, and G. W. Procop, “Evaluation of ColorPAC *Giardia/Cryptosporidium* rapid assay and ProSpect *Giardia/Cryptosporidium* microplate assay for detection of *Giardia* and *Cryptosporidium* in fecal specimens,” *Journal of Clinical Microbiology*, vol. 39, no. 12, pp. 4523–4525, 2001.

[212] L. S. Garcia and R. Y. Shimizu, “Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens,” *Journal of Clinical Microbiology*, vol. 35, no. 6, pp. 1526–1529, 1997.

[213] R. J. Ten Hove, M. van Esbroeck, T. Vervoort, J. van den Ende, L. van Lieshout, and J. J. Verweij, “Molecular diagnostics of intestinal parasites in returning travellers,” *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 28, no. 9, pp. 1045–1053, 2009.

[214] H. Rotterdam and P. Tsang, “Gastrointestinal disease in the immunocompromised patient,” *Human Pathology*, vol. 25, no. 11, pp. 1123–1140, 1994.

[215] R. Weber, B. Ledergerber, R. Zbinden et al., “Enteric infections and diarrhea in human-virus-infected persons: prospective community-based cohort study,” *Archives of Internal Medicine*, vol. 159, no. 13, pp. 1473–1480, 1999.

[216] J. A. Montero, J. T. Sinnott, D. A. Holt, and C. Lloyd, “Biliary *cryptosporidiosis: current concepts*,” *Infections in Medicine*, vol. 18, no. 6, pp. 312–316, 2001.

[217] M. Scaglia, S. Gatti, P. Bassi, P. L. Viale, S. Novati, and S. Ranieri, “Intestinal co-infection by *Cyclospora* sp. and *Cryptosporidium parvum: first report in an AIDS patient*,” *Parasite*, vol. 1, no. 4, pp. 387–390, 1994.

[218] D. Reijasse, N. Patye-Mariad de Serre, D. Canioni et al., “Cytotoxic T cells in AIDS colonic cryptosporidiosis,” *Journal of Clinical Pathology*, vol. 54, no. 4, pp. 298–303, 2001.

[219] Y. M. Miao, F. M. Awad-El-Kariem, C. Franzen et al., “Eradication of cryptosporidia and microsporidia following successful antiretroviral therapy,” *Journal of Acquired Immune Deficiency Syndromes*, vol. 25, no. 2, pp. 124–129, 2000.

[220] N. A. Foudraine, G. J. Weaverling, T. van Cool et al., “Improvement of chronic diarrhea in patients with advanced HIV-1 infection during potent antiretroviral therapy,” *AIDS*, vol. 12, no. 1, pp. 35–41, 1998.

[221] E. Pozio and M. A. G. Morales, “The impact of HIV-protease inhibitors on opportunistic parasites,” *Trends in Parasitology*, vol. 21, no. 2, pp. 58–63, 2005.

[222] W. Gatei, J. Greensill, R. W. Ashford et al., “Molecular analysis of the 18S rRNA gene of *Cryptosporidium* parasites from patients with or without human immunodeficiency virus infections living in Kenya, Malawi, Brazil, the United Kingdom, and Vietnam,” *Journal of Clinical Microbiology*, vol. 41, no. 4, pp. 1458–1462, 2003.

[223] U. Morgan, R. Weber, L. Xiao et al., “Molecular characterization of *Cryptosporidium* isolates obtained from human immunodeficiency virus-infected individuals living in Switzerland, Kenya, and the United States,” *Journal of Clinical Microbiology*, vol. 38, no. 3, pp. 1180–1183, 2000.

[224] M. T. Llorente, A. Clavel, M. P. Góñi et al., “Genetic characterization of *Cryptosporidium* species from humans in Spain,” *Parasitology International*, vol. 56, no. 3, pp. 201–205, 2007.

[225] O. Brandonisio, P. Maggi, M. A. Panaro, L. A. Bramante, A. Di Coste, and G. Angaran, “Prevalence of cryptosporidiosis in HIV-infected patients with diarrhoeal illness,” *European Journal of Epidemiology*, vol. 9, no. 2, pp. 190–194, 1993.

[226] E. Pozio, G. Rezza, A. Boschini et al., “Clinical cryptosporidiosis and human immunodeficiency virus (HIV)-induced immunosuppression: findings from a longitudinal study of HIV-positive and HIV-negative former injection drug users,” *The Journal of Infectious Diseases*, vol. 176, no. 4, pp. 969–975, 1997.

[227] P. Rossi, F. Rivasi, M. Codeluppi et al., “Gastric involvement in AIDS-associated cryptosporidiosis,” *Gut*, vol. 43, no. 4, pp. 476–477, 1998.

[228] O. Brandonisio, P. Maggi, M. A. Panaro et al., “Intestinal protozoa in HIV-infected patients in Apulia, South Italy,” *Clinical Microbiology and Infection*, vol. 12, no. 2, pp. 489–501, 1999.

