Clinical Significance of Enteric Protozoa in the Immunosuppressed Human Population

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INTRODUCTION

Parasitic diseases continue to cause significant morbidity and mortality throughout the world irrespective of the patient’s immune status. It is estimated that there are approximately 340 parasite species capable of infecting humans, with the majority of the 3 billion people currently infected residing in developing regions of the world (99). Enteric protozoan parasites remain the most commonly encountered parasitic diseases and continue to cause significant morbidity and mortality.

Risk factors for acquisition of parasitic infections are the same in both immunocompetent (IC) and immunosuppressed (IS) individuals. What, then, is the role of the immune system in parasitic infections? Through local and systemic responses, the immune system plays an integral part in modifying the establishment of infection, controlling disease once it is established, limiting the severity and dissemination of the disease, and assisting in clearance or control of the parasite. Thus, IS hosts are more likely to acquire infection after exposure, have more severe disease once the infection is established, have disseminated infection rather than localized infection, and be unable to clear parasites with chronic carriage states. These all lead to, and account for, the greater morbidity and mortality in these patients. However, the majority of IS individuals do not differ from IC hosts in their presentation, with the major determinants of clinical severity and outcome of parasitic infection in IS patients being the degree of immune deficiency. Furthermore, with immune reconstitution through effective therapy or withdrawal of immunosuppressive agents, these patients are more likely to behave like IC hosts.

The number of IS individuals worldwide continues to increase each year as the human immunodeficiency virus (HIV) pandemic continues to spread unabated in many parts of the world, with an estimated 14,000 new infections occurring daily (19). What compounds this problem is that the majority of these new infections continue to occur in regions of the world where access to active therapy is limited and thus patients progress to profound immunosuppression (i.e., AIDS). In contrast, in more developed nations the numbers of IS individuals continue to increase as a result of medical interventions with more aggressive immunosuppressive therapies for immune-mediated disorders and hematopoietic and solid organ transplants, with approximately 1 million transplants performed annually (17).

A summary of studies reporting the worldwide prevalence of pathogenic enteric protozoa in HIV-infected persons is given in Table 1, and treatment options for enteric protozoal infections are listed in Table 2. Examples of stained enteric protozoa are shown in Fig. 1.

IMMUNITY

The immune system can simplistically be divided into three components, namely, nonspecific immunity (e.g., skin and other mucosal barriers), the innate immune system (soluble factors and cells), and the adaptive immune system. The innate system is limited to pattern recognition immune responses based on a diverse array of sensors (e.g., Toll-like receptors or complement) which detect invading pathogens. In contrast, the adaptive immune system is able to undergo maturation. This is represented by increased affinity antibody production (immunoglobulin G [IgG] as opposed to initial IgM antibodies) and cell-mediated
Immunity with T-cell memory. Most pathogens have developed ways of evading the protection provided by the immune system. Thus, infection and/or persistent infection/carriage is established by the interplay between the host (immune system) and the pathogen. In the IS patient, changes often occur in favor of the pathogen. The advantage to the pathogen depends on the component of the immune system that is defective. Any component of the immune system can be functionally or genetically abnormal as a result of acquired (e.g., through HIV infection, lymphomas, or high-dose steroids or other immune-suppressive medications) or congenital illnesses, with more than 120 congenital immunodeficiencies described to date that either affect humoral immunity or compromise T-cell function (19). Immunosuppression may also occur in malnourished persons, patients undergoing chemotherapy for malignancy, and those receiving immunosuppressive therapy. However, for parasitic infections, cell-mediated (T-cell) abnormalities predominate. These patients tend to have an increased risk of acquiring common pathogens (e.g., cryptosporidia) with delayed coccidian clearance. With profound cell-mediated defects, reactivation of previously controlled pathogens (e.g., toxoplasmas) can occur. In addition, these patients are at risk of infection by “nonpathogenic” parasites (those that do not cause disease in normal hosts). With reconstitution of the cell-mediated immunity, the risk of parasitic infections reverts to that for a normal host.

HIV infection is the most common immunodeficiency state worldwide, with the hallmark of infection being depletion of CD4+ T lymphocytes, essential components of the cell-mediated immune system. After acquisition of HIV through genital secretions, the virus infects local macrophages using the CD4 receptor to mediate cell entry. These cells are transported to local lymph nodes, and viral replication commences. This is followed 1 to 3 weeks later with a seroconversion syndrome similar to a glandular fever-like illness, characterized by high viral replication and a decline in CD4+ T-cell counts. Both cellular and humoral responses ensue, which reduce the viremia, and the CD4+ T-cell count reverts to normal. This is followed by an asymptomatic phase lasting from 1 to 15 years or longer, which is characterized by viral replication and variable CD4+ T-cell loss of approximately 25 to 60 cells/μL per year. Once the CD4+ T-cell count drops below 200 cells/μL, patients are considered to have developed AIDS, with the risk of an AIDS-defining illness or opportunistic infection significantly increasing.

Developing an infection with enteric protozoan parasites is dependent on absolute CD4+ T-cell counts, with lower counts associated with more severe disease, more atypical disease, and a greater risk of disseminated disease (16, 36, 207, 237, 280). In addition, at counts of less than 200 (i.e., profound immunosuppression), HIV-infected patients are at risk from specific opportunistic protozoan pathogens which are usually unable to establish infection in IC hosts.

### OPPORTUNISTIC INFECTIONS

Opportunistic infections are generally restricted to severely IS individuals and are considered AIDS-defining illnesses in HIV-infected patients, as they almost always occur when the CD4+ T-cell count falls below 200 cells/μL. Furthermore, with effective therapy and immune reconstitution, i.e., a rise of CD4+ T cells to above 200 cells/μL, the risk of these infections virtually disappears.

