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Chapter

Immune Dysfunction during Enteric Protozoal Infection: The Current Trends

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Abstract

Enteric protozoa usually cause severe morbidity and mortality in humans. Protozoal infections contribute to the high burden of infectious diseases. Despite recent advances in the epidemiology, diagnostic tool, molecular biology, and treatment of protozoan illnesses, gaps in knowledge still exist; hence, protozoal infections require further research. We are describing here some important enteric protozoal infections along with the immune dysfunction produced by them. Genus-1. Entamoeba; 2. Giardia; 3. Cryptosporidium; 4. Cyclospora; 5. Cystoisospora; 6. Dientamoeba; 7. Blastocystis; 8. Balantidium.

Keywords: parasite, protozoa, trophozoites, cysts, molecular characterization, laboratory diagnosis, treatment, vaccine

1. Introduction

Parasites are living organisms, which live in or upon another organism and derive nutrients directly from it, without giving any benefit to host. There are three main classes of parasites: protozoa, helminthes, and ectoparasites.

In the current chapter, we are going to discuss about pathogenic protozoan parasites responsible for intestinal infections and the immune system disturbance produced by them. Protozoa are unicellular organisms classified as eukaryotes. Protozoa responsible for intestinal infections are *Entamoeba*, *Giardia*, *Cryptosporidium*, *Cyclospora*, *Cystoisospora*, *Dientamoeba*, *Blastocystis*, and *Balantidium*. These enteric protozoa are associated with diarrheal illnesses in humans, with some causing severe debilitating illnesses, especially in immunosuppressed populations [1].

Enteric protozoa have been given much attention in developing countries, because of their poor sanitary conditions and the unavailability of effective water treatment, which provide a suitable environment for their transmission [2–4]. In more industrialized settings, less focus has been placed on the impact of protozoal infection, presumably because of better health standards. As a result of underdiagnosis and lack of monitoring programs, reliable data are not available for the estimation of the protozoal disease burden in developed settings [5]. The major focus in developed countries is on bacterial and viral infections; hence, in operational surveillance systems, only a few or no parasitic protozoa are included [6, 7]. However, evidence suggests that some protozoa, like *Entamoeba* spp., *Cryptosporidium*, and

Giardia, are isolated frequently from diarrheal patients in developing countries (8). Others, such as *Blastocystis* spp. and *Dientamoeba fragilis*, are isolated mainly in developed countries [8, 9]. Despite the lower prevalence of parasitic diseases in developed countries, they may result in a greater economic burden on the country.

Many protozoal diseases appearing and reappearing in developed countries presented as a public health problem in developing countries long before. The factors that influence the emergence and reemergence of the protozoal disease are similar to other diseases like change in the parasite or host that favor increased transmission, environment, and demographic change that favor increased human-parasite contact and increased the recognition of previously existing problem due to availability of more advanced diagnostic techniques.

There is lack of sensitive diagnostic techniques to detect parasite in clinical specimens, and hence, the estimation of parasite prevalence is difficult, and the carrier stages and subclinical infections of parasites are often not diagnosed [10]. So the development of technologies that can simultaneously detect several protozoa in stool is the current need of the hour [11, 12]. For the sensitive, accurate, and simultaneous detection of protozoan parasites, the molecular techniques are the most promising methods in comparison to conventional staining and microscopy methods [12]. Unfortunately, the molecular methods are not used routinely for the detection of parasitic protozoa because these are costly and labor-intensive [13]. Much effort must now be placed in the development of inexpensive molecular tools for routine laboratory applications.

2. Enteric protozoal infections

The enteric protozoa that are considered as a major public health problem are *Entamoeba histolytica*, *Giardia intestinalis*, *Cryptosporidium* spp., *Dientamoeba fragilis*, *Cyclospora cayetanensis*, *Blastocystis* spp., *Cystoisospora belli*, and *Balantidium coli*.

2.1 *Entamoeba* species

2.1.1 Introduction

Genus *Entamoeba* is divided into six species that have been described in humans, including *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, *Entamoeba polecki*, *Entamoeba coli*, and *Entamoeba hartmanni*. Among these, *E. histolytica* is the only pathogenic species [10, 14, 15]. Infection of *E. histolytica* is mainly seen in immigrants from or travelers to areas of endemicity, male having sex with male (MSM), HIV-infected patients, and institutionalized populations [16]. Millions of people are infected with *E. histolytica,* and more than 55,000 people die each year globally due to amoebic colitis, a leading cause of diarrhea [17].

2.1.2 Epidemiology

Globally, the highest burden of amebiasis is seen in tropical and subtropical areas of developing countries because of their improper hygiene and water sanitation. It was estimated that *E. histolytica* affect millions of people and responsible for the death of more than 50,000 people each year globally [1]. Amebiasis is also seen in the developed world, mostly in returning travelers or immigrants from endemic countries [18].

It has been noticed that the prevalence of *E. histolytica/dispar* has increased to 36.6% in developed world from 2000 to 2015 [19] in comparison to 1986 in which it was 20% as per Boston report [20].

2.1.3 Pathogenesis

Transmission—Amebiasis is acquired by the ingestion of cyst via the fecal-oral route, but now it is found that fecal contact can occur during sexual contact also (MSM–male having sex with men).

Life cycle—Following ingestion cyst converts into trophozoites known as invasive form of *Entamoeba histolytica.* In asymptomatic individuals, cysts and trophozoites pass into the stool without invading intestinal mucosa, whereas in a few cases, it invade intestinal mucosa and lead to invasive manifestations of *Entamoeba histolytica* like dysentery, amoebic colitis, and ameboma. After invading intestinal mucosa, trophozoites can travel to other organ via bloodstream and manifest as brain abscess and liver abscess [21, 22]. Males are more commonly affected from this invasive disease than females (**Figure 1**).

Pathophysiology—The virulence factors of *E. histolytica* are its enzymes and proteases that help in the invasion of the epithelial cells, penetrating the intestinal mucosa and degradation of the extracellular matrix proteins [23, 24]. More recently it is thought that some individuals are genetically resistant to infection, while malnourished children are more susceptible, and a polymorphism in the **leptin receptor** is found to be associated with increased susceptibility to amebiasis [24, 25].

2.1.4 Immune dysfunction

In some cases, *E. histolytica* colonizes the colon by high-affinity binding to MUC2 mucin without producing any symptoms, whereas in some it causes an

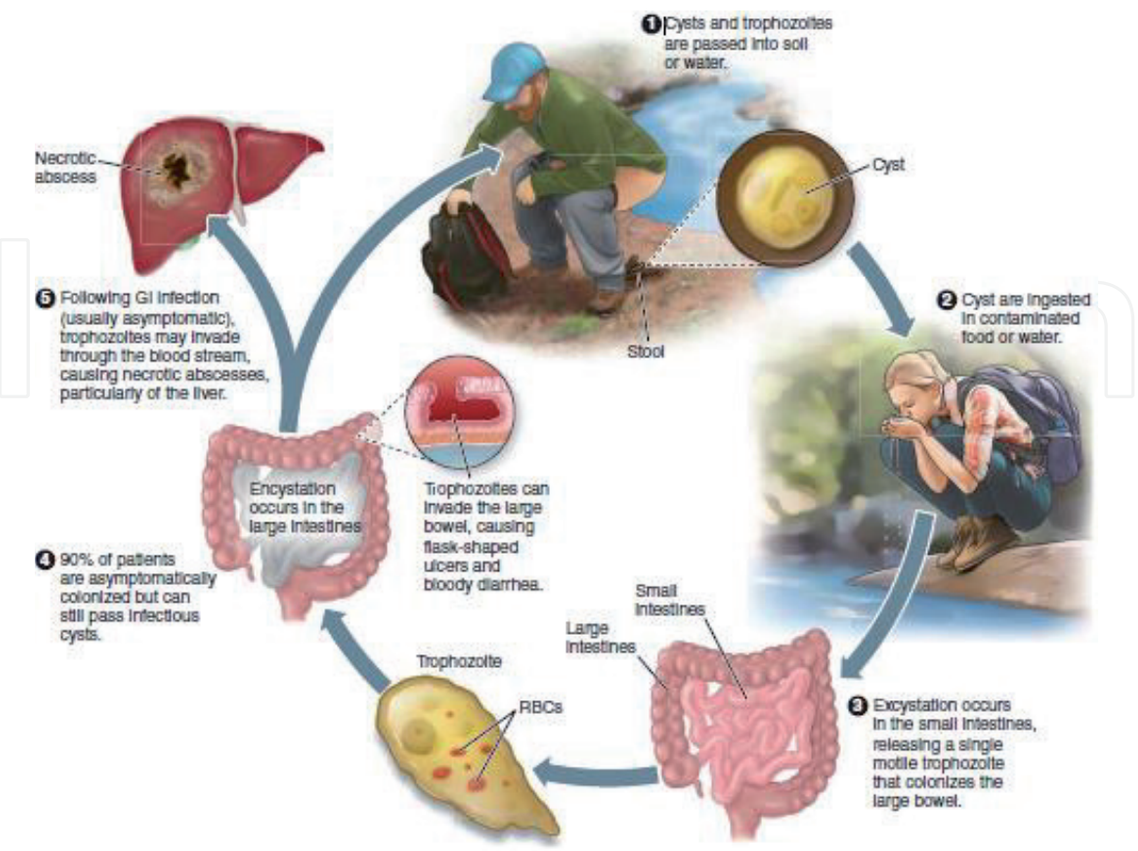

Figure 1.
Life cycle of Entamoeba histolytica*. Courtesy: Ref. [110], Fig. 218–1.*

aggressive inflammatory response upon invasion of the colonic mucosa. The parasite has cysteine protease that cleaves C-terminus of MUC2 that dissolves the mucus layer followed by binding of *E. histolytica* to the mucosal epithelium. As a result, the host mounts a pro-inflammatory response that causes tissue damage and participates in disease pathogenesis. *E. histolytica* escapes the host immune system by mechanisms that are not completely understood. The parasite can destroy effector immune cells by inducing neutrophil apoptosis and suppressing respiratory burst. It also suppresses the production of nitric oxide (NO) from macrophages. Following the adherence of *E. histolytica* to the host cells, multiple cytotoxic effects occur that can promote cell death through phagocytosis and apoptosis, which might play critical roles in immune evasion [26].

