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Enteric Protozoa in the Developed World: a Public Health Perspective

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INTRODUCTION

Parasitic diseases contribute significantly to the burden of infectious diseases worldwide. While most infections and death from parasitic diseases affect people in developing countries, they also cause significant illness in developed countries (305). In 2004, the WHO reported that diarrheal disease affected far more individuals than any other illness, even in regions that include high-income countries (468). Several species of enteric protozoa are associated with diarrheal illnesses in humans, with some causing severe debilitating illness, especially in immunosuppressed populations (228, 382, 390, 406, 410). Protozoan-related morbidity and mortality in both humans and animals worldwide are well documented (89, 94, 181, 222). Other protozoa have caused significant amounts of disease in livestock, often associated with losses in production, resulting in millions of dollars of losses in the food and livestock industry (288, 383, 431, 437, 438). Other impacts of parasitic infections include reduced worker productivity, reduced commodity yields, effects on income, and impacts on food security (70, 288). Several enteric protozoa cause zoonotic (transmitted from animals to humans) illnesses associated with livestock and domestic pets, and more recently, the prominence of open farms and petting zoos has featured in several zoonotic outbreaks and transmission to humans (55, 66, 146, 290, 340). Human-to-animal transmission of parasites is also becoming an emerging issue of public health and veterinary significance (89, 432). As humans and their livestock move further into wildlife domains, parasitic disease might represent a serious threat to wildlife, which in turn may act as reservoirs and/or amplifiers of emerging and exotic diseases for humans and their livestock (429, 432).

Much attention has been paid to enteric protozoa in human infections in developing countries, where poor sanitary conditions and the unavailability of effective water treatment have sustained conditions for their transmission (21, 110, 178, 180, 278, 349, 410). Climate change is predicted to influence changes in precipitation quantity, intensity, frequency, and duration and subsequently affect environmental conditions that predispose developing countries to the transmission of waterborne disease (181). Less focus has been placed on the impact of these changes in more industrialized settings, presumably because of better health standards. Therefore, estimation of the disease burden is often complicated by a lack of reliable data as a result of underdiagnosis and the lack of monitoring programs (288). However, despite the lower prevalence of parasitic diseases in industrialized countries, they may potentially result in a greater economic burden due to higher income, medical, and treatment costs (438).

In many developed countries, only a few or no parasitic proto-

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zoa are included in operational surveillance systems, as the major focus is on bacterial and viral infections. Where these systems exist, they are used mainly as indicators for identifying outbreaks of food-borne and waterborne diseases and in institutional settings (72, 73, 231, 463, 476–478). However, evidence suggests that while some enteric protozoa, such as *Entamoeba* spp., *Cryptosporidium*, and *Giardia*, are isolated frequently from diarrheal patients in developing regions such as Asia and sub-Saharan Africa (126, 284, 363), others, such as *Blastocystis* spp. and *Dientamoeba fragilis*, are isolated mainly in developed countries (390; S. M. Fletcher, Y. Li, D. Stark, and J. Ellis, presented at the Communicable Disease Control Conference, Canberra, ACT, Australia, 2011). In developed settings, however, enteric protozoa are often ignored as a cause of diarrhea due to better hygiene conditions. In many cases, the sick person may not seek medical attention, and even if he or she does, a stool specimen is not routinely requested from persons with diarrheal illnesses (170, 357, 427, 446, 476). For example, during an outbreak of gastroenteritis linked to a water supply in Austria in 2006, there was no identification of pathogenic microorganisms in stool samples from affected patients, and no parasite etiology was considered in this large outbreak (269). This could have been due to the fact that test requests by general practitioners may not have complied with existing knowledge of the gastroenteritis etiology at that time (427, 446).

In industrialized settings, the prevalence of protozoan illnesses is frequently captured by several surveillance systems, including outbreak surveillance, passive and active surveillance of notifiable diseases, and laboratory-based surveillance (258, 260, 298). Estimates of parasite prevalence are sometimes affected by the lack of sensitive diagnostic techniques to detect them in clinical specimens, while carrier stages and subclinical infections are often not diagnosed (236, 460). The development of technologies that can simultaneously detect several protozoa in stool is desirable in industrialized settings (386, 427). This includes the development of molecular markers for the detection of outbreaks, for source attribution, and to estimate their contribution to the overall burden of infectious diseases (236, 318, 426, 463). Current opinion suggests that molecular techniques are the most promising methods for the sensitive, accurate, and simultaneous detection of protozoan parasites in comparison to conventional staining and microscopy methods (386, 387), with much benefit to the water industry and public health (7, 40, 47, 48, 375). Unfortunately, molecular methods can be quite costly and labor-intensive and thus are not used routinely for the detection of parasitic protozoa, even in resource-rich settings (341). Much effort must now be placed on developing inexpensive molecular tools for routine laboratory applications in industrialized settings.

This review seeks to discuss the public health impact of common enteric protozoa associated with diarrheal illnesses in industrialized settings. The role that protozoa play in human and animal infections is discussed, along with the implications of climate change and the importance of water quality management to their prevention and control. The main enteric protozoa considered of public health significance and covered here are *Cryptosporidium* spp., *Giardia intestinalis*, *Entamoeba histolytica*, *Dientamoeba fragilis*, *Cyclospora cayetanensis*, *Blastocystis* spp., *Cystoisospora belli*, and *Balantidium coli*. Two microsporidian species, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, are also included.

Distribution in Developed Countries

One report (Fletcher et al., presented at the Communicable Disease Control Conference, Canberra, ACT, Australia, 2011) estimated that an enteric pathogen is isolated in an average of 40.9% (95% confidence interval [CI], 33.4 to 48.8%) of diarrheal cases in developed countries, among which enteric parasites represent less than 1% (95% CI, 1.1 to 3.5%) of cases. However, enteric parasites—mainly protozoa—are isolated from 1% to 65% of patients with diarrhea in various settings. The relative prevalences of enteric protozoa reported in several developed countries in outbreak and nonoutbreak settings among humans are reported in Table 1 (1, 11, 12, 24, 60, 85, 90, 96, 128, 144, 184, 192, 193, 204, 215, 225, 247, 248, 255, 264, 265, 301, 335, 355, 370, 395, 416, 417, 425, 442, 444, 452, 467). *Giardia intestinalis* (0.2% to 29.2% of cases), *Cryptosporidium* spp. (0.1% to 9.1% of cases), *Entamoeba* spp. (0.2% to 12.5% of cases), and *Cyclospora cayetanensis* (0.2% to 4.3% of cases) were the most common protozoa reported in developed settings. The common protozoa, however, were rivalled in prevalence by *Blastocystis* spp. (0.4% to 18.1%) and *D. fragilis* (0.4% to 6.3%). Both have the potential to cause illness but are more frequently associated with asymptomatic infection. Nevertheless, evidence from the literature suggests that *Blastocystis* spp. and *D. fragilis* have relatively high prevalences in developed settings (395; Fletcher et al., presented at the Communicable Disease Control Conference, Canberra, ACT, Australia, 2011). Infections are associated with recent travel to developing regions (394), with immigrants and refugees (164, 273), and with domestic transmission (387). The prevalence rates of *D. fragilis* vary widely, from 0.4% to 42%, and the incidence of this parasite was found to be second only to that of *Blastocystis* spp. and of similar or greater magnitude to those of the more commonly diagnosed parasites *Giardia*, *Cryptosporidium* spp., and *Entamoeba* spp. in many developed regions when diagnostic methods were implemented for these species. Several reports have also identified *D. fragilis* as the most common pathogenic protozoan found in stool when appropriate diagnostic methods are used (389). It is therefore recommended that both *Blastocystis* spp. and *D. fragilis* be considered in the differential diagnosis of gastrointestinal infections in developed settings (26).

Asymptomatic carriage of protozoan parasites is also common in developed countries, as several types have been isolated from healthy individuals without diarrhea (0.5% to 16.5%) (90, 301). For example, about 90% of individuals infected with *Entamoeba* are colonized by the nonpathogenic species *Entamoeba dispar*, and as such, they are asymptomatic; this is true even for immunosuppressed populations (14, 385). Table 2 presents an epidemiological summary of several protozoa that have been implicated in human illnesses in developed countries, and examples of stained enteric protozoa are shown in Fig. 1. Recommended treatment regimens for enteric protozoa are presented in Table 3, based on international standards (218, 390).

EPIDEMIOLOGY, DIAGNOSIS, AND TREATMENT

Cryptosporidium Species

Cryptosporidium was first recognized as an important cause of infection in AIDS patients (410). It is now well recognized and accounts for about 20% of diarrheal episodes in children in developing countries and up to 9% of episodes in developed settings and causes a considerable amount of diarrheal illness in young

TABLE 1 Prevalence of protozoa as described by various studies from developed countries

Reference	Study location; period	Cases and samples	Lab method(s)	Overall pathogen isolation rate (%)	No. of samples	Parasites detected (%)[a,b]
184	Melbourne, Australia; 1997	Fecal specimens from community-based asymptomatic individuals	Modified iron-hemotoxylin stain to detect *Giardia*, *Cryptosporidium*, *Blastocystis*, *Entamoeba*, and other protozoa	11.8	1,091	Protozoa (8.3), *Giardia* spp. (1.6), *Blastocystis* spp. (6.0), *Cryptosporidium* (0.4), *D. fragilis* (0.4)
335	Helsinki, Finland; 1985-1986	Diarrhea in adult out- and inpatients attending three health centers and a municipal hospital	Formalin-ether concentration method and modified Ziehl-Neelsen method for *Cryptosporidium*	35.2	253	*G. intestinalis* (2.0), *Cryptosporidium* sp. (2.8), *E. histolytica/E. dispar* (0.4) (majority of isolates were imported from other countries)
452	Madrid, Spain; 1980–1983	Fecal samples from infected persons (50% children) in hospital	Microscopic examination for parasites[c]	46.1	5,022 cases	*Giardia intestinalis* (2.7), *Entamoeba histolytica/E. dispar* (<1)
247	Ljubljana, Slovenia; 1992-1993	Patients with diarrhea	Permanent staining, Gomori's trichrome modification for *Blastocystis* sp., and safranin-methylene blue stain for *Cryptosporidium*, with bright-field microscopy	3.7 (39/1,066 patients)	1,066 cases, 150 controls	*Blastocystis* spp. (3.7 [cases] and <1 [controls])
11	Population of England; 1993-1996	Fecal samples from patients with diarrhea and controls	Novel real-time PCR for *Giardia* sp., real-time nested PCR for *Cryptosporidium* sp.	20 (population), 51 (pathogen isolated)	2,422 cases, 2,205 controls	Cases: *Giardia* (2), *Cryptosporidium* spp. (2); controls: *Giardia* (1), *Cryptosporidium* spp. (0.5)
396	Sydney, Australia; 2003–2006	HIV-positive and HIV-negative MSM with diarrhea, presenting at general medical practice (GP)	Molecular methods, modified iron-hematoxylin stain, and a carbol-fuschin staining step to detect coccidian parasites	23.98 (448 pathogens isolated)	1,868 total, 628 from HIV-negative patients, 618 from HIV-positive patients, 622 from non-MSM	*Entamoeba histolytica/E. dispar* complex (2.9), *Cryptosporidium* species (1), *Giardia intestinalis* (2.9), *Dientamoeba fragilis* (0.8), *Blastocystis* spp.(16.9)
425	Northeast region of England; 2003–2005	Humans	*Cryptosporidium* screen using auramine staining, microscopic examination for OCP	NA	279 outbreaks, 2,889 cases tested	*Cryptosporidium* (3.0)
90	Ankara, Turkey; 2005–2007	Hospital patients presenting with IBS, inflammatory bowel disease (5), and chronic diarrhea	Native Lugol's, trichrome, and Kinyoun's acid-fast staining; genomic DNA preparation; subtyping by PCR with STS primers	18.1 (cases), 16.7% (controls)	105 cases, 96 controls	*Blastocystis* (18 [cases]) and 16.7 [controls])
442	Uppsala, Sweden; 1981	Hospital inpatients and outpatient Swedish children of <15 years of age with acute gastroenteritis	Parasitic studies, formalin-ether concentration method; *Cryptosporidium* detected by light microscopy and Kinyoun's acid-fast staining[d]	68	416 cases, 200 controls	*Giardia intestinalis* (1 [cases] and 1 [controls])
355	Brisbane, QLD, Australia; circa 1992	260 nonhospital patients with diarrhea sent to a private laboratory in Brisbane	Fecal culture and microscopy (trichrome staining for identification by oil immersion microscopy)	15	260 specimens	*G. intestinalis* (1.5), *Blastocystis* spp. (10.8), *G. intestinalis* (1.5)
416	Stockholm, Sweden; 1996-1997	Consecutive adult patients of >15 years of age with diarrhea and healthy control subjects	OCP by direct microscopy and *Cryptosporidium* oocysts by a modified Ziehl-Nielsen technique	56 (cases), 16 (controls), 8 (protozoa)	851 cases, 203 controls	Cases: *E. histolytica* (1), *G. intestinalis* (2), *Blastocystis* spp. (4), *Cryptosporidium* (2), *microsporidia* (<1); controls: *E. histolytica* (<1), *Blastocystis* spp. (9)
417	42 specialized travel or tropical medicine sites located on six continents; 1996–2005	Returned travelers diagnosed with an "infectious gastrointestinal disease"	Best available reference diagnostics; there was considerable heterogeneity between centers in terms of availability and sophistication of diagnostic (e.g., molecular) testing	39 (65% of isolates were parasitic)	7,442; 2,902 tested for parasites	*Giardia* (27.9), *E. histolytica* (12.5), *Dientamoeba fragilis* (4.0), *Cryptosporidium* spp. (1.1), *Cyclospora* (1.1), *Cystoisospora belli* (0.1)
12	26 U.S. states and two Canadian provinces; 1996	Fecal samples from infected and noninfected persons	Formalin-ethyl acetate concentration sedimentation procedure and confirmatory permanent smears when necessary		5,250 cases	*C. cayetanensis* (4.3); note that 91% of infected patients had gastroenteritis symptoms