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**TABLE 1. Worldwide prevalence of pathogenic enteric protozoa in HIV-infected persons**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Organism</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>Apulia, southern Italy</td>
<td>Cryptosporidium parvum</td>
<td>21.54</td>
</tr>
<tr>
<td>60</td>
<td>France</td>
<td>Cryptosporidium sp.</td>
<td>37.3</td>
</tr>
<tr>
<td>82</td>
<td>Cuba</td>
<td>Cryptosporidium sp.</td>
<td>11.9</td>
</tr>
<tr>
<td>118</td>
<td>Seoul, South Korea</td>
<td>Cryptosporidium parvum</td>
<td>10.5</td>
</tr>
<tr>
<td>122</td>
<td>Ethiopia</td>
<td>Entamoeba histolytica</td>
<td>10.3</td>
</tr>
<tr>
<td>179</td>
<td>San Pedro Sula, Honduras</td>
<td>Entamoeba histolytica</td>
<td>5.8</td>
</tr>
<tr>
<td>192</td>
<td>Northern India</td>
<td>Entamoeba histolytica</td>
<td>7.7</td>
</tr>
<tr>
<td>229</td>
<td>Northern India</td>
<td>Entamoeba histolytica</td>
<td>7.7</td>
</tr>
<tr>
<td>240</td>
<td>Cameroon</td>
<td>Cryptosporidium</td>
<td>3.8</td>
</tr>
<tr>
<td>254</td>
<td>Sydney, Australia</td>
<td>Cryptosporidium parvum</td>
<td>3.2</td>
</tr>
<tr>
<td>289</td>
<td>Iran</td>
<td>Giardia lamblia</td>
<td>7.3</td>
</tr>
<tr>
<td>36</td>
<td>Uganda</td>
<td>Entamoeba histolytica</td>
<td>1.4</td>
</tr>
<tr>
<td>174</td>
<td>Guinea-Bissau</td>
<td>Cryptosporidium parvum</td>
<td>25</td>
</tr>
<tr>
<td>53</td>
<td>Brazil</td>
<td>Giardia lamblia</td>
<td>16</td>
</tr>
<tr>
<td>83</td>
<td>Los Angeles, CA</td>
<td>Cryptosporidium parvum</td>
<td>17.7</td>
</tr>
</tbody>
</table>
Cyclospora

*Cyclospora cayetanensis* is a coccidian protozoan most commonly found in developing regions of the world. However outbreaks of cyclosporiasis have been reported from North America (137, 138, 181) and Europe (76). The sources of infection in all these cases were traced to vegetables imported from areas of endemicity. Humans are the only known host for this parasite (99). Infection is via the fecal-oral route, though oocysts are not infective immediately after excretion in the feces and require sporulation in the environment to become infective.

Generally patients present with a rapid-onset, self-limiting diarrhea (178). With progressive immune suppression (CD4+ T cell counts of <200 cells/μL in HIV-infected individuals) prolonged carriage occurs, resulting in frequent severe relapses which may last from 4 to 7 weeks (11, 90, 139). These recurrences in turn result in severe malnutrition and significant morbidity and mortality in HIV-coinfected patients (201).

Episodes of diarrhea associated with *C. cayetanensis* infections have also been documented in other IS hosts, with cases of prolonged diarrhea in patients with compensated idiopathic hepatic cirrhosis, protein energy malnutrition, Hodgkin’s lym-

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**TABLE 2. Treatment options for infections with enteric protozoa**

<table>
<thead>
<tr>
<th>Intestinal parasite</th>
<th>Antimicrobial therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptosporidium</em></td>
<td>Nitazoxanide (500 mg twice a day for 14 days), albendazole (400 mg twice a day for 7–14 days), or paromomycin (500 mg four times a day for 7–14 days)</td>
</tr>
<tr>
<td><em>Cyclospora cayetanensis</em></td>
<td>Co-trimoxazole (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)</td>
</tr>
<tr>
<td><em>Dientamoeba fragilis</em></td>
<td>Iodoquinol (650 mg three time a day for 20 days), metronidazole (500–750 mg three time a day for 10 days), or paromomycin (25–35 mg/kg/day for 7 days) (271)</td>
</tr>
<tr>
<td>E. bieneusi</td>
<td>Albendazole (400 mg twice a day for 28 days) or fumagillin (20 mg three time a day for 14 days)</td>
</tr>
<tr>
<td>E. intestinalis</td>
<td>Albendazole (400 mg twice a day for 28 days)</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>Metronidazole (750–800 mg three times a day for 6–10 days) or tinidazole (2 g once daily for 10 days) followed by paromomycin (500 mg three times a day for 7 days)</td>
</tr>
<tr>
<td><em>Giardia intestinalis</em></td>
<td>Metronidazole (2 g daily for 3 days) or tinidazole (2-g single dose)</td>
</tr>
<tr>
<td><em>Isospora belli</em></td>
<td>Co-trimoxazole (160 mg trimethoprim plus 800 mg sulfamethoxazole, four times a day for 10 days) or ciprofloxacin (500 mg twice a day for 7 days)</td>
</tr>
</tbody>
</table>

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**FIG. 1.** Photomicrographs of enteric protozoa stained with a modified iron-hematoxylin stain (incorporating a carbol fuschin staining step). (A) *Cyclospora* oocysts; (B) *Cryptosporidium* oocysts; (C) *Dientamoeba fragilis* binucleated trophozoite; (D) *Dientamoeba fragilis* uninucleated trophozoite; (E) *Entamoeba histolytica* cysts; (F) *Entamoeba histolytica* trophozoite; (G) *Giardia* cysts; (H) *Giardia* trophozoites. Bars represent 10 μm.
phoma, and acute lymphoblastic leukemia being described (133, 215, 287).

Disseminated infections are uncommon, with several described cases involving the biliary tract in HIV-infected individuals (112, 291). Diagnosis was confirmed by histological confirmation of *C. cayetanensis* in the gallbladder epithelium following cholecystectomy for acalculous cholecystitis (291). Although *C. cayetanensis* oocysts have been observed in respiratory samples, it remains unclear whether these caused pulmonary disease, as no respiratory tissue samples were obtained (109).