2.1.5 Diagnosis

For the early diagnosis of invasive and extraintestinal amebiasis, a high level of clinical suspicion is necessary [27].

Morphological similarities of the pathogenic species *E. histolytica* to the non-pathogenic species *E. dispar* and *E. moshkovskii* make the diagnosis difficult. Hence, microscopy is usually considered insufficient for the differentiation of these species [28]. In microscopic techniques, trichrome-stained smears and wet mount preparations of stool specimens are used in routine for the identification of *Entamoeba* spp. [29]. Other methods for *Entamoeba* identification include culture, antigen-based, and molecular tests.

In culture methods, polyxenic culture media like Balamuth's medium and axenic culture media like diamond's medium are used. A new axenic culture medium **CLUPS** for the growth of *E. histolytica* was also there which is superior to previously used **PEHPS** media [30]. However, cultivation is more sensitive than microscopy, but these methods are time-consuming, not cost-effective, and hence not routinely utilized by most diagnostic laboratories. Cultivation can effectively distinguish between *E. dispar* and *E. histolytica* [31].

Enzyme immunoassay (EI) kits are commercially available that detect *E. histolytica* or differentiate between *E. histolytica* and *E. dispar* by detecting 170 kDa of lectin antigen.

A rapid immunochromatographic testing (ICT) assay is available that detects antigens of *E. histolytica* and *E. dispar* (29 kDa surface antigen) in stool; however, this assay cannot differentiate between *E. histolytica* and *E. dispar* [29]. This assay also detects antigens of *Giardia* (alpha 1 Giardia antigen) and *Cryptosporidium* (protein disulfide isomerase antigen).

For the detection of *E. histolytica* antibodies in human serum serological methods such as latex agglutination, indirect hemagglutination (IHA), immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA) are highly sensitive [10, 32]. However, in endemic areas serology is of limited use because of the difficulty in distinguishing between past and present infections [10].

Molecular assays (like PCR) for the detection and differentiation of *Entamoeba* species are now considered as the gold standard for diagnosis of *Entamoeba* [28, 33]. When species differentiation is difficult or not possible than *E. histolytica/E. dispar* complex should be reported.

BioFire FilmArray system is the fully automated PCR system which can detect bacterial, viral, parasitic (*E. histolytica*, *Giardia*, *Cyclospora*, *Cryptosporidium)* diarrheal pathogen by using gastrointestinal panel [34].

For the diagnosis of invasive disease, a combination of microscopy, culture, and serology should be used with a PCR assay or with abdominal imaging (when PCR is unavailable) [32].

2.1.6 Treatment

In asymptomatic carriers of *E. histolytica*, luminal agents should be used to minimize the spread of disease. The treatment of choice is different for intestinal and invasive disease; hence, diagnosis is important before treatment. Metronidazole, a tissue amoebicide, is highly effective and used for invasive amoebic disease along with a luminal agent for the elimination of intestinal colonization [35]. Other agents like tinidazole and ornidazole can also be used for the treatment of invasive disease. For the treatment of intestinal and asymptomatic infections, the luminal agents paromomycin, iodoquinol, and diloxanide furoate are strictly recommended, as they are effective in eliminating cysts from the intestinal tract [36].

Regimen: asymptomatic carriage—luminal agent, Idoquinolone 650 mg tid for 20 days or Paromomycine 500 mg tid for 10 days.

Acute colitis—Metronidazole 750 PO or IV for 5–10 days or tinidazole 2 g/d PO for 3 days Plus luminal agent as above.

Amebic liver abscess—Metronidazole 750 PO or IV for 5–10 days or tinidazole 2 g/d PO once or ornidazole 2 g/d PO once Plus luminal agent as above.

2.1.7 Prevention and control

By avoiding the consumption of contaminated food and water and by treating the asymptomatic person, the infection can be prevented.

Certain **vaccines** against *E. histolytica* are under trial like colonization blocking vaccine targeting SREHP, 170 kDa subunit of lectin antigen, and 29 kDa cysteine-rich protein. Till now no vaccines against *E. histolytica* are licensed for human use [37, 38].

2.2 *Giardia intestinalis*

2.2.1 Introduction

Giardia intestinalis also known as *Giardia duodenalis* and *Giardia lamblia* is a common cause of parasitic diarrhea. It belongs to the flagellate group and first discovered by Leeuwenhoek in 1681 in his own stool.

2.2.2 Epidemiology

Giardia lamblia is distributed worldwide and reported from both temperate and tropical countries of the world. The prevalence of *Giardia* in low- and high-income countries is 4–43% and 1–7%, respectively [39, 40]. It was reported that *Giardia* affect approximately 2% of adults and 8% of children in developed countries [41].

Because of the increasing burden of illness from *Cryptosporidium* spp. and *Giardia* and their ability to impair development and socioeconomic improvements, they were included in the WHO Neglected Diseases initiative in 2004 [14].

2.2.3 Pathogenesis

Transmission—The most important mode of infection is fecal-oral route, but various studies have also found evidence of zoonotic transmission [42]. Giardiasis presents mainly as acute or chronic diarrhea associated with abdominal pain, nausea, malabsorption, and weight loss. *Giardia* infection can lead to growth retardation in malnourished children [43] and zinc deficiency in school-aged children [44]. *Giardia* infection is also common in dogs and cats [45]. They are

also a potential source of human infection that may be acquired through handling, sleeping together, licking, and kissing, as the zoonotic genotypes of *Giardia* were isolated from cats and dogs [46]. Giardiasis is also associated with waterborne disease outbreaks and is related to travel-associated diarrhea [47].

Life cycle—Cyst is the infective form of *Giardia* and ingestion of as low as 10 cysts can initiate infection. After ingestion excystation occurs in the small intestine, and its cyst converts into trophozoites. These trophozoites do not disseminate hematogenously and remain in the small bowel. It can either attach to the intestinal epithelium via suckling disc or remain free in the intestinal lumen. Trophozoites convert into cyst form, and both trophozoite and cysts are excreted in feces, but only the cysts are capable of surviving in the outside environment and disease transmission (**Figure 2**).

Pathophysiology—After attachment to the intestinal epithelium, it feed on the mucus secretions and lead to apoptosis of enterocyte; epithelial detection tests are used for screening and are more sensitive than routine microscopic examination. Loss of brush border is also seen.

All of these consequences manifest as lactose intolerance and malabsorption syndrome.

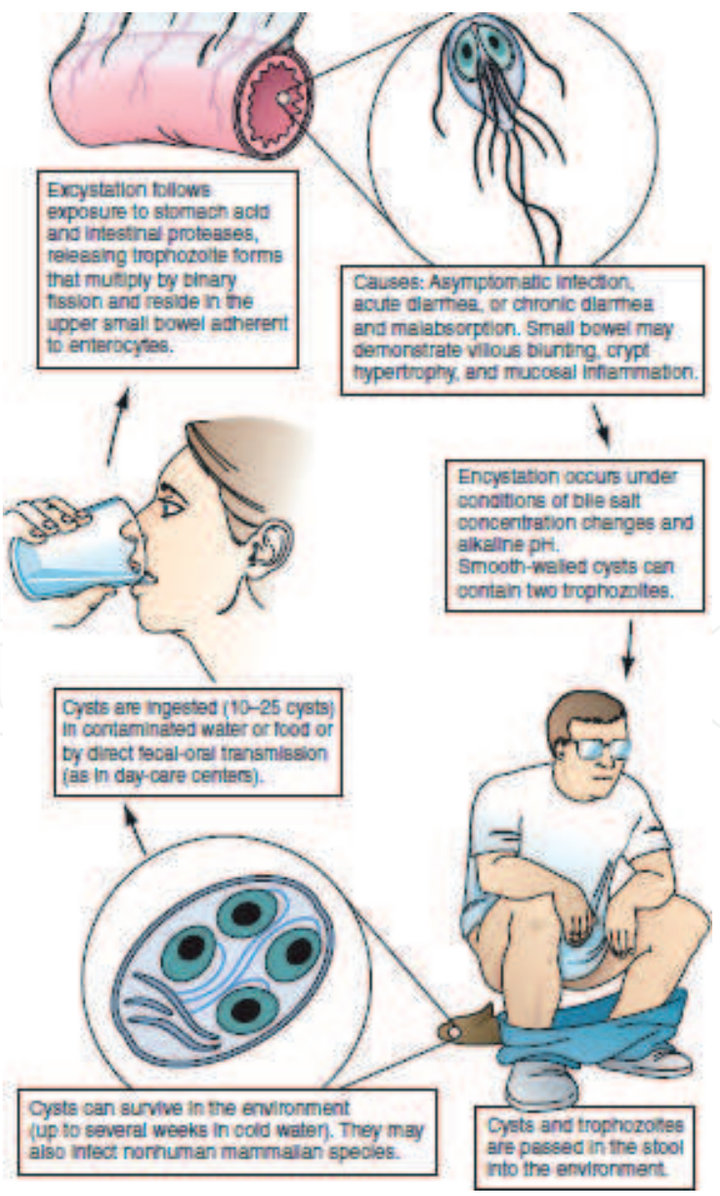

Figure 2.
Life cycle of Giardia. *Courtesy: Ref. [111], Fig. 224–1.*

2.2.4 Immune dysfunction

Both B cell-mediated antibody production and T cell-mediated immune responses play an in important role in protection against *Giardiasis*. Humans with immunodeficiency disorders like common variable immunodeficiency (CVID) and impaired IgA function have an increased risk of developing chronic *Giardia* infections. People living in endemic areas are less prone to infection or reinfection, indicating that acquired immunity exists.