144	Noumea, New Caledonia; 1990-1991	Patients of all ages with diarrhea	Formalin-ether concentrates/smears stained with merthiolate-iodine-formaldehyde solution and examined microscopically[e]	40.4	2,088 cases	*E. histolytica/dispar* (3.5), *Giardia intestinalis* (7.8)
60	Republic of Korea; 2004–2006	Diarrheal patients hospitalized in 96 hospitals	Enzyme immunoassay kit	36.8	76,652 cases	Protozoa (2.3), *Cryptosporidium* spp. (0.8), *Entamoeba histolytica/E. dispar*[f] (0.6), *Giardia* (1.7)
81	Netherlands; 1996–1999	Stool samples from patients who consulted with a GP regarding gastroenteritis	Microscopic examination of SAF-fixated samples[g]	37.5 (cases), 9.8 (controls)	857 cases, 574 controls	Cases: *G. intestinalis* (5.4), *Cryptosporidium* spp. (2.1), *Cyclospora* (0.2), *Entamoeba* spp. (0.9), *D. fragilis* (10.3); controls: *G. intestinalis* (3.3), *Cryptosporidium* (0.2), *Cyclospora* (0.2), *Entamoeba* spp. (0.7), *D. fragilis* (14.6)
192	Gyeonggi-do, South Korea; 2004–2006	Gastroenteritis patients in hospital	ELISA	3.4	6,071 cases	Protozoa (3.4), *G. intestinalis* (2.5), *E. histolytica/E. dispar* (0.4), *Cryptosporidium parvum* (0.4)
301	16 Danish counties, Denmark; 2000-2001	Stool samples from children of less than 5 years of age from hospitals and general practice offices	Microscopy[h] plus Ziehl-Neelsen acid-fast staining for *Cryptosporidium* and *Cyclospora* sp.	54 (cases), 22 (controls)	424 cases, 866 controls	Cases: *G. intestinalis* (<1), *Cryptosporidium* (1.7), *Blastocystis* spp. (<1); controls: *G. intestinalis* (<1), *Blastocystis* spp. (1.3)
24	Melbourne, Victoria, Australia; 1980–1993	Hospitalized children (0 to 14 years old)	Microscopy[i] for OCP, modified acid-fast smears for cryptosporidia	56.6	3,785	*Cryptosporidium* (0.5), *G. intestinalis* (0.3)
264	Sydney, Australia; 2001	412 children under 6 years of age with diarrhea, either hospitalized or outpatients at The Sydney Children's Hospital	*Giardia*-specific enzyme immunoassay and microscopy with modified Ziehl-Neelsen stain for cryptosporidia	33	412	*Cryptosporidium* spp. (2.4), *G. intestinalis* (2.7)
370	Melbourne, Australia; 1997–1999	Fecal samples from family units (community) of at least two children (≤15 years old) and two adults each	A modified iron-hematoxylin stain was used to detect *Giardia* and *Cryptosporidium* in concentrated specimens	25	791	*Cryptosporidium* spp. (1.6), *Giardia intestinalis* (2.5)
193	Neusiedl am See, rural eastern Austria; January to December 2007	Patients who consulted general practitioners for gastroenteritis	*Giardia intestinalis* plus *Cryptosporidium parvum* (RIDA Quick *Cryptosporidium/Giardia* Combi test)	23.2	306	Protozoa (1.3), *Cryptosporidium* spp. (1.6), *Giardia intestinalis* (2.5)
128	Canadian hospital morbidity database (HMDB); 1995–2004	Population hospitalized in Canada for gastorintestinal illness (acute, chronic, and rehabilitation care)	Not given	21.7	927,645 hospitalizations	*Entamoeba* spp. (0.05), *Giardia intestinalis* (0.15), *Cryptosporidium* spp. (0.08)
467	England; 1993–1996	Patients with diarrhea in the community and seen by GP	Microscopy[j] (12)	24	1,262	Protozoa (1.6), *Cryptosporidium* spp. (1.3), *Giardia intestinalis* (0.32)
204	Berlin, Germany; 2005–2007	Patients of ≥18 years of age hospitalized with community-acquired gastroenteritis	SAF fixation-concentration and microscopy for OCP; direct immunofluorescence antibody test for *G. intestinalis* and *Cryptosporidium*; Kinyoun's staining method for *Cryptosporidium*, *C. cayetanensis*, and *I. belli*	59.8	132	*Blastocystis* spp. (7.6), *Giardia intestinalis* (7.6)
255	Parma, Italy; 1983-1984	Hospitalized patients with acute enteritis	Microscopy	26.2	797	Protozoa (3.1), *Giardia intestinalis* (1.4)
225	Seattle, WA, USA; 1998–2001	Children with diarrhea who presented to a pediatric emergency department	Trichrome stains and formalin-ethyl acetate sedimentation for OCP[D] and fluorescence antibody testing for *Giardia* and *Cryptosporidium* sp.	19.5	656 (from 1,626 patients)	Protozoa (2.13), *Blastocystis* spp. (1.1), *Cryptosporidium* spp. (0.15), *Giardia* spp. (0.46), *E. histolytica/E. dispar* (0.15)

(Continued on following page)

TABLE 1 (Continued)

Reference	Study location; period	Cases and samples	Lab method(s)	Overall pathogen isolation rate (%)	No. of samples	Parasites detected (%)[a,b]
265	United States; 1996-1997	National surveillance data for food-borne illness cases	Not given	Not given	Estimated 38,629,641 cases	Parasites (6.6), *Cryptosporidium parvum* (0.8), *Cyclospora cayetanensis* (0.4), *Giardia intestinalis* (5.2)
215	Helsinki, Finland; 1982-1983	Fecal samples sent by GPs for routine parasitological examination	Ritchie's formalin ether concentration method, used along with a modified Ziehl-Neelsen method	Not given	154	*Cryptosporidium* spp. (9.1), *Giardia* (29.2), *Blastocystis* spp. (13), *E. histolytica/E. dispar* (2)
1	Tenerife, Canary Islands, Spain; ~2004	Clinical samples (156 stools)	Light microscopy, staining with Weber's chromotrope and PCR-hybridization for the identification of *Enterocytozoon bieneusi*	11.54	156	Protozoa (11.54), *E. bieneusi* (11.54)
96	United States (10 cities); 1998-1999	Diarrheal stool specimens from HIV-infected patients in 3 U.S. hospitals and a database	Moura's quick-hot Gram chromotrope technique, modified trichrome blue stain (confirmed by chromotrope 2R staining and oil immersion microscopy)	1.5	737	Microsporidian species (1.5)
248	Vigo, Spain; ~2001	Elderly HIV-negative patients; 47 of 60 had diarrhea	Light microscopy with Weber's chromotrope-based stain and PCR-hybridization	17.02	60	*E. bieneusi* (17.02)
448	Central area of Brussels, Belgium; 2002-2003	Patients suspected of suffering from a parasitic gastrointestinal illness	Bright-field microscopy and PCR	37	1,207 stool samples from 448 outpatients	*D. fragilis* (6.3), *G. intestinalis* (7.1), *C. parvum* (1.6%), *E. hystolytica* (0.2%), *Blastocystis* sp. (9.8%), *Entamoeba coli* (5.4%)

[a] In some reports, some patients had multiple pathogens isolated; hence, the percentages may not add up to 100%.

[b] The designation *E. histolytica* is reported as *E. histolytica/E. dispar* for consistency, unless the study's methodology indicated clearly that the test was specific for *E. histolytica*.

[c] Incomplete techniques and no permanent stained smears used. The methods were described as follows. "Wet mounts of 5,022 freshly passed stools were examined for leukocytes or parasites or both. The samples which were not immediately processed were stored at 4degrees Celcius for a maximum of 36 h."

[d] Permanent stains were not performed for routine investigations of protozoa.

[e] No permanent stains performed. Although the authors indicate the presence of *E. histolytica*, there is nothing in the article to indicate that the organisms were confirmed as *E. histolytica* versus *E. histolytica/E. dispar*.

[f] A Ridascreen immunoassay kit was used, and this test detects *E. dispar* trophozoites. However, the authors reported that the organisms were *E. histolytica*.

[g] Microscopic examination included a combination of (i) a wet film (iodine stained or unstained); (ii) Ridley concentration (iodine staining); (iii) modified Ziehl-Neelsen staining of Ridley concentrate; and (iv) permanent staining by hematoxylin.

[h] Microscopy of concentration sediment wet mount for protozoa only, other than special stains for coccidia.

[i] Microscopy for wet mount examination only, other than special stains for coccidia.

[j] Microscopy not defined.

TABLE 2 Summary of the epidemiology of pathogenic protozoa associated with human illness

Parasite	Disease symptom(s)	Primary host(s)	Mode(s) of transmission	Susceptible individuals
Cryptosporidium spp.	Diarrhea	Humans, other mammals, and birds	Oocysts in water and on uncooked or undercooked food; person to person; zoonotic	Animal handlers, travelers, MSM, caterers, day care staff
Cyclospora cayetanensis	Diarrhea	Humans and other mammals	Oocysts in water and on uncooked or undercooked food; person to person	Travelers to nonindustrialized countries (South America); major food and water outbreak risk
Giardia intestinalis	Diarrhea, malabsorption	Humans, other mammals, and birds	Cysts in water and on uncooked or undercooked food; person to person; zoonotic	Young adults, MSM, day care staff
Entamoeba histolytica	Dysentery, liver abscess	Humans and other mammals	Cysts in water and on uncooked or undercooked food; person to person; zoonotic	Immigrants/travelers to areas of endemicity, MSM, HIV patients, and institutionalized persons
Blastocystis sp.	Abdominal pain and diarrhea	Humans and other mammals	Cysts in untreated or minimally treated water and on uncooked or undercooked food; person to person; zoonotic	Anyone, especially in child care centers or other institutional settings
Dientamoeba fragilis	Diarrhea	Humans	Fecal-oral; uncertain	Children and adults, both immunocompetent and immunosuppressed populations
Cystoisospora belli	Diarrhea	Humans	Oocysts in contaminated water or food; person to person	Travelers to nonindustrialized countries, AIDS patients, and indigenous populations (United States)
Balantidium coli	Diarrhea, dysentery	Humans, pigs, nonhuman primates, cats, rodents	Cysts in untreated or minimally treated water and on uncooked or undercooked food; person to person; zoonotic	People living in close proximity to pigs, travelers to nonindustrialized countries (Southeast Asia, Western Pacific Islands, rural South America)
Microsporidia	Persistent diarrhea	Humans and other mammals	Ingestion of spores; person to person; zoonotic	Immunosuppressed and HIV/AIDS patients, immunocompetent patients, travelers

farm animals worldwide (340, 473). The environmentally resistant oocysts are fully sporulated when they are excreted in feces, and therefore they are immediately infectious (116, 441, 477). Infections are usually characterized by self-limiting diarrhea associated with severe abdominal pain in both immunosuppressed and immunocompetent persons, especially HIV-infected persons and children worldwide (16, 116, 390). The duration of clinical symptoms is highly dependent on the person's immunological competence (235). It is still an important cause of potentially life-threatening diarrhea in HIV-infected patients with limited access or poor compliance with highly active antiretroviral therapy (HAART) (228, 326). *Cryptosporidium* spp. are of importance to transplant patients, especially in regions where the organisms are endemic, where they can cause life-threatening prolonged diarrhea, dehydration, and malabsorption in transplant recipients (100). Species of *Cryptosporidium* and subtype families of *Cryptosporidium hominis* have been shown to induce different clinical manifestations and have different potentials to cause outbreaks (473). In developed countries, transmission occurs from person to person, especially in day care settings and between men who have sex with men (MSM), as well as through waterborne and zoonotic infections (473). *Cryptosporidium* spp. have been identified as a common cause of diarrhea in persons from developed countries visiting less developed areas (343, 473, 474, 476). Animal models have demonstrated the role of *Cryptosporidium parvum* in the formation of polyps and adenocarcinoma lesions in the guts of dexamethasone (Dex)-treated severe combined immunodeficiency (SCID) mice, suggesting the need to investigate whether a similar *C. parvum*-induced gastrointestinal cancer occurs in humans (50–52, 74, 459).

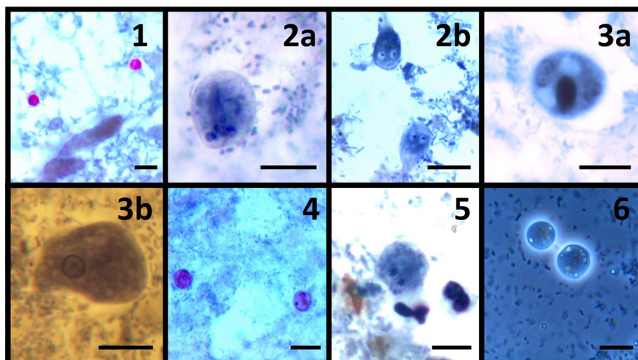

FIG 1 Photomicrographs of six enteric protozoa. Plates 1 to 5 were stained with a modified iron-hematoxylin stain (incorporating a carbol fuschin staining step). Plate 6 was a wet preparation. (1) *Cryptosporidium* oocysts; (2a) *Giardia intestinalis* cysts; (2b) *Giardia intestinalis* trophozoite; (3a) *Entamoeba histolytica* cyst; (3b) *Entamoeba histolytica* trophozoite; (4) *Cyclospora cayetanensis* oocysts; (5) *Dientamoeba fragilis* binucleated trophozoite; (6) *Blastocystis* oocysts. Bars, 10 μm. (All graphics by Damien Stark.)