Diagnosis of *Cyclospora* oocysts may be problematic, as most laboratories fail to recognize them in direct fecal smears. Special stains such as modified acid-fast auramine or modified iron-hematoxylin are usually required for definitive diagnosis. Other methods used are autofluorescence under UV epifluorescence (99) and DNA amplification by PCR (171). Histopathological examination of jejunal biopsy specimens from infected individuals showed mild to moderate acute inflammation of the lamina propria and surface epithelial disarray.

*Cyclosporiasis* in patients can be treated effectively with a 10-day course of trimethoprim-sulfamethoxazole. Maintenance therapy with either trimethoprim-sulfamethoxazole or sulfadoxine-pyrimethamine is required to prevent recurrent disease (275). Alternatives include nitazoxanide (a new thiazolide antiparasitic agent), combination therapy with both pyrimethamine and leucovorin, or ciprofloxacin (295).

Data on the immune response to *C. cayetanensis* and mechanisms of immunity to this pathogen and how these relate to various immune deficiencies are lacking. However, previous exposure to *Cyclospora* may confer some resistance against challenge infection (98).

**Cryptosporidium**

*Cryptosporidium* species have a worldwide distribution, and the ability to infect a large range of vertebrate hosts (208, 212, 223, 236, 263). *Cryptosporidium parvum* and *Cryptosporidium hominis* are the species most commonly associated with human cryptosporidiosis (149), though infections with other species such as *Cryptosporidium felis* and *Cryptosporidium meleagris* (121, 224) have been reported, particularly in IS patients (149, 198). Infection is via the fecal-oral route.

Studies from London and Nairobi demonstrate that cryptosporidiosis remains the most common opportunistic enteric protozoal disease encountered in IS HIV-infected patients (78, 203). This is supported by a recent prospective, comparative study comparing the prevalences of enteric protozoa among HIV-positive and -negative men in Australia (254). A total of 1,868 patients submitted stool specimens over a 36-month period for examination for the presence of enteric parasites. In this study *C. parvum* cases occurred exclusively in HIV-positive patients. Other IS groups at significant risk of severe cryptosporidiosis include those diagnosed with non-Hodgkin’s lymphoma, leukemia, lymphoproliferative disease, or protein energy malnutrition (51, 133, 205, 215). Patients receiving immunosuppressive drugs for organ transplants or cancers are also at risk of prolonged, potentially life-threatening persistent diarrhea (67, 140, 238), as are IS patients undergoing hemodialysis (244). *Cryptosporidium* infection with serious clinical symptoms has also been observed in IS patients with hyper-IgM syndrome and primary CD4 lymphopenia (286). *Cryptosporidium parvum* infections have also been reported in transplant patients undergoing immunosuppressive therapy. One case study reports *C. parvum*-related sclerosing cholangitis in a 40-year-old renal transplant patient. After reduction of immunosuppression, *C. parvum* was cleared from her stool, with a marked improvement in diarrhea and general health (1).

Patients that are not IS tend to present with a self-limiting diarrhea, which may last for several weeks to months even in IC individuals. The highest burden of disease occurs in children under 5 years of age (2). Infection in the IS individual is usually associated with chronic diarrhea and wasting and can be life threatening (2, 197, 203). Disseminated infection has been described, predominantly involving the biliary tract with cases of ascending cholangitis (24, 29, 49, 288). Other manifestations include pulmonary cryptosporidiosis (163, 218). These infections may occur in the absence of gastrointestinal involvement (218).

Laboratory diagnosis of cryptosporidiosis traditionally relies on special staining techniques, such as modified acid-fast auramine or modified iron-hematoxylin, and microscopy (99). Other alternative diagnostic techniques have also been employed. Several commercial companies have developed rapid diagnostic tests that are simple to perform and can be completed in less time than traditional methods for detecting *Cryptosporidium*; these rapid tests include lateral-flow immunoassays, immunochromatographic assays, and direct fluorescent-antibody tests (151). The use of enzyme-linked immunosorbent assay (ELISA) for the detection of *Cryptosporidium* antigen in stools has been described (147), and PCR assays have been developed for the specific detection of *Cryptosporidium* species in stools. A TaqMan PCR assay targeting the 18S ribosomal DNA (rDNA) has allowed sensitive detection of *Cryptosporidium* species (155). Various other PCR assays for the detection of *Cryptosporidium* species in stool specimens have also been developed (18, 107, 136, 293). While being more expensive and time-consuming, PCR and ELISA have shown superior sensitivity for the detection of *Cryptosporidium* species compared to conventional staining and microscopy (158, 199).

Based upon the paucity of evidence, the effectiveness of any therapeutic agent in the treatment of cryptosporidiosis has yet to be confirmed. However, the drugs paromomycin, azithromycin (218), and nitazoxanide (249) have been used to treat cryptosporidiosis in HIV-infected patients and have been shown to reduce the parasite load. Resolution of cryptosporidiosis can be maintained with effective highly active antiretroviral therapy (HAART) (191, 218, 242, 292). There is also evidence to suggest that some antiretroviral compounds used in HAART may have a direct inhibitory effect on *Cryptosporidium* (197).

Both humoral and cellular immune mechanisms appear to be necessary for protection against *Cryptosporidium*. The exacerbation of disease by immune suppression reflects the role of the immune system in controlling replication of the organism. IS patients with cryptosporidiosis fail to develop a significant serological response to the organism (184); in contrast, seroepidemiological studies have indicated that between 30 and 80% of IC patients will elicit an antibody...
response (26). Reports of chronic cryptosporidiosis in children with congenital Ig deficiency but intact cellular immunity highlight the importance of antibodies in the immune response against cryptosporidium (172). Murine models have shown that gamma interferon mediates an important protective innate response against Cryptosporidium infection (188). Studies suggest that CD4+ cells in the gut epithelium also play a role in controlling infection, along with Th1 and Th2 cytokine production (187, 267).