According to a study, cellular immune response to *Giardia* can be understood by murine models. The mice model shows that in the absence of $CD4^+$ T cells, poor control or chronic infection to giardia occurs, indicating that these cells are crucial for the murine defense against *G. lamblia*. Cytokines like tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ) has been shown to be important for determining the parasite load and the duration of an infection.

Cytokines that are secreted by $CD4^+$ T cells were found in the mice infected with *Giardia*, indicating that a range of T_H responses may contribute to the protection against *Giardia*.

In another study, it was found that following infection of *G. lamblia*, there is an upregulation of peroxisome proliferator-activated receptor alpha and interleukin-17A (IL-17A) [48]. IL-17A was also found to be upregulated in another mouse study also, where IL-17A and its receptor were important for defense against the parasite [49]. Hence, on the basis of these studies, IL-17A may be linked to protection against *Giardia*.

Some human studies for *Giardia*-specific immune responses were also there. In one study, IFN-γ was secreted, and cells were proliferating after the stimulation of $CD4^+$ T cells with *Giardia* trophozoites, suggesting that *Giardia*-specific proliferation of $CD4^+$ T cells exists in humans as well [50].

The relative importance of the cytokines IL-17A, IFN-γ, TNF-α, IL-4, and IL-10 in the T cell response to *Giardia* infection in humans has not been determined [51].

2.2.5 Diagnosis

In outline the diagnosis of *Giardia* is based on the microscopic detection of *Giardia* cysts or trophozoites in a stool specimen by using wet mount, iodine mount, and trichrome stain method.

The string test (entro-test) may be useful for revealing *Giardia* trophozoites from duodenal sample [52].

Antigen detection assays that are available for *G. intestinalis* include EIAs, ELISAs, and direct fluorescent-antibody tests [53, 54]. For the cultivation of Giardia axenic media like diamond media can be used [55].

Molecular methods have higher sensitivity than conventional methods; however, many of them are still not commercially available [10, 52]. The use of real-time PCR is increasing for the detection of *Giardia*. Conventional single, nested, and multiplex PCRs have also been developed [10, 52].

2.2.6 Treatment

Metronidazole 250 mg TDS for 5 days or tinidazole 2 g once PO has been used as the therapy of choice. Due to the emergence of resistant isolates of *Giardia*, treatment failures and clinical relapses have been known to occur [43]. Alternatively, nitazoxanide 500 mg BD for 3 days can be used. Other drugs like furazolidone, albendazole, and paromomycin are also effective against giardiasis.

2.2.7 Prevention and control

By improving food and personal hygiene and by treating asymptomatic person, *Giardia* infection can be prevented.

One vaccine (GiardiaVax) has been licensed for dogs and cats in the United States [56, 57]. No human vaccines are currently available [57, 58].

2.3 *Cryptosporidium* species

2.3.1 Introduction

It is the coccidian parasite which causes self-limiting illness in immunocompetent person but can lead to severe disease in immunocompromised (AIDS) person. Two species of *Cryptosporidium—C. parvum* and *C. hominis*—found to be associated with human disease.

Epidemiology—The distribution of *Cryptosporidium* spp. is seen worldwide, and it mainly affects or causes severe illness in children and immunocompromised population. The prevalence of cryptosporidiosis in children of developing and developed nations was found to be 20 and 9%, respectively [59]. In adult population the change in prevalence of *Cryptosporidium* spp. was seen in the year 2000; before its prevalence was 3.8% in Los Angeles, and after 2000 it increased to 13% in the developed world [19].

Different clinical manifestations are produced by species of *Cryptosporidium* and subtype families of *Cryptosporidium hominis* [60].

2.3.2 Pathogenesis

Transmission—It is a waterborne disease transmitted via the fecal-oral route, by ingestion of contaminated salads, contaminated water supply, or recreational water, swimming in public pools, and it is observed that it can be transmitted from person to person, men who have sex with men, as well as through zoonotic infections [60]. *Cryptosporidium* spp. have a wide host range including humans, domestic pets, and wildlife [61]. Zoonotic transmission occurs by direct contact with infected animals or their feces or indirectly through the consumption of contaminated water [61, 62].

Life cycle—The environmentally resistant oocysts are produced by *Cryptosporidium*, and they sporulate when excreted in feces, and therefore, these oocysts are immediately infectious [63]. After ingestion of oocyst, excystation occurs in the intestine to liberate sporozoite which further develops into meront (with four merozoite). Meront form converts into macrogamete and microgametes that lead to zygote and finally unsporulated oocyst excreted into the feces.

Pathophysiology—In the small intestine, *Cryptosporidium* present inside the intracellular vacuoles and no characteristic changes were found on biopsy. Infection of *Cryptosporidium* is characterized by self-limiting diarrhea along with abdominal pain, dehydration, and malabsorption. It is an important infection in both immunosuppressed (especially HIV-infected) and in transplant recipients [64] **Figure 3** (Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease; 8th ed. Philadelphia. Elsevier saunder's 2015 Fig. 284–1. Life cycle of Cryptosporidium, p. 3174).

2.3.3 Immune dysfunction

The occurrence of cryptosporidiosis is closely related to the immune status of its host. It primarily affects the infants and immunocompromised individuals. In

recent years, several studies have highlighted the importance of innate immunity in cryptosporidiosis. Intestinal epithelial cells play a key role in cryptosporidiosis as these are the exclusive host cell for the replication of the parasite and also participate in the protective immune response. Epithelial cells produce chemokines and attract immune cells to the infected area. They also release antimicrobial peptides with parasiticidal activity and induce apoptosis. Intestinal dendritic cells induce adaptive immunity and control *Cryptosporidium parvum* infection in the early stage [65].

Both innate and adaptive immune responses are important for controlling cryptosporidiosis. Innate immune responses are mediated by Toll-like receptor pathways, antimicrobial peptides, prostaglandins, mannose-binding lectin, cytokines, and chemokines. Cell-mediated responses, particularly those involving $CD4^+$ T cells and IFN-γ, play an important role. The parasite has developed several escape mechanisms to slow down these protective mechanisms [66].

2.3.4 Diagnosis

Routinely the diagnosis of cryptosporidiosis is done by the identification of oocysts in stool by microscopic examination. The staining and preservation technique enhance the sensitivity of the test. The staining technique for *Cryptosporidium* spp. includes modified Ziehl-Neelsen technique, Kinyoun's acid-fast staining technique, modified Sheather's flotation technique, and the iron-hematoxylin staining technique. Out of these staining techniques, modified Ziehl-Neelsen technique is widely used [67, 68].

Antigen detection assay is also used widely in the diagnosis of cryptosporidiosis; it is more effective in cases where oocyst numbers are low. These assays are thought to be more sensitive than conventional staining [69, 70]. Other tests like fluorescence

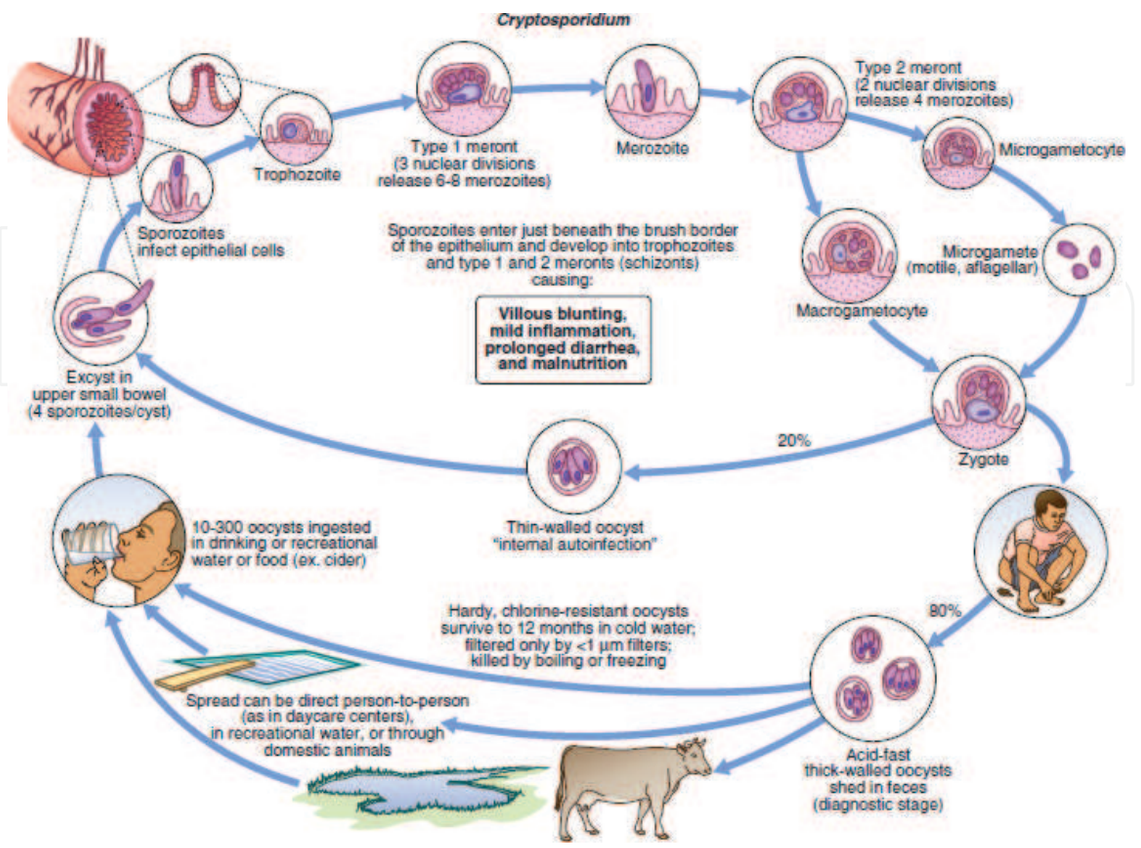

Figure 3.
Life cycle of Cryptosporidium. Courtesy: Ref. [112], Fig. 284–1.

microscopy and direct fluorescent-antibody (DFA) assay have been used for the detection of *Cryptosporidium* oocysts with relatively high specificities (96–100%) and sensitivities (98.5–100%) [70, 71]. Molecular methods for the detection and differentiation of *Cryptosporidium* spp. include nested PCR, real-time PCR, multiplex real-time PCR, reverse transcription-quantitative real-time PCR, and multiplex tandem real-time PCR [12, 72]. More recently, an automated multiplex tandem PCR is used; it is based on **a robotic platform** and used to detect *Cryptosporidium* spp. and coinfecting diarrheal pathogens from the human fecal genomic sample [73]. Some new methods such as **reverse line blot (RLB) hybridization**, loop-mediated isothermal amplification method, and nucleic acid sequence-based amplification (NASBA) methods that amplify RNA from either RNA or DNA templates are also used for the diagnosis of *Cryptosporidium* spp. [72, 74]. Another new technique for the detection of *Cryptosporidium* spp. includes **next-generation sequencing, DNA microarray,** etc. which are also available [70] (**Figure 4**).