In developed countries, sporadic outbreaks due to fecal-oral transmission have been reported among children attending playgroups and day care centers and by ingestion of contaminated salads, contaminated water supply, or recreational water, contact with sick animals, swimming in public pools, and person-to-person transmission (55, 66, 325, 340). Epidemiological variations have been observed in the geographical, seasonal, and socioeconomic effects of the distribution of *Cryptosporidium* spp. in humans that may influence the sources and routes of transmission (473). *Cryptosporidium* spp. have a wide host range and cause infections in humans, livestock, domestic pets, and wildlife, among all four classes of vertebrates, and, most likely, in all mammalian species (55, 89, 290, 345, 441). Most animals are not infected with human-pathogenic species and thus play no role in zoonotic transmission of cryptosporidiosis. However, zoonotic transmission from direct contact with infected animals or their

TABLE 3 Treatment options for infections with enteric protozoa[a]

Intestinal parasite or disease	Antimicrobial therapy (dosing)
Blastocystis spp.	Nitazoxanide (500 mg twice a day for 3 days)[b]
Cryptosporidium spp.	Nitazoxanide (500 mg twice a day for 3–14 days); for AIDS-associated infections, include HAART
Cyclospora cayetanensis	Cotrimoxazole (TMP-SMX; 160 mg trimethoprim plus 800 mg sulfamethoxazole, twice a day for 7 days), pyrimethamine (50–75 mg daily) and leucovorin (5–10 mg daily), or ciprofloxacin (500 mg twice a day); for non-AIDS patients receiving TMP-SMX, use 1 double-strength tablet orally twice daily for 7–10 days; for AIDS patients receiving TMP-SMX, use 1 double-strength tablet orally four times daily for 10 days, followed by twice a day for 3 weeks
Dientamoeba fragilis	Iodoquinol (650 mg three times a day for 20 days), metronidazole (500–750 mg three times a day for 10 days), or paromomycin (25–35 mg/kg of body weight/day for 7 days) (271); for treatment failures, tetracycline (500 mg orally four times daily for 10 days) plus iodoquinol (650 mg orally three times daily for 10 days)
Entamoeba histolytica invasive disease (amoebic colitis, amoebic liver abscess, or disseminated disease)	Luminal agent, i.e. paromomycin (8–12 mg/kg or 500 mg orally three times a day for 7 days) or iodoquinol (650 mg orally three times a day for 20 days)
Entamoeba histolytica/E. dispar complex intestinal disease	Paromomycin (8–12 mg/kg or 500 mg three times a day for 7 days) or iodoquinol (650 mg orally three times a day for 20 days)
Giardia intestinalis	Metronidazole (250 mg daily for 3 days), tinidazole (2-g single dose), or albendazole (200–400 mg twice a day for 5 days)
Cystoisospora belli	Cotrimoxazole (160 mg trimethoprim plus 800 mg sulfamethoxazole, four times a day for 10 days) or ciprofloxacin (500 mg twice a day for 7 days); for non-AIDS patients receiving TMP-SMX, use 1 double-strength tablet orally twice daily for 10 days; for AIDS patients receiving TMP-SMX, use 1 double-strength tablet orally four times daily for 10 days, followed by twice a day for 3 weeks
Microsporidia	
E. bieneusi[c]	Fumagillin (20 mg three times a day for 14 days)
E. intestinalis	Albendazole (400 mg twice a day for 28 days)

[a] Based on CDC guidelines (149, 218, 391).
[b] Treatment for *Blastocystis* spp. is considered controversial, but infection is eradicated by treatment.
[c] Albendazole is not very effective to eradicate infection but may alleviate symptoms. Fumagillin is effective but has serious side effects.

feces (55, 89, 345, 476) or indirectly through the consumption of contaminated water (63, 66, 327, 476) can occur.

The small size and subtle staining characteristics of *Cryptosporidium* spp. have contributed to the difficulties of identifying these parasites in routine stool preparations (116, 375). The diagnosis of cryptosporidiosis is generally undertaken by identification of oocysts in stool of the host/patient. Traditionally, the identification of *Cryptosporidium* oocysts was based on microscopic examination (300, 330). However, the identification of morphological characters of *Cryptosporidium* is unreliable and relatively time-consuming, even with light microscopy (209). Staining and preservation methods have been used to enhance the sensitivity of tests. Unfortunately, these do not identify species, and their analytical sensitivity can be poor, especially for samples containing small numbers of oocysts (138, 253, 329). The modified Ziehl-Neelsen technique (413) is used widely; however, one recent study found that it was less sensitive than PCR (75.7%; 95% CI = 68.3 to 81.8%) but was highly specific (100%; 95% CI = 96.5 to 100%) (56). Kinyoun's acid-fast staining technique (102), modified Sheather's flotation technique (76, 294, 296), and the iron-hematoxylin staining technique (119) have also been described.

Antigen detection by immunoassays has been used widely in the diagnosis of cryptosporidiosis, as these assays are thought to be more sensitive than conventional staining and more effective in cases where oocyst numbers are low (139, 141, 329). An evaluation of the Meridian Premier *Cryptosporidium* test and the Alexon ProSpecT *Cryptosporidium* microplate assay found that both systems performed according to the manufacturers' values for sensitivity and specificity (for the Meridian test, 91 and 99%, respectively; and for the Alexon test, 97 and 100%, respectively) (139). Highly rapid immunoassays have also been developed that can be used with fresh, frozen, or unfixed human fecal specimens. For example, the Biosite Diagnostics (San Diego, CA) Triage rapid qualitative enzyme immunoassay (EIA) for the detection of *Giardia*, *E. histolytica*/*E. dispar*, and *Cryptosporidium* antigens has been demonstrated to have a 98.3% sensitivity and 99.7% sensitivity in the detection of *Cryptosporidium* spp. from stool specimens (140). A multicenter French study evaluated the sensitivity and specificity of four immunochromatographic (ICT) assays and found that RIDAQuick (mean sensitivity, 73.3%), Remel Xpect (mean sensitivity, 74.1%), and ImmunoCard STAT (mean sensitivity, 73.3%) were fairly sensitive for detecting *C. parvum* and *C. hominis* but were very limited in detecting other *Cryptosporidium* spp. (3). Fluorescence microscopy and direct fluorescent-antibody (DFA) assay have been used with relatively high specificities (96 to 100%) and sensitivities (98.5 to 100%) for the detection of *Cryptosporidium* oocysts in clinical and environmental samples (139, 158, 209). However, the sensitivity and specificity of monoclonal antibody (MAb)-based DFA assays have been affected by different factors, such as the purity of the *Cryptosporidium* antigen originally used to raise the MAb (139, 158, 209, 214, 329).

Molecular methods have been developed for the detection and differentiation of *Cryptosporidium* spp. at the species/genotype and subtype levels (9, 168, 290, 372, 386). These methods, including a nested PCR (348), real-time PCR, multiplex real-time PCR, reverse transcription–quantitative real-time PCR (372, 455), and

multiplex tandem real-time PCR (386), have been used for identification of species in diarrheal stools in relatively short time frames. More recently, an automated multiplex tandem PCR using a robotic platform to simultaneously detect *Cryptosporidium* spp. and coinfecting diarrheal pathogens from human fecal genomic samples was described. The assay was rapid (taking <2 h, with ~5 to 10 min of technical work following the extraction of genomic DNA), and due to automation, data analysis required little molecular biological expertise, making it well suited to various diagnostic facilities and settings (e.g., hospitals or quarantine facilities) (210). Other tools, based on the *Cryptosporidium* oocyst wall protein (COWP) gene, have been used to amplify DNAs of *C. parvum*, *C. hominis*, *Cryptosporidium meleagridis*, and species and genotypes closely related to *C. parvum* (9, 242). However, it has been suggested that these methods have limited usefulness in genotyping *Cryptosporidium* spp. in animals because of their narrow specificity (342, 473). The disadvantage of some PCR tools is that they are designed to detect the dominant *Cryptosporidium* genotype in the specimen and require a substantial amount of PCR product to be visible on an agarose gel, and when specific genotyping and subtyping tools are used, they fail to detect concurrent infections with mixed *Cryptosporidium* species/genotypes or fail to detect other divergent species/genotypes (168). New methods such as reverse line blot (RLB) hybridization, isothermal methods such as the loop-mediated isothermal amplification method (319, 372), and nucleic acid sequence-based amplification (NASBA) methods that amplify RNA from either RNA or DNA templates also provide additional diagnostic platforms for the detection of *Cryptosporidium* spp. (154, 372).

Cryptosporidiosis is usually self-limiting in immunocompetent persons, requiring little or no treatment, but it is especially challenging to treat among high-risk immunosuppressed groups (39, 83). Nitazoxanide has proven effective in the treatment of immunocompetent patients (133); however, a higher dosage and longer duration of treatment have been indicated for immunosuppressed patients (39, 129). Rehydration fluids and nutritional management may be required for immunosuppressed persons with dehydration (57, 83).

Dientamoeba fragilis

There has been much debate about the pathogenicity of *Dientamoeba fragilis* (26, 390). Unlike the case for other protozoa, a cyst stage has not been demonstrated, and trophozoites degenerate within hours of being passed in stool (26, 391–393). Although the mode of transmission remains unknown, based on high rates of coinfection with *Enterobius vermicularis*, it was suggested previously that infection occurs via the pinworm vector (25, 153, 191). Recent studies have discounted this idea (229). However, high rates of coinfection with other enteric pathogens and protozoa suggest that transmission occurs directly via the fecal-oral route (26, 191). Infection may be acute or chronic and has been reported in both children and adults and both immunocompetent and immunosuppressed populations (26, 127, 361, 389, 392, 444). The most common clinical symptoms include abdominal pain, persistent diarrhea, loss of appetite, weight loss, and flatulence (22, 355, 389, 444). These symptoms are akin to those of irritable bowel syndrome (IBS), so *D. fragilis* should be considered in the differential diagnosis of IBS (399). *Dientamoeba fragilis* infection also presents with similar symptoms to those of *Giardia* infection and is estimated to occur with a similar or greater prevalence to that of *Giardia* infection in Belgium (444) and Australia (127, 355, 394, 395, 401). *Dientamoeba fragilis* trophozoites have been reported in nonhuman primates, but the limited host range suggests that human infection may not involve transmission from other animal species (396).

Traditional diagnosis relies on the microscopic detection of trophozoites in fresh or fixed stool specimens. The demonstration of the characteristic nuclear structure of *D. fragilis* needed for a definitive diagnosis cannot be achieved with unstained fecal material (392); as such, prompt fixation and permanent staining are necessary for definitive diagnosis (355, 388, 391). Microscopy of fixed smears with permanent staining (modified iron-hematoxylin or trichrome staining) (127, 393, 403) is considered the gold standard for diagnosis of *D. fragilis* infection, but this method is time-consuming and relatively insensitive compared to molecular methods (391). More recently, diagnostic tests based on various conventional and real-time PCRs to detect the small-subunit (SSU) rRNA gene of *D. fragilis* have been developed to facilitate rapid, sensitive, and specific diagnosis in fresh stools (386, 388, 401, 455), and the successful culture and cryopreservation of viable *D. fragilis* trophozoites from clinical specimens have been achieved (27, 355). It was demonstrated that when microscopy is combined with other methods, the success of detection of *D. fragilis* is increased significantly (26, 386, 388). However, while molecular methods are more sensitive than microscopy and staining, many of the necessary products are not commercially available and the methods are not employed routinely, even in developed countries.

Treatment is recommended in symptomatic patients and asymptomatic family members to prevent reinfection (153, 444). Various antimicrobial therapies have resulted in the successful clearance of *D. fragilis* and total resolution of gastrointestinal symptoms in infected patients (152, 387, 445). Paromomycin, secnidazole, iodoquinol, tetracycline, ornidazole, and metronidazole have been used successfully to treat *D. fragilis* infections (22, 152, 305, 445). However, there is emerging evidence of treatment failures of metronidazole among *D. fragilis* isolates, suggesting the increased need for combination therapy for these protozoa (22, 389). Combination therapy has been effective in the complete eradication of the parasite and in resolution of symptoms (387, 389).

Entamoeba Species

Six species of the genus *Entamoeba* have been described in humans, including *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, *Entamoeba poleki* (also called *Entamoeba chattoni*), *Entamoeba coli*, and *Entamoeba hartmanii*. Among these, *E. histolytica* is the only pathogenic species (201, 236, 351). Improvements in the understanding of the biochemical, immunological, and genetic differences of the members of this genus have resulted in the confirmation of three species, *E. histolytica*, *E. dispar*, and *E. moshkovskii*, that are morphologically identical in both their cyst and trophozoite stages (41, 169). The vast majority (about 90%) of individuals infected with *Entamoeba* spp. are colonized by the nonpathogenic strain *E. dispar* (324, 385). In developed countries, infections of *E. histolytica* (a true pathogen) are largely confined to immigrants from or travelers to areas of endemicity, MSM, HIV-infected patients, and institutionalized populations (164, 174, 315). The WHO reports that approximately 500 million people worldwide are infected annually with *E. histolytica*,

resulting in symptomatic illnesses and death in about 50 million and 100,000 persons, respectively (470). However, it is believed that since 90% (450 million) of infections are due to *E. dispar*, while 10% (or 50 million) are infections with *E. histolytica*, the worldwide incidence of invasive disease is more likely to be 5 million cases annually, with global mortality still at 100,000 per annum (201). Approximately 4 to 10% of carriers infected with *E. histolytica* develop clinical disease within a year, and amoebic dysentery is considered the third leading cause of death from parasitic disease worldwide (178, 280, 385). *Entamoeba histolytica* causes a range of disease manifestations, including (i) dysentery or dysenteric syndrome, characterized by small volumes of bloody, mucoid stools without fecal leukocytes; (ii) amoebic colitis, characterized by ulcerations of the colonic mucosa with typical flask-shaped abscesses; (iii) amoeboma, the formation of a fibrotic mass in the intestinal wall; and (iv) invasive disease, resulting in amoebic abscesses in the brain, lung, or liver (84, 315, 324, 449). Amoebic brain abscess occurs when *Entamoeba histolytica* trophozoites travel to extraintestinal tissues through the bloodstream, invading the central nervous system and producing amoebic brain abscesses that are frequently lethal (257). Other rare forms of amoebiasis have been reported, including cutaneous amoebiasis (amoebiasis cutis), arising as a complication of amoebic dysentery (232, 323). This usually results from contamination of damaged skin or continuous contact with exudates containing virulent trophozoites that stick to the traumatized skin, such as in the perianal or perigenital area, resulting in lysis of the skin and subcutaneous tissue and, subsequently, ulceration and necrosis (36, 323, 454).

