

Review article

WATER-BORNE PROTOZOAN PARASITIC INFECTIONS

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Abstract

Contamination of drinking water by human and animal excreta is a major public health problem the world over. A number of serious protozoan parasitic infections are transmitted to humans by way of contaminated drinking water. Water used by humans for purposes other than drinking such as washing, bathing, swimming, recreational and irrigation activities could also transmit waterborne parasitic pathogenic agents resulting in disease.

Pathogenic protozoan parasitic agents that could be transmitted by drinking water are, *Entamoeba histolytica*, *Giardia intestinalis*, *Cryptosporidium parvum*, *Isospora belli*, *Blastocystis hominis*, *Cyclospora cayetanensis* and Microsporidia. *Naegleria fowleri* and *Acanthamoeba* spp. are generally associated with water related recreational activities.

The review includes a brief historical account, clinical features and the mode of exposure / association with water, under each parasite listed.

Key words – waterborne, outbreaks, protozoan, immunocompromized, diarrhoea, swimming

Water-borne protozoan parasitic infections

Introduction

The most prevalent health hazard associated with drinking water is contamination. This could occur directly or indirectly by human or animal excreta. Water used by humans for purposes other than drinking such as washing, irrigation and recreation could also easily convey pathogenic agents to humans. These agents include viruses, bacteria protozoans and helminths.

Drinking water plays a major role in the transmission of several important protozoan parasitic infections, namely. *Entamoeba histolytica*, *Giardia intestinalis* (syn. *lamblia*), *Cryptosporidium parvum*, *Isospora belli*, *Blastocystis hominis*, *Cyclospora cayetanensis*, and Microsporidia. *Naegleria fowleri* and *Acanthamoeba* spp. are generally associated with water related recreational activities such as swimming. Parasitic agents such as *Acanthamoeba* spp. gain access to the human body by way of inhalation. The organisms are known to be present in droplets of water coming out of showers and air conditioning systems.

The positive effects of public health measures instituted several decades ago are now being reflected in the improved life expectancy and falling death rates worldwide. An increasing elderly population and an ever growing population of immunocompromised persons as a result of cancer chemotherapy, organ transplantation and HIV infections have become a global phenomenon. These populations constitute the high risk groups for emerging and re-emerging infections. Opportunistic infections such as cryptosporidiosis and cyclosporiasis illustrate this point.

Protozoan parasites are the most commonly identified pathogenic agents in waterborne disease outbreaks. This review is confined to information on waterborne protozoan parasitic agents, some of which are well known while others are considered emerging.

Detailed records of waterborne parasitic infections in countries like Sri Lanka are non-existent. Published reports from elsewhere illustrate the seriousness and the magnitude of waterborne protozoan parasitic infections (3).

Entamoeba histolytica and *Entamoeba dispar*

Amoebiasis refers to the infection of the gastrointestinal tract by *Entamoeba histolytica*, a protozoan parasite that is capable of invading the large intestinal mucosa. It has the potential to spread to other organs, mainly the liver. The trophozoite (growing stage) of the parasite was first described by Losch in 1873. Losch saw the parasite in the stools of a young Russian with chronic dysentery. Few years later Councilman and LeFleur (1891) provided clinical evidence to show that there was an association between the presence of the organism and dysentery (1). Quincke and Roos first described the cyst, stage that is responsible for transmission, in 1893 (2).

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Schoudinn gave the name *Entamoeba histolytica* to the organism in 1903. Brumpt first suggested the possibility that the parasite existed as a member of a complex of two species in 1925, following studies on the four-nucleated cyst (3). He differentiated the two species into a non-pathogenic, non-invasive form and an invasive pathogenic form. The name *Entamoeba histolytica* was retained for the pathogenic invasive form while the non-invasive, non-pathogenic species was named *Entamoeba dispar*. This division did not receive support from scientists and the species name *dispar* went into oblivion. The non-pathogenic form of the parasite was considered a different strain of *Entamoeba histolytica* which had the capability of reverting back to the pathogenic state under certain circumstances. It took more than half a century and a series of elegant laboratory investigations by two persons, Sargeant and Williams, to demonstrate the pathogenic and the non-pathogenic strains of *Entamoeba histolytica* based on the isoenzyme patterns of parasites from different geographical locations (4). Brumpt's original differentiation was re-examined by Clark and Diamond, this time using biochemical, immunological and genetic studies (5, 6, 7). They re-described the invasive form retaining the name *Entamoeba histolytica* and separated the non-pathogenic form into a new species – *Entamoeba dispar* with no invasive potential. However, the two species are morphologically similar and are capable of colonising the human large gut.