2.3.5 Treatment

In immunocompetent persons, cryptosporidiosis is usually self-limiting, requiring little or no treatment, but in immunosuppressed person, treatment is required [75]. Nitazoxanide 500 mg BD for 3 days has proven to be effective against cryptosporidiosis. In patients with dehydration, rehydration fluids and nutritional management may be required.

Nitazoxanide is the only licensed drug for the treatment of cryptosporidiosis, and it is given only in persons after the first year of life and with healthy immune systems. Recently trials are going on clofazimine, a drug used for leprosy, to know its effectiveness against *Cryptosporidium* spp. [76].

2.3.6 Prevention and control

Prevention can be done by avoiding exposure to infectious oocyst in human or animal feces, proper hand washing, and improved personal hygiene. No vaccines are available for use against *Cryptosporidium* spp. [76].

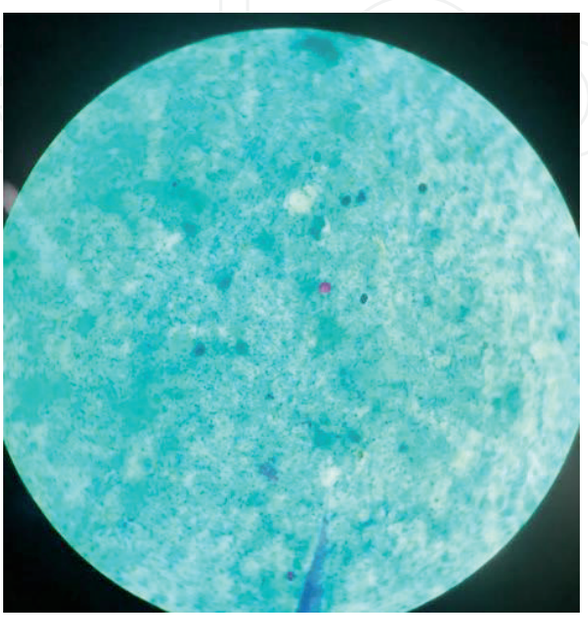

Figure 4.
Oocyst of Cryptosporidium *on modified ZN stain.*

2.4 *Cyclospora*

2.4.1 Introduction

This is a coccidian parasite, and the only species of this genus found in humans is *Cyclospora cayetanensis*. Similar to *Cryptosporidium, Cyclospora* is also associated with severe illness in immunocompromised patients in comparison to immunocompetent patients.

Epidemiology—Human cyclosporiasis is distributed worldwide, and cases are reported both from tropical and subtropical areas of the world. According to a study from China, the prevalence of *Cyclospora* spp. from 2007 to 2009 was 0.70%, whereas in the United Kingdom at the same time, the prevalence was 0.07% [77].

2.4.2 Pathogenesis

Transmission—An important feature of the *C. cayetanensis* is that the direct fecal-oral transmission from fresh stool is not seen because the oocysts require days to weeks outside the host to sporulate and become infectious.

Life cycle and pathophysiology—Similar to *cryptosporidium* spp.

Clinical feature—Infection due to *Cyclospora* manifest as persistent diarrhea, fever, bloating, flatulence, abdominal cramps, constipation, and fatigue, weight loss, etc.

Immune dysfunction—similar to *Cryptosporidium* spp.

2.4.3 Diagnosis

Routinely the diagnosis of *Cyclospora* is done by using microscopic methods. *Cyclospora* oocyst produces autofluorescence (white-blue) under an epifluorescence microscope [78, 79]. By demonstration of *Cyclospora* oocysts, autofluorescence and staining characteristics diagnosis can be made. Oocysts appear as acid-fast round or ovoid very small (8–10 μm) structures. The diameter measurement of oocyst is important to differentiate it from smaller *Cryptosporidium* oocysts, which measure 4–6 μm in size. The procedures used to diagnose *Cyclospora* includes 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 [80].

Molecular techniques have been developed like various PCR tools that target the internal transcribed spacer region, which use primers for the 18S rRNA gene [79]. These tools include conventional PCR, reverse transcriptase PCR in combination with agarose gel electrophoresis, and nested PCR [79]. *Cyclospora* is included in the BioFire FilmArray systems GIT panel; hence, syndromic diagnosis can be made.

2.4.4 Treatment

Laboratory diagnosis is important as the treatment of *Cyclospora* is different from the other protozoa of similar presentation. Trimethoprim-sulfamethoxazole (also known as co-trimoxazole) 160/800 mg BD for 7–10 days is the drug of choice for cyclosporiasis.

2.4.5 Prevention and control

No vaccine is available till now against *Cyclospora*; by avoiding contaminated food and water it can be prevented.

2.5 *Cystoisospora belli* (formerly *Isospora belli*)

2.5.1 Introduction

Cystoisospora belli is responsible for intestinal disease in several mammalian hosts. It is also a coccidian parasite similar to *Cryptosporidium* and *Cyclospora*. It is also found to be associated with more severe illness in immunocompromised host.

2.5.2 Epidemiology

Very little is known about the epidemiology and prevalence of *Cystoisospora belli*. The worldwide reported prevalence of *Cystoisospora belli* was 2.5–26.1%, and it is mostly seen in HIV-positive individuals [18].

2.5.3 Pathogenesis

Transmission—Infection occurs via the ingestion of mature sporulated oocysts present in contaminated food or water.

Life cycle and pathophysiology—Similar to *Cryptosporidium* spp.

Clinical feature—Infection is manifested as watery diarrhea, abdominal cramps, anorexia, and weight loss, and it is almost indistinguishable from cryptosporidiosis. It is more common in AIDS patients and causes traveler's diarrhea in travelers to developing countries. In HIV-infected patients, infection of *Cystoisospora belli* may lead to chronic diarrhea, acalculous cholecystitis cholangiopathy, and extraintestinal infection [81]. Other *Cystoisospora* species causes of diarrhea in domestic animals, like *Cystoisospora suis*, lead to severe diarrheal illness in pigs [82].

Immune dysfunction—Similar to *cryptosporidium* spp.

Diagnosis is made by demonstration of oocyst by direct microscopic examination of the feces, with acid-fast staining or fluorescent staining with auramine and rhodamine. Oocysts of *Cystoisospora belli* are large (20–23 μm × 10–19 μm) and ellipsoidal in shape in comparison to oocyst of *Cryptosporidium* which is round and 4–6 μm in diameter.

Autofluorescence can be seen, but this property is not consistent in *Cystoisospora*.

In molecular techniques PCRs using SSU rRNA primer have shown excellent sensitivity and specificity for the detection of *C. belli* in fecal samples. But these tests are not commercially available [83, 84] (**Figure 5**).

Treatment is done by giving oral co-trimoxazole 160/800 mg BD for 10 days and in case of HIV-infected patients three times daily for 3 weeks. Other drugs like ciprofloxacin, pyrimethamine, and folinic acid are good alternative.

2.5.4 Prevention and control

To date no vaccines are available for *Cystoisospora belli*; however, trials are going on to identify the suitable candidate of parasite for vaccine preparation [85]. Hence, prevention can be done by avoiding contaminated food and water consumption.

2.6 *Dientamoeba fragilis*

2.6.1 Introduction

It was initially considered as amoeba but recently classified under ameboflagellate as it has internal flagellum. It mostly involves the lumen of the cecum and upper colon of the human intestine.

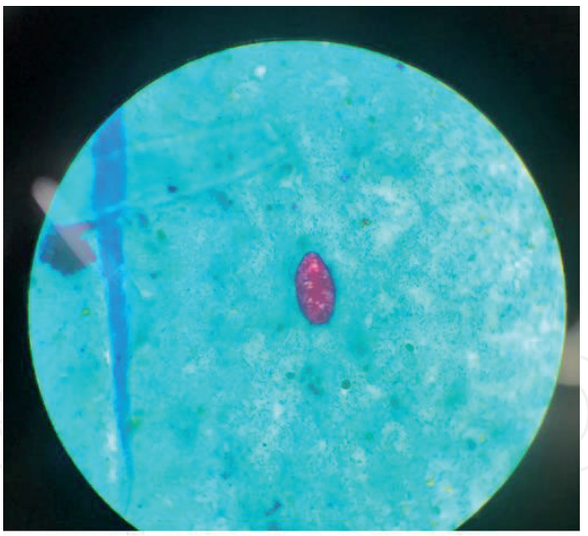

Figure 5.
Oocyst of Cystoisospora *in modified ZN stain.*

Epidemiology—The reported prevalence of *Dientamoeba fragilis* in the year of 2015 was around 1–70% in developed settings [86]. It varies globally as in United States and Australia; the reported prevalence is 5%, and in Netherland it was around 8% [87].