[229] D. Dionisio, “Cryptosporidiosis in HIV-infected patients,” *Journal of Postgraduate Medicine*, vol. 16, no. 4, pp. 103–107, 1980.

[230] V. A. Moore, and T. K. Graczyk, “Risks of recreational exposure to waterborne pathogens among persons with HIV/AIDS in Baltimore, Maryland,” *American Journal of Public Health*, vol. 90, no. 6, pp. 911–912, 2000.

[231] J. W. Liao and M. Assmar, “Cryptosporidiosis in immunocompromised patients in the Islamic Republic of Iran,” *Journal of Environmental Health*, vol. 15, no. 3, pp. 124–125, 2003.

[232] M. A. Blanco, A. Iborra, A. Vargas, E. Nse, L. Mba, and I. Fuentes, “Molecular characterization of *Cryptosporidium* isolates from humans in Equatorial Guinea,” *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 103, no. 12, pp. 1282–1284, 2009.
[297] J. Macey, L. Lior, A. Johnston et al., “Outbreak of diarrheal illness in attendees at a Ukrainian dance festival, Dauphin, Manitoba–May 2001,” Canada Communicable Disease Report, vol. 28, no. 17, pp. 141–145, 2002.

[298] A. Hajdu, L. Vold, T. A. Østmo et al., “Investigation of Swedish cases reveals an outbreak of cryptosporidiosis at a Norwegian hotel with possible links to in-house water systems,” BMC Infectious Diseases, vol. 8, article 152, 2008.

[299] R. Gait, R. H. Soutar, M. Hanson, C. Fraser, and R. Chalmers, “Outbreak of cryptosporidiosis among veterinary students,” The Veterinary Record, vol. 162, no. 26, pp. 843–845, 2008.

[300] S. O. Brockmann, C. Drewec, C. Wagner-Wiening et al., “Serological and epidemiological analysis of an outbreak of gastroenteritis among military recruits in Germany caused by Cryptosporidium parvum,” Infection, vol. 36, no. 5, pp. 450–457, 2008.

[301] H. Yoshida, M. Matsuo, T. Miyoshi et al., “An outbreak of cryptosporidiosis suspected to be related to contaminated food, October 2006, Sakai City, Japan,” Japanese Journal of Infectious Diseases, vol. 60, no. 6, pp. 405–407, 2007.

[302] N. Pandak, K. Zeljka, and A. Cvitkovic, “A family outbreak of cryptosporidiosis: probable nosocomial infection and person-to-person transmission,” Wiener Klinische Wochenschrift, vol. 118, no. 15-16, pp. 485–487, 2006.

[303] K. M. Kiang, J. M. Scheftel, F. T. Leano et al., “Recurrent outbreaks of cryptosporidiosis associated with calves among students at an educational farm programme, Minnesota, 2003,” Epidemiology and Infection, vol. 134, no. 4, pp. 878–886, 2006.

[304] G. Preiser, L. Preiser, and L. Madeo, “An outbreak of cryptosporidiosis among veterinary science students who work with calves,” Journal of American College Health, vol. 51, no. 5, pp. 213–215, 2003.

[305] F. Dalle, P. Roz, G. Dautin et al., “Molecular characterization of isolates of waterborne Cryptosporidium spp. Collected during an outbreak of gastroenteritis in South Burgundy, France,” Journal of Clinical Microbiology, vol. 41, no. 6, pp. 2690–2693, 2003.

[306] R. H. Ashbolt, D. J. Coleman, A. Misrachi, J. M. Conti, and M. D. Kirk, “An outbreak of cryptosporidiosis associated with an animal nursery at a regional fair,” Communicable Diseases Intelligence, vol. 27, no. 2, pp. 244–249, 2003.

[307] E. M. D. N. Gonçalves, A. J. da Silva, M. B. D. P. Eduardo et al., “Multilocus genotyping of Cryptosporidium hominis associated with diarrhea outbreak in a day care unit in São Paulo,” Clinics, vol. 61, no. 2, pp. 119–126, 2006.

[308] A. D. Howe, S. Forster, S. Morton et al., “Cryptosporidium oocysts in a water supply associated with a cryptosporidiosis outbreak,” Emerging Infectious Diseases, vol. 8, no. 6, pp. 619–624, 2002.

[309] P. R. Hunter, D. C. Wilkinson, I. R. Lake et al., “Microsatellite typing of Cryptosporidium parvum in isolates from a waterborne outbreak,” Journal of Clinical Microbiology, vol. 46, no. 11, pp. 3866–3867, 2008.

[310] A. Egorov, F. Frost, T. Muller, E. Naumova, A. Tereschenko, and T. Ford, “Serological evidence of Cryptosporidium infections in a Russian city and evaluation of risk factors for infections,” Annals of Epidemiology, vol. 14, no. 2, pp. 129–136, 2004.

[311] E. S. Quiroz, C. Bern, J. R. MacArthur et al., “An outbreak of cryptosporidiosis linked to a foodhandler,” The Journal of Infectious Diseases, vol. 181, no. 2, pp. 695–700, 2000.