The acceptance of *Entamoeba dispar* as a separate non-pathogenic species has had a profound impact on the current knowledge of the epidemiology of amoebiasis. This is mainly because the large number of asymptomatic infections worldwide is now attributed to the non-pathogenic *Entamoeba dispar*. Together, *E. histolytica* and *E. dispar* infect about 10% of the world's population (8). However, infections with *E. dispar* is much more common so that the true prevalence of invasive amoebiasis worldwide would be around 1%. As parasitic causes of death, only malaria and schistosomiasis surpass amoebiasis (9). *E. histolytica* is capable of infecting any part of the human body but the most common form of extraintestinal amoebiasis is amoebic liver abscess. This clinical entity which results from migration of amoebic trophozoites from the colon to the liver via the portal circulation, is 10

times more common in adults than in children and 3 times commoner in males (10).

Clinical features of amoebiasis

The incubation period varies from a few days to months depending on the endemicity of the infection in the locality. In highly endemic areas for pathogenic *Entamoeba* species, the onset is usually rapid. This is the usual picture in waterborne infections. Generally, the onset is gradual with prodromal episodes of diarrhoea and abdominal cramps. The hallmark of amoebic colitis is diarrhoea with blood and mucus. These symptoms may continue for weeks and are accompanied by nausea, anorexia and weight loss. In extraintestinal amoebiasis the signs and symptoms are related to the organ or tissue affected.

Mode of exposure / Association with water

Humans are the primary reservoirs of the infection with *E. histolytica*. Hence contamination of water supplies (both drinking and recreational) with domestic sewage helps in the transmission of the infection. Several outbreaks of infection have been traced to sewage contamination of drinking water (11). Contaminated swimming pool water has also been incriminated as a source of infection (11). The chances of water supplies getting contaminated with amoebic cysts are greater in the tropics where carrier rates are high and environmental sanitation poor. The cysts remain viable for months in water at 0°C, 3 days at 30°C, 30 minutes at 45°C and 5 minutes at 50°C (11). The cysts are highly resistant to chlorination (12).

Acanthamoeba spp.

Acanthamoeba is a free-living amoeba capable of causing severe disease in humans. The members of the genus *Acanthamoeba* were formally referred to as *Hartmannella* (Alexieieff 1912). In 1975, Sawyer and Griffin proposed the new family *Acanthamoebidae* for the amoebae that possess acanthopodia (spines) (13). Until recent times members of *Hartmannella* (*Acanthamoebidae*) were known to be pathogenic to mammals although they had been isolated from the throat (14) and the nasal passage (15) of children. In 1982, Cleland *et al* reported the first case of chronic amoebic meningoencephalitis in a Nigerian patient whose cerebrospinal fluid harboured the organism (16). *Acanthamoeba* species can also cause severe eye lesions in humans. Keratitis and uveitis are

common manifestations due to this organism (17). Serum antibodies against *Acanthamoeba* spp. are quite common in keeping with the ubiquitous nature of the parasite (18). The organism has been isolated and cultured from normal flora of the upper respiratory tract of humans (19, 20). This implies that the infection is common and non-pathogenic in immunocompetent persons.

Clinical features of *Acanthamoeba* infections

The incubation period varies with a number of host factors. Mild injuries to the eye predispose to infections rapidly. Swimming while wearing contact lenses is also considered a predisposing host factor. Contact lens wearers may develop keratitis. *Acanthamoeba* also causes granulomatous amoebic encephalitis, a chronic central nervous system disease, in immunocompromised persons.

