GLOBAL WATER PATHOGEN PROJECT

**PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND
EPIDEMIOLOGY ASPECTS**

MICROSPORIDIA

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Summary

Microsporidia are obligate, intracellular, spore-forming parasites, now classified as fungi. Of over 1200 species in 144 genera, most infect insects, birds and fish, but 17 species are known to infect humans. Spores that infect humans are usually 1-4 µm in size. Human microsporidiosis has been a worldwide emerging and opportunistic infection in children, AIDS patients, organ transplant recipients, and travelers, being associated with clinical symptoms such as diarrhea, keratitis, myositis, and bronchitis. Fecal-oral transmission of microsporidia spores is thought to be the major cause for most infections while waterborne and foodborne transmission also occurs. Treatment of microsporidiosis is generally achieved with medications like albendazole and fumagillin.

Human-pathogenic microsporidia such as *Enterocytozoon bieneusi* and *Encephalitozoon* spp. have been detected with molecular methods in various waters including irrigation water, recreation water, drinking water, river water, and wastewater. Microsporidian spores are resistant to environmental degradation and may retain infectivity when stored at low temperature in fresh or marine waters. Spores of some species stored in water at 4°C remain infectious up to two years.

Diatomaceous earth filters used in swimming pools are reportedly inefficient in removing spores. Microsporidia in water can be inactivated by ultraviolet radiation and ozone although spores of some species are highly resistant to chlorine disinfection. Conventional and alternative (i.e., wetlands) wastewater treatment systems have shown variable efficacy in the removal of spores. Enterococci have been suggested as an indicator for the presence of microsporidian spores in wastewater. Wastewater processing end-products like biosolids and effluents may still contain microsporidian spores requiring further processing, e.g., ultrasonic treatment and quicklime stabilization.

1.0 Epidemiology of the Disease and Pathogen(s)

1.1 Global Burden of Disease

Microsporidia are increasingly recognized as opportunistic infectious agents worldwide in both developed and developing countries (Stark et al., 2009). In developed countries, the occurrence of microsporidiosis in HIV/AIDS patients is progressively decreasing probably due to access to highly active antiretroviral therapy (HAART) (Weber et al., 1999; van Hal et al., 2007). However, in some developing countries with limited access to HAART the HIV pandemic continues to spread and microsporidiosis in AIDS patients still remains high (Fayer and Santin, 2014).

In developed countries prevalence rates for enteric microsporidiosis in HIV-seropositive persons with diarrhea

ranged from 2% to 78% varying by degree of immunosuppression, antiviral treatment history, geographical locations, methods of detection, and experiences of diagnosticians, whereas in HIV-seropositive persons without diarrhea infections ranged from 1.4% to 4.3% (Didier et al., 2004; Matos et al., 2012). In the majority of the studies performed in developed countries, *Enterocytozoon bieneusi* was the species detected most often, followed by *Encephalitozoon intestinalis* (Lobo et al., 2012). Recently, a 10-year study in Portugal involving 856 patients observed that *E. bieneusi* was more common in the HIV-seropositive group of patients (7.0-39%) than in the HIV-seronegative group (5.1-15%) (Lobo et al., 2012). In developing countries, *E. bieneusi* prevalence rates were reported between 2.5% and 51% in HIV-seropositive adult patients with diarrhea and in 4.6% of patients without diarrhea, and ranged from 5.35% to 58.1% in HIV-seronegative persons with and without diarrhea (Matos et al., 2012). In children, *E. bieneusi* prevalence ranged from 17.4% to 76.9% in HIV-seropositive children with diarrhea, and from 0.8% to 22.5% in the immunocompetent or apparently immunocompetent groups of children with or without diarrhea (Matos et al., 2012).

In individuals not infected with HIV, seroprevalence rates ranged from 1.3% to 22% among blood donors, pregnant women, slaughterhouse workers, and persons with unknown causes of diarrhea (Didier, 2005). Results from microscopy and PCR-based diagnostic studies presented similar prevalence rates for microsporidiosis in individuals not infected with HIV in the range of 3.3 to 10% in travelers, 5.9 - 17.4% in children with and without diarrhea, and at 17.0% in elderly persons (Didier, 2005).

In HIV-infected patients, microsporidial infection is recognized as an increasingly important cause of morbidity and is responsible for significant gastrointestinal and disseminated diseases (Didier, 2005; Chacin-Bonilla et al., 2006). Many early reports have associated this infection with chronic diarrhea in HIV-infected patients throughout the world (Weber and Bryan, 1994; Asmuth et al., 1994; Kotler and Orenstein, 1998; Bern et al., 2005). Persistent diarrhea, malabsorption, and wasting, which are the most common clinical manifestations associated with the infection in patients with AIDS, are observed in those with ≤ 100 CD4 cells/mm³. A cause and effect between microsporidiosis and diarrhea was reasonably well-accepted but was complicated by concerns about the direct effect of HIV itself, declining immune status, and the presence of other intestinal pathogens on the intestinal mucosa (Didier and Weiss, 2011). For example, although one study documented significant morbidity and high mortality among patients with intestinal microsporidiosis, the lack of a control group made it impossible to assess the disease burden attributable to microsporidiosis itself, as opposed to that of severe immunosuppression (Dascomb et al., 1999).

1.2 Taxonomic Classification of the Agent (s)

Microsporidia are unicellular, intracellular, eukaryotic parasites that were previously considered to be among the

earliest-branching eukaryotes based on the presence of prokaryote-like ribosomes, and the absence of mitochondria, Golgi, and peroxisomes. However, they were recently reclassified with the fungi based on the presence of chitin in the spore wall, identification of a mitochondrial hsp70 gene, and phylogenetic analyses of a long-branch attraction artefact of fast-evolving genes. In addition, the microsporidian genome was observed to be highly reduced and compact, for example, the *Encephalitozoon cuniculi* genome is 2.9 Mb and consists of approximately 2000 genes, suggesting that microsporidia are highly efficient parasites (Didier, 2005; Didier and Weiss, 2006). These parasites have a unique mechanism of host cell infection.

The infectious stage, or spore, contains a coiled polar tube, also called a polar filament that everts under appropriate conditions (change in pH or osmotic pressure) and essentially injects the spore cytoplasm through the polar filament into the host cell (Didier and Weiss, 2011; Bouzahzah and Weiss, 2010). The spores of the microsporidia species that infect humans are relatively small (Figure 1), measuring 1.0 - 3.0 μm $\times$ 1.5 - 4.0 μm ; these are surrounded by a glycoprotein outer layer and a chitinous inner layer that provide protection from the environment (Franzen and Muller, 1999).

Figure 1. Morphology of microsporidia (<http://www.cdc.gov/dpdx/microsporidiosis/gallery.html>). (a) Spores of *Tubulinosema acridophagus* in (BAL) specimens, stained with Chromotrope 2R stain. (b) Microsporidia spores from a corneal section, stained with modified trichrome. (c) Electron micrograph of an *Enterocytozoon bienewsi* spore. Arrows indicate the double rows of polar tubule coils in cross section which characterize a mature *E. bienewsi* spore. (d) Monoclonal antibody-based immunofluorescence identification of *Encephalitozoon hellem*

The life cycle of microsporidia in the host is shown in Figure 2. The infective form of microsporidia is the resistant spore and it can survive for a long time in the environment (stage 1). The spore extrudes its polar tubule and infects the host cell (stage 2). The spore injects the infective sporoplasm into the eukaryotic host cell through the polar tubule (stage 3). Inside the cell, the sporoplasm undergoes extensive multiplication either by merogony (binary fission) or schizogony (multiple fission) (stage 4). This development can occur either in

direct contact with the host cell cytoplasm (e.g., *E. bienewsi*) or inside a vacuole termed parasitophorous vacuole (e.g., *E. intestinalis*). Either free in the cytoplasm or inside a parasitophorous vacuole, microsporidia develop by sporogony to mature spores (stage 5). During sporogony, a thick wall is formed around the spore, which provides resistance to adverse environmental conditions. When the spores increase in number and completely fill the host cell cytoplasm, the cell membrane is disrupted and releases the spores to the surroundings (stage 6). These free mature spores can infect new cells thus continuing the cycle.