2.6.2 Pathogenesis

Many debates are there for the pathogenicity of *D. fragilis* [88, 89]. Previously it was thought that in *D. fragilis*, cyst stage has not been found [89], but recently both the **cyst and precyst stage** have been identified [90]. The nuclear chromatin of trophozoite of *D. fragilis* is fragmented into 3–5 granules; hence, it is known as fragilis. Although the mode of transmission remains unknown, previously it was thought that infection occurs via the pinworm vector because of its high rates of coinfection with *Enterobius vermicularis* [91]. But according to recent studies, it is found that transmission occurs directly via the fecal-oral route [89].

Clinical symptoms of *Dientamoeba fragilis* infection include abdominal pain, persistent diarrhea, loss of appetite, weight loss, and flatulence. Symptoms of *D. fragilis* are similar to those of irritable bowel syndrome (IBS); hence, it should be considered in the differential diagnosis of IBS [92].

2.6.3 Immune dysfunction

Dientamoeba fragilis is often described as "neglected parasite" [93]. Recently, several studies have occurred on the life cycle and molecular biology of the organism, but the knowledge on immune response against the pathogen is lacking. Because of the close relativity of *Dientamoeba fragilis* to histomonads, the immunological research on *H. meleagridis* can give an indication to the immunological responsiveness of host against *D. fragilis* [94].

2.6.4 Diagnosis

It is based on the microscopic detection of trophozoites in fresh or fixed stool specimens. The trophozoites degrade soon after passing in the stool; hence, the examination should be done immediately. Modified iron-hematoxylin or trichrome staining of fixed smears is considered the gold standard for the diagnosis of

D. fragilis infection [95]. But in comparison to molecular methods, this method is time-consuming and relatively insensitive [96]. The demonstration of the characteristic nuclear structure is needed for the definitive diagnosis of *D. fragilis* which cannot be seen in unstained fecal material. Hence, fixation and staining are necessary for definitive diagnosis of *D. fragilis* [96].

Antigen detection by rapid fecal immunoassays like enzyme immunoassays, fluorescent antibody, and rapid cartridge formats is previously under trial, but now enzyme immunoassay and immunofluorescence tests are available for antigen detection [90].

Antibody detection can be done by using an indirect immunofluorescence method [90].

For the cultivation of *D. fragilis*, no axenic cultures exist, and it will grow well in xenic cultures. For growth of *D. fragilis*, Loeffler's slope medium and modified Earle's balanced salt solution media are used [90].

Recently, the more advanced tests based on various conventional and real-time PCRs are used to detect the small subunit (SSU) rRNA gene of *D. fragilis.* These tests are more rapid, sensitive, and specific for the diagnosis in fresh stools [96]. Molecular methods are more sensitive than microscopy and staining but still not commercially available. *Dientamoeba fragilis* is not included in the gastrointestinal panel of BioFire FilmArray system [90].

2.6.5 Treatment

For the treatment of *D. fragilis* paromomycin, secnidazole, iodoquinol, tetracycline, ornidazole, and metronidazole have been used successfully. The treatment is recommended both in symptomatic patients and asymptomatic family members to prevent reinfection. There are few studies which show emergence of resistance or treatment failures of metronidazole therapy; it suggests that the combination therapy should be used for these protozoa [97]. For the complete eradication of the parasite and in resolution of symptoms, combination therapy is effective [97].

2.6.6 Prevention and control

By improving personal hygiene, *Dientamoeba* infection can be prevented. No vaccines available yet for *Dientamoeba fragilis*.

2.7 *Blastocystis* species

2.7.1 Introduction

Blastocystis spp. are commonly isolated from stools of humans and animals [13]. There are numerous subtypes of *Blastocystis* spp., but it is unclear whether any of these subtypes are specific to humans disease. It was found that *Blastocystis* sp. subtype 3 is most commonly associated with illness in human [13, 98]. It appears in various morphological forms like vacuolar, granular, amoeboid, and cyst forms, and less frequently avacuolar and multivacuolar cells and cells containing filament-like inclusions [99, 100].

Epidemiology—The prevalence of *Blastocystis* spp. in developed countries is 0.5–23% and in developing countries 22–100%. The prevalence varies in different geographical are according to the socioeconomic status of that area [101].

2.7.2 Pathogenesis

Transmission is usually taken place via the fecal-oral route through the consumption of cyst. These cysts may be waterborne or foodborne. There are many zoonotic subtypes of *Blastocystis* spp. which supports the increased potential for zoonotic transmission [102].

Life cycle—Infection is acquired through the consumption of cyst, and it converts to vacuolar, granular, and amoeboid form in human intestine. Cyst form is excreted in the feces and contaminates the food and water ingested by other animals.

Clinical features of *Blastocystis* spp. infection include mostly asymptomatic or nausea, anorexia, abdominal pain, flatulence, and acute or chronic diarrhea. *Blastocystis* spp. have been found to be the most common enteric organisms isolated from diarrheal patients but have been reported as noninfectious pathogens or a member of normal gut microbiota [103].

2.7.3 Immune dysfunction

According to a study, the incubation of *Blastocystis* sp. ST1 cells or culture filtrates induces the production of proinflammatory cytokines interleukin (IL)-8 and granulocyte-macrophage colony-stimulating factor. It suggests that the parasite was able to modulate the host immune response. *Blastocystis* sp. releases cysteine proteases, which induce human colonic epithelial cells (HT84) for the production of IL-8. Cysteine proteases of *Blastocystis* sp. are involved in parasite survival in vivo and represent virulence factors. *Blastocystis* sp. also secretes protease, which is able to cleave human secretory immunoglobulin A. Apart from proteases, hydrolases and protease inhibitors were predicted to be secreted by *Blastocystis* sp. which participates in the blastocystosis physiopathology and its mechanisms for immune evasion [104].

2.7.4 Diagnosis

The diagnosis of infection with *Blastocystis* spp. is done by using wet mount smears, iodine staining, trichrome staining, or iron-hematoxylin staining and detecting the vacuolar, granular, amoebic, or cystic form in stool samples [13, 100]. Trichrome staining of fecal smears or xenic in vitro culture systems has better sensitivity [98]. Subtype 7 which is a slower-growing subtype of *Blastocystis* spp. may be missed with this procedure [98].

For antibody detection in serum ELISA and immunofluorescent assay are also available. ELISA for the antigen detection in the stool is also introduced recently [105].

In molecular techniques, PCR using the SSU rRNA gene is being used increasingly for the detection of *Blastocystis* spp. with increased sensitivity, and it is more commonly used method nowadays [13]. Based on SSU rRNA 17, subtypes of *Blastocystis* spp. were identified out of which ST3 is the commonest worldwide [106] (**Figure 6**).

2.7.5 Treatment

Drugs used for the treatment of pathogenic *Blastocystis* spp. include metronidazole, co-trimoxazole, nitazoxanide, and a combination of paromomycin and metronidazole. Among these metronidazole is the drug of choice, although failures of this drug in eradicating the organism are common [107].

2.7.6 Prevention and control

Prevention can be done by avoiding the contaminated food and water consumption; no vaccines are available for *Blastocystis* spp. till now.

2.8 *Balantidium coli*

2.8.1 Introduction

Balantidium coli is the largest protozoan that infects humans. It belongs to the ciliate group. The whole body of the organism is covered with a row of cilia which is responsible for the rotary motility of *B. coli* (**Figure 7**).

2.8.2 Epidemiology

It mostly occurred in tropical and subtropical areas of developing countries. The estimated prevalence of *B. coli* worldwide is 1% as the human infection with this protozoa is rare [108].

2.8.3 Pathogenesis

Transmission—Pigs are the natural host for *B. coli*. Human acquires infection by ingestion of cysts present in water and undercooked food (fecal-oral route).

Life cycle—After ingestion of cyst, it converts into trophozoite in the large intestine of human. Both cyst and trophozoite are excreted into the feces, but in external environment, trophozoite disintegrates and cyst will survive. It contaminates the water and food, and the life cycle continues.

Clinical feature—Infections of *B. coli* are mostly asymptomatic, but in symptomatic patients, mild diarrhea and abdominal discomfort have been reported. In a few patients fulminating acute balantidiasis, with intestinal perforation, can

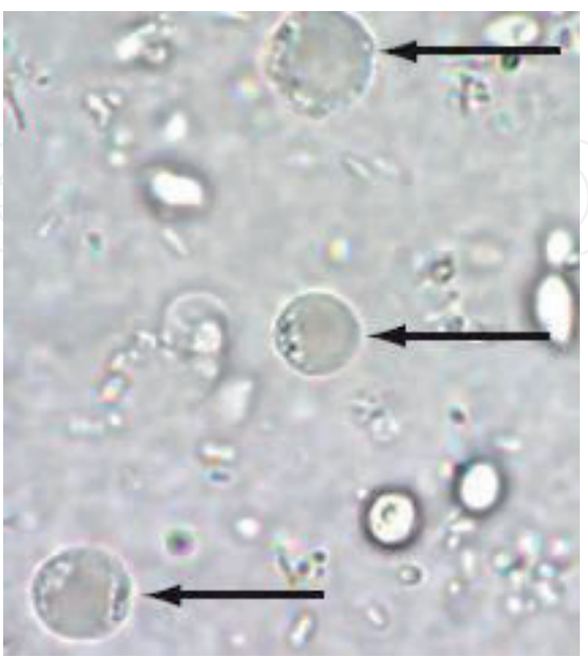

Figure 6.
Cysts of Blastocystis hominis *on wet mount. Courtesy: Ref. [113], Fig. 285–5.*

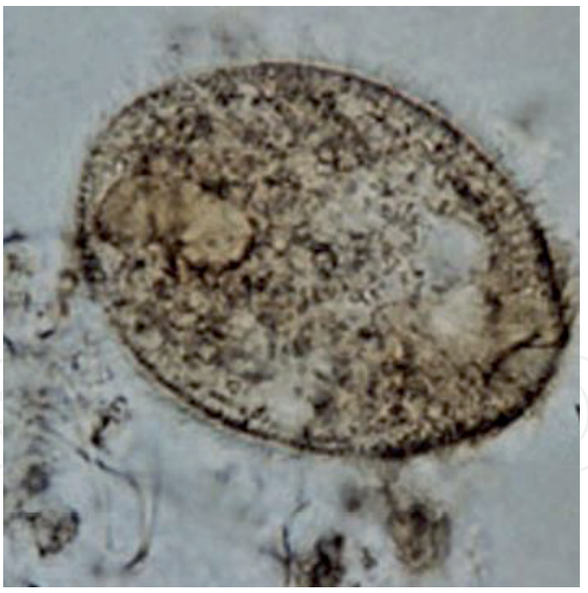

Figure 7.
Trophozoite of B. coli demonstrating cilia on the surface. Courtesy: Ref. [114], Fig. 285–4.

develop which has a case fatality rate of about 30%. Fulminating dysentery, resembling amoebic dysentery, can also occur in *B. coli* infection [108].