Mode of exposure / Association with water

Acanthamoebae are ubiquitous in nature. They have been found in tap, fresh, coastal and bottled mineral water, contact lens solutions, eyewash stations, soil, dust, air, sewage, heating, ventilation and air conditioning units, dialysis machines and dental units, hot tubs and gastrointestinal washings (20, 21, 22). The infection can easily be transmitted via drinking water, water in swimming pools and natural water bodies. Infections leading to keratitis occur during warm weather swimming in lakes and ponds while wearing contact lenses (17, 18). Home made contact lens cleaning solutions and washing of eyes with household water following minor injuries are also common modes of transmission. Cysts of Acanthamoebae are highly resistant to chlorination (12).

Naegleria fowleri

Naegleria fowleri is a free-living amoeba. It is the aetiological agent of primary amoebic meningoencephalitis. The organism has been known for quite sometime but was confused with other genera. A case reported by Derrick where the amoeba described resembled *Iodamoeba buetschlii* was probably *N. fowleri* (23, 24, 25). This is now considered to be the first publication describing *N. fowleri* infection in man. To date nearly 200 cases have been reported worldwide. The numbers seem insignificant but the exposure to infection is much more common as seen by the widespread presence of antibodies to *Naegleria* species (18, 26).

Clinical features of *Naegleria fowleri* infection

The incubation period is about 2 to 7 days. The onset of meningoencephalitis is sudden with headache, fever, nausea, vomiting and signs of pharyngitis.

Mode of exposure / Association with water

N. fowleri is a thermophilic amoeba and has a cosmopolitan distribution in surface waters naturally heated by the sun. They tolerate temperatures ranging between 40 - 45°C. The organism can also thrive in industrial cooling water towers and geothermal springs (27). *N. fowleri* may be found in poorly chlorinated swimming pool water, artificial lakes and in warm water near discharge outlets of power plants (20). Large numbers of organisms have been found in water with high content of iron and manganese (3). Some species of *Naegleria* interact with *Legionella* species and have been incriminated for the dissemination of *Legionella* in water (3).

Giardia lamblia

Leeuwenhoek first saw this flagellate protozoan parasite in 1681 in his own stools. The credit for the first description goes to Lamble (1859) who called it *Cercomonas intestinalis*. Stiles in 1915 created the binomial *Giardia lamblia* in honour of Giard of Paris and Lamble of Prague. Giardiasis is the most commonly diagnosed protozoan flagellate infection of the intestinal tract (29). Using stool microscopy, detection rates vary between 2 and 5% in industrialised countries and between 20 and 30% in developing countries (3).

Clinical features of giardiasis

The incubation period is about 2 weeks but may be prolonged to several months. Watery or fatty diarrhoea is the main symptom which is worse in the morning. Diarrhoea is often described as explosive with offensive stools. There is no blood or pus in the stools. Some experience dyspepsia with dull upper epigastric pain.

Mode of exposure / Association with water

Epidemic giardiasis associated with drinking water has been reported from the USA, Canada, England and Sweden (30, 31, 32, 33). Drinking water has been incriminated for giardiasis in travelers, particularly in the former Soviet Union (34). Animal reservoirs of the infection are known (11). *Giardia* cysts are highly resistant to ordinary disinfection (11). In the USA,

backpackers and picnickers who drink stream water are at risk. *Giardia* has also been incriminated for swimming pool associated outbreaks of gastroenteritis (35).

Cryptosporidium parvum

The genus name *Cryptosporidium* was proposed by Tyzzer in 1910 for the organisms he frequently encountered in the gastric glands of laboratory mice. Tyzzer established the new species *Cryptosporidium parvum* in 1912 for organisms which exclusively infected the intestinal epithelial cells of mice. The organism is a coccidian like *Toxoplasma gondii*. It was only in 1976 that the first cases of cryptosporidiosis were reported in humans (36). In persons with normal immune functions, the infection is either asymptomatic or self-limiting. In immunocompromised persons, the infection takes a serious course with intractable diarrhoea. People with a CD4 cell counts over 180 cells / mm³ have been shown to clear the infection spontaneously while those with lower counts had persistent disease (37). The parasite is capable of infecting a wide range of animal species as well as humans. The lack of host specificity has been demonstrated in cross transmission studies (38). A study carried out in a paediatric unit in Sri Lanka has shown that about 6% of acute diarrhoea in children is associated with *C.parvum* (39).