Figure 2. Life cycle of *Enterocytozoon* and *Encephalitozoon* species of microsporidia in humans (<http://www.cdc.gov/dpdx/microsporidiosis/index.html>)

"Microsporidia" is a nontaxonomic name used to describe parasites belonging to the phylum Microspora, which includes over 1200 species in 144 genera that infect members of all animal phyla, including insects, mammals, birds, reptiles, and fish, and 17 species identified associated with infections in immunocompromised and immunocompetent persons (Table 1) (Fayer and Santin, 2014; Didier and Weiss, 2006). *Enterocytozoon bienewsi* and the *Encephalitozoon* species (*E. cuniculi*, *E. intestinalis*, and *E. hellem*) are the most prevalent microsporidia identified in humans. Of these four species, *E. bienewsi* is the most frequently diagnosed species in humans worldwide, mainly associated with chronic diarrhea and

wasting syndrome. Up to date, 204 *E. bienewsi* genotypes have been identified based on nucleotide sequence polymorphisms in the 243 bp region of the ribosomal internal transcriber spacer (ITS), including 52 in humans, 34 in both human and animals, 106 host-adapted genotypes in specific animal groups, and 18 novel genotypes in water for which the host is still unknown (Santin, 2015). Additionally, four and three genotypes have been described based on the ITS sequences for *E. cuniculi* and *E. hellem*, respectively (Talabani et al., 2010; Didier et al., 1995; Mathis et al., 1999). For *E. intestinalis*, genotyping data are still not available (Liguory et al., 2000).

Table 1. Species of microsporidia infecting humans

Microsporidia species	Sites of infection	Likely source
<i>Encephalitozoon (syn. Nosema) cuniculi</i>	Systemic, eye, respiratory tract, urinary tract, liver, peritoneum, brain	Mammals
<i>Enterocytozoon bienersi</i>	Intestine, biliary tract, respiratory tract	Mammals, birds
<i>Encephalitozoon hellem</i>	Eye, respiratory tract, urinary tract, systemic	Birds, fruit bats
<i>Encephalitozoon (syn. Septata) intestinalis</i>	Intestine, biliary tract, respiratory tract, bone, skin, systemic	Mammals
<i>Microsporidium africanum (syn. Nosema sp.)</i>	Eye	Unknown
<i>Microsporidium ceylonensis (syn. Nosema sp.)</i>	Eye	Unknown
<i>Microsporidium sp. (similar to Endoreticulatus spp.)</i>	Bone	Unknown
<i>Pleistophora ronniae (syn. Pleistophora sp.)</i>	Muscle	Unknown
<i>Trachipleistophora hominis</i>	Muscle, eye	Unknown
<i>Trachipleistophora anthropolopthera</i>	Systemic, eye	Unknown
<i>Tubulinosema sp.</i>	Muscle	Insect
<i>Anncaliia (syns. Nosema and Brachiola) algerae</i>	Eye, muscle	Insect
<i>Anncaliia (syns. Nosema and Brachiola) connori</i>	Systemic	Unknown
<i>Anncaliia (Brachiola) vesicularum</i>	Muscle	Unknown
<i>Nosema ocularum</i>	Eye	Unknown
<i>Nosema sp.</i>	Eye	Unknown
<i>Vittaforma corneae (syn. Nosema corneum)</i>	Eye, urinary tract	Unknown

1.3 Transmission

Human microsporidiosis has been a worldwide emerging and opportunistic infection in AIDS patients, children, organ transplant recipients, travelers, contact lens wearers and the elderly (Didier, 2005). Species of microsporidia infecting humans have been identified in water sources as well as in domestic, wild, and food-producing farm animals (Didier, 2005). Humans obtain these parasites after exposures to infected persons (anthroponotic transmission) or animals (zoonotic transmission) or ingestion of contaminated food or water (foodborne or waterborne transmission).

1.3.1 Routes of transmission

Microsporidia of interest in the context of this chapter are spread by the fecal-oral route but are also considered ubiquitous organisms in the environment, with a number of potential reservoirs of microsporidia species that can be transmitted to humans which include other infected humans, animals, and water. Infected animals shed spores with feces and urine into the environment, and since primary microsporidia infections commonly occur in the respiratory and intestinal tracts of infected individuals, probable modes of transmission include direct contact with infected persons or animals or contaminated fomites, inhalation of contaminated aerosols, and ingestion of contaminated food or water (Didier, 2005).

The fecal-oral transmission of microsporidian spores is thought to be the major cause for most human infections (Didier and Weiss, 2011).

Vertical transmission of microsporidiosis from mother to offspring has been described in rodents, rabbits, carnivores, and non-human primates, but it has not been observed in humans (Fayer and Santin, 2014; Didier et al., 2004). Some reports suggested that person-to-person transmission of microsporidia might occur through homosexual practices or intravenous drug use, but additional confirmation is required (Fayer and Santin, 2014).

Zoonotic transmission of microsporidia is likely because a wide range of animals are infected with species and genotypes of microsporidia that infect humans, and among the risk factors associated with microsporidiosis in HIV-infected individuals were contact with animals and eating undercooked meat (Didier, 2005). Direct evidence for zoonotic transmission of microsporidiosis was reported in a 2-year-old child who was infected with a unique *E. bienersi* genotype Peru 16 of guinea pig origin after being exposed to a few guinea pigs infected with the same genotype in the same household (Cama et al., 2007).

Water is the most likely source for transmission of microsporidian spores. Several characteristics of microsporidia indicate the probability of waterborne transmission. Many human-pathogenic microsporidia species also can infect animals and could be excreted with urine and feces of infected animals to contaminate water supplies (Didier et al., 2004). By molecular methods, human-pathogenic microsporidia such as *E. bienersi* and *Encephalitozoon* spp. have been detected in various waters worldwide (Fayer and Santin, 2014). In addition, microsporidian spores are generally resistant to environment and survive for extended periods of time in water, the spores are relatively small and not easily trapped by filtration, and the infectious dose is probably low (Franzen and Muller, 1999).

Microsporidia are of concern for foodborne transmission as a result of food globalization, however, relatively few studies have documented the presence of microsporidia in foods (Fayer and Santin, 2014; Didier et al., 2004). In Poland, Sweden, Costa Rica, and Egypt, various vegetables and fruits such as lettuce, celery, cilantro, mung beans, strawberries, and raspberries purchased from local markets were identified containing human-pathogenic microsporidia species (Decraene et al., 2012; Jedrzejewski et al., 2007; Calvo et al., 2004; Mossallam, 2010). In 2009, an outbreak of microsporidiosis was found associated with contaminated food served at conference in a hotel in Sweden (Decraene et al., 2012).