While asymptomatic *E. histolytica* infections are equally distributed between the genders, invasive disease is more common in men (which may be due to a male-related susceptibility to invasive disease). Higher rates of carriage have been observed in HIV-infected MSM in the Asia Pacific region (194, 203, 280, 400). Patients with amoebic colitis present with a gradual onset of bloody or profuse watery diarrhea and abdominal pain and tenderness (324, 385). Symptoms may last for several weeks, and fulminant necrotizing colitis, the most severe form of intestinal disease, is often fatal (174, 324, 379). In children, <40% of patients present with fever and rectal bleeding without diarrhea. Some patients develop fulminant amoebic colitis, with profuse bloody diarrhea, fever, pronounced leucocytosis, and widespread abdominal pain, often with peritoneal signs (280, 399).

The virulence of pathogenic *E. histolytica* is based on its ability to secrete enzymes and proteases that contribute to the invasion of the epithelial cells penetrating the intestinal mucosa and to degradation of the extracellular matrix proteins and subsequently to interfere with the host's humoral immune response (14, 315). More recently, the emergence of clear roles for human and parasite genetics and environmental factors in the virulence of *E. histolytica* has increased our understanding of infections. It is thought that some persons are genetically resistant to infection, while malnourished children are more susceptible, and a polymorphism in the leptin receptor increases susceptibility to amoebiasis in both adults and children (14, 277, 332). The evidence suggests that not all strains are capable of causing liver abscess, and the observed higher incidence in men may be a result of gender-based differences in the complement system (379). For example, higher gamma interferon and functional natural killer T cell levels in females might underlie their resistance to liver abscess (280, 332). Based on the many presentations of amoebiasis, a high level of clinical suspicion is necessary for the early diagnosis of invasive and extraintestinal amoebiasis (221, 454). The specific diagnosis of *E. histolytica* infection is important in order to minimize undue treatment of individuals infected with nonpathogenic species of *Entamoeba* (131).

Diagnosis is difficult based on the fact that the pathogenic species *E. histolytica* is morphologically identical to the nonpathogenic species *E. dispar* and *E. moshkovskii*; hence, microscopy is generally considered insufficient for differentiation of these species (397). Various methods are employed in the diagnosis of *Entamoeba*. In many countries, microscopy is widely used for protozoan identification (131, 204, 249). Microscopic techniques utilized in the clinical laboratory include the screening of wet preparations, concentrated samples, and permanently stained smears for the identification of *E. histolytica/E. dispar/E. moshkovskii* in feces (131). The CDC recommends that in order to maximize the recovery of cysts, stool samples in formalin or other fixatives should be concentrated prior to microscopic examination (49). Wet mount preparations and trichrome-stained smears of stool specimens are routinely used for identification of *E. histolytica/E. dispar* (49). Various fixatives utilized for the preservation of the morphology of the parasites can be used in conjunction with various stains. The pathogenic species *E. histolytica* can sometimes be differentiated from the nonpathogenic, morphologically identical species by microscopy on the basis of ingested red blood cells within the cytoplasm of the trophozoites; however, this phenomenon does not occur commonly and is rarely seen in clinical samples (49, 131). Therefore, when a definitive diagnosis is not possible via microscopy, the presence of the *E. histolytica/E. dispar/E. moshkovskii* complex should be reported (49). Microscopy is less reliable at identifying *Entamoeba* species than culture or antigen-based and molecular tests (179, 410).

While cultivation is more sensitive than microscopy and isoenzyme analysis can effectively distinguish between *E. dispar* and *E. histolytica*, these methods are time-consuming, are not cost-effective, and are not routinely utilized by most diagnostic laboratories (132, 177). EIA kits are commercially available that detect *E. histolytica* only, while others detect both *E. histolytica* and *E. dispar* (49, 131, 460). A rapid ICT assay is available that detects antigens of *E. histolytica* and *E. dispar* in stool; however, this assay does not distinguish between *E. histolytica* and *E. dispar* (49). This assay also detects antigens of *Giardia* and *Cryptosporidium.* Borderline positive results and questionable negative results obtained by this technique should be confirmed further by additional testing. This assay is quick and easy to perform, and no special equipment is needed (49). EIAs and rapid ICT tests require the use of fresh or frozen stool specimens and cannot be used with the majority of preserved specimens because antigens are lost during the concentration procedure (49, 397). However, some newer single-vial collection systems that utilize nontraditional fixatives can be used in these assays. Many stool antigen assays have been shown to be as sensitive and specific as culture with isoenzyme analysis and outperform microscopy for the detection of *E. histolytica* in areas of endemicity (177, 179, 316). One study found that in comparison to PCR, some stool antigen tests lacked sensitivity but were highly specific in diagnosing *E. histolytica/E. dispar* in areas where these parasites are not endemic (460), while another study found that antigen detection tests can be both rapid and technically simple to perform (177).

A number of molecular assays have been described in the scien-

tific literature for detection and/or differentiation of *Entamoeba* species and are now considered the gold standard for diagnosis (177, 397). Both conventional and real-time PCRs have proven to be more sensitive and specific than microscopic examination for the detection of *E. histolytica* and *E. dispar* in single stool samples (455, 460). In developed settings, PCR is more useful for detection of *E. histolytica* in stools than antigen detection tests due to the higher sensitivities observed for PCR and the reduced chance of cross-reactivity with other *Entamoeba* species (167, 397, 460). However, PCR has yet to become the mainstream for the detection of parasites in the clinical laboratory, even in developed countries, due to the need for specialized equipment, dedicated molecular areas, specified workflow, and associated costs. It must be emphasized that when species differentiation is not possible, the *E. histolytica/E. dispar* complex should be reported.

Serological methods such as indirect hemagglutination (IHA), latex agglutination, immunoelectrophoresis, immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA) are highly sensitive at detecting *E. histolytica* antibodies in human serum and are useful for detecting invasive disease (41, 131, 236). However, serology is of limited use in areas of endemicity because of the difficulty in distinguishing between past and present infections (167, 236); hence, it is of more value in nonendemic settings in establishing the diagnosis of *E. histolytica* infection if antibodies are present (460). A combination of microscopy, culture, and serology should be complemented with a PCR assay or with abdominal imaging (when PCR is unavailable) for detection of invasive disease (41, 131). Diagnosis of amoebic brain abscesses usually requires computerized axial tomography scans of the brain (257). Identification of *E. histolytica* antigens in tissue aspirates or PCR analysis of tissue aspirates is also effective (390).

All infections with *E. histolytica* should be treated because of its potential for causing invasive disease and the risks to public health (110, 324, 385). Asymptomatic carriers should be treated with a luminal agent to minimize the spread of disease. The treatment of choice differs for intestinal and invasive disease; hence, diagnosis is important before treatment begins. Metronidazole is a highly effective tissue amoebicide and is used in the treatment of invasive amoebic disease. However, a luminal agent should be used in combination, as treatment does not eliminate intestinal colonization in up to 50% of patients with invasive amoebiasis, resulting in relapse of invasive infection months later (174, 222, 236). Other nitroimidazole derivatives, such as tinidazole and ornidazole, are equally effective for treatment of invasive disease (218, 324). The luminal agents paromomycin, iodoquinol, and diloxanide furoate are strictly recommended for treatment of patients with intestinal and asymptomatic infections, as they are effective in eliminating cysts from the intestinal tract (174, 218).

Giardia intestinalis

Giardia intestinalis (syn. *Giardia duodenalis* and *Giardia lamblia*) is a common cause of parasitic diarrhea, with prevalences ranging from 2 to 7% in developed countries to 20 to 30% in most developing countries worldwide (134, 207). Because of the burden of illness from *Cryptosporidium* spp. and *Giardia*, their ability to impair development and socioeconomic improvements, and their associations with poverty, they were included in the WHO Neglected Diseases Initiative in 2004 (354). *Giardia intestinalis* consists of seven genetically distinct genotypes, designated A to G, but a novel lineage designated assemblage H has been identified in marine vertebrates (233). Assemblages A and B infect mammalian species and are the only two assemblages known to infect humans; hence, they are considered to be zoonotic (118, 134, 146, 196, 236, 262, 433). Recent developments in the study of protein coding capacities have found genomic differences between strains WB (assemblage A) and GS (assemblage B), which may explain some of the observed biological and clinical differences between the two isolates. These observations led to the suggestion that *Giardia* assemblages A and B may be two different species (134, 207, 285).

It has been suggested that *Giardia* trophozoites may remain in the small intestine for weeks to years (315). However, this is unsupported, since many long-term infections are suspected to represent either persisting abdominal symptoms elicited post-*Giardia* infection or reinfections (176, 196, 484). The fecal-oral route still remains the most important mode of infection (33), and various studies have found evidence of zoonotic transmission (89, 118, 237). The symptoms of giardiasis can be variable, but it presents mainly as acute or chronic diarrhea associated with abdominal pain, nausea, malabsorption, and weight loss. In malnourished children, infection can lead to growth retardation, and asymptomatic illness is also possible (37, 40). There have been a few reports suggesting that giardiasis may be a risk factor for zinc deficiency in school-aged children (80, 328). High rates of asymptomatic carriage of *Giardia* have been reported for humans and animals in developing settings (213).

In developed settings, *Giardia intestinalis* has also been isolated from various animals, including livestock (17, 31, 65), fish (475), and nonhuman primates (262). *Giardia* infestation is common in dogs and cats, with prevalences of approximately 8% to 16% and 4% to 11%, respectively (44, 302). While there is limited evidence for direct transmission from companion animals to people, rare infections and the isolation of zoonotic genotypes from cats and dogs suggest that they are a potential source of human infection that may be acquired through handling, sleeping together, licking, and kissing (61, 308, 346, 424, 480). Giardiasis is frequently associated with waterborne and day care center disease outbreaks and is related to travel-associated diarrhea (471). A study of travel- and migration-associated illnesses in Europe revealed that *G. intestinalis* was the second most common pathogen and the most common parasite as a cause of gastrointestinal illnesses (122). *Giardia* is frequently isolated from diarrheal stools from MSM, with or without HIV/AIDS, in whom transmission is most likely via the fecal-oral route (33, 324). In the United States, it is suspected that seasonal peaks are related to increased use of recreational water venues, such as lakes, rivers, swimming pools, and water parks, in summer months (188).

Diagnosis is usually based on the microscopic detection of *Giardia* cysts or trophozoites in a stool specimen. Stools may be examined either directly as fresh smears, preserved in formalin or polyvinyl alcohol and stained with iodine, trichrome, or hematoxylin, or after formalin-ethyl acetate concentration (222, 236, 262, 289, 305, 457, 465). Several antigen detection assays are available for *G. intestinalis*, including EIAs, ELISAs, and monoclonal antibody and direct fluorescent-antibody tests, which are widely used (130, 140, 141, 145, 234, 431). Many have proven to be cost- and labor-effective, and many of them are designed for the sensitive and specific testing of several protozoa simultaneously (140, 141, 465). These antigen detection tests are useful in screening settings and are more sensitive than routine microscopic examinations for

detection of ova, cysts, and parasites (OCP) (137). For example, one rapid qualitative EIA has been demonstrated to have a 95.9% sensitivity and 97.4% sensitivity for the detection of *Giardia* in stool specimens (140). The use of real-time PCR as a tool for the detection of *Giardia* is increasing in developed settings. Conventional single, nested, and multiplex PCRs have also been developed but are utilized more often in specialized centers involved in molecular studies of *Giardia* (40, 236, 475). These molecular methods have proven to provide higher sensitivity than conventional methods; however, many of them are still not commercially available (236). The use of PCR in molecular epidemiology studies has advanced the knowledge of the population structure of *Giardia*, which has improved the understanding of zoonotic transmission and the molecular epidemiology of giardiasis (430).

Treatment is usually the same for both immunosuppressed and immunocompetent patients (83). Metronidazole or tinidazole has been used as the therapy of choice against giardiasis; however, treatment failures and clinical relapses have been known to occur (37) and could be due to the emergence of resistant isolates of *Giardia* (37). Furazolidone, albendazole, nitazoxanide, and paromomycin are suitable alternatives when available (83). One vaccine (GiardiaVax) has been licensed for use in the United States to prevent clinical disease in dogs and to significantly reduce the incidence, severity, and duration of cyst shedding in cats (267). However, more recent data have shown limited vaccine efficacy (15), and no human vaccines are currently available (434).

Cyclospora cayetanensis

Cyclospora cayetanensis has emerged as an important cause of endemic or epidemic diarrheal illness in children and adults worldwide (53). *Cyclospora cayetanensis* is the only species of this genus found in humans and is host specific (79). An important feature of the biology of *C. cayetanensis* is that oocysts excreted in feces require days to weeks outside the host to sporulate and to become infectious; hence, direct fecal-oral transmission from relatively fresh stool does not occur (189, 259). Clinical illness is characterized by persistent diarrhea, bloating, flatulence, abdominal cramps, constipation, and fatigue (305). Illness associated with travel to nonindustrialized countries has been reported in the United Kingdom (291) and Netherlands (456) and is a common cause of illness among returned international travelers (417). However, non-travel-related and waterborne cases of cyclosporiasis have been reported in several developed countries (12, 450). Food-borne outbreaks have been reported in North America (189, 291, 450) and Germany (92). Infections in immunosuppressed patients have been reported in Turkey (350), and although symptoms may be similar to those in immunocompetent individuals, they may be prolonged (410). Of the 1,110 laboratory-confirmed cases of sporadic cyclosporiasis captured by U.S. disease surveillance over the 1997-2008 period, approximately one-third of cases occurred in persons with a known history of international travel, the vast majority of whom traveled to countries in Southern and Central America (173). Domestically acquired cases were concentrated in time (late spring and summer) and were probably linked to an undetected outbreak (173).