2.8.4 Immune dysfunction

Immune response in *B. coli* is poorly understood due to the lack of studies available. It was found that infection of *B. coli* is more severe in immunocompromised patients.

2.8.5 Diagnosis

B. coli is diagnosed by its large size cysts (50–70 μm) and trophozoite (30–200 μm × 40–70 μm) and rotatory motility which is demonstrated by wet mount slide preparations. Trophozoites of *B. coli* are visible even with hand lens and, sometimes with the naked eye, in freshly collected diarrheic stools as well as in bronchoalveolar wash fluid [108]. Collection of stool samples should be done for several days because the excretion of parasites can be erratic [108].

Balantidium coli can be cultivated on Boeck and Drbohlav egg serum media and Balamuth media. It can also be cultivated on Pavlova xenic culture media, and the growth of trophozoites can be obtained within 72 h on this media [109].

2.8.6 Treatment

B. coli is treated with tetracycline or metronidazole:

- Tetracycline—500 mg qid for 10 days
- Metronidazole 750 mg tds for 5–7 days

2.8.7 Prevention and control

By avoiding contamination of food and water with pig and human feces and by treating the carrier shedding the cyst, the infection can be prevented. No vaccines are available till now for *Balantidium coli* infection.

3. Conclusion

Enteric protozoa are responsible for severe morbidity and mortality in humans. Protozoal infections contribute to the high burden of infectious diseases. Despite recent advances in the epidemiology, diagnostic tool, molecular biology, and treatment of protozoan illnesses, gaps in knowledge still exist; hence, protozoal infections require further research. Both humoral and cellular immunities play an important role in the protection of host against enteric protozoal infection. Protozoa have some virulence factor or mechanism to evade the immune system of the host, for the production of disease. An already dysfunctional host immune system (immunocompromised conditions) helps the protozoa to cause easy and more serious disease. Among these enteric protozoal infection *Entamoeba histolytica*, *Giardia intestinalis*, *Cryptosporidium*, *Cyclospora*, *Cystoisospora belli*, *Dientamoeba fragilis*, *Blastocystis*, and *Balantidium coli* are common. In the recent years, increasing trends of *Entamoeba histolytica*, *Giardia intestinalis*, and *Cryptosporidium* is seen both in developing and developed countries. Blastocystis is an emerging infection to humans. Mostly enteric protozoal infections are acquired through fecal-oral route and present with abdominal pain and fever mainly. Some of them can lead to anemia and malabsorption syndrome. If not diagnosed or treated timely, enteric protozoal infection can be fatal. Diagnosis for most of them is based on microscopy,but recently many molecular methods are available to diagnose them. By using multiplex PCR, BioFire Gastrointestinal panel, common diarrheal illness-causing organisms can be identified in a shorter time span. ELISA and immunofluorescent assay are also available for some of them, e.g., *Blastocystis* spp. and *Dientamoeba fragilis*. For the treatment of protozoal infection, drugs like metronidazole, paromomycin, diloxanide furoate, nitazoxanide, cotrimoxazole, etc. are used. The infection can be prevented by good personal hygiene and by avoiding food and water contamination. Till date, no vaccines are available for human use against these protozoal infections.

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References

[1] Kucerova Z, Sokolova O, Demyanov A, Kvac M, Sak B, Kvetonova D, et al. Microsporidiosis and cryptosporidiosis in HIV/AIDS patients in St. Petersburg, Russia: Serological identification of microsporidia and *Cryptosporidium parvum* in sera samples from HIV/AIDS patients. AIDS Research and Human Retroviruses. 2011;**27**(1):13-15

[2] Bakheit MA, Torra D, Palomino LA, Thekisoe OM, Mbati PA, Ongerth J, et al. Sensitive and specific detection of *Cryptosporidium* species in PCR-negative samples by loop-mediated isothermal DNA amplification and confirmation of generated LAMP products by sequencing. Veterinary Parasitology. 2008;**158**(1-2):11-22

[3] Escobedo A, Almirall P, Robertson L, Franco R, Hanevik K, Morch K, et al. Giardiasis: The ever-present threat of a neglected disease. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders). 2010;**10**(5):329-348

[4] Mor SM, Tzipori S. Cryptosporidiosis in children in Sub-Saharan Africa: A lingering challenge. Clinical Infectious Diseases. 2008;**47**(7):915-921

[5] Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, et al. Food-borne diseases—The challenges of 20 years ago still persist while new ones continue to emerge. International Journal of Food Microbiology. 2010;**139**:S3-S15

[6] Waldron LS, Ferrari BC, Cheung-Kwok-Sang C, Beggs PJ, Stephens N, Power ML. Molecular epidemiology and spatial distribution of a waterborne cryptosporidiosis outbreak in Australia. Applied and Environmental Microbiology. 2011;**77**(21):7766-7771

[7] Yoder JS, Hlavsa MC, Craun GF, Hill V, Roberts V, Yu PA, et al. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events–United States, 2005-2006. MMWR Surveillance Summaries. 2008;**57**(9):1-29

[8] Shah N, DuPont HL, Ramsey DJ. Global etiology of travelers' diarrhea: Systematic review from 1973 to the present. The American Journal of Tropical Medicine and Hygiene. 2009;**80**(4):609-614

[9] Fletcher SM, Stark D, Ellis J. Prevalence of gastrointestinal pathogens in Sub-Saharan Africa: Systematic review and meta-analysis. Journal of Public Health in Africa. 2011;**2**(2)

[10] Lebbad M. Molecular Diagnosis and Characterization of Two Intestinal Protozoa: *Entamoeba histolytica* & *Giardia intestinalis*. Institutionen för mikrobiologi, tumör-och cellbiologi/ Department of Microbiology, Tumor and Cell Biology. Karolinska Institutet; 2010

[11] Hove RJ. Molecular Detection of Intestinal Parasites for Clinical Diagnosis and Epidemiology. Doctoral dissertation, Department of Parasitology/Epi-Diag, Faculty of Medicine, Leiden University Medical Center (LUMC), Leiden University; 2009

[12] Stark D, Al-Qassab S, Barratt J, Stanley K, Roberts T, Marriott D, et al. Evaluation of multiplex tandem real-time PCR for detection of *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba histolytica*, and *Giardia intestinalis* in clinical stool samples. Journal of Clinical Microbiology. 2011;**49**(1):257-262

[13] Roberts T, Barratt J, Harkness J, Ellis J, Stark D. Comparison of microscopy, culture, and conventional polymerase chain reaction

for detection of *Blastocystis* sp. in clinical stool samples. The American Journal of Tropical Medicine and Hygiene. 2011;**84**(2):308-312

[14] Santos HLC, Bandea R, Martins LAF, De Macedo HW, Peralta RHS, Peralta JM, et al. Differential identification of *Entamoeba* spp. based on the analysis of 18S rRNA. Parasitology Research. 2010;**106**(4):883-888

[15] Saleh R, El-zahraa F, Gad M, Ashour A, Soliman M, El-Senousy W, et al. Molecular detection of *Entamoeba histolytica* in fresh vegetables and irrigation. Egyptian Journal of Aquatic Biology and Fisheries. 2019;**22**(5 (Special Issue)):551-561

[16] Gualdieri L, Rinaldi L, Petrullo L, Morgoglione M, Maurelli M, Musella V, et al. Intestinal parasites in immigrants in the city of Naples (southern Italy). Acta Tropica. 2011;**117**(3):196-201

[17] Shirley D-AT, Farr L, Watanabe K, Moonah S. A review of the global burden, new diagnostics, and current therapeutics for amebiasis. In: Open Forum Infectious Diseases. USA: Oxford University Press; 2018

[18] Ghoshal U, Jain V, Tejan N, Kalra SK, Ranjan P, Sinha R, et al. A road less travelled: Clinical comparison of HIV seropositive and seronegative patients with cystoisosporiasis—An 11-year experience from a tertiary care centre in Northern India. Indian Journal of Medical Microbiology. 2018;**36**(4):508

[19] Momba M, Ebdon J, Kamika I, Verbyla M. Using indicators to assess microbial treatment and disinfection efficacy. In: Global Water Pathogen Project. East Lansing, Michigan: Michigan State University, UNESCO; 2019

[20] Walsh JA. Problems in recognition and diagnosis of amebiasis: Estimation of the global magnitude of morbidity and mortality. Reviews of Infectious Diseases. 1986;**8**(2):228-238

[21] Maldonado-Barrera CA, del Rosario C-EM, Muñoz-Fernández L, Victoria-Hernández JA, Campos-Rodríguez R, Talamás-Rohana P, et al. Clinical case of cerebral amebiasis caused by *E. histolytica*. Parasitology Research. 2012;**110**(3):1291-1296

[22] Tamer GS, Öncel S, Gökbulut S, Arisoy ES. A rare case of multilocus brain abscess due to *Entamoeba histolytica* infection in a child. Saudi Medical Journal. 2015;**36**(3):356

[23] Fletcher SM, Stark D, Harkness J, Ellis J. Enteric protozoa in the developed world: A public health perspective. Clinical Microbiology Reviews. 2012;**25**(3):420-449