Concerns for vectorborne transmission of microsporidia also were raised recently because some microsporidia species infecting insects are capable of causing disseminated infection in humans. *Anncaliia algerae* has been reported to cause superficial corneal lesion, vocal cord, and muscle infections in humans (Visvesvara et al., 1999; Cali et al., 2010; Coyle et al., 2004). Because it infects many mosquito genera worldwide, it can be highly prevalent in the general environment and thus a potential source of exposure for humans (Fayer and Santin, 2014).

1.3.2 Reservoirs

Although our understanding of reservoirs of microsporidia is still limited, potential sources are beginning to be identified using molecular epidemiology for both humans and animals (Santin and Fayer, 2011). Over a thousand species of microsporidia infect a wide range of animals, including vertebrate and invertebrate animals. Numerous vertebrate hosts have been identified for *E. bienersi*, including humans, non-human primates, and diverse farm, companion, and wild animals. Among the hosts are humans, macaques, marmosets, pigs, cattle, horses, llamas, kudus, dogs, cats, foxes, raccoons, otters, guinea pigs, beavers, rabbits, muskrats, falcons, and other birds (Fayer and Santin, 2014). Invertebrate hosts such as insects were also identified as potential sources of microsporidia in human infections. These include *A. algerae*, *Anncaliia vesicularum* and *Trachipleistophora hominis* that can infect many genera of insects and patients with AIDS, supporting the possibility for vectorborne transmission of microsporidiosis (Fayer and Santin, 2014; Didier et al., 2004).

1.3.3 Incubation period

In healthy persons, microsporidiosis by *E. bienersi* is characterized by self-limiting diarrhea usually of less than one month's duration (Didier, 2005), but the incubation period for human microsporidiosis still remains undetermined due to the lack of available data. Only one cohort study in Sweden describing a foodborne outbreak in 2009 (in a conference in a hotel) associated with *E. bienersi*, has documented incubation periods for cases of microsporidiosis (Decraene et al., 2012). The distribution of case-patients over time indicates a point-source outbreak

but is broad, reflecting a wide range of incubation periods. The median incubation time between the date of the conference and illness onset in case-patients was 9 days for all case-patients (range 0 - 21 days) and 7 days (range 3 - 15 days) for the four microbiologically confirmed case-patients, with the latter (3 - 15 days) more reliable and comparable to other protozoan parasites such as *Cryptosporidium* (1 - 12 days) and *Giardia* (3 - 25 days) (Decraene et al., 2012).

1.3.4 Period of communicability

Currently little is known for the period of communicability of microsporidia. It may vary depending on when spores disappear from the discharges. A study in Thailand on children with asymptomatic *E. bienersi* infections suggested the spore shedding pattern and intensity in these children was variable. Mean spore concentrations in the stool samples from these children ranged from 2.4×10^2 to 1.2×10^5 spores per gram. Light microscopy could detect spores in stool specimens for 9 - 33 days, while PCR was able to detect *E. bienersi* in stool specimens for 3 - 40 days longer (Mungthin et al., 2005). In some cases of the immunocompetent travelers with chronic traveler's diarrhea (3 - 6 weeks), spore shedding still continued after the resolution of clinical gastrointestinal symptoms (Wichro et al., 2005).

1.3.5 Population susceptibility

During the past 30 years, opportunistic infections due to microsporidia species have been recognized predominantly in severely immunocompromised patients with AIDS. Cases of microsporidiosis in immunocompromised persons not infected with HIV as well as in immunocompetent persons also have been reported, including travelers, children, organ transplant recipients, contact lens wearers, cancer patients, and the elderly (Didier and Weiss, 2011). Clinical symptoms and disease associated with microsporidiosis vary with the species causing the infection and the status of the host's immune system.

In HIV-positive patients, the most common clinical manifestation is chronic diarrhea and wasting due to enteric infection, but the spectrum of disease due to different species of microsporidia is broad and includes hepatitis, peritonitis, keratoconjunctivitis, sinusitis, bronchitis, pneumonia, cystitis, nephritis, myositis, encephalitis, and other cerebral infections (Franzen and Muller, 2001). In non-HIV-infected populations, the etiologic agent in nearly 50% of diarrhea cases is undetermined, and it is likely that microsporidia may contribute since these infections are difficult to diagnose or methods to deliberately look for microsporidia are not applied. Furthermore, several species of microsporidia cause systemic infections with a broad range of clinical syndromes and often are overlooked or not considered for diagnosis. In some healthy immunocompetent persons such as travelers, diarrhea tended to be self-limiting and lasting 3-6 weeks (Barratt et al., 2010). Children especially young children during the ages of 18-24 months have been found

to be infected with microsporidia, primarily *E. bienersi*, and developed persistent diarrhea (Pagornrat et al., 2009; Tumwine et al., 2002). Organ transplant recipients who were given immunosuppressive therapy and became infected with *E. bienersi* and *Encephalitozoon* spp. likewise developed fatigue, fever, nausea and diarrhea (Kotler and Orenstein, 1998; Gumbo et al., 1999). It was also reported that the elderly appeared to be more susceptible to microsporidiosis due to decreasing immune competency associated with aging (Lores et al., 2002).

1.4 Population and Individual Control Measures

1.4.1 Hygiene measures - hand washing, disinfection

Individuals at risk for developing life-threatening microsporidiosis such as AIDS patients or organ transplant recipients, have been urged to drink bottled or boiled water and to wash hands appropriately (Mofenson et al., 2009; Kaplan et al., 2009). Other preventive strategies include thorough cooking of meat, fish, and seafood, as well as thorough washing fruits and vegetables prior to ingestion. Since animals are known to be infected with microsporidian species that infect humans, limited exposure to animals suspected of carrying microsporidiosis may be warranted. A wide range of strategies have been applied to reduce the viability and potential infectivity of microsporidia present in the environment. Boiling for at least 5 min can kill *E. cuniculi* spores in water, and application of disinfectants including quaternary ammonium, 70% ethanol, formaldehyde (0.3 or 1%), phenolic derivatives, 1% hydrogen peroxide, chloramine, sodium hydroxide, or amphoteric surfactants for 30 min at 22 °C also can completely destroy *E. cuniculi* spores (Didier et al., 2004; Waller, 1979).

1.4.2 Drug therapy

Currently, the two primarily administered drugs for treating microsporidiosis in animals and humans include albendazole and fumagillin (Didier et al., 2004). Albendazole is a benzimidazole that inhibits tubulin polymerization and also has been used as an anthelmintic and antifungal agent. This drug is effective against *Encephalitozoon* species of microsporidia that infect mammals including humans, but is only variably effective against *E. bienersi* (Conteas et al., 2000; Gross, 2003). Although albendazole is likely less effective against *E. bienersi*, there are reports of success with albendazole

therapy in immunosuppressed patients (Metge et al., 2000). Fumagillin is an antibiotic produced by the fungus *Aspergillus fumigatus* and is highly effective when used topically to treat keratoconjunctivitis due to *Encephalitozoon* species (Conteas et al., 2000; Chan et al., 2003). When administered systemically to humans for treatment of intestinal microsporidiosis at a dose of 20 mg orally three times daily, fumagillin was highly effective against *E. bienersi*, but caused neutropenia and thrombocytopenia in some patients (Champion et al., 2010; Molina et al., 2002). A semi-synthetic analogue of fumagillin named TNP-470 (also named AGM-1470) appears to be less toxic in laboratory animals and was observed to be as effective as fumagillin against several species of microsporidia in tissue culture and in infected athymic mice (Didier et al., 2006; Coyle et al., 1998; Didier, 1997). Additional drugs with variable results that have been reported for treating microsporidiosis in humans include atovaquone, azithromycin, furazolidone, itraconazole, metronidazole, nitazoxanide, octreotide, sinefungin, and sulfa drugs (Didier et al., 2004). In AIDS patients, the highly active antiretroviral therapy (HAART) regimen resulted in HIV reduction and concomitant improvement in CD4⁺ T cell levels with a subsequent reduction in the prevalence of many opportunistic infections including microsporidiosis (Conteas et al., 2000; Carr et al., 1998).