Isospora belli

Isospora belli is a coccidian parasite that has a global distribution limited to mainly tropical regions in developing countries where it is endemic (especially Africa, the Middle East, and South America). The parasite invades the intestinal epithelium, where it completes its life cycle in the cytoplasm of the enterocyte (234). Unsporulated oocysts are excreted in feces and mature outside the host, where they develop into infective sporulated oocysts. Infection is then acquired through ingestion of these infective oocysts. Immunodeficiency was shown to increase the susceptibility to infection with I. belli (117), which accounted for up to 20% of cases of diarrhea in AIDS patients (46, 65).

The parasite may cause acute self-limiting diarrhea, fever, and abdominal pain that usually resolves spontaneously in a normal host. In severely IS patients, severe chronic diarrhea is often reported and has been associated with fulminant diarrheal diarrhea leading to a wasting syndrome and sometimes death in AIDS patients (46).

Diarrhea associated with I. belli infections has also been reported in patients with other immunosuppressive diseases, such as lymphoblastic leukemia (148, 283), adult T-cell leukemia (115, 159), Hodgkin's disease (225), non-Hodgkin's lymphoma (234), lymphoproliferative disorders (159, 214), and renal transplant recipients (169), and in a liver transplant patient with chronic diarrhea (15). Patients with malabsorption syndrome, particularly malnourished children, are also at increased risk of chronic isosporiasis (21, 168).

As with other opportunistic protozoal pathogens, clinical manifestations are dependent on the balance between the immune system and the virulence of the organism, with chronic carriage states (“latency”) and clinically symptomatic reactivation during periods of severe immunodeficiency (152), as reported for a recent case of reactivation 8 years following acquisition (234).

Diagnosis is by direct visualization of the oocyst in feces; however, for some patients microscopic examination of stool samples may remain negative, even in cases of severe diarrhea (65) Therefore, a mucosal biopsy may be required for a definitive diagnosis. Detection of I. belli by PCR has been used as an additional diagnostic tool in clinical laboratories. Conventional PCR using primers based on 18S rDNA sequences shows excellent sensitivity and specificity (202). Recently, a real-time PCR targeting the internal transcribed spacer 2 region of the rRNA gene for the detection of Isospora belli DNA in fecal samples was developed. This real-time assay achieved 100% specificity and sensitivity (266).

Isospora infection responds promptly to antimicrobial therapy. Co-trimoxazole, sulfadiazine, and pyrimethamine have all been used successfully to treat isosporiasis (65, 219, 283). A number of randomized control trials conducted in areas of endemity have shown a very high efficacy of co-trimoxazole against I. belli (275), with a 7- to 10-day course usually being efficacious in treating the infection (234). Maintenance or secondary prophylaxis should be used to prevent relapses (275).

Once again, there are limited data in the scientific literature in regard to the mechanisms of immunity to this pathogen, and it is evident that more research is needed in this area.

Microsporidia

The term microsporidia is used as general nomenclature for the obligate intracellular parasites belonging to the phylum Microsporidia. Microsporidia were originally classified as protozoa, though it has been recently suggested that they are more closely related to fungi and as such should no longer be considered protozoa (135). However, as the precise relationship between the microsporidia and fungi is yet to be defined, microsporidia are included here as protozoa.

Thirteen hundred species of microsporidia belonging to 160 genera and infecting a wide range of vertebrate and invertebrate hosts have been described. Fourteen microsporidian species have been identified as human pathogens: Anncalia algerae, Anncalia connori, Anncalia vesicularum, Encephalitozoon cuniculi, Encephalitozoon hellem, Encephalitozoon intestinalis (syn. Septata intestinalis), Enterocytozoon bieneusi, Microsporidium ceylonensis, Microsporidium africanum, Noema oculatum, Pleistophora ronneafiei, Trachipleistophora hominis, Trachipleistophora anthropophthera, and Vittaforma cornea. Of these, E. bieneusi and E. intestinalis are the two most common causes of human enteric disease.

Microsporidia are recognized as opportunistic infectious agents worldwide in both developed and developing countries. Human microsporidiosis represents an important disease, occurring mainly but not exclusively in severely immunocompromised patients with AIDS. A recent study examined a total of 893 fecal specimens from hospitalized patients for microsporidia using a modification of the Gram-chromotrope stain technique. One hundred sixteen patients (13.0%) were positive for microsporidia, and approximately one-third of the patients were IC individuals. Microsporidiosis was commonly observed in children aged 0 to 6 years (26.4%) and adults aged ≥31 years (57.2%). Among the IS group, microsporidiosis were observed to be more prevalent in patients with hematological malignancy or a combination of malignancy and diabetes mellitus (210). What role microsporidia play in causing gastrointestinal symptoms in IC populations is unclear. However, another recent study found that microsporidia were more prevalent in IC individuals than in HIV-infected patients, with none of the IC group displaying gastrointestinal symptoms (209).

The mode of transmission for the various microsporidia remains obscure. It is accepted that infection occurs via the fecal-oral route for intestinal microsporidia such as E. bieneusi and E. intestinalis, as spores of these species are shed in the feces, but they can also often be shed in the urine from disseminated infections of the kidney. This is supported by the finding of E. bieneusi and E. intestinalis spores in recreational...
bathing water alongside oocysts of other enteric pathogens (94, 95, 114).

*Enterocytozoon bieneusi* is the most common microsporidian in humans (130) and the second most prevalent cause of diarrhea in IC patients, after *Cryptosporidium* (32). However, in countries with access to HAART, the prevalence of microsporidial infections has declined (105). In a study from Australia, van Hal et al. (273) found that the total incidence of intestinal microsporidiosis in HIV-infected patients declined from 11% in 1995 to 0% from 2004 onwards. These dramatic changes have been attributed to restoration of cell-mediated immunity and possibly secondary to the direct antiparasitic activity of some components of HAART (i.e., protease inhibitors) (228). In contrast, in developing countries with limited access to HAART, the incidence of microsporidiosis remains high (33).