Standard laboratory procedures for ova and parasites do not identify *Cyclospora*; therefore, the laboratory must be notified when *Cyclospora* is being considered (368, 461).

Diagnosis is made by the demonstration of *Cyclospora* oocysts by examination of their autofluorescence and staining characteristics. Oocysts appear as acid-fast round or ovoid structures and autofluoresce white-blue under an epifluorescence microscope, using a 330- to 380-nm dichromatic (DM) excitation filter, or blue or green with a 450- to 490-nm DM filter (53, 137, 305, 306, 330, 456). However, detection is often difficult because the pathogen is very small, measuring 8 to 10 μm in diameter; hence, oocyst measurement is important to differentiate the oocysts of this species from smaller *Cryptosporidium* oocysts, which measure 4 to 6 μm (137, 259, 330). The oocysts can be seen in concentrated or nonconcentrated feces by light microscopy using various stains, but the disadvantage is that *C. cayetanensis* oocysts stain only variably with Gram, Giemsa, and hematoxylin-eosin stains and results are variable with modified Ziehl-Neelsen staining (259, 368, 456). A safranin-based stain has been described that uniformly stains oocysts of *Cyclospora* a brilliant reddish orange when the smears are heated in a microwave oven prior to staining (305, 461). Other routine procedures in use include concentration by the formalin-ethyl acetate technique followed by either (i) UV epifluorescence and bright-field microscopy, (ii) examination of a modified acid-fast-stained stool slide, or (iii) examination using a modified safranin-based technique (151, 307, 368, 377, 461). Samples can be stored in 2.5% potassium dichromate for 14 days at temperatures ranging from 22 to 37°C for sporulation or molecular detection (306, 377). In comparison to UV detection, the sensitivity of the acid-fast technique is about 78% (93).

Various molecular techniques have been developed for the identification of *Cyclospora*. These include spectrophotometry-based detection with an oligonucleotide ligation assay. Various PCR tools have been used that target the internal transcribed spacer region, using primers for the 18S rRNA gene (93, 306). These include conventional PCR, reverse transcriptase PCR in combination with agarose gel electrophoresis, and nested PCR (281, 304, 306, 314, 450). Some of these techniques for the identification of *C. cayetanensis* are time-consuming, labor-intensive, and subject to problems of contamination; hence, further studies are needed for the development of highly sensitive rapid tests for this protozoan. On a cautionary note, it was reported that PCRs for *Cyclospora* cross-amplify DNAs from other coccidia, especially those belonging to the genus *Eimeria.* This cross-reactivity with *Eimeria* is problematic mainly in outbreak settings and with environmental specimens, since human infections by *Eimeria* are currently unknown (259, 314). A restriction fragment length polymorphism (RFLP) protocol is required to distinguish between these species in environmental samples (259).

Laboratory diagnosis is important before empirical treatment commences, since the organism requires different treatment from that for some of the other protozoa of similar presentation (368). Trimethoprim-sulfamethoxazole (TMP-SMZ; also known as cotrimoxazole) is the drug of choice for managing cyclosporiasis. Infection can be treated effectively with cotrimoxazole for 10 days, with results occurring in a few days (218, 222, 306). Oral or intravenous rehydration may be appropriate, based on the degree of dehydration (368).

Blastocystis Species

Blastocystis spp. are commonly found in stools from humans and numerous animal hosts (341, 418). There is considerable genetic heterogeneity within *Blastocystis* organisms, and currently, human, mammalian, avian, and reptilian isolates have been assigned to 1 of 13 subtypes (312, 341, 384, 402). While it is unclear whether

any of these subtypes are specific to human disease, *Blastocystis* sp. subtype 3 is most commonly associated with illness in human prevalence studies (90, 341, 418, 479). The name *Blastocystis hominis* refers to approximately 10 different genetically diverse populations that are all morphologically identical and cannot be differentiated on the basis of microscopy alone (406, 435). In many reports, *B. hominis* refers to the parasite isolated from humans and *Blastocystis* spp. represent those isolated from other animal hosts (293). Based on the discrepancies in the use of these terms, several experts in the field have come to a consensus that based on published small-subunit rRNA gene analyses, all mammalian and avian isolates should be designated *Blastocystis* spp. and assigned to one of nine subtypes (408). Knowledge about the life cycle of *Blastocystis* spp. remains elusive, and various morphological forms have been described (82, 320). These are the vacuolar, granular, amoeboid, and cyst forms, and other, less frequently encountered forms are the avacuolar and multivacuolar cells and cells containing filament-like inclusions (191, 274, 371, 409, 419).

The role that *Blastocystis* spp. play in eliciting gastrointestinal pathology and symptoms remains uncertain and controversial (191, 421). Boorom et al. argue that there may be some pathogenic variants of *Blastocystis* spp. and that the limitations in their detection by existing diagnostic methods may have added to the controversy about their pathogenicity (32). Another thought is that the amoeboid form of *Blastocystis* spp. could be either an indicator of or contributor to the pathogenicity of these protozoa in patients exhibiting symptoms (422), but this suggestion remains unsupported (341, 420). However, more recent *in vivo* and *in vitro* studies suggest that some of the "strains" or potentially different species of this organism may be pathogenic (32, 399, 418, 479). The genetic and antigenic diversity among *Blastocystis* spp. complicates the epidemiological understanding of these parasites; however, emerging evidence—despite still being controversial—suggests that pathogenicity may be subtype dependent (419, 421). It is thought that the cyst is probably the infectious form and is transmitted by the fecal-oral route: when the parasite passes down the intestinal tract, small vesicles within its cytoplasm coalesce into a vacuole, eventually forming a cyst which is passed into the environment in the stool of the host (191, 274, 371).

Clinical features of illness which have been attributed to *Blastocystis* spp. include nausea, anorexia, abdominal pain, flatulence, and acute or chronic diarrhea (216, 381). In several instances, *Blastocystis* spp. have been the most common enteric organisms isolated from diarrheal patients but have been reported as noninfectious pathogens (422, 438, 444). *Blastocystis* spp. are the most common enteric protozoa isolated from diarrheal patients in most developed countries (247, 309, 395, 416). For example, they are commonly isolated from immigrants in Italy (164), associated with chronic gastrointestinal illness of unknown etiology (216), and often associated with IBS-like symptoms (32, 90, 91, 211).

However, numerous studies have found no link between *Blastocystis* spp. and active disease (90, 91, 404, 420). It has also been argued that identification of *Blastocystis* from patient samples is not clinically significant but should be used as a marker of potential exposure to other pathogenic protozoa (315). Another study suggested that immunosuppression plays an important role in the display of clinical symptoms (418). More recently, clinical and molecular investigations demonstrated an association with *Blastocystis* spp. in IBS patients that supports a pathogenic role of this parasite in Latin America (211). However, other studies have failed to establish an association between *Blastocystis* spp. and IBS (334, 414, 440). It has been suggested that since IBS is a functional disorder that can be caused by various microbiological, genetic, and environmental factors, these factors could also explain the discrepancy in the literature (321). Cysts may be waterborne, food borne, or passed from person to person, especially in child care centers or other institutional settings. It has also been suggested that since many zoonotic subtypes exist, there is increased potential for zoonotic transmission (191, 420, 438).

The diagnosis of infection with *Blastocystis* spp. is usually based on the detection of the vacuolar, granular, amoebic, or cystic form in stool samples, using wet mount smears, iodine staining, trichrome staining, or iron-hematoxylin staining (309, 341, 390, 402, 404, 416, 481). However, identification by direct wet mounts is difficult and has resulted in false-negative results due to the polymorphic nature of *Blastocystis* spp. (404, 419). It has been suggested that trichrome-stained fecal smears or xenic *in vitro* culture systems offer the best sensitivity (90, 418). However, slower-growing subtypes (e.g., subtype 7) may be missed with this procedure (418). The formol ether concentration technique (FECT), which is commonly used for laboratory identification of OCP, is not recommended for *Blastocystis* due to its inability to isolate *Blastocystis* spp. (309, 404, 419).

Several molecular techniques with increased sensitivity have been developed (341). Advancements in the sequence analysis of *Blastocystis*-specific PCR products and subtype-specific PCR primers have led to progress in identifying several subtypes (268, 312, 341, 402, 418, 451). PCR using the SSU rRNA gene is being used increasingly for detection of *Blastocystis* spp. Despite being more costly, PCRs have demonstrated much higher sensitivities than more commonly used methods such as permanent staining (48%) (211, 341), direct light microscopy (29%), and xenic *in vitro* stool culture analysis (52%) (320). Owing to the wide genetic diversity among *Blastocystis* spp., the choice of primers is crucial from a diagnostic perspective (341).

Although disagreement about the pathogenicity of *Blastocystis* spp. still exists, treatment is indicated when there is no alternative explanation for symptoms (109, 218). Metronidazole is the suggested drug of choice, although failures of this drug in eradicating the organism are common (275, 407, 409). Cotrimoxazole, nitazoxanide, and a combination of paromomycin and metronidazole have also been used (222, 485). A recent study found extensive differences in drug sensitivities among two clinically important zoonotic subtypes (subtypes 4 and 7) and identified four new potential therapeutic options against *Blastocystis* spp., namely, mefloquine, cotrimoxazole (trimethoprim-sulfamethoxazole) (1:2), ornidazole, and furazolidone; the study also confirmed the antiprotozoal activities of 10 compounds already reported to be effective against *Blastocystis* spp. (272). It is clear that antimicrobial eradication of *Blastocystis* spp. is possible; however, because of the difficulty of the problem, a reevaluation of current treatment regimens is required, with a view to defining clear new treatments. Consideration should also be given to the fact that improvements in symptoms after treatment with metronidazole or trimethoprim-sulfamethoxazole may be due to clinical effectiveness of these drugs against coinfecting pathogens (71). There is therefore a need for further investigations to better understand drug efficacy, resistance, and reinfection issues (407).

Cystoisospora belli (formerly *Isospora belli*)

Cystoisospora belli causes intestinal disease in several mammalian hosts (42). Infections are thought to be acquired through the ingestion of mature environmentally resistant sporulated oocysts in contaminated food or water, although good evidence for the source of infection in most infected patients is limited (109, 219). Infection is almost indistinguishable from cryptosporidiosis (208) and is usually self-limiting and characterized by watery diarrhea, abdominal cramps, anorexia, and weight loss (175, 311). *Cystoisospora belli* is often implicated in traveler's diarrhea in travelers to developing countries with high levels of endemicity (2, 156, 311, 313, 427). It is more common in AIDS patients (165, 230, 453), other immunocompromised patients (163, 227, 266, 336, 344), and indigenous populations in the United States (291, 315). In HIV-infected patients, infection may be characterized by chronic diarrhea, acalculous cholecystitis cholangiopathy, and extraintestinal infection (337, 453). Other *Cystoisospora* species are important causes of diarrhea in domestic animals (108), and *Cystoisospora suis* is an economically important parasite causing severe diarrheal illness in pigs (8, 212, 282).

Diagnosis is made by direct microscopic observation of the oocyst in feces, with acid-fast staining, since the oocysts are large (20 to 23 μm by 10 to 19 μm) and morphologically distinctive. Diagnosis has also been done from mucosal biopsy specimens (227, 453). Molecular techniques such as conventional and real-time PCR can be used to augment diagnosis where possible, since these methods can be more sensitive in detecting infection (283). However, while PCRs have shown excellent sensitivity and specificity for the detection of *C. belli* in fecal samples, these are neither widely nor commercially available (283, 337, 347, 390, 427, 453). Infection usually responds to treatment with oral cotrimoxazole, and where resistance or intolerance exists, ciprofloxacin is a good alternative (109).

Balantidium coli

Balantidium coli is a ciliate and is the largest protozoan that infects humans (114, 383). Pigs are thought to be the natural host of this parasite, despite showing no clinical disease (111, 359). Infections in humans are acquired via the fecal-oral route from the ingestion of cysts present in untreated or minimally treated water and on uncooked or undercooked food (114, 374). Human infection is common mainly in communities that live in close proximity to pigs (114, 359). The vast majority of infections are asymptomatic, but mild diarrhea and abdominal discomfort have been reported in symptomatic patients. A few patients develop fulminating acute balantidiasis, with intestinal perforation leading to a case fatality rate of about 30%, or fulminating dysentery associated with hemorrhage and shock, resembling amoebic dysentery (120, 359). Balantidiasis is uncommon in developed countries, but infections have been reported as far north as Sweden, Finland, and Northern Russia, with the highest prevalence rates in tropical and subtropical regions (383). In developed countries, clinicians and laboratory technologists should consider balantidiasis as an alternate diagnosis for patients presenting with watery diarrhea who have a recent travel history to developing countries, especially those in Southeast Asia, the Western Pacific islands, and rural South America (111, 120, 359, 360).

Because of its large size (cysts are 50 to 70 μm; trophozoites are 30 to 200 μm by 40 to 70 μm) and spiraling motility, *Balantidium* is easily recognized in wet mount slide preparations. Trophozoites are visible with a hand lens, and sometimes with the naked eye, in freshly collected diarrheic stools (114, 359) as well as in bronchoalveolar wash fluid; cysts are more common in formed stools. Collection of stool samples over several days is recommended because excretion of parasites can be erratic (359). Treatment is done with tetracycline or metronidazole for 5 or 10 days, respectively (109).