2.0 Environmental Occurrence and Persistence

Spores of microsporidia have been detected in surface water, groundwater, and tertiary agricultural effluent (Dowd et al., 1998; Thurston-Enriquez et al., 2002; Coupe et al., 2006), and the spores are usually resistant to environmental degradation and may retain infectivity for a long time when stored at low temperature in water (Koudela et al., 1999; Li et al., 2003; Shadduck et al., 1978), thus posing a contamination risk to drinking, recreational, and agricultural water supplies. One report of a presumed waterborne outbreak with microsporidia suggested that the source of microsporidiosis infections was probably linked to drinking water in France; most cases lived within an area supplied by one of the three local water distribution subsystems (Cotte et al., 1999). Some reported sources of possible waterborne microsporidia are listed in Tables 2 and 3. Studies are still underway to define the true risks associated with waterborne transmission of microsporidiosis as well as to standardize methods of detection and removal of microsporidia from water sources.

Table 2. Detection of waterborne microsporidia from sewage and wastewater plants

Area	Species	Matrix	Detection Methods	References
China (Four cities)	<i>E. bienersi</i>	Raw sewage from WWTPs ^a	PCR	Li et al., 2012

Area	Species	Matrix	Detection Methods	References
Ireland	<i>E. bienewisi</i> , <i>E. intestinalis</i>	Activated sludge from WWTPs	FISH	Graczyk et al., 2007
Ireland	<i>E. bienewisi</i> , <i>E. intestinalis</i>	Biosolids from WWTPs	FISH	Graczyk et al., 2007
Ireland (Keadue)	<i>E. bienewisi</i>	Wetland influent from a WWTP	FISH, PCR	Graczyk et al., 2009
Ireland (Keadue)	<i>E. bienewisi</i>	Wetland effluent from a WWTP	FISH, PCR	Graczyk et al., 2009
Northwestern Ireland	<i>E. bienewisi</i> , <i>E. intestinalis</i> , <i>E. hellem</i>	Raw sewage	FISH	Cheng et al., 2011
Northwestern Ireland	<i>E. bienewisi</i> , <i>E. intestinalis</i> , <i>E. hellem</i>	Secondary effluents	FISH	Cheng et al., 2011
Northwestern Ireland	<i>E. hellem</i> , <i>E. bienewisi</i>	Wetland effluents from WWTPs	FISH, PCR	Graczyk et al., 2009
Northwestern Ireland	<i>E. bienewisi</i> , <i>E. hellem</i>	Sewage sludge from a WWTP	FISH	Cheng et al., 2011
Northwestern Ireland	<i>E. bienewisi</i> , <i>E. intestinalis</i> , <i>E. hellem</i>	Biosolids from WWTPs	FISH	Cheng et al., 2011
Poland	<i>E. bienewisi</i> , <i>E. intestinalis</i>	Sewage sludge from WWTPs	FISH	Graczyk et al., 2007
Spain (Central)	<i>E. bienewisi</i> , <i>E. intestinalis</i> , <i>E. cuniculi</i> , <i>A. algerae</i>	Raw sewage and treated wastewater; DWTPs ^b , water from four river basins	Chromotrope stain, PCR	Galvan et al., 2013
Spain (Galicia)	Unknown species	Raw sewage	Chromotrope stain	Izquierdo et al., 2011
Spain (Galicia)	Unknown species	Secondary effluent from a WWTP	Chromotrope stain	Izquierdo et al., 2011
Spain (Galicia)	Unknown species	Influent from DWTPs	Chromotrope stain	Izquierdo et al., 2011

Area	Species	Matrix	Detection Methods	References
Tunisia	<i>E. bienewisi</i>	Treated wastewater from WWTPs	PCR	Ben Ayed et al., 2012
Tunisia	<i>E. bienewisi</i>	Raw sewage	PCR	Ben Ayed et al., 2012
Tunisia	<i>E. bienewisi</i>	Dry and dehydrated sludge from WWTPs	PCR	Ben Ayed et al., 2012
USA ^c (Nebraska)	<i>E. cuniculi</i>	Wastewater from livestock production facilities	PCR	Kahler and Thurston-Enriquez, 2007
USA	<i>E. intestinalis</i>	Raw sewage	PCR	Dowd et al., 1998
USA	<i>E. intestinalis</i> , <i>V. corneae</i>	Tertiary effluent from a WWTP	PCR	Dowd et al., 1998

^a WWTP wastewater treatment plant; ^b DWTP drinking water treatment plant

Host affected ^cPigs;

Table 3. Detection of waterborne *microsporidia* in a variety of surface and ground waters

Area	Species	Matrix	Detection Methods	References
China (Shanghai)	<i>E. bienewisi</i>	Huangpu River	PCR	Hu et al, 2014
China ^a	<i>E. bienewisi</i>	Recreational lake in a public park	PCR	Ye et al., 2012
Costa Rica	<i>Pleistophora</i> spp. (89% homology)	Irrigation water from a canal	PCR	Thurston-Enriquez et al., 2002
France	<i>E. bienewisi</i> (98% homology)	River Seine	Uvitex 2B stain, PCR	Sparfel et al., 1997
France	<i>E. bienewisi</i>	River Seine	Uvitex 2B stain, PCR	Fournier et al., 2000
France	<i>E. bienewisi</i>	RRA	PCR	Coupe et al., 2006
France	<i>E. bienewisi</i>	Recreational lake	PCR	Coupe et al., 2006
France	Unknown species	Swimming pools	PCR	Fournier et al., 2002
Ghana	Unknown species	Sachet drinking water	Trichrome stain	Kwakye-Nuako et al., 2007
Guatemala	<i>E. intestinalis</i>	Water for drinking	PCR	Dowd et al., 2003

Area	Species	Matrix	Detection Methods	References
Ireland ^b	<i>E. bieneusi</i> , <i>E. intestinalis</i>	Shannon River	FISH, PCR	Graczyk et al., 2004
Ireland ^c	<i>E. bieneusi</i> , <i>E. intestinalis</i> , <i>E. hellem</i>	Three river-basin districts	FISH	Lucy et al., 2008
Mexico	<i>E. intestinalis</i> (94% homology)	Irrigation water from a river	PCR	Thurston-Enriquez et al., 2002
Spain (Central)	<i>E. bieneusi</i> , <i>E. intestinalis</i> , <i>E. cuniculi</i> , <i>A. algerae</i>	Raw and finished water from WWTPs and DWTPs, water from four river basins	Chromotrope stain, PCR	Galvan et al., 2013
Spain (Galicia)	<i>E. intestinalis</i>	RRA	Chromotrope stain, PCR	Izquierdo et al., 2011
USA	<i>E. bieneusi</i> , <i>E. intestinalis</i>	Surface water	PCR	Dowd et al., 1998
USA	<i>E. intestinalis</i>	Ground water	PCR	Dowd et al., 1998
USA ^d (New York)	<i>E. bieneusi</i>	Stormwater	PCR	Guo et al., 2014