[24] Anaya-Velázquez F, Padilla-Vaca F. Virulence of *Entamoeba histolytica*: A challenge for human health research. Future Microbiology. 2011;**6**(3):255-258

[25] Mondal D, Haque R, Sack RB, Kirkpatrick BD, Petri WA Jr. Attribution of malnutrition to cause-specific diarrheal illness: Evidence from a prospective study of preschool children in Mirpur, Dhaka, Bangladesh. The American Journal of Tropical Medicine and Hygiene. 2009;**80**(5):824-826

[26] Begum S, Quach J, Chadee K. Immune evasion mechanisms of *Entamoeba histolytica*: Progression to disease. Frontiers in Microbiology. 2015;**6**:1394

[27] Verma GK, Sharma NL, Shanker V, Mahajan VK, Kaushik R, Verma S, et al. *Amoebiasis cutis*: Clinical suspicion is the key to early diagnosis. The Australasian Journal of Dermatology. 2010;**51**(1):52-55

[28] Stark D. *Entamoeba histolytica*. In: PCR for Clinical Microbiology. Springer; 2010. pp. 363-367

[29] Pritt BS, Clark CG. Amebiasis. In: Mayo Clinic Proceedings; Elsevier. 2008

[30] Gonzalez-Salazar F, Meester I, Guzmán De La Garza F, La Garza-Salinas D, Sampayo-Reyes A, Garza-Gonzalez J, et al. "CLUPS": A new culture medium for the axenic growth of *Entamoeba histolytica*. Journal of Parasitology Research. 2018:133-137. DOI: http://dx.doi.org/10.5772/intechopen.91698

[31] Fotedar R, Stark D, Marriott D, Ellis J, Harkness J. *Entamoeba moshkovskii* infections in Sydney, Australia. European Journal of Clinical Microbiology & Infectious Diseases. 2008;**27**(2):133-137

[32] Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Laboratory diagnostic techniques for *Entamoeba* species. Clinical Microbiology Reviews. 2007;**20**(3):511-532

[33] Soares NM, Azevedo HC, Pacheco FT, de Souza JN, Del-Rei RP, Teixeira MC, et al. A cross-sectional study of *Entamoeba histolytica*/dispar/moshkovskii complex in Salvador, Bahia, Brazil. BioMed Research International. 2019:1-7

[34] Piralla A, Lunghi G, Ardissino G, Girello A, Premoli M, Bava E, et al. FilmArray™ GI panel performance for the diagnosis of acute gastroenteritis or hemorrhagic diarrhea. BMC Microbiology. 2017;**17**(1):111

[35] Kelly P. Protozoal gastrointestinal infections. Medicine. 2013;**41**(12):705-708

[36] Kappagoda S, Singh U, Blackburn BG. Antiparasitic therapy. In: Mayo Clinic Proceedings; Elsevier. 2011

[37] Quach J, St-Pierre J, Chadee K. The future for vaccine development against *Entamoeba histolytica*. Human Vaccines & Immunotherapeutics. 2014;**10**(6):1514-1521

[38] Roncolato EC, Teixeira JE, Barbosa JE, Ramalho LNZ, Huston CD. Immunization with the *Entamoeba histolytica* surface metalloprotease EhMSP-1 protects hamsters from amebic liver abscess. Infection and Immunity. 2015;**83**(2):713-720

[39] Waldram A, Vivancos R, Hartley C, Lamden K. Prevalence of Giardia infection in households of Giardia cases and risk factors for household transmission. BMC Infectious Diseases. 2017;**17**(1):486

[40] Leder K, Weller DPF. Giardiasis: Epidemiology, clinical manifestations, and diagnosis.. UpToDate. [Accessed on: 15 March 2019]

[41] Dunn N, Juergens AL. Giardiasis. Treasure Island, FL: StatPearls; 2019

[42] Dixon B, Parrington L, Cook A, Pintar K, Pollari F, Kelton D, et al. The potential for zoonotic transmission of *Giardia duodenalis* and *Cryptosporidium* spp. from beef and dairy cattle in Ontario, Canada. Veterinary Parasitology. 2011;**175**(1-2):20-26

[43] Busatti HG, Santos JF, Gomes MA. The old and new therapeutic approaches to the treatment of giardiasis: Where are we? Biologics: Targets & Therapy. 2009;**3**:273

[44] Demirci M, Delibas N, Altuntas I, Oktem F, Yönden Z. Serum iron, zinc and copper levels and lipid peroxidation in children with chronic giardiasis. Journal of Health, Population, and Nutrition. 2003:72-75

[45] Olson ME, Leonard NJ, Strout J. Prevalence and diagnosis of Giardia infection in dogs and cats using a fecal antigen test and fecal smear. The Canadian Veterinary Journal. 2010;**51**(6):640

[46] Yoshiuchi R, Matsubayashi M, Kimata I, Furuya M, Tani H, Sasai K.

Survey and molecular characterization of Cryptosporidium and *Giardia* spp. in owned companion animal, dogs and cats, in Japan. Veterinary Parasitology. 2010;**174**(3-4):313-316

[47] Wilson N, Baker M, Edwards R, Simmons G. Case-case analysis of enteric diseases with routine surveillance data: Potential use and example results. Epidemiologic Perspectives & Innovations. 2008;**5**(1):6

[48] Dreesen L, De Bosscher K, Grit G, Staels B, Lubberts E, Bauge E, et al. *Giardia muris* infection in mice is associated with a protective interleukin 17A response and induction of peroxisome proliferator-activated receptor alpha. Infection and Immunity. 2014;**82**(8):3333-3340

[49] Dann SM, Manthey CF, Le C, Miyamoto Y, Gima L, Abrahim A, et al. IL-17A promotes protective IgA responses and expression of other potential effectors against the lumen-dwelling enteric parasite Giardia. Experimental Parasitology. 2015;**156**:68-78

[50] Ebert E. Giardia induces proliferation and interferon γ production by intestinal lymphocytes. Gut. 1999;**44**(3):342-346

[51] Saghaug CS, Sørnes S, Peirasmaki D, Svärd S, Langeland N, Hanevik K. Human memory CD4+ T cell immune responses against *Giardia lamblia*. Clinical and Vaccine Immunology. 2016;**23**(1):11-18

[52] Hooshyar H, Rostamkhani P, Arbabi M, Delavari M. *Giardia lamblia* infection: Review of current diagnostic strategies. Gastroenterology and Hepatology from Bed to Bench. 2019;**12**(1):3

[53] Foronda P, Bargues M, Abreu-Acosta N, Periago M, Valero M, Valladares B, et al. Identification of genotypes of *Giardia intestinalis* of human isolates in Egypt. Parasitology Research. 2008;**103**(5):1177-1181

[54] Barbecho JM, Bowman DD, Liotta JL. Comparative performance of reference laboratory tests and in-clinic tests for Giardia in canine feces. Parasites & Vectors. 2018;**11**(1):444

[55] Garcia LS. Diagnostic medical parasitology: An update. Clinical Microbiology Newsletter. 1994;**16**(14):105-110

[56] Meeusen EN, Walker J, Peters A, Pastoret P-P, Jungersen G. Current status of veterinary vaccines. Clinical Microbiology Reviews. 2007;**20**(3):489-510

[57] Serradell MC, Saura A, Rupil LL, Gargantini PR, Faya MI, Furlan PJ, et al. Vaccination of domestic animals with a novel oral vaccine prevents *Giardia* infections, alleviates signs of giardiasis and reduces transmission to humans. NPJ Vaccines. 2016;**1**:16018

[58] Tian H-F, Chen B, Wen J-F. Giardiasis, drug resistance, and new target discovery. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders). 2010;**10**(4):295-302

[59] Elwin K, Hadfield SJ, Robinson G, Crouch ND, Chalmers RM. *Cryptosporidium viatorum* n. sp. (Apicomplexa: Cryptosporidiidae) among travellers returning to Great Britain from the Indian subcontinent, 2007-2011. International Journal for Parasitology. 2012;**42**(7):675-682

[60] Xiao L. Molecular epidemiology of cryptosporidiosis: An update. Experimental Parasitology. 2010;**124**(1):80-89

[61] Clarke SH. Reducing the public health risk of cryptosporidiosis by

optimizing treatment processes at a military water system. US Army Medical Department Journal. 2011;**12**:50-61

[62] Chalmers RM, Smith RP, Hadfield SJ, Elwin K, Giles M. Zoonotic linkage and variation in *Cryptosporidium parvum* from patients in the United Kingdom. Parasitology Research. 2011;**108**(5):1321-1325

[63] Yoder JS, Harral C, Beach MJ. Control CfD, Prevention. Giardiasis surveillance—United States, 2006-2008. MMWR Surveillance Summaries. 2010;**59**(6):15-25

[64] Areeshi M, Dove W, Papaventsis D, Gatei W, Combe P, Grosjean P, et al. *Cryptosporidium* species causing acute diarrhoea in children in Antananarivo, Madagascar. Annals of Tropical Medicine and Parasitology. 2008;**102**(4):309-315

[65] Laurent F, Lacroix-Lamandé S. Innate immune responses play a key role in controlling infection of the intestinal epithelium by *Cryptosporidium*. International Journal for Parasitology. 2017;**47**(12):711-721

[66] Borad A, Ward H. Human immune responses in cryptosporidiosis. Future Microbiology. 2010;**5**(3):507-519

[67] Khurana S, Chaudhary P. Laboratory diagnosis of cryptosporidiosis. Tropical Parasitology. 2018;**8**(1):2

[68] Mirhashemi ME, Zintl A, Grant T, Lucy FE, Mulcahy G, De Waal T. Comparison of diagnostic techniques for the detection of *Cryptosporidium* oocysts in animal samples. Experimental Parasitology. 2015;**151**:14-20

[69] Quilez J, Sánchez-Acedo C, Clavel A, Del Cacho E, Lopez-Bernad F. Prevalence of *Cryptosporidium* infections in pigs in Aragon (northeastern Spain). Veterinary Parasitology. 1996;**67**(1-2):83-88