Intestinal Microsporidiosis (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*)

Enterocytozoon bieneusi and *Encephalitozoon intestinalis* are species of microsporidia that cause enteric disease. These parasites, historically considered spore-forming protozoa, are now classified as fungi (69, 150, 157). However, based on their importance, especially in immunocompromised populations, they are included in this review. Intestinal microsporidiosis was first identified in AIDS patients, with *E. bieneusi* being the more common of the two causative species (81, 398). Many identical genotypes of *E. bieneusi* from humans and animals are known (81), raising concerns over waterborne, food-borne, and zoonotic transmission. The importance of this parasite in human infections increased as it became a cause of opportunistic infections in HIV/AIDS and other immunosuppressed patients during the 1980s (6, 29, 114, 248, 315). However, with increasing access to antiretroviral therapy, infection rates in HIV/AIDS patients have reduced significantly (390, 448), with prevalences ranging from 0 to 42% (13). Infections have also been found in non-HIV/AIDS and immunocompetent patients (1, 248) and in persons with corneal infections (263), and there are also many infections thought to be associated with protracted traveler's diarrhea, during or after travel (156). Infections with *Encephalitozoon* spp. are believed to occur through ingestion or inhalation of spores (86), with the primary infection developing in the epithelium of the small intestinal or respiratory tract (86). Severe ulceration of the small bowel is associated with mucosal atrophy and acute and chronic inflammation (13). In the case of *E. bieneusi*, infection occurs mainly through ingestion of spores, which subsequently develop within the epithelial cells lining the duodenum and jejunum of the small intestine (86). Gastrointestinal symptoms include persistent diarrhea, abdominal pain, and weight loss, especially in immunosuppressed individuals (86). In otherwise healthy individuals such as travelers, *E. bieneusi* infection has resulted in self-limiting diarrhea of approximately 1 month (86). Fecal-oral transmission is implicated most often as the means of infection (96).

The diagnosis of intestinal microsporidiosis is difficult and is usually based on microscopic detection of the spores in stool samples, requiring special fluorescent or trichrome stains to detect the small spores (114, 200, 222). However, the detection of the spores and species determination can be difficult due to their small size (222), requiring reliable immunological diagnostics to supplement PCR or histochemistry when sporadic spore shedding is suspected (88). One study found that microscopy and staining demonstrated low sensitivity for the detection of microsporidian species compared with the calcofluor white technique and staining with DAPI (4′,6-diamidino-2-phenylindole), which demonstrated a higher sensitivity and specificity (97.12% and 98.55%, respectively) (439). However, the disadvantage of calcofluor white staining is that it requires a fluorescence microscope and is a nonspecific stain leading to the fluorescence of other organisms and

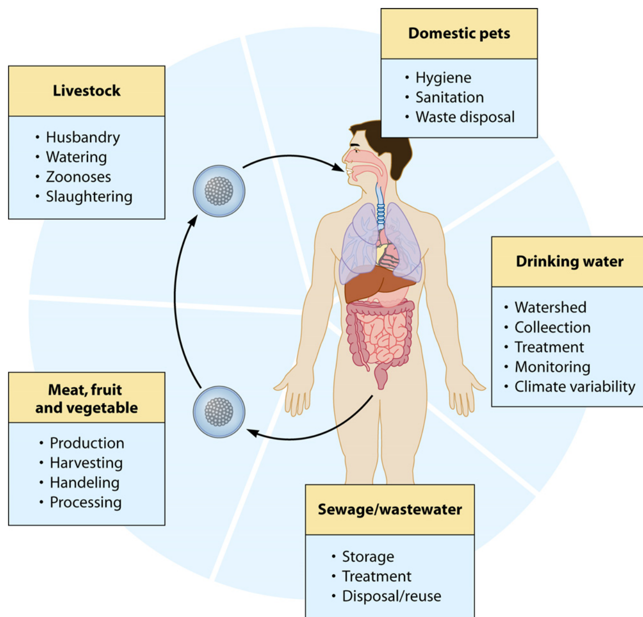

FIG 2 Complex interactions in the transmission and control of enteric protozoal infections. Infectious parasites are transmitted to humans through several routes, including contaminated food and water, inadequately treated sewage/sewage products, and livestock and domestic pet handling. Prevention and control strategies can be implemented at different levels of food production, liquid waste management, water quality control, and livestock and pet handling processes.

artifacts which can be mistaken for microsporidia (78, 423). PCR-based methods have now been used successfully for detection of microsporidian infections, exhibiting excellent sensitivity and specificity (186, 248, 349).

Where treatment is indicated, albendazole, administered at 400 mg every 12 h for 1 month, is effective against *Encephalitozoon* species (13, 114, 222). *Enterocytozoon bieneusi* is less responsive to albendazole, and while symptoms may improve, microsporidian spore shedding is likely to continue (136). Fumagillin is the only treatment that has demonstrated consistent efficacy against *E. bieneusi* infection; however, neutropenia and thrombocytopenia have been reported as major side effects in both HIV-infected patients and transplant recipients, requiring monitoring of tacrolimus levels (58, 276). Supportive fluid therapy in immunocompromised patients and early initiation of HAART in HIV-infected patients are crucial to help restore the immune status of the host (87, 114, 117). Significant reductions have been seen in the incidence of intestinal microsporidiosis in HIV-infected patients since the introduction of HAART (398, 448).

MODES OF TRANSMISSION

Any discussion about the importance of enteric protozoa in public health cannot be complete without the inclusion of their modes of transmission, as these routes play a significant role in their widespread dissemination and affect people from all walks of life (Fig. 2). Interventions aimed at prevention and control of these routes of transmission have proven effective in various settings in reducing the incidence and prevalence of diarrheal illnesses. Food- and waterborne transmission is the main focus of this section.

Food-Borne Transmission

The majority of the enteric protozoa discussed herein can be transmitted by food. Although the risk of obtaining a food-borne protozoan infection is lower in developed settings, the significance and impact of these infections cannot be ignored (125, 183, 222, 288). In developed countries, large-scale food production, distribution, retailing, and importation of raw food ingredients increase the risk for the spread of food-borne infectious disease and often result in costly recalls (250). Intestinal protozoa can contaminate food through a variety of routes (42). Food can become contaminated with parasites during the production stage, from contaminated irrigation water, soil, untreated manure, or biosolids used as fertilizers (54). Food may also become contaminated during the harvesting, handling, and preparation processes, from cross-contamination with soiled implements, animal manure, or contaminated water used for preparation or by the hands of the food handlers themselves (79, 157, 174). The risk of food-borne transmission is increased when food is consumed raw, undercooked, or in a semicooked form (198, 322). Food becomes contaminated directly from feces, soil, irrigation water, sewage, and human handling during various phases of the food production chain (115, 288, 383).

Food-borne outbreaks cost individuals and families thousands of dollars in medical costs and lost wages. The food industry suffers financially even more due to recalls of food products, litigation, and, sometimes, closure (38, 184, 197). In recognizing the public health and trade implications of pathogens in fresh fruit and vegetables, the FAO and WHO have provided scientific advice to the Codex Committee on Food Hygiene (CCFH) on the fresh produce commodities of greatest concern from a global perspective (113). The submission looked at microbial hazards in produce that is marketed fresh, and often ready-to-eat, throughout the production-to-consumption continuum, with the development of specific management guidance (113).

Cryptosporidium, *C. cayetanensis*, and *Giardia* are the main enteric protozoa associated with food-borne infections in developed countries (291). A U.S. report for the years 2000 to 2008 indicated that a significant proportion of laboratory-confirmed cases of food-borne parasitic diseases were due to *G. intestinalis* (356). In addition, of a total of 53 cases of seafood-associated outbreaks of parasitic infection reported in the United States from 1973 to 2006, *Giardia* was responsible for 43%; more than half (55%) of these were associated with the consumption of fish (199). It was reported that from 1990 to 2000, there were 11 food-borne outbreaks of cyclosporiasis in North America that affected at least 3,600 people (259). Almost all cases of *C. cayetanensis* infection in the United States are food borne (173, 356). Several cases of food-borne cyclosporiasis related to the consumption of salads have also been reported in European countries (92, 198, 350). According to one U.S. report, source attribution is often affected by insufficient numbers of cases for definitive case-control studies and a lack of tools for molecular epidemiology (173). Approximately 10% of *Cryptosporidium* infections are ascribed to food-borne transmission (171), and outbreaks have been associated with eating raw produce or salads (66, 187, 322, 476). Food-borne outbreaks associated with imported foods have been reported in several industrialized countries (92, 189, 271, 291, 322, 450). It is therefore important to have adequate food safety standards in place to govern locally produced and imported foods, which is

essential to the safety of the food supply chain (220). In addition, industrialized countries should give priority to the institution of sensitive disease surveillance systems and the development of molecular methods for linking cases of protozoan infections (162, 173, 258).

Waterborne Transmission

The role of water resources in the transmission of enteric protozoa cannot be overemphasized, especially where there is the potential for water supply contamination. Despite ongoing investment in better sanitation infrastructure, water quality, and environmental protection legislations and the subsequent reduction in pathogen loads in public water supplies, waterborne disease outbreaks still pose significant risks to human health in developed countries (34). Small water supplies in rural communities in developed countries may be more vulnerable to contamination than the larger public water supplies, resulting in outbreaks of infectious disease (195, 317). In the United States, it is suspected that seasonal peaks of cryptosporidiosis are related to increased use of recreational water venues such as lakes, rivers, swimming pools, and water parks in summer months (188, 463). *Cryptosporidium hominis* subtype IbA10G2 was the causative agent identified in the largest waterborne outbreak of cryptosporidiosis reported in Australia to date and was associated with public swimming pools in densely populated coastal cities (463). Surveillance-based data indicate a significant increase in onset of cryptosporidiosis and giardiasis during summer through early fall in the United States (476, 477). These are likely associated with increased outdoor activities and increased exposures, such as camping and use of communal swimming venues (e.g., lakes, rivers, swimming pools, and water parks) by young children, during the summer recreational water season (476, 477). *Cryptosporidium* spp. remain the leading cause of diarrheal illness outbreaks in treated recreational water venues in the United States (476). One study determined that acute diarrheal illness associated with small community water supplies could cost an estimated $4.671 billion (95% CI, $1.721 to $9.592 billion), with the capital costs of intervention amounting to $13.703 billion (95% CI, $6.670 to $20.735 billion) and those of postinfectious IBS resulting from these acute gastroenteritis outbreaks amounting to $11.896 billion (95% CI, $3.118 to $22.657 billion) (195).

The oocysts of several protozoa are highly resistant to chlorination, a conventional water treatment method (43). It is suspected that when water becomes contaminated, oocysts can become trapped by chlorine and UV-resistant biofilms in water pipelines and constantly shed into the water supply (68, 123, 466). While availability of potable water and water treatment standards are generally better in developed countries, remote and isolated areas often have deficient water systems, resulting in waterborne outbreaks, for example, in South Bass Island, OH (303), and in France (135). *Giardia intestinalis* and *Cryptosporidium* spp. have been implicated in several large waterborne outbreaks in the United States (72, 252, 303, 352), Norway (297), Australia (295, 463), and elsewhere (360).

Many countries worldwide have legislation or follow standard guidelines to monitor microbial indicator organisms to determine the microbiological quality of water (68, 317). Traditionally, fecal indicator bacteria (FIB)—total coliforms, fecal coliforms, and enterococci—are used as microbial indicators, and there are very few reports outlining the use of protozoa as indicators of fecal pollution (190, 269, 310, 353). Unfortunately, many waterborne pathogens are still difficult to detect, and despite advances in molecular diagnostics, such methods are not widely available or used even in developed countries (123). Increased water shortages in some developed countries have caused authorities to resort to the use of recycled water from highly contaminated sources such as sewage (67, 239). The increased use of biosolids from sewage as soil conditioners as a means of sustainable disposal has raised issues about the potential for the transmission of infectious pathogens, including the protozoa *G. intestinalis*, *Cryptosporidium* spp., *Balantidium*, *Entamoeba* spp., *Blastocystis* spp., and *Dientamoeba fragilis* (23, 104, 217). These issues have been the subject of extensive debate in many countries, such as Australia, New Zealand, the United States, and countries in the European Union, highlighting an extensive gap in knowledge on the spread of enteric protozoa through recycled sewage and biosolids (67, 243).

Potable water. A water supply can become contaminated by sewage and runoff from farms, fecal matter from animal activity, or decomposing animal carcasses washed into water bodies (154). In properly operated conventional water treatment plants, most protozoa are excluded from drinking water by their size (<30 μm). However, due to their smaller size (range, 1 to 17 μm), *G. intestinalis* cysts, *Cryptosporidium* oocysts, and the spores of microsporidia have been able to penetrate water treatment systems and have been detected in aquatic environments (415). While waterborne infections are not as widespread in many developed countries, climatic events that can tax treatment plant operations or inadequate, interrupted, and intermittent treatment can occur, resulting in large outbreaks in the community (297, 338). In a Spanish study, *Cryptosporidium* oocysts were found in 15.4% to 63.5% of various raw surface water samples, 30.8% of treated water from small treatment facilities, and 26.8% of chlorinated tap water. *Giardia* cysts were found in 26.9% to 92.3% of various raw surface water samples, 19.2% of treated water from small treatment facilities, and 26.8% of chlorinated tap water. The presence of *Cryptosporidium* and *G. intestinalis* was significantly associated with the turbidity levels of the samples and with increased count levels for total coliforms and *Escherichia coli* ($P < 0.01$) (46).