Hosts affected ^aMonkeys; ^b Zebra mussels; ^c Marine mussels, duck mussels, zebra mussels ; ^d Wildlife

Table 4. Genotypes of *E. bieneusi* identified in wastewater

Area	Genotypes Identified (GenBank Accession Number)	Water Type	Reported Hosts in the Literature	References
China	WW1 (JQ863269) WW2 (JQ863270) WW3 (JQ863271) WW4 (JQ863272) WW5 (JQ863273) WW6 (JQ863274) WW7 (JQ863275) WW8 (JQ863276) WW9 (JQ863277)	Raw sewage from WWTPsa	Unknown	Li et al., 2012
China	EbpA (AF076040)	Raw sewage and river water	Human, cattle, horse, mouse, pig	Hu et al., 2014; Li et al., 2012
China	EbpB (AF076041)	River water	Pig	Hu et al., 2014

Area	Genotypes Identified (GenBank Accession Number)	Water Type	Reported Hosts in the Literature	References
China	EbpC (AF076042)	Raw sewage, river water, recreational lake water	Human, beaver, fox, muskrat, rhesus macaque, otter, pig, raccoon	Hu et al., 2014; Li et al., 2012; Ye et al., 2012
China	EbpD (AF076043)	Raw sewage	Human, pig	Li et al., 2012
China	PtEbIV (DQ885580)	Raw sewage	Cat	Li et al., 2012
China	PtEbIX (DQ885585)	Raw sewage ; river water	Dog	Hu et al., 2014; Li et al., 2012
China	PigEBITS7 (AF348475)	Raw sewage	Human, pig	Li et al., 2012
China	PigEBITS8 (AF348476)	Raw sewage	Pig	Li et al., 2012
China	WL14 (AY237222)	Raw sewage	Muskrat	Li et al., 2012
China, Tunisia	BEB6 (EU153584)	Raw sewage and treated wastewater from WWTPs a	Cattle, goat	Li et al., 2012; Ben Ayed et al., 2012
China, Tunisia	WL12 (AY237220)	Raw sewage and treated wastewater	Human, beaver, otter	Li et al., 2012; Ben Ayed et al., 2012
China, Tunisia	Peru6 (AY371281)	Raw sewage and treated wastewater	Human, bird, cattle, dog	Kahler et al., 2007; Ben Ayed et al., 2012
China, Tunisia	Peru8 (AY371283)	Raw sewage and treated wastewater, river water	Human, chicken, mouse	Hu et al., 2014; Li et al., 2012; Ben Ayed et al., 2012
China, Tunisia	Peru11 (AY371286)	Raw sewage and treated wastewater river water, recreational lake water	Human, baboon, rhesus macaque	Hu et al., 2014; Li et al., 2012; Ben Ayed et al., 2012 Ye et al., 2012
China, Ireland, Tunisia	TypeIV (AF242478)	Raw sewage and treated wastewater, wetland irecreational lake water	Human, cat, cattle, dog, rhesus macaque	Li et al., 2012; Ben Ayed et al., 2012; Graczyk et al., 2009; Ye et al., 2012
China, Spain	C (AF101199)	Raw sewage and treated wastewater	Human, mouse	Li et al., 2012; Galvan et al., 2013

Area	Genotypes Identified (GenBank Accession Number)	Water Type	Reported Hosts in the Literature	References
China, Spain, Tunisia	D (AF101200)	Raw sewage and treated wastewater, river water	Human, beaver, baboon, cattle, dog, falcon, fox, horse, mouse, muskrat, pig, raccoon, rhesus macaque	Hu et al., 2014; Li et al., 2012; Galvan et al., 2013; Ben Ayed et al., 2012
China, Tunisia, United States	WL4 (AY237212)	Raw sewage and treated wastewater, stormwater	Muskrat	Li et al., 2012; Ben Ayed et al., 2012; Guo et al., 2014
Spain	D-like (DQ836345)	Raw sewage and treated wastewater, raw water from a DWTPb, river water	Cat	Galvan et al., 2013
Tunisia	BEB3 (AY331007)	Raw sewage and treated wastewater	Cattle	Ben Ayed et al., 2012
Tunisia	WL1 (AY237209)	Raw sewage and treated wastewater	Raccoon	Ben Ayed et al., 2012
Tunisia	WL2 (AY237210)	Raw sewage and treated wastewater	Raccoon	Ben Ayed et al., 2012

Source: Modified from the reference Fayer and Santin, 2014. ^a WWTP wastewater treatment plant; ^b DWTP drinking water treatment plant

2.1 Detection Methods

Light microscopic examination of stained clinical smears, especially the fecal samples, is an inexpensive method of detecting microsporidian spores even though it does not allow identification of microsporidia to the species level. The most widely used staining technique is the Chromotrope 2R method or its modifications (Mohammed et al., 2009; Behera et al., 2008). This technique stains the spore and the spore wall a bright pinkish red. Often, a belt-like stripe, which also stains pinkish red, is seen in the middle of the spore. Transmission electron microscopy (TEM) has long been the gold standard for identifying microsporidia based on observing the polar filament in the organisms, and is still important for observing and describing the ultrastructural features of developing and mature organisms but is too expensive, time-consuming, and insensitive for routine diagnosis (Weber et al., 2000). Fluorescent brightener (e.g. Calcofluor White, Uvitex 2B, Fungifluor) and immunofluorescence antibody stains are used commonly to detect microsporidian spores using the epifluorescent microscope (Didier et al., 2004; Marshall et al., 1997).

During the past 20 years, PCR-based molecular methods for amplifying gene targets of the microsporidia have been developed and increasingly applied to improve the sensitivity and species-specificity for diagnosing microsporidiosis (Fayer and Santin, 2014; Didier et al., 2004). Compared to traditional microscopy-based methods, these molecular methods can offer potential advantages such as increased sensitivity, greater specificity, faster time-to-result, and greater ease of interpretation by non-specialists (Ghosh and Weiss, 2009). Currently there are molecular methods available for identification and characterization of *E. bienersi*, *E. intestinalis*, *E. hellem* and *E. cuniculi* using species-specific PCR and sequencing analyses, mostly based on the analysis of ribosomal internal transcriber spacer (ITS) nucleotide sequences (Fayer and Santin, 2014; Didier and Weiss, 2006; Franzen and Muller, 1999).

In response to the Safe Drinking Water Amendments of 1999, the United States Environmental Protection Agency published methods 1622 and 1623 for identifying and determining the concentration of the waterborne parasites, *Cryptosporidium* spp. and *Giardia duodenalis*, and these approaches also are being applied for detecting and

identifying microsporidia (<http://www.epa.gov/nerlcwww/>). The general scheme of these procedures utilizes pre-enrichment filtration followed by immunomagnetic bead separation (IMS) assay and detection by immunofluorescence antibody staining (FA) (Didier et al., 2004). Variations on this approach for detecting microsporidia in water samples include application of the IMS followed by PCR (Dowd et al., 1999; Sorel et al., 2003), water filtration followed by PCR (Sparfel et al., 1997), calcium carbonate flocculation followed by PCR (Hu et al., 2014), concentration of microsporidia by continuous separation channel centrifugation (Borchardt and Spencer, 2002), and sedimentation followed by fluorescent in situ hybridization (FISH) using species-specific fluorochrome-labelled probes (Cheng et al., 2011; Lucy et al., 2008).