The clinical manifestations of microsporidiosis are very diverse and vary according to the causal species, with diarrhea being the most common. In the case of *E. bieneusi* infection, the disease is usually confined to the gastrointestinal tract, though disseminated infection can occur. In HIV patients with *E. bieneusi* infection, chronic diarrhea associated with wasting syndromes and cachexia are common (32, 130). Dissemination to the hepatobiliary system with cholangitis (29, 226), to the maxillary sinus with invasive sinusitis (131, 272), and to the respiratory system with pulmonary infections (28, 66) have all been reported for *E. bieneusi/HIV* coinfections. Intestinal disease resulting from *E. bieneusi* infection has also been reported in heart-lung, liver, and renal transplant recipients undergoing immunosuppressive therapy (110, 116, 190, 231, 248).

*Encephalitozoon* spp. are the second most common microsporidian in humans and the most common cause of disseminated microsporidiosis (130, 153, 164, 268, 278, 279). There are three species within this genus that are known to cause human disease: *E. cuniculi*, *E. hellem*, and *E. intestinalis*. While *E. intestinalis* can infect most tissues, this species is usually associated with enteric disease (14, 81).

Traditionally, light microscopy based on modified trichrome stains (e.g., Ryan’s stain) has been used by most laboratories for the diagnosis of enteric microsporidiosis (70). Immunofluorescence tests using the chitin binding fluorochromes Uvitec 2B, Fungifluor, calcofluor white, and Fungigual A are also available and offer a sensitive and rapid method for detecting microsporidial spores in stool, intestinal fluid, biopsy imprint, and tissue specimens, even from archived material (5, 58, 87, 97, 200, 294) Molecularly based PCR assays (63, 77, 96) are also available for the diagnosis of *E. bieneusi* and *E. intestinalis* infection. The advantage of molecular testing over microscopic methods is that it allows for determination of the species of microsporidia. A comparison of PCR and an immunofluorescent-antibody test (IFAT) for the detection of *E. bieneusi* and *E. intestinalis* found that the two techniques had similar sensitivities, though IFAT was cheaper and more rapid (6). However, there are currently no commercially available or FDA-approved IFAT kits. Staining of tissue sections by the Warthin-Starry staining method was assessed as an effective diagnostic tool for the microscopic detection of microsporidia and demonstrated better diagnostic capabilities than the hematoxylin and eosin stain (88, 89).

Management of microsporidial infection most often includes oral treatment with the drug albendazole (23, 52, 85, 105, 193). Albendazole has demonstrated good antimicrosporidial activity against *Encephalitozoon* spp., particularly *E. hellem* (69, 71) (in vivo and in vitro), though it is only partially active against *E. bieneusi* (57, 73, 85, 105, 157, 162, 193). The drug nitazoxanide has demonstrated activity against *E. bieneusi*, with the symptoms of one patient receiving nitazoxanide therapy resolving in the absence of antiretroviral therapy (22). The drug fumagillin has also demonstrated good anti- *Enterocytozoon bieneusi* activity (40, 57, 195), although various adverse side effects have been documented (56, 141, 194, 195). The drug furazolidone has also demonstrated a degree of anti- *Enterocytozoon* activity (74). As with most opportunistic infections, HAART plays a key role in eradicating microsporidia in HIV-infected patients (105), and effective HAART is likely to reduce the incidence of microsporidial infections in the future.

Cell-mediated immunity appears to be critical for protection against the microsporidia. An effective Th1 cytokine response is important in the immune response to infection. However, the role of humoral immune responses in human microsporidial infections is yet to be fully elucidated (72).

**OTHER PATHOGENIC ENTERIC PARASITES**

Infections with the following pathogenic enteric parasites generally occur in both IC and IS patients. Although greater rates of carriage of some of these pathogens (i.e., *Entamoeba histolytica*) are associated with HIV-infected patients, this probably reflects the higher opportunity acquisition risk secondary to various sexual practices rather than the immune deficiency per se.

**Blastocystis spp.**

*Blastocystis* spp. are enteric unicellular parasites that are the most frequently reported parasite in human fecal samples (261). Although traditionally classified as a protozoan parasite, recently, due to new molecular techniques, *Blastocystis* has been shown to be closely related to the stramenopiles. However, due to it being nonmotile and not possessing flagella, in contrast to other stramenopiles, it has been placed in a new class, class Blastocystea, in the subphylum Opalinata, infra-kingdom Heterokonta, subkingdom Chromobiota, kingdom Chromista (45).

*Blastocystis* exhibits extensive genetic diversity, and this has been documented using numerous molecular techniques. Currently there are at least nine subtypes (genotypes) within *Blastocystis*. Recent studies have shown that no group exclusive to humans exists and that all clades have been detected in human stool (222). Consequently, human isolates of *Blastocystis* that were commonly referred to as *Blastocystis hominis* should be called *Blastocystis* spp. due to there not being a single subtype specific to humans.

Laboratory diagnosis of *Blastocystis* relies on several methods for identification, including light microscopy of fresh samples from wet preparations and concentrates, permanently stained fixed fecal smears, culture, and molecular techniques (55, 260, 261).

There is still much controversy surrounding *Blastocystis* and its pathogenicity in humans. There are conflicting reports in the scientific literature, where many authors view *Blastocystis*...
as a pathogen, (10, 41, 176, 177) while many other reports doubt the role of *Blastocystis* in human infection (175, 270). The most common symptoms associated with this parasite are diarrhea, abdominal pain, and vomiting. As no recognized animal model exists for *Blastocystis*, Koch’s postulates are un-able to be fulfilled in order to confirm or exclude the pathogenic nature of this organism. While the organism has a global distribution, the prevalence is higher in developing countries (261). Acquisition of the organism is thought to occur as a result of frequent animal-human, human-human, and human-animal transmission, with reports of carriage of *Blastocystis* in mammals, birds, amphibians, and even insects (264, 265).