A review of documented waterborne disease outbreaks in the United States from 1971 to 2006 found that parasites accounted for about 18% of outbreaks (72). *Giardia intestinalis* was the sole pathogen identified in 86.0% (123) of the 143 drinking water outbreaks of known parasitic etiology, affecting 28,127 individuals (6.3% of the 449,959 cases) (72). On the other hand, *Cryptosporidium* was responsible for only 13 outbreaks (9.1%), but these outbreaks were responsible for the vast majority (421,301 cases [93.6%]) of the total cases. The majority of cryptosporidiosis (403,000 cases) cases were attributed to a single outbreak of *C. hominis* in Milwaukee, WI (72, 252). *Entamoeba* spp. and *C. cayetanensis* were responsible for two and one outbreak, respectively, with fewer cases, while coinfections were also identified (*Cryptosporidium*-*Giardia* and *Giardia*-*Entamoeba* spp.) as the cause of two and one outbreak, respectively (72).

More recently, concern has been raised about the role of biofilms in drinking water distribution networks and the role they play in becoming transient or long-term habitats for hygienically relevant microorganisms, including parasitic protozoa (e.g., *Cryptosporidium*). These organisms can attach to preexisting biofilms, where they become integrated and survive for days to weeks or even longer, depending on the biology and ecology of the or-

ganism and the environmental conditions, remaining undetected by conventional detection methods (472). It has been suggested that protozoa may act as protective environments for pathogenic bacteria, protecting them from disinfection and promoting extended survival under these conditions (378). Further study is therefore important to determine the role of biofilms in drinking water systems and their potential for long-term harborage of enteric protozoa and other pathogenic organisms and subsequent health risks to humans (378, 472).

Rain/roof water has been used worldwide, especially where water scarcity is a problem, as an alternative water source for drinking and various nonpotable uses (4, 239). This generally involves installing rainwater tanks to collect roof water from residential dwellings for uses such as drinking, cooking, irrigation, showering, clothes laundering, and toilet flushing (4, 103, 458). Despite the obvious benefits from using rainwater, there are public health risks, including the transmission of zoonotic bacterial and protozoan pathogens from bird and animal droppings via individual and communal rainwater systems (4, 458). The Australian guidelines indicate that roof water should be harvested in a way that minimizes health and environmental risks or, at a minimum, reduces these risks to acceptable levels (103). In countries where the microbiological quality of rainwater is assessed, it is based mainly on the three bacterial indicators (coliforms, *E. coli*, and enterococci) that are commonly detected in harvested rainwater. Their presence may be an indication of the potential for contamination with environmentally resistant cysts or oocysts of protozoa (46, 240, 246). A report from South East Queensland, Australia, found *Giardia* in 10% of roof-harvested rainwater samples (5). Based on the assessment of the risk of infection associated with various types of exposure to rainwater, it was reported that although the risk of infection from the use of rainwater for showering and garden hosing was well below the threshold value of one extra infection per 10,000 persons per year, the risk of infection from ingesting *G. intestinalis* via drinking exceeded this threshold value and indicated that if undisinfected rainwater was ingested by drinking, then the incidence of giardiasis would be expected to range from 1.0×10^1 to 6.5×10^1 cases (with a mean of 1.6×10^1 cases based on Monte Carlo analysis) per 10,000 persons per year (5). These findings indicate the need for disinfection of rainwater for drinking purposes (5). Giardiasis is a frequently diagnosed waterborne disease and is a major public health concern for water utilities in developed nations (196, 238, 430).

Evidence for contamination of groundwater with protozoa was found in France. *Cryptosporidium* oocysts were detected in 78% of both surface water and groundwater samples, while *Giardia* cysts were found in 22% and 8% of surface water and groundwater (sinkhole, spring, and well bore) samples, respectively (223). *Cryptosporidium* oocysts were transported from the sinkhole to the spring and the well bore, suggesting that oocysts are subject to storage and remobilization in karst conduits (223, 269).

Wastewater and biosolids. Increased urban populations, industrialization, and urbanization have resulted in the overwhelming production of sewage sludge (23). Wastewater treatment processes should reduce the number of pathogens in the wastewater by concentrating them with the solids in the sludge. Although some treatment processes are designed specifically to inactivate pathogens, many are not, hence the potential risk for pathogen survival in sewage sludge and its by-products (155). The most widely available and recommended option is land application of sewage sludge to cropping land as a soil conditioner and fertilizer (23). However, by virtue of its origin, sewage sludge is a reservoir for enteric pathogens. One major risk is that biosolids may contain ova and cysts of parasites and enteric bacteria and viruses (23, 67). Health risks may result from the consumption of food and water contaminated with sewage or biosolids (369) or crops grown on biosolid-enriched soil (286, 287). In managing the beneficial reuse of biosolids on land, a balance is achieved between treating contaminants in the biosolids and retaining the biosolids' nutrient value. The U.S. EPA legislation for the safe use of biosolids (known as the Part 503 Rule) was promulgated in 1993 and used operational standards intended to reduce pathogens to levels that are not expected to cause adverse health effects (105). Similar guidelines have been established in Europe, the United Kingdom, and Australia (104, 369). The proponents of the use of biosolids in this way suggest that for it to be considered a public health risk, a plausible transmission route should exist between the source (biosolids) and the susceptible community. The pathogenic protozoa would need to be present in a sufficient infectious dose, survive the sewage/biosolids treatment processes, survive through storage and land application, enter into the water supply system, and bypass drinking water treatment (67, 159, 369). Gerba et al. concluded that the probability of emerging parasites such as microsporidia and *Cyclospora* surviving various biosolids treatments was low, since they were unlikely to survive the temperatures achieved in anaerobic digestion and do not survive well under low-moisture conditions (142). However, another study detected *Cryptosporidium* oocysts in 10% and *Giardia* cysts in 35% of the samples of sludge sanitized by quicklime or peat and after 30 weeks of composting (339). In addition, many practitioners and researchers do not encourage the reuse of recycled sewage as potable water because pathogens such as *Giardia* are not always effectively removed and because of the possibility of malfunctioning of reverse osmosis (RO) systems—deemed the most effective in removing viruses—as often as 5 days a year (67).

Biosolids generated at wastewater treatment plants (WWTP), along with food waste, pet excrement (i.e., dog and cat feces), and human excrement in absorbent products (e.g., disposable baby napkins for children and adults and feminine hygiene products), are considered a potential source of infectious microorganisms in municipal solid waste (MSW) (143). The majority of protozoan parasites (97%) are expected to come from pet feces; however, the evidence suggests that enteric protozoa would be expected to be inactivated by temperatures above 45°C and that most landfills achieve temperatures of 38°C to 78°C (143). There is still much uncertainty about the survival of enteric protozoa in leachate from unlined landfills and the resultant contamination of underground aquifers and the water supply. One of the most recent studies to evaluate this found the presence of viable *Giardia* in large concentrations in leachate (143).

IMPLICATIONS OF CLIMATE-RELATED CHANGES

According to the Stern review, climate change is a serious global threat and demands an urgent global response (411). Climatologists and health experts have postulated that climate change will impact human health, and this is best understood by the identification of existing vulnerabilities of the ecosystem to climate variability (59, 97). Vulnerabilities include highly complex interactions between changing weather, ecosystem changes, microbial and parasitic evolution, and technological and societal adapta-

tions (59, 98). The main impact to health may arise from changes in weather patterns associated with extreme weather events such as excessive rainfall, flooding, and droughts (172, 206). These events have the ability to significantly increase the risk of infectious diseases due to the abundance and distribution of disease agents in the environment (97, 279, 333). Some experts have projected that the following water-related risks are likely to increase in both developed and developing countries as a direct result of climate change: (i) excess precipitation and floods may result in increased runoff and turbidity and decreased effectiveness of water treatment (59); (ii) heavy rain, snow, or ice melt may flush animal manure, human sewage, and wildlife and pet droppings into surface water or groundwater reservoirs, leading to contamination of drinking water sources (59, 77); (iii) droughts may lead to reduced water availability, lower water pressure, compaction contributing to increased runoff when rain eventually does fall (59), and increased use of alternative water sources for domestic purposes and irrigation, increasing the likelihood of contamination of water and food (435); (iv) a decreased food supply associated with extended dry spells and drought in some countries may lead to increased importation of substandard foods, resulting in the introduction of food safety hazards such as enteric protozoa at various stages of the food chain, from primary production through to consumption (435); and (v) there may be increased risk for the contamination of recreational waters and exacerbation of the presence of biological contaminants in marine environments, leading to seafood contamination (261).

The greatest impact of climate change is expected to be on less developed countries, and small island states are particularly vulnerable to the effects of extreme weather, sea level rise, and increased temperatures (411, 413). In the context of infectious diseases, the smallest and poorest populations could be affected most seriously due to already vulnerable economies and public health infrastructure, inadequate fresh water resources, and poor sanitation and hygiene (99, 182). Health consequences such as diarrheal diseases, malnutrition, and malaria are projected to pose the largest risks to future populations, especially young children (97). Projections suggest that although low-income countries will be most vulnerable to adverse effects, requiring additional human and financial resources, high-income countries will also be affected adversely, with the severe impact on the local economy (97, 411). Generally speaking, the risk of diarrheal disease in developed countries may be mediated by more stringent public health measures for sewage disposal, water treatment, and hygiene. However, indigenous populations, institutionalized persons, and the immunosuppressed will remain at extremely high risk (206). There is therefore need for more research into the role of this phenomenon in the spread of enteric infections in developed settings.

The challenges of food-borne, waterborne, and zoonotic protozoan diseases associated with climate change are expected to increase, with a need for active surveillance systems, some of which have already been initiated by several developed countries (172, 206). Studies conducted in the United States (77), Taiwan (62), New Zealand (35), Australia (172), Bangladesh (182), Canada (428), and China (483) have found increased diarrhea-associated morbidity related to increased temperature and extreme rainfall days, although in Australia there were no differences in climate-associated increases in *Salmonella* infection rates between subtropical and tropical regions (482). Several authors have suggested that the seasonal incidence of infection with some enteric protozoa may be affected by increased rainfall, increased pollution from farm waste, or animal husbandry practices (172, 206). Exposure to floodwaters and swimming in lakes and ponds with elevated pathogen levels are other risk factors to be noted (99). Management of diarrheal illnesses in this complex setting will require dialogue and collaboration between public health, water treatment, veterinary, and food safety experts worldwide (288). The rate of climate change, the degree of its effect, and its impact on infectious diseases, including parasitic diseases, may differ from country to country (224, 362). These conclusions are therefore drawn based on scientific plausibility, and countries should seek to establish proper surveillance networks to connect epidemic intelligence and infectious disease surveillance with meteorological, entomological, water quality, remote sensing, and other data for multivariate analyses and predictions (362).

PREVENTION AND CONTROL OF PROTOZOAN INFECTIONS

The ubiquitous nature, small size, and abundance in the environment of protozoan parasites make them difficult to control. Health care providers and public health personnel should provide adequate instructions to infected patients and carriers on how to avoid spreading the infection to others and should employ infection control precautions for hospitalized and institutionalized patients. The following are suggested controls based on proven intervention measures that can be applied in various situations.

General Measures

In domestic settings, simple prevention and control measures are effective against most enteric parasites. These include personal hygiene; hand washing after using or handling the toilet, changing diapers, or caring for a person with diarrhea and before preparing or handling food (366); proper disposal of excreta; and washing of soiled materials such as clothing or bedding. The use of adequately treated water, for example, by boiling, for drinking, food preparation, and washing of fruits and vegetables is essential (57). In the food industry, some protozoa get into food through fecal contamination of raw materials and inadequate treatment of the food before consumption or through posttreatment contamination. This is true for the oocysts or sporocysts of *Cryptosporidium* spp. and *C. cayetanensis* (291, 374). Control measures must therefore be aimed at all aspects of the food chain, including minimizing dissemination of cysts and oocysts in the farming environment and via human waste management (373). This may include abstinence from using untreated human and animal manure in farming.

Food hygiene and management practices in food service and catering industries should include restricting the purchase of raw materials to suppliers with good agricultural practices (95) and having adequate facilities for washing and cleaning of raw fruits and vegetables before storage and before preparation (220). A system for identifying and controlling microbial hazards, such as the Hazard Analysis Critical Control Point (HACCP), should be employed by food and water production companies, in industries or sectors that use fresh produce, and in operations in which contaminated water or ingredients could end up in the product (79, 172, 202).

Persons working in high-risk environments such as schools, day care centers, food-handling facilities, and aged-care institutions should be educated properly on their risk of infection (297), and where infection occurs, persons should be excluded for up to 48 h

after the last diarrhea episode (10). Infected persons such as travelers, campers, and hikers should refrain from passing of feces into rivers and streams and should not use recreational waters such as swimming pools and public baths for up to 2 weeks after symptoms cease (57). *Cryptosporidium* spp. and *Giardia intestinalis* in swimming pools are difficult to control (325). Open farms and petting zoos should have guidelines in place for the handling of animals and should ensure that adequate hand-washing facilities are in place for visitors and staff. Wilderness backpackers and travelers to developing countries must exercise precautions with food and drinking water safety (331). Basic hygiene, including hand washing and cleaning of cookware, is essential in the prevention of diarrhea (10). Long-term groups, including military, missionary, aid worker, volunteer, and tourist groups, should seek travel advice. They should also take with them adequate supplies to treat water for domestic use (e.g., filtration and boiling), as chlorination by itself cannot remove the oocysts of protozoa such as *Cryptosporidium* spp. and *C. cayetanensis* from water (12, 19). Routine boiling should be used to augment chlorination in less developed settings (331). Since domestic house flies can play a role in the efficient transmission of human protozoan parasites, care must be taken with food exposed to flies (160). In the case of cyclosporiasis, it is important that it be considered by microbiologists and physicians as a possible cause of illnesses in cases of prolonged diarrhea that are not associated with travel, as well as in food-borne outbreaks associated with imported foods (306).