2.2 Data on Occurrence

Many species of microsporidia that infect humans, such as *E. bienersi*, *E. intestinalis*, *E. hellem*, *E. cuniculi*, *A. algerae*, and *Vittaforma corneae*, have been identified in various water sources including ground water, surface water, ditch water, irrigation water, recreation water, drinking water, and wastewater (Tables 2 and 3). In addition, *E. bienersi* spores were identified in the feces of fur-bearing animals that are closely associated with surface water (Sulaiman et al., 2003), and an increased rate of microsporidiosis was reported among people living near water distribution subsystems in France (Cotte et al., 1999). Data on molecular characterization of *E. bienersi* in environmental samples are presented in Table 4.

Table 5. Genotypes of *E. bienersi* identified in surface water

Area	Genotype identified (GenBank Accession Number)	Water Type	Hosts Affected	References
China	RWSH1 (KM496311) RWSH2 (KM496312) RWSH3 (KM496313) RWSH4 (KM496314) RWSH5 (KM496315) RWSH6 (KM496316)	River	Unknown	Hu et al., 2014
China	LW1 (JX000571)	Recreational lake	Human, pig, wild boar	Ye et al., 2012
China	WL15 (AY237223)	Recreational lake	Human, beaver, fox, horse, muskrat, rhesus macaque, raccoon	Ye et al., 2012
China	PigEBITS4 (AF348472) CS-8 (KF607054)	River	Pig	Hu et al., 2014
China	G (AF135834)	River	Pig, wild boar, horse	Hu et al., 2014
China	O (AF267145)	River	Human, pig	Hu et al., 2014
United States	WL6 (AY237214)	Stormwater	Muskrat, raccoon, squirrel, woodchuck	Guo et al., 2014
United States	SW1 (KF591677) SW2 (KF591678) SW3 (KF591680)	Stormwater	Unknown	Guo et al., 2014

2.2.1 Excreta in environment

Mean spore concentrations in the stool samples of children ranged from 2.4×10^2 to 1.2×10^5 spores per gram (reference 33). The excretion of microsporidian spores in feces from humans and animals could potentially pose a significant health threat when feces and wastewater come in contact with susceptible humans or animals from direct contact or various land manure management practices. Manure generated by livestock may be stored or treated in holding ponds or lagoons, thus there is a significant possibility that human-pathogenic microsporidia present in these systems could be transmitted to humans (Kahler and Thurston-Enriquez, 2007). Livestock manure

and wastewater are often used to fertilize crops through spray irrigation or direct land application, which may contaminate the crops and pose a risk of infection to consumers who come in contact with or eat the products of these crops (Thurston-Enriquez et al., 2002). Runoff events from these manure-impacted fields may also transport microsporidia spores to surface water and soil (Kahler and Thurston-Enriquez, 2007). Additionally, because spores of human-pathogenic microsporidia species are similar in size to bacteria, they could potentially be transported through the soil to contaminate groundwater (Dowd et al., 1998). Subsequently, these pathogens could contaminate drinking and recreational water, and food irrigated with contaminated water or in contact with contaminated soil

(Kahler and Thurston-Enriquez, 2007). For instance in Ireland, the most common source of waterborne microsporidia are agricultural lands which contain animal feces from grazing herds and spreading of stored waste from winter-housing and human sewage sludge end products spread on agricultural land (Lucy et al., 2008).

2.2.2 Sewage and wastewater

Monitoring of raw wastewater for pathogens has been used in surveillance of a few bacterial, viral and parasitic pathogens in urban communities (Li et al., 2012; Feng et al., 2009; Talebi et al., 2008). The high concentration of pathogens in raw sewage facilities and the detection of these pathogens via the analysis of a small number of samples can provide a quick overall picture of the disease transmission at the community level, although some zoonotic pathogens in wastewater may also come from domestic animals (Li et al., 2012). A total of 386 raw wastewater samples were collected from wastewater treatment plants (WWTPs) from four cities in China, and *E. bienewsi* was detected in more than 90% of the samples from Shanghai, Qingdao, and Wuhan, and in 62.1% of the samples from Nanjing (Li et al., 2012). In the 338 *E. bienewsi*-positive samples, a total of 23 ITS genotypes were seen. Genotype D was the most prevalent, being found in 82.5% of the samples.

Wastewater treatment plants play an important role in reducing the microbes in sewage (Fayer and Santin, 2014). After treatment, liquid and suspended particles are discharged into surface waters or onto land as biosolids. Any pathogens surviving treatment can pose a waterborne public health risk or as a contaminant of biosolids applied as fertilizer. In the United States, 14 water concentrates were examined and one sample each of *E. intestinalis* and *V. corneae* was detected in tertiary sewage effluent (Dowd et al., 1998). In northwestern Spain, 16 water samples from 8 WWTPs in Galicia were examined for microsporidia by microscopy using Weber's chromotrope-based stain. Microsporidian spores were detected in one sample in the final effluent water from one WWTP. In the other WWTPs, spores were seen in influent water but not in effluent water (Izquierdo et al., 2011). In central Spain, an annual prevalence rate of microsporidia in water samples were 61% in seven WWTPs, with 64.3% (36/56) and 57.1% (32/56) in raw and finished water, respectively. Four human-pathogenic species of microsporidia were detected, including *E. bienewsi* (C, D, and D-like genotypes), *E. intestinalis*, *E. cuniculi* (genotypes I and III), and *A. algerae* (Galvan et al., 2013). In Tunisia, 110 raw wastewater samples and 110 treated wastewater samples from 18 WWTPs were examined for *E. bienewsi* by PCR (Ben Ayed et al., 2012). Eighty-six (78.2%) raw wastewater samples from 17 WWTPs and 49 (44.5%) treated wastewater samples from 16 WWTPs were positive for *E. bienewsi*, with genotypes D and Type IV being the most prevalent genotypes. Analysis of 209 ITS sequences of *E. bienewsi* obtained from those wastewater samples in the 18 WWTPs showed a similar genetic diversity, regardless of the geographical location (Ben Ayed et al., 2012). In Keadue, Ireland, *E. bienewsi* spores from a WWTP were

identified in a single influent wetland water sample at a concentration of 10 spores/L and in two of three wetland effluent samples at a mean concentration of 185 spores/L (Graczyk et al., 2009). In northwestern Ireland, wastewater samples from four constructed horizontal wetlands were tested qualitatively and quantitatively for human-pathogenic microsporidia. Multiplex FISH identified *E. hellem* and *E. bienewsi* spores which were further confirmed by 18S rRNA PCR. The overall prevalence of *E. hellem*-positive samples (75.8%) was significantly higher than that of *E. bienewsi*-positive samples (51.5%). The average concentration of *E. hellem* spores in wetland effluents of 29 ± 13.3 spores/L was significantly higher than that of *E. bienewsi* (7 ± 1.9 spores/L) in effluents (Graczyk et al., 2009). Also in northwestern Ireland, wastewater samples from four WWTPs were examined by multiplex FISH once in April and once in July, and *E. bienewsi*, *E. hellem*, and *E. intestinalis* spores were detected in effluent water from 3, 3, and 1 WWTP, respectively (Cheng et al., 2011). One study in the United States has also estimated the presence of human-pathogenic microsporidia in environmental samples collected from livestock production facilities in eastern Nebraska. *Encephalitozoon cuniculi* was identified in one of the wastewater samples that originated from an outdoor swine lagoon that received wastewater from nursery pig and adult swine houses (Kahler and Thurston-Enriquez, 2007).