Clinical features of cryptosporidiosis

The incubation period varies between 5 to 28 days. Symptoms vary from self limiting acute diarrhoea to severe prolonged diarrhoea with dehydration. In immunocompromised patients, the parasite causes intractable watery diarrhoea resulting in malabsorption and wasting.

Mode of exposure / Association with water

Outbreaks of cryptosporidiosis have been associated with untreated drinking water, water treated by chlorination only and water subjected to conventional treatment (coagulation, sedimentation, sand filtration and chlorination) (12). This is mainly due to the extremely small size of the oocyst (4 – 6 µm), which is responsible for transmission of the infection. Among the protozoans under consideration, *C.parvum* has the smallest and most chlorine resistant infective stage (oocyst), and hence, the easiest to be transmitted via drinking water. Historically, the massive outbreak in Milwaukee, USA, is the largest even reported where drinking water was found to be the source of infection

(35). Swimming pool and recreational water have been incriminated for outbreaks of cryptosporidiosis (40, 41, 42).

Isospora belli

The two species *Isospora hominis* and *Isospora belli*, both coccidian parasites, were thought to be similar until it was discovered that they have markedly different life cycles. The former is now classified under *Sarcocystidae*. Wenyon named *Isospora belli* in 1923. Virchow is credited with the first description of the parasite in 1860 in the intestine of a man at autopsy. Wenyon and others encountered the parasite frequently in soldiers during war times. It is now thought to be the only *Isospora* species that infects humans, the parasite's only known hosts (43). The infection has been reported from both developed and developing countries (3). Intestinal signs and symptoms are unusual in immunocompetent persons. Outbreaks of diarrhoea due to the parasite have been reported in mental wards, day care centres, World War 11 veterans from the Pacific and HIV infected persons (3, 44).

Clinical features of *Isospora belli* infection

Most infections are asymptomatic. The main feature of *I.belli* infection is diarrhoea and, in those who have mild symptoms, the infection is usually self-limiting. In immunocompromised persons, diarrhoea takes a prolonged course leading to malabsorption and wasting.

Mode of exposure / Association with water

Poor environmental and personal sanitation leading to contamination of drinking water and other food with oocysts is the currently accepted explanation for the spread of the infection (3).

Blastocystis hominis

B.hominis was classified a yeast by Brumpt (1912). In 1967, Zierdt suggested that the organism was a protozoan and later considered it a potential pathogen (45, 46). Although its pathogenic role is not accepted universally, some studies suggest that it could lead to intestinal disturbances (47, 48). The infection is also known to take an aggravated course in immunocompromised persons (49).

Clinical features of blastocystosis

Diarrhoea is the main symptom. It is usually self-limiting but in some, particularly in the immunocompromised, the infection takes a prolonged course with anorexia, flatulence, colicky abdominal pain and low grade fever.

Mode of exposure / Association with water

The infective stage of the parasite is the cyst. Drinking water is the major mode of transmission (50). Drinking water sources could get contaminated with cysts of *B.hominis* in domestic sewage. In a recent study in Thailand, blastocystosis was shown to be significantly associated with unboiled, unfiltered drinking water (51).

Cyclospora cayetanensis

Eimer first noticed *Cyclospora* in 1870 in the intestines of moles. The organism has subsequently been recorded from moles, other rodents and snakes. The first human cases were from Papua New Guinea. *Cyclospora*-like organisms were isolated from three patients but were thought to be organisms belonging to different *Isospora* species (52). From 1985 onwards, organisms, 8 – 10 µm in size, staining red with modified acid fast stains have been isolated frequently from humans. At one time they were described as cyanobacteria-like bodies (blue green algae) or coccidian like bodies (3). These were implicated for frequent diarrhoeal episodes. The coccidian like bodies resembled closely the unsporulated coccidian organisms in diarrhoeal stools of Peruvian children and attempts were made to 'sporulate' them. Based on the morphology of the sporulated bodies, the organisms were then placed in the genus *Cyclospora* (3). Severe *Cyclospora* infections have been described in patients with AIDS (53, 54).