What role *Blastocystis* plays in gastrointestinal disease in IS hosts is also unclear. There have been several studies of the prevalence of intestinal parasites in HIV-infected patients, with most finding higher rates of *Blastocystis* carriage. Several studies from Africa showed *Blastocystis* infection to be at a higher rate in HIV-positive patients than in negative controls. A study in Senegal found *Blastocystis* only in HIV-infected patients, with all but one suffering from diarrhea and with no other pathogens found in the samples. This study suggested that *Blastocystis* may be considered an opportunistic parasite (104). Another African study in an Ethiopian teaching hospital found there to be an incidence of *Blastocystis* infection of 14.1% in HIV/AIDS patients. There were no statistically significant differences in the prevalence of parasites among cases and controls except for *Blastocystis*, which was significantly higher in HIV/AIDS patients, which shows that it may be a possible pathogen in immunocompromised patients (122). A study in Iran showed that the occurrence of parasites in HIV-infected patients was not as high as seen in African countries, with an infection rate of only 18.4%. Of the parasites seen in that study, though, *Blastocystis* was the second most prevalent at 4.4%, with most of these cases being seen in patients with diarrhea (289). There are also insufficient data on the immunology of the parasite and the interactions with IS hosts.

**Dientamoeba fragilis**

*Dientamoeba fragilis* is a trichomonad parasite. Humans are probably the definitive host of this parasite even though *D. fragilis* trophozoites have been reported in nonhuman primates, including macaques (132, 167), baboons (204), and gorillas (255).

There is little understanding of the pathogenesis and pathology resulting from *D. fragilis* infection, and understanding is further hampered by the lack of a suitable animal model (75, 161, 166). However, acute and chronic diarrhea with associated abdominal pain has been documented in IC children and adults (61, 253, 285).

The impact of immune deficiency on *D. fragilis* infection and disease remains unclear. A study from Argentina found higher rates of *D. fragilis* infections in IS patients, in contrast to an Australian study, which found no difference in carriage rates in HIV-infected patients and the general population (189, 254). Clearly, further study of all aspects of this infection and in all patient groups is warranted.

Diagnosis of *D. fragilis* is based on prompt fixation and permanent staining, as trophozoites degenerate within hours of being passed and demonstration of the characteristic nuclear structure is achieved by use of permanently stained preparations only (284). However, as many laboratories do not routinely perform permanent staining, the incidence of *D. fragilis* is likely to be underestimated. Newer molecular techniques, such as conventional and real-time PCR, targeting the 18S rDNA have been developed (251–253, 257).

Multiple differing antimicrobial agents have been used for treatment of *D. fragilis* with varying success. These include doxycycline, iodoquinol, metronidazole, and seccnidazole (25, 108, 150, 230). However, there is no general consensus on what is best practice in treating *D. fragilis* infections. There are also no data available on the mechanisms of immunity for this parasite.

**Entamoeba histolytica**

*Entamoeba histolytica* is a nonflagellated amoeboid protozoan parasite. The genus *Entamoeba* includes six species (*E. histolytica, Entamoeba dispar, Entamoeba moshkovskii, Entamoeba polecki, Entamoeba coli, and Entamoeba hartmanni*) that are capable of infecting the intestinal lumen of humans. All these species are considered commensal organisms and rarely (if ever) cause intestinal disease in humans, with the exception of the pathogenic *E. histolytica*, for which humans are the primary reservoir (258). *Entamoeba histolytica* is an invasive pathogen and the causative agent of amoebiasis, with approximately 50 million cases acquired annually in the developing world (274). What role *E. moshkovskii* plays in human infections is yet to be adequately defined, most likely in part due to diagnostic confusion with *E. histolytica* and *E. dispar*. Although previous studies have not shown any association between *E. moshkovskii* in clinical samples and disease (54), recent studies have reported *E. moshkovskii* as an enteropathogen in patients presenting with gastrointestinal symptoms (92, 93, 221). There have also been no adequate studies examining the pathogenic potential of this organism in IS groups, and it is clear that further study is needed to assess the true pathogenicity of this organism.

The factors that control the pathogenesis of *E. histolytica* are not completely understood. However, key features are the ability of the organism to lyse host cells and cause tissue destruction, with induced immune responses occurring in invasive disease (3). *Entamoeba histolytica* acquisition is via the fecal-oral route. In confined populations (e.g., men who have sex with men [MSM] visiting sex-on-premises venues), carriage rates are significantly higher than in the general population once the organism has been established secondary to various oral-anal sexual practices (173, 254, 274). As the risks for HIV acquisition and parasite acquisition in these environments overlap, apparent associations between *E. histolytica* and immunosuppression exist. However, in an Australian study Entamoeba infection was found to be more prevalent in HIV-negative than in HIV-positive MSM with diarrhea. In the United States *E. histolytica* rates in AIDS patients remain low, with amoebiasis being diagnosed more frequently in individuals exposed to HIV through male-male intercourse (182). Thus, it is probable that HIV-positive individuals are no more susceptible to gastrointestinal symptoms than HIV-negative individuals.

Following an incubation period that can vary greatly, rang-
ing from days to years, individuals infected with *E. histolytica* present with symptoms of abdominal pain, tenderness, and diarrhea with >10 bowel movements/day, corresponding with colitis and ulcerative disease on histopathological examination. Bowel complications occur in 1 to 4% of patients. Invasive or extraintestinal disease is uncommon and may be present in 0.1 to 1% of symptomatic patients, with the liver being the most common site involved (>50%) (259). The lungs are the second most common site of invasive amoebiasis (245). Invasive amoebiasis involving the heart (59, 111, 245), brain (20, 143, 277), and genitourinary tract (38, 183, 217) has also been reported. Generally, when clinical symptoms develop they are limited to the gastrointestinal tract. However, the likelihood of developing invasive amoebiasis is increased in the presence of HIV infection (144–146, 245), with higher rates of amoebic colitis (144, 213) and amoebic liver abscesses (35, 142, 160) reported. Furthermore, seroprevalence studies, which are considered a sensitive marker of previous invasion, suggest that immunosuppression increases the risk of disseminated disease, with significantly higher rates of antibody positivity in HIV-positive patients (50, 269).