2.2.3 Sludge

Since 2000, there has been an increase both in the overall percentage and volumes of sewage sludges spread on Irish farmland, with an estimated 45,543 tonnes spread in 2005 and 86,411 tonnes in 2007 (Cheng et al., 2011; Lucy et al., 2008). These large volumes of spread biosolids increase the risk of pathogens like microsporidia spores being introduced via surface runoff into waters used for drinking-water (Lucy et al., 2008). One study in Ireland examined *E. intestinalis*, *E. hellem*, *E. cuniculi*, and *E. bienewsi* in sewage sludge during the activation secondary treatment process and in the corresponding sewage sludge end products from four urban WWTPs by multiplex FISH. There was a significant reduction of microsporidia concentration in dewatered and biologically stabilized sewage sludge cake compared to that in the corresponding sludge samples collected during the activation process. *Enterocytozoon bienewsi* was found in activated sewage sludge samples from all WWTPs, and the mean concentration (224 spores/L) was significantly higher than the mean concentration of *E. intestinalis* (51 spores/L). The overwhelming majority of spores identified in sewage sludge-derived samples were viable, and the fraction of nonviable pathogens constituted no more than 1% (Graczyk et al., 2007). Also in Ireland, sewage sludge cake samples from three WWTPs and biological-treated liquid sewage sludge samples from one WWTP were examined for viable microsporidian spores by multiplex FISH once in April and once in July. For the biosolids, viable *E. bienewsi* spores were not only the most frequently detected but in general had the highest concentration in all WWTPs in April and July. The highest concentrations of *E. bienewsi*, *E.*

intestinalis, and *E. hellem* spores in sewage sludge cake samples were 19 spores/g, 16 spores/g, and 32 spores/g, respectively. The liquid sewage sludge samples from one WWTP contained the lowest microsporidian loadings with *E. bienewsi* concentrations of 450 spores/L in April and 1,000 spores/L in July, and *E. hellem* concentration of 400 spores/L in April and zero in July. *E. intestinalis* was not identified in these sludge samples (Cheng et al., 2011). In Tunisia, 12 sludge samples were collected from 5 WWTPs, and *E. bienewsi* was identified by PCR in one dry sludge sample and seven dehydrated sludge samples (Ben Ayed et al., 2012). In Poland, solid waste landfill leachate samples from a municipal landfill and sewage sludge samples from two urban WWTPs were quantitatively tested for viable microsporidian spores by multiplex FISH. The solid waste landfill leachate samples were tested positive for *E. bienewsi* (mean = 12.4 ± 1.0 spores/g), and both *E. bienewsi* and *E. intestinalis* were identified in the sewage sludge samples: WWTP1 (*E. bienewsi*, mean = 21.6 ± 3.3 spores/g; *E. intestinalis*, mean = 10.6 ± 1.4 spores/g); WWTP2 (*E. bienewsi*, mean = 13.3 ± 1.2 spores/g; *E. intestinalis*, mean = 25.7 ± 3.4 spores/g). Sewage sludge samples contained significantly more microsporidian spores than the landfill leachate samples (Graczyk et al., 2007).

2.2.4 Surface waters

Human-pathogenic microsporidian spores have been detected in various surface waters, including river water, lake water, storm water, and recreational water. In France, one report examined microsporidian spores in surface waters from the River Seine by light microscopy and PCR, and documented for the first time the detection of *E. bienewsi* spores in one sample although its sequence showed 98% homology with a published *E. bienewsi* sequence (Sparfel et al., 1997). In 25 other water samples collected from the River Seine, 64% were positive for microsporidia by nested PCR. Unexpectedly, *E. bienewsi* was identified in only one sample, and unknown microsporidia species were identified in eight samples with highest scores of homology with *V. corneae* or *Pleistophora* sp. (Fournier et al., 2000). In the United States, four surface water samples were examined, *E. bienewsi* and *E. intestinalis* were detected by PCR in one and two samples, respectively (Dowd et al., 1999). In New York City, 67 stormwater samples were collected from a drinking source watershed and 58.2% were positive for *E. bienewsi*, including two known genotypes (WL4 and WL6) and three new genotypes (SW1, SW2, and SW3), with WL4 being the most common genotype in 23 samples (Guo et al., 2014). In central Spain, an annual prevalence rate of microsporidia in water samples was 50% in six locations of influence on four river basins, with *E. bienewsi* genotype D-like detected in these samples (Galvan et al., 2013). In China, to investigate the impact of the pig carcass incident on microbial water quality, 178 river water samples were collected from the upper Huangpu River, and 31.5% were PCR-positive for *E. bienewsi*. Seventeen *E. bienewsi* genotypes were found belonging to 11 established genotypes (EbpC, EbpA, D, CS-8, PtEb IX, Peru 8, Peru 11, PigEBITS4, EbpB, G, O) and six new ones (RWSH1 to RWSH6), with EbpC being the most common genotype in 37 samples. Most of the *E.*

bienewsi genotypes belonged to pig-adapted Groups 1d and 1e, suggesting that dead pigs contributed significantly to *E. bienewsi* contamination in the Huangpu River (Hu et al., 2014).

Exposure to recreational bathing waters can also play a role in epidemiology of microsporidiosis. In France, 48 water samples were collected during a one-year period in six swimming pools in Paris, and one sample was positive for microsporidia with unknown species which was close to an insect microsporidia *Endoreticulatus schubergi* (Fournier et al., 2002). In another study in France near Paris, 57 water samples were collected monthly for one year from two recreational lakes and three river sites where bathing and boating are frequent, and *E. bienewsi* was detected by PCR in one lake sample and one river sample (Coupe et al., 2006). In northwestern Spain, six water samples from recreational river areas (RRAs) in Galicia were examined for microsporidia by microscopy and PCR. Microsporidian spores were detected in only one sample and were identified as *E. intestinalis* (Izquierdo et al., 2011). At a public park in China, 23 water samples were collected in a small lake where rhesus monkeys frequently bathed, *E. bienewsi* was detected in 13 (56.5%) samples, including the human-pathogenic genotypes of Peru11, W15, EbpC, and Type IV, thus posing a potential public health risk for microsporidiosis through drinking or recreational water by microsporidia of rhesus monkey origin (Ye et al., 2012). In order to protect public health, it is recommended to: (a) introduce bather number limits to recreational areas; (b) prevent diapered children from entering beach water; (c) advise people with gastroenteritis to avoid bathing; and (d) use showers prior to and after bathing (Graczyk et al., 2010). Whenever possible, recreational bathing areas should be located away from and upstream of point sources of contamination (Stewart et al., 2002).