Water and Wastewater Quality Control

Drinking water supply. The benefits of supplying safe water cannot be overemphasized (256, 365). Hunter and colleagues estimated that the cost benefit of annual maintenance coupled with the benefits of reducing the impact of postgastroenteritis IBS from waterborne infections was nearly 10 times (9.87; 95% CI, 3.34 to 20.49), suggesting that the health benefits of improving the management of water supply systems outweigh the costs (195). Conventional water treatment involves a series of steps, including coagulation, flocculation, clarification, sedimentation, medium or membrane filtration, and disinfection (using chlorine/chlorimination, ozone, or UV), aimed at improving the microbiological quality of water (30, 75). The challenge for the physical removal of cysts and oocysts in the water treatment process is their small size, and the general rule is that the smaller the cysts and oocysts, the more difficult it is to remove them using conventional water treatment technology. *E. histolytica* cysts range in size from 10 μm to 20 μm, *G. intestinalis* cysts are 8 to 12 μm by 7 to 10 μm, and *C. parvum* oocysts range in size from 4 μm to 6 μm, making *C. parvum* oocysts the most difficult of these parasites to physically filter from water (131, 236). The efficacy of the coagulation, sedimentation, and filtration processes for the removal of protozoan cysts and oocysts in water treatment systems is particularly important and ideally should be preceded by pretreatment methods such as those aimed at the reduction of disinfectant and disinfection by-product (DBP) levels (30). Unfortunately, conventional treatment processes are prone to vulnerabilities that affect their efficacy in the removal of protozoan cysts and oocysts (30, 358, 364; S. Li, Z. Ran, C. Cui, and Y. Yuan, presented at the International Conference on Computer Distributed Control and Intelligent Environmental Monitoring [CDCIEM], 2011). However, there is evidence that significant improvement in conventional water treatment standards has occurred in the past 20 years, with full conventional treatment effective at reducing the prevalence of oocysts by a factor of up to 114 (95% CI = 80.64 to 160.78) (64). A study in Spain found that approximately 40% of samples of the effluents from drinking water treatment plants contained *Cryptosporidium* oocysts and *Giardia* cysts after undergoing coagulation, flocculation, and clarification through sedimentation, filtration, and disinfection (chlorination) processes and that about 90% of the organisms in the drinking water remained viable after treatment (48). A U.S. study found that *Cryptosporidium* oocyst and *Giardia* cyst removal across conventional treatment was influenced by the initial pathogen concentrations and level of turbidity. The study found that the higher the initial amount of contamination, the larger was the amount of pathogen removal observed with coagulation by alum, and higher raw water turbidity appeared to result in a higher log removal level for both *Cryptosporidium* oocysts and *Giardia* cysts (20).

The U.S. Army recommends that improving flocculation mixing intensities and flow distribution throughout the water treatment plant will assist in the prevention of outbreaks of cryptosporidiosis in keeping with U.S. EPA standards (63). A study identified that polyaluminum chloride (PACl) is an alternative coagulant that improves floc formation and sedimentation, producing significantly less sludge (63). A pilot-scale test of the electrofiltration process in a drinking water treatment plant was demonstrated to be effective in the removal of waterborne particles of <4 μm and can be pursued further for large-scale use, with the added benefit of reducing or eliminating the need for coagulants and the subsequent residual/scum formation (245). Hence, careful selection of methods for the inactivation of protozoa in water treatments is necessary, as demonstrated by several studies (30, 358, 364; Li et al., presented at CDCIEM, 2011). However, significant removal of protozoan cysts or oocysts has been achieved during filtration processes as water passes through a porous medium or membrane, resulting in up to 1.7 to 3.6 $\log_{10}$ reductions of oocysts, depending on the filter medium used (75). It is therefore recommended that combinations of treatments and multiple-barrier approaches to optimize water treatment through a synergistic effect be employed (107, 226, 358, 364).

On a small scale, point-of-use (POU) water purification technologies (such as chlorination with safe storage, solar UV treatment, ceramic filtration, or biosand filtration [BSF]) (338, 380), employed in small communities or at the household level, are recommended for reducing risks of enteric infectious agents transmitted by drinking water, especially for pregnant women, institutionalized toddlers and elderly, and immunosuppressed populations (338, 436). On a larger scale, a combination of physical methods that affect survival or removal of protozoan parasites should be employed (67, 243). These include freezing, heating, filtration, sedimentation, UV light irradiation (67, 239), high pressure, and ultrasound (23). Some experts suggest that ozone is a more effective chemical disinfectant than chlorine or chlorine dioxide for inactivation of protozoan parasites in drinking and recreational waters (161, 469; Li et al., presented at CDCIEM, 2011). Biosand filtration has been used widely, with good effect at removing the common protozoa in developed countries and at low cost in developing countries (68, 358, 410, 436). HACCP principles have been applied in some countries as a means of providing potable water free of microbiological health hazards (166). Slow sand filtration, solar technology, and membrane technology are

effective methods for reducing pollution from rainwater to be used as a safe drinking water supply (185).

Sewage and wastewater by-products. The first step in the control of microbial risks from sewage and wastewater is the institution of proper legislation and guidelines for those involved. These should apply even at the household level, where sludge, including livestock wastes, may be added to compost (217), as well as to storage and treatment of such wastes (79). Studies have shown high concentrations of both *Cryptosporidium* oocysts and *Giardia* cysts detected in effluents from WWTPs after going through secondary and tertiary treatment (48). Due to the potential risks to public health, recycled wastewater should be approved for use only under circumstances where other options are technically or economically infeasible and only for industrial purposes using separated pipelines (67, 239).

Wastewater treatment reduces the number of pathogens in the wastewater by concentrating them with the solids in the sludge. Although some treatment processes are designed specifically to inactivate pathogens, many are not, and the actual mechanisms of microbial inactivation are not fully understood for all processes (155). One study evaluated six sewage sludge hygienization processes, including closed reactor and open windrow composting, and sludge sanitation by quicklime or peat addition. The study found that while these processes were effective in removing indicator bacteria (for example, fecal coliforms), cysts or oocysts of pathogenic protozoa survived the majority of these processes (339). The lime stabilization method has also been used successfully to destabilize fecal coliforms, *Salmonella*, adenovirus type 5, and rotavirus after only 2 h of treatment in biosolids. However, in this model, *Cryptosporidium* oocysts and *Ascaris lumbricoides* ova remained viable following 72 h of liming (28).

Several emerging methods for wastewater treatment involve membrane and filtration technologies. Pressure-driven membrane processes (microfiltration, ultrafiltration, nanofiltration, and reverse osmosis) are now commonly used (367, 447). Among these methods, ultrafiltration is quite efficient in the removal of suspended particles and colloids, turbidity, algae, bacteria, parasites, and viruses for clarification and disinfection purposes and, as such, can be used to replace several of these steps in the conventional treatment process (447). Another method is the use of membrane bioreactors (MBRs) that combine suspended biomass, similar to that in the conventional activated sludge process, with immersed microfiltration or ultrafiltration membranes that replace gravity sedimentation and clarify the wastewater effluent, producing high-quality effluent suitable for unrestricted irrigation and other industrial applications (364). Membrane technologies are prone to fouling caused by microbe-generated extracellular polymeric substances such as proteins and natural organic matter. Therefore, next-generation nonfouling membranes with much narrower pore size distributions than those derived from immersion precipitation, in addition to fouling-resistant surfaces, are needed for improved contaminant retention without intensive chemical treatment and while reducing the need for subsequent decontamination (364, 367).

Protozoan parasites form part of the microbial ecology in wastewater that is responsible for purification processes and improving the quality of the effluent, by maintaining the density of dispersed bacterial populations by predation (254). The persistence of these pathogenic parasites in biosolids emphasizes the need for guidelines and regulations to govern the use of biosolids (23). Bean et al. suggested that *Cryptosporidium* oocysts should be considered an indicator for evaluating biosolids intended for land application (28). Australia, the United States, and the European Union have developed regulations to control pathogen risk arising from land application of biosolids, based on the concept of multiple barriers to the prevention of transmission. The barriers are aimed at protecting human health, food quality, and the environment from potential microbial and chemical contaminants and include (i) treatment to reduce pathogen content and vector attraction, (ii) restrictions on crops grown on land to which biosolids have been applied, and (iii) minimum intervals following application and grazing or harvesting (104, 105, 112, 155, 464). The availability of multiple barriers, such as constructed barriers, catchment barriers, and dilution in the catchment area, reduces the risk of waterborne transmission of protozoa from sewage effluent (443).

Detection of Protozoa in Water Samples

Detection of protozoa in water samples is important for the monitoring of contaminants and for the prevention and control of parasitic infections from water. Their small size and dispersion in water and the difficulty in efficiently concentrating oocysts from environmental samples while excluding extraneous materials make it difficult to detect protozoa in water by traditional methods, with detection requiring well-trained and experienced personnel (45, 47). The biggest problem with identifying protozoa in water supplies is that cultivation from cysts or oocysts is difficult and it is almost impossible to concentrate oocysts from environmental samples while limiting the presence of extraneous materials. As a result, enteric bacteria are traditionally used as surrogate markers for fecal contamination of water sources (47). However, the development of new assays and methods for the purification and concentration of cysts or oocysts by the use of immunomagnetic separation (IMS) has addressed this problem (375). Currently, the U.S. EPA and other such bodies commonly use detection methods for isolation of protozoa, from either large (up to 1,000 liters) or small (10 to 50 liters) samples, involving four sequential steps: (i) the filtration of water, resulting in the cysts or oocysts and extraneous materials being retained on the filter; (ii) the elution and separation process, involving purification and concentration of cysts or oocysts by IMS and discarding of the extraneous material; (iii) staining with specific fluorescent antibodies (FA); and (iv) enumeration using fluorescence and differential interference contrast microscopy (45, 48, 106, 375). Other techniques that can also be used for cyst or oocyst purification purposes include density gradient, saturated-salt flotation, and continuous flow centrifugation, continuous flow filtration, and flow cytometry with cell sorting (45, 251, 299). Additional molecular methods are currently available for the identification of protozoa in water samples by detection of pathogen-specific genes or SSU rRNA (292, 375). For example, nested PCR-RFLP assays are used to target different loci (1 and 2) of the hypervariable region of the 18S rRNA gene for identification of different *Cryptosporidium* spp. in water (292). Other PCRs have been developed to amplify the COWP gene (9). Species of microsporidia have been identified in various water sources by use of Weber's chromotrope-based stain, with positive samples analyzed by PCR (200). Several authors suggest that molecular techniques are the most promising methods for the sensitive, accurate, and simultaneous detection of waterborne protozoa, with much benefit to the water industry and

public health (47, 101), compared with conventional staining and microscopy (205, 386, 387).

Another approach is the use of alternative bioindicators together with conventional fecal markers to identify the source of fecal pollution and associated pathogens in water (353). Water can become contaminated by point sources (raw sewage, storm water, wastewater effluent, and industrial sources) and non-point-source discharges (agriculture, forestry, wildlife, and urban runoff). The identification of the source of fecal contamination and pathogens could aid in the management and remediation of water resources when other control strategies are not feasible (353). However, some scientists suggest that fecal indicators do not adequately indicate the presence of all fecal pathogens in natural waters because their presence is affected by different environmental conditions (147, 241, 310). Since the cysts and oocysts of enteric protozoa are hardy and chlorine resistant and can survive for longer periods in water, these could be evaluated for use as alternative fecal indicators (310). The WHO suggests that the spores of the anaerobic bacteria *Clostridium perfringens* and *Bacillus* spp. can be used to evaluate the effectiveness of protozoan cyst or oocyst removal by sewage/water treatment, since they are highly resistant in the environment and their vegetative cells do not reproduce in aquatic sediments (18).

Evaluating the presence of fecal indicator organisms in water or the bioaccumulation of encysted protozoa in shellfish has value for identifying source attribution for surveillance and in outbreak settings (115). Bivalve shellfish have been used as bioindicators of fecal protozoan contamination in water bodies. For example, *Cryptosporidium* and *Giardia* in clams (*Corbicula fluminea*) have been detected by PCR and DFA assays (270) or IFA (148). When it is not possible or practical to test for individual protozoan parasites, it may be useful to use a combination of fecal indicator source tracking methods to enhance identification of contaminants of public health interest in surface waters (121, 270, 353).

CONCLUSIONS

Enteric protozoa continue to contribute to the burden from preventable infectious diseases affecting humans and animal health in industrialized settings. *Giardia intestinalis*, *Cryptosporidium* spp., and *Entamoeba* spp. are the most commonly reported protozoa associated with enteric infections and are associated mainly with food- and waterborne outbreaks. Others, such as *Cyclospora cayetanensis*, *Dientamoeba fragilis*, *Balantidium coli*, *Cystoisospora belli*, and *Blastocystis* spp., are emerging as important causes of illness, with serious implications for travelers to developing regions, immunocompromised populations, and young children. Although public health measures in most developed countries are more stringent than those in developing settings, minority groups, institutionalized persons, and the immunocompromised remain at extremely high risk, which can be extended to the rest of the population, and hence they should be considered a public health priority. Furthermore, the challenges of protozoan diseases transmitted by food, water, and animals are expected to increase as a result of complex interactions between human and animal hosts, fueled by the emerging effects of climate change and urbanization and the need to increase food production, reuse of gray water, and biosolids. Advancements in molecular diagnostics and the use of molecular epidemiology have the potential to identify previously undetected species and zoonotic serotypes that cause illnesses in both animals and humans and are useful for surveillance and outbreak control. Although there is much evidence for some protozoa as a cause of illness in humans and/or animals, there is still need for the development of better diagnostic methods for the rapid and sensitive detection of others. There is therefore a need for much more research into these issues to better understand the potential impact of climate change on the incidence and prevalence of parasitic diseases and how governments can act now to mitigate their effects. The management of protozoan infections in this complex environment, coupled with the projected effects of climate change, will require an increase in the allocation of research and development funding and a multidisciplinary approach.

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