*Entamoeba dispar*, the usually free-living (occasionally pathogenic) *Entamoeba moshkovskii*, and *E. histolytica* are morphologically identical, though genetic differences have confirmed the separation of these three as independent species (62, 68, 250). Due to this conserved morphology, stained smears of stool specimens are insufficient for differentiation of these species. Staining of fixed fecal smears with iron-hematoxylin or Ziehl-Neelsen stain (47, 154) can determine the presence of the *Entamoeba histolytica/E. dispar/E. moshkovskii* complex within a stool specimen, while other techniques such as PCR or ELISA must be employed for differentiation. A number of PCR assays are available for detection and/or differentiation of *Entamoeba* species (62, 127, 239). A unique approach to diagnosis of *E. histolytica* infection is described by Britten et al. (37), which makes use of DNA probes conjugated to an antibody-detectable protein tag. Immunological assays are also useful and often employed. Commercial antigen capture and antibody-detecting ELISA kits are also available (34, 128, 129, 165), along with rapid lateral-flow cartridge tests (101). While some authors suggest that the commercial antigen capture ELISA kits are preferable to the use of PCR due to the rapidity and simplicity of these kits (84), other authors suggest that PCR is more useful than antigen capture ELISA for detection of *E. histolytica* in stools due to the higher sensitivities observed in PCR and the reduced chance of cross-reactivity with other *Entamoeba* species (256).

A number of different serological assays for the detection of *E. histolytica* antibodies are commercially available. Indirect hemagglutination assays, latex agglutination assays, complement fixation assays, indirect immunofluorescence assays, and ELISAs have all been developed (91). Serological testing may be helpful from a diagnostic perspective in certain situations. In patients suspected of having extraintestinal disease, serological tests are warranted; however, testing in patients with intestinal disease is normally not recommended. The serological tests are specific but have varying sensitivity depending on the presence or absence of invasive disease and the type of invasive disease. The sensitivity for serology is 100% in patients with amoebic liver abscess and 82% in those with invasive intestinal disease (91). Antibody testing to diagnose carriage or noninvasive disease is unhelpful, as the sensitivity is low at around 8% (91). Serum IgG antibodies persist for years, and antibody titers can remain high for years after successful therapy and/or eradication of the organism. This limits the usefulness of the test in areas where infection is endemic and people have been exposed to *E. histolytica*, due to the inability of serological tests to distinguish past from current infection (91). However, serological tests may be helpful from a diagnostic perspective in industrialized countries, where infections and exposure to *E. histolytica* are uncommon.

Diagnosis of invasive disease is usually based on patient symptoms (e.g., localized pain) followed by radiographic studies to identify the presence of lesions or organomegaly (245). To identify *E. histolytica* as the causative agent of a tissue abscess or lesion, PCR of tissue aspirates or identification of *E. histolytica* antigens in tissue aspirates is required (4, 220). A possible prediction of invasive disease can be made based on the level of Ig subtypes present in the circulation. One study noted that in the event of invasive disease, anti-*E. histolytica* IgG and IgA levels were comparatively high, while much lower anti-*E. histolytica* IgM and IgE antibody levels were detected (246).

Drug treatments for amoebiasis include paromomycin, diloxanide furoate, and iodoquinol. These drugs are all effective at treating luminal amoebiasis, although they are ineffective against invasive amoebiasis (85). Nitroimidazole derivatives such as metronidazole, secnidazole, tinidazole, and ornidazole are effective for treatment of invasive amoebiasis though less effective against luminal disease (85, 119). For treatment of luminal and extraintestinal amoebiasis, Farthing (85) suggests a 5-day course of metronidazole plus a 10-day course of diloxanide furoate or a 7- to 10-day course of paromomycin for the treatment of invasive amoebiasis.

Innate immune responses are the initial mechanisms that are responsible for limiting *E. histolytica* infection and invasive disease. *Entamoeba histolytica* has been shown to evade complement-mediated lysis (232), and extracellular cytotoxic proteases of *E. histolytica* are capable of degrading the complement anaphylatoxins C3a and C5a (233). Intestinal epithelial cells have been shown to initiate an inflammatory response to *E. histolytica* infection, and nuclear factor κB and proinflammatory cytokines also play a significant role in host defense (243).

**Giardia intestinalis**

*Giardia intestinalis* (synonyms, “*Giardia lamblia*” and “*Giardia duodenalis*”) is a common and ubiquitous flagellated protozoan parasite with a worldwide distribution. *Giardia* species are parasites of mammals and other animals, including reptiles and birds (7, 42–44, 123–126). Humans become infected by ingestion of cysts, which develop into trophozoites after excystation. Infections occur in both developed and developing regions of the world (241).

An Australian study found that HIV-infected patients were as likely to have *Giardia* as HIV-negative MSM (254). In this study, 3% of HIV-negative MSM were infected, compared to 4.5% of HIV-positive MSM and 1.5% of the general popula-
tion. This suggests that, as with other enteric parasites, sexual practices lead to higher *Giardia* carriage rates (83, 196).

In developing countries the burden of disease remains high in IC individuals, especially in children less than 10 years of age, with *Giardia* considered normal gut flora in children in developing countries (134).

After acquisition, approximately 50% of people clear *Giardia* without any untoward effects, 5 to 15% of people shed cysts asymptomatically, and the remainder develop an acute and/or chronic infection (7, 241). The acute infection lasts for days to weeks and is accompanied by nausea and the sudden onset of explosive, watery, foul-smelling diarrhea. The acute phase is often followed by a subacute or chronic phase. Chronic symptoms may last for years and be continuous, intermittent, sporadic, or recurrent, with episodes of diarrhea or loose stools. Between bouts of diarrhea the patient may have normal stools. However, abdominal discomfort may be continuous and independent of the alteration in bowel habits (271).

Symptoms of giardiasis in HIV-infected individuals appear to be similar to, and no more severe than, those of giardiasis in HIV-negative individuals, with asymptomatic infection occurring commonly in the presence of HIV (39, 196). With progressive immunosuppression following reduced CD4+ counts, the risk of symptomatic *Giardia* infections is increased (12, 79). Despite this, giardiasis is not considered a major cause of enteritis in HIV-infected patients (12, 196).