Clinical features of *Cyclospora cayetanensis* infection

The symptoms are similar to those produced by cryptosporidiosis. In patients with AIDS, the infection may last for several months.

Mode of exposure / Association with water

Although proven cases of waterborne *Cyclospora* infections are few, it is thought to be the major mode of transmission of the parasite. The prolonged sporulation time (1 – 2 weeks) also supports this view. *Cyclospora* infection acquired via chlorinated drinking water has been reported (55). Reports of drinking untreated water or reconstituted milk have been obtained from patients with infection (56, 57, 58). In Chicago, in an outbreak in a hospital where 20 resident doctors contracted the infection, the source was traced to the rooftop water reservoir

(59). A child who acquired the infection following a swim in lake Michigan has been reported (3). The infection has also been reported in Nepal and the organism has been demonstrated in sewage water (60). Another indication of the association of *Cyclospora* with water is found in a report from Indonesia where the incidence of diarrhoea due to this organism was highest in expatriates during the rainy season (61).

Microsporidia

'Microsporidia' is a non-taxonomic term used to designate members of the order Microsporidia of the phylum *Microspora*. The organisms are ubiquitous and are known to infect a wide range of vertebrates. The first documented human case was in 1985 (3). Five genera have been reported to be pathogenic to humans. These are, *Encephalitozoon*, *Enterocytozoon*, *Septata*, *Pleistophora* and *Vittaforma*. Based on DNA analysis, *Septata intestinalis* may now be placed in the genus *Encephalitozoon* and, based on the ultra structure, *Nosema corneae* is now called *Vittaforma corneae* (62).

Clinical features of *Microspora* infection

The incubation period is not known. This is because the exact route of infection has not yet been characterised. The infection leads to chronic diarrhoea, dehydration and weight loss. The clinical course is severe in the immunocompromised. The parasites may also cause keratitis, hepatitis, myositis and nervous system disease.

Mode of exposure / Association with water

Microsporidia are commonly found in ditch and other surface waters. Most of them have zoonotic origins but *E. bienersi* is thought to be a natural human parasite (3). Under normal environmental conditions, these organisms are able to survive for long periods of time. At 4°C Microsporidia can remain viable in water for about a year (3). Food and water contaminated with spores are thought to be the most probable modes of transmission (40).

Quality assurance of drinking and recreational water

Drinking water supplies should be sufficient in quantity, wholesome and non-injurious to health. Microbiological testing provides a sensitive indicator of the effectiveness of the source protection, treatment and distribution. With regards to detection of parasites and their stages

in water, the currently available laboratory techniques are grossly inadequate. For detection of cysts of *Giardia* and oocysts of *Cryptosporidium*, methods are being developed in industrialised countries. Research on these lines has not even been attempted in developing countries. Even the bacteriological quality testing for which the techniques are available are not being carried out properly in poor countries. Many are still without treated pipe borne drinking water. With regards to recreational waters, almost nothing has been done in the way of parasitological testing, with chlorination being the only treatment. Protozoan cysts and coccidian oocysts are highly resistant to chlorination and this procedure therefore will not help in preventing transmission (11, 12). Giardiasis and cryptosporidiosis are the most commonly recognised parasitic protozoan infections associated with recreational water (40).

For poor developing countries, the only avenue open currently is to improve the diagnostic laboratory techniques so that emerging infections and their patterns could be methodically monitored. These records will point to outbreaks of possible waterborne parasitic infections in a precise locality or in a high-risk group. Until such time these countries acquire the technology and the resources to monitor parasitological quality of water, the early detection and treatment of those who are infected remain the only solution. When monitoring waterborne parasitic infections the fact that many have zoonotic origins should not be forgotten.

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