[70] Cunha FS, Peralta RHS, Peralta JM. New insights into the detection and molecular characterization of Cryptosporidium with emphasis in Brazilian studies: A review. Revista do Instituto de Medicina Tropical de São Paulo. 2019;**61**:30

[71] Jex A, Smith H, Monis P, Campbell B, Gasser R. Cryptosporidium—Biotechnological advances in the detection, diagnosis and analysis of genetic variation. Biotechnology Advances. 2008;**26**(4):304-317

[72] Skotarczak B. Progress in the molecular methods for the detection and genetic characterization of *Cryptosporidium* in water samples. Annals of Agricultural and Environmental Medicine. 2010;**17**(1):1-8

[73] Jex AR, Stanley KK, Lo W, Littman R, Verweij JJ, Campbell BE, et al. Detection of diarrhoeal pathogens in human faeces using an automated, robotic platform. Molecular and Cellular Probes. 2012;**26**(1):11-15

[74] Plutzer J, Tomor B. The role of aquatic birds in the environmental dissemination of human pathogenic *Giardia duodenalis* cysts and *Cryptosporidium* oocysts in Hungary. Parasitology International. 2009;**58**(3):227-231

[75] Cabada MM, White AC Jr. Treatment of cryptosporidiosis: Do we know what we think we know? Current Opinion in Infectious Diseases. 2010;**23**(5):494-499

[76] Nachipo P, Hermann D, Quinnan G, Gordon MA, Van Voorhis WC, Tam P-YI. Evaluating the safety, tolerability, pharmacokinetics and efficacy of clofazimine in cryptosporidiosis (CRYPTOFAZ): Study protocol for a randomized controlled trial. Trials. 2018;**19**(1):456

[77] Zhou Y, Lv B, Wang Q, Wang R, Jian F, Zhang L, et al. Prevalence and molecular characterization of *Cyclospora cayetanensis*, Henan, China. Emerging Infectious Diseases. 2011;**17**(10):1887

[78] Chacín-Bonilla L. Epidemiology of *Cyclospora cayetanensis*: A review focusing in endemic areas. Acta Tropica. 2010;**115**(3):181-193

[79] Ortega YR, Sanchez R. Update on *Cyclospora cayetanensis*, a food-borne and waterborne parasite. Clinical Microbiology Reviews. 2010;**23**(1):218-234

[80] Smith HV. Cyclospora. In: Foodborne Diseases. Switzerland: Springer Humana Press; 2007. pp. 277-301

[81] Resende DV, Pedrosa AL, Correia D, Cabrine-Santos M, Lages-Silva E, Meira WS, et al. Polymorphisms in the 18S rDNA gene of *Cystoisospora belli* and clinical features of cystoisosporosis in HIV-infected patients. Parasitology Research. 2011;**108**(3):679-685

[82] Tadesse T, Tilahun B, Mengistu S, Alemu S, Zeryehun T, Kefyalew D. Prevalence and species distribution of ectoparasite of domestic dogs in Jimma town, Oromia regional state, southwest Ethiopia. Age. 2019;**93**(24.22):0.770

[83] Murphy SC, Hoogestraat DR, SenGupta DJ, Prentice J, Chakrapani A, Cookson BT. Molecular diagnosis of cystoisosporiasis using extended-range PCR screening. The Journal of Molecular Diagnostics. 2011;**13**(3):359-362

[84] Basyoni MM, Elghobary HAF. Genotypic identification of Cystoisospora in immunocompromised patients using Tm-variation analysis. The Korean Journal of Parasitology. 2017;**55**(6):601

[85] Palmieri N, Shrestha A, Ruttkowski B, Beck T, Vogl C, Tomley F, et al. The genome of the protozoan parasite *Cystoisospora suis* and a reverse vaccinology approach to identify vaccine candidates. International Journal for Parasitology. 2017;**47**(4):189-202

[86] Ögren J, Dienus O, Löfgren S, Einemo I-M, Iveroth P, Matussek A. *Dientamoeba fragilis* prevalence coincides with gastrointestinal symptoms in children less than 11 years old in Sweden. European Journal of Clinical Microbiology & Infectious Diseases. 2015;**34**(10):1995-1998

[87] Leder K, Weller DPF, Weller PF, Baron EL. *Blastocystis species*. UpToDate. 2019

[88] Stark D, Barratt J, Van Hal S, Marriott D, Harkness J, Ellis J. Clinical significance of enteric protozoa in the immunosuppressed human population. Clinical Microbiology Reviews. 2009;**22**(4):634-650

[89] Barratt JL, Harkness J, Marriott D, Ellis JT, Stark D. A review of *Dientamoeba fragilis* carriage in humans: Several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. Gut Microbes. 2011;**2**(1):3-12

[90] Garcia LS. *Dientamoeba fragilis*, one of the neglected intestinal Protozoa. Journal of Clinical Microbiology. 2016;**54**(9):2243-2250

[91] Barratt JL, Harkness J, Marriott D, Ellis JT, Stark D. The ambiguous life of *Dientamoeba fragilis*: The need to investigate current hypotheses on transmission. Parasitology. 2011;**138**(5):557-572

[92] Stark D, Van Hal S, Marriott D, Ellis J, Harkness J. Irritable bowel syndrome: A review on the role of intestinal protozoa and the importance of their detection and diagnosis. International Journal for Parasitology. 2007;**37**(1):11-20

[93] Stark D, Barratt J, Chan D, Ellis JT. *Dientamoeba fragilis*, the neglected trichomonad of the human bowel. Clinical Microbiology Reviews. 2016;**29**(3):553-580

[94] Mitra T, Kidane FA, Hess M, Liebhart D. Unravelling the immunity of poultry against the extracellular protozoan parasite *Histomonas meleagridis* is a cornerstone for vaccine development: A review. Frontiers in Immunology. 2018;**9**:2518

[95] Fletcher SM. Gastrointestinal illnesses caused by microbes in Sydney. Australia: OPUS; 2013

[96] Stark D, Barratt J, Roberts T, Marriott D, Harkness J, Ellis J. Comparison of microscopy, two xenic culture techniques, conventional and real-time PCR for the detection of *Dientamoeba fragilis* in clinical stool samples. European Journal of Clinical Microbiology & Infectious Diseases. 2010;**29**(4):411-416

[97] Stark D, Barratt J, Roberts T, Marriott D, Harkness J, Ellis J. A review of the clinical presentation of dientamoebiasis. The American Journal of Tropical Medicine and Hygiene. 2010;**82**(4):614-619

[98] Tan KS, Mirza H, Teo JD, Wu B, MacAry PA. Current views on the clinical relevance of *Blastocystis* spp. Current Infectious Disease Reports. 2010;**12**(1):28-35

[99] Poirier P, Wawrzyniak I, Albert A, El Alaoui H, Delbac F, Livrelli V. Development and evaluation of a real-time PCR assay for detection and quantification of Blastocystis parasites in human stool samples: Prospective study of patients with hematological malignancies. Journal of Clinical Microbiology. 2011;**49**(3):975-983

[100] Elghareeb AS, Younis MS, El Fakahany AF, Nagaty IM, Nagib MM. Laboratory diagnosis of *Blastocystis* spp. in diarrheic patients. Tropical Parasitology. 2015;**5**(1):36

[101] Seyer A, Karasartova D, Ruh E, Güreser AS, Turgal E, Imir T, et al. Epidemiology and prevalence of *Blastocystis* spp. in North Cyprus. The American Journal of Tropical Medicine and Hygiene. 2017;**96**(5):1164-1170

[102] Torgerson PR, Macpherson CN. The socioeconomic burden of parasitic zoonoses: Global trends. Veterinary Parasitology. 2011;**182**(1):79-95

[103] Yason JA, Liang YR, Png CW, Zhang Y, Tan KSW. Interactions between a pathogenic Blastocystis subtype and gut microbiota: In vitro and in vivo studies. Microbiome. 2019;**7**(1):30

[104] Wawrzyniak I, Poirier P, Viscogliosi E, Dionigia M, Texier C, Delbac F, et al. Blastocystis, an unrecognized parasite: An overview of pathogenesis and diagnosis. Therapeutic Advances in Infectious Disease. 2013;**1**(5):167-178

[105] Dogruman-Al F, Turk S, Adiyaman-Korkmaz G, Hananel A, Levi L, Kopelowitz J, et al. A novel ELISA test for laboratory diagnosis of *Blastocystis* spp. in human stool specimens. Parasitology Research. 2015;**114**(2):495-500

[106] Zulfa F, Sari IP, Kurniawan A. Association of Blastocystis subtypes with diarrhea in children. Journal of Physics: Conference Series. 2017;**884**:1742-6596

[107] Stensvold CR, Smith HV, Nagel R, Olsen KE, Traub RJ. Eradication of Blastocystis carriage with antimicrobials: Reality or delusion? Journal of Clinical Gastroenterology. 2010;**44**(2):85-90

[108] Schuster FL, Ramirez-Avila L. Current world status of *Balantidium coli*. Clinical Microbiology Reviews. 2008;**21**(4):626-638

[109] Barbosa AS, Cardozo ML, Dib LV, Fonseca ABM, Uchôa CMA, Bastos OMP, et al. Comparative study of three xenic media culture for cultivation of *Balantidium coli* strains. Revista Brasileira de Parasitologia Veterinária. 2018;**27**:20-26

[110] Fauci J et al. Harrison's Principles of Internal Medicine. 20th ed. New York, London, Chicago: McGraw Hill Education; 2019. p. 1569

[111] Fauci J et al. Harrison's Principles of Internal Medicine. 20th ed. New York, London, Chicago: McGraw Hill Education; 2019. p. 1616

[112] Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease. 8th ed. Philadelphia: Elsevier Saunder's; 2015. p. 3174

[113] Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease. 8th ed. Philadelphia: Elsevier Saunder's; 2015. p. 3190

[114] Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease. 8th ed. Philadelphia: Elsevier Saunder's; 2015. p. 3189