Although there is little in the scientific literature in regard to *Giardia* infections in IS individuals, a number of studies have shown that *Giardia* is more prevalent in the stools of hypogammaglobulinemic patients than in those of IC hosts (86, 235). It has also been shown that the majority (>90%) of hypogammaglobulinemic patients passing *Giardia* cysts are symptomatic, with chronic diarrhea (30). Symptomatic giardiasis has been observed in X-linked infantile congenital hypogammaglobulinemia (Bruton’s syndrome) and also in the common variable (late-onset) acquired hypogammaglobulinemia (30). IS patients, including nephrotic syndrome children receiving corticosteroids, protein-calorie malnutrition patients, patients with cases of marasmic kwashiorkor, and lymphoma patients, were found to be significantly more at risk for *Giardia* infection (211).

Enzyme immunoassays, immunochromatographic assays, and direct-fluorescence assays for detection of *G. intestinalis* in stool have been available in the form of commercial kits for several years (113, 216, 282). These kits are commonly used in diagnostic laboratories. Compared to microscopy, the coproantigen assays are less time-consuming and easier to perform. However, conflicting data in the exist in the scientific literature in regard to the performance of these rapid assays. Some researchers have reported excellent sensitivity and specificity (48, 100–102), while others have reported the rapid tests to be generally less sensitive than conventional microscopic methods (216, 282). A number of PCR assays are also available for the detection of *Giardia* in stool specimens (8, 106, 120, 276). *Giardia* infection can also be diagnosed microscopically by identification of cysts and trophozoites in stained or unstained fecal smears. *Giardia* cysts and trophozoites have a unique morphology, different from that of most other protozoa, and thus can be identified by trained lab staff in a simple wet preparation of the fecal specimen.

*Giardia* infection can be treated with metronidazole, quinacrine, furazolidone, paromomycin, tinidazole, nitrozoxamide, and ornidazole (13, 85, 103, 196). Albendazole has also demonstrated good anti-giardial activity and therapeutic efficacy (103). Gupta et al. (119) suggest that metronidazole is the drug of choice for treating giardiasis and advise a dosage of 15 mg/kg/day in three divided doses for 5 days or 2 g daily for 3 days.

Antigiardial host defenses are B-cell dependent, with secretory IgA antibodies playing an important role (80). T cells also appear to be important in the intestinal elimination of the organism. However, patients with marked T-cell deficiencies do not exhibit an increased susceptibility to giardiasis (281).

**CONCLUSIONS**

Gastrointestinal disease, such as diarrhea, abdominal pain, and irritable bowel syndrome, are common in the world’s population. For example, an estimated 1.6 to 2.5 million deaths of children under 5 years old are caused by diarrhea globally (170, 180), with approximately 73% of these deaths being in just 15 developing countries (27). In addition, diarrhea is also a major cause of morbidity and death in HIV-infected individuals (227). In coming years there is likely to be an increase in the number of HIV/AIDS deaths, with worrying projections of 6.5 million deaths in 2030 and HIV/AIDS being the main burden of disease in some developing countries by 2015 (185). In developed countries the number of IS individuals continue to increase each year with more patients undergoing hematopoietic and solid organ transplants and more aggressive treatments instigated with immunosuppressive therapies. Furthermore, although diarrheal diseases may be declining due to improvements in treatment, the total population of people continues to grow, leaving diarrheal diseases remaining as a major problem, especially in the developing world. It is thus likely that the clinical significance of enteric protozoa in the IS population will continue to grow globally.

Each year, many studies of infectious diseases and their interaction with the immune system are published. Central to most of this research is a knockout mouse, which has assumed an important role in studies investigating how the immune system functions not only in response to microbes but also in other disease conditions (186, 247). Indeed, advances in our understanding of the mechanisms by which pathogens cause diarrhea have been greatly enhanced by the availability of immune-suppressed mice generated by knockout technologies (206). Unfortunately, our knowledge of the human immune system and the way it interacts with knockout technologies is much more limited, despite the recognition that enteric parasites are commonly associated with the onset of diarrhea in IS patients. CD4 counts play an incredibly important role in the presentation of diarrhea as well as in the control of protozoa in HIV-infected individuals. For example, chronic diarrhea is typically associated with lower CD4 counts than acute diarrhea. In addition, at counts of less than 200, HIV-infected patients are at risk from specific opportunistic protozoan pathogens which are usually unable to establish infection in IC hosts (207, 280).

Advances in the diagnosis of infectious diseases occur regularly, although the first form of diagnosis of parasite infections is still by light microscopy of stool by an experienced
microscopist. Commercially available fecal immunoassays now not only are widely available for the majority of enteric protozoa but also provide a cost-effective alternative to traditional diagnosis of protozoan enteric parasites. These kits are rapid and easy to use and are far more practical for most laboratories than PCR assays. A confirmatory test such as PCR may be run subsequently. Ironically, even in today’s age of modern technology being available in the developed world, the cause of gastroenteritis may still go undiagnosed in about 50% of cases (9). Even in IC individuals, the presence of parasites, including *D. fragilis*, is normally overlooked in cases of diarrhea, as the provision of appropriate technology in diagnostic laboratories is not yet commonplace or routine. Nine parasitic protozoan species or genera (*B. hominis*, *Cryptosporidium spp.*, *Cyclospora spp.*, *D. fragilis*, *E. histolytica*, *E. bieneusi*, *E. intestinalis*, *G. intestinalis*, and *I. belli*) are recognized as a cause of gastrointestinal disease (primarily diarrhea), with three additional species (*E. dispar*, *E. moshkovskii*, and *E. polecki*) warranting further investigation into their role as pathogens of the human gastrointestinal tract.

Several of the parasite species discussed here are zoonotic, and animals may be the reservoir and source of the parasitic species. Exposure to pets and other animals is a recognized risk factor for acquisition of enteric parasites causing diarrhea (79). Infection is likely to be acquired via ingestion (fecal-oral transmission), with contaminated food or water representing the likely vehicle of transmission (64, 156). In at-risk groups, sexual practices may also represent a contributor to the transmission of parasites (254).

REFERENCES


290. Reference deleted.


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