2.2.5 Groundwaters

It has been shown that enteroviruses and enteric bacteria can be transported over long distances in the subsurface, where they could eventually contaminate drinking wells, but very limited studies have looked at the transport of protozoan parasites in relation to groundwater (Zychowski and Bryndal, 2015; Hynds et al., 2014). This is because most protozoa of clinical import are too large to be transported for any great distance in the terrestrial subsurface. Microsporidia, because of their small size similar to most bacteria, may not be strained by soil matrices as readily as other parasites. Thus their small size increases their potential to be subsurface and groundwater contaminants (Dowd et al., 1998). In the United States, the presence of *E. intestinalis* was confirmed for the first time in groundwater. Six groundwater samples collected from wells were examined, and five samples showed positive for microsporidia by immunofluorescence assay analysis and one sample was identified as *E. intestinalis* by PCR. The detection of microsporidia in groundwater samples indicates that there may be the potential for subsurface transport of these parasites (Dowd et al., 1998). A recent study in Taiwan showed correlation between taking baths in hot springs and the incidence of microsporidial keratitis

in nine patients infected with *V. corneae*, but whether there are factors present in the hot springs that make attendees more susceptible to microsporidial invasion still requires further investigation (Fan et al., 2012).

2.2.6 Drinking waters

Drinking water outbreaks of waterborne pathogens might be associated with heavy rainfall and flooding. In Ireland, heavy periods of rainfall lead to: (1) runoff of slurries and sewage sludges from agricultural land into drainage channels and surface waters; (2) storm water overflows in WWTPs with subsequent release of untreated wastewater; and (3) increased turbidity in reservoirs used for drinking water (Lucy et al., 2008). In Guatemala, 12 water concentrates were collected in various water sources used for public consumption in rural areas around the city of Guatemala, and *E. intestinalis* was detected in six water samples by PCR (Dowd et al., 2003). One study in Ghana examined pathogenic parasites in 27 different brands of sachet drinking water samples purchased from local vendors, and identified microsporidia spores in 51.2% by microscopy, which should be confirmed by molecular methods in further studies (Kwakye-Nuako et al., 2007). Drinking water treatment plants (DWTPs) are charged with producing potable water free of pathogens and toxins at extremely low levels deemed safe for public health. In northwestern Spain, 16 water samples from 8 DWTPs in Galicia were examined for microsporidia and two samples showed positive results by microscopy, and the spores were detected only in influent water but not in effluent water from DWTPs (Izquierdo et al., 2011). In central Spain, an annual prevalence rate of microsporidia in water samples were 27% in four DWTPs, with 35.5% (11/31) and 18.8% (6/32) in raw and finished water, respectively. *E. bienersi* genotype D-like was detected in a DWTP (Galvan et al., 2013).

2.2.7 Seawater

Microsporidian spores have been found throughout the bathing season in marine recreational waters. In the United States, recreational beach water samples were collected during 11 consecutive summer weeks from the Chesapeake Bay in Maryland, including 27 weekend samples and 33 weekday samples. Both the numbers of bathers and water turbidity levels on weekends were significantly greater than those on weekdays. The proportion of water samples containing microsporidian spores were significantly higher in weekend water samples (59%) than in weekday samples (30%), and the concentrations of spores also were significantly higher on weekends (mean = 4.8 ± 0.9 spores/L) when compared to weekdays (mean = 1.8 ± 0.6 spores/L). Microsporidian spores were represented mostly by *E. bienersi*, whereas *E. intestinalis* spores were detected in a single weekend water sample (Graczyk et al., 2007; 2010). In Ireland, coastal waters were surveyed for the human waterborne enteropathogens by utilizing bivalve mussel species as biomonitors, and it showed that the coastal bay with raw urban sewage discharge was 100% positive for *E. bienersi*, *E. intestinalis*, and *E. hellem* (Lucy et al., 2008).

2.2.8 Soil

It has been suggested that the soil of public parks presents an important source of infections of intestinal parasites which may have a significant impact on public health (Giacometti et al., 2000; Martinez-Moreno et al., 2007). Children might be the main group affected because they play in playgrounds and can accidentally ingest the pathogens from contaminated soil (Dado et al., 2012). In Hong Kong, China, a study reported a series of microsporidial keratoconjunctivitis in pediatric and teenage healthy individuals with cluster infection after mud exposure in the game of ruby. PCR testing was positive for the small subunit rRNA gene of *V. corneae* in three out of five eyes. The cluster infection of microsporidia in these cases may be attributed to exposure to contaminated soil during the contact sport (Kwok et al., 2013). Two studies in Singapore also identified an increasing incidence of microsporidial keratitis with a strong correlation with prior soil exposure (Loh et al., 2009; Tan et al., 2013). In North America, soil, sand, and compost samples from popular urban sites in Vancouver (Canada) and Seattle (USA) suggested that there was a wide diversity of microsporidian species in these samples. The majority of microsporidian species were obtained from soil (17 species), compost (10 species), and sand (2 species), but none are known to infect humans. In addition, the distribution for each species was usually restricted to a particular site, and that only a small number of species appear to be more widespread, thus highlighting the diversity of Microsporidia in common, urban habitats (Ardila-Garcia et al., 2013). In Korea, 34 farm soil samples were collected from seven different localities along the western side of the Korean Peninsula, and *E. hellem* (genotype 1B) was identified in three samples (8.8%) by real-time PCR and nucleotide sequencing. The number of spores detected from the soil samples ranged from 9,275 to 16,455 per g of soil. The study concluded that the occurrence of *E. hellem* in these farm soil samples might come from livestock farms (Kim et al., 2015).

2.2.9 Irrigation water and on crops

Irrigation waters may be contaminated either by the introduction of sewage or by runoff from nonpoint sources. Rain events may carry fecal contamination from agricultural, domestic, and wild animals (including birds) into canals, river waters, and wells that serve as sources of irrigation water (De Roeve, 1998). The use of irrigation waters to mix insecticides and fungicides that are sprayed directly onto crops increases the risk of surface contamination by pathogenic microorganisms and raises concern for food safety (Thurston-Enriquez et al., 2002). In one study, water from canals, lakes, and rivers used directly for irrigation of crops was collected in the United States and several Central American countries including Costa Rica, Panama, and Mexico. Twenty-eight percent of the irrigation water samples (7/25) were tested as positive for microsporidia by PCR, with one or more positive samples from each country. Two positive samples were sequenced and subsequent database homology comparisons allowed the presumptive identification of two human pathogenic species, *E. intestinalis* (94% homology) and

mleistophoraspp. (89% homology), but neither of them was close enough to determine the actual identity. These findings indicated the presence of microsporidia in irrigation waters used in the production of crops traditionally consumed raw, and suggested that there may be a risk of infection to consumers who come in contact with or eat these products (Thurston-Enriquez et al., 2002).

2.2.10 Fish and shellfish

It has been reported that molluscan shellfish are capable of recovering and concentrating environmentally derived pathogens and can be used as a monitoring tool for the sanitary assessment of water quality. In Ireland, zebra mussels were collected at eight sites across the Shannon River drainage area, where intensive agricultural entities and human activities that can potentially deliver anthropozoonotic pathogens to surface water via runoff or wastewater disposal are found. By multiplex FISH and PCR, *E. intestinalis* was detected at two sites, and the mean concentration of *E. intestinalis* spores varied from seven to eight per mussel. Spores of *E. bienersi* were identified in 4 of 19 (21%) mussels, and the concentration ranged 3 - 12 spores/mussel. Approximately 80% of microsporidia were viable and thus capable of initiating human infection. The study has documented for the first time the detection of human-pathogenic *E. intestinalis* and *E. bienersi* spores in molluscan shellfish. Although the actual transmission route of the two microsporidia species is unknown, it is quite possible that infectious spores of human or animal origin passed to the environment with feces or urine contaminating the Shannon River (Graczyk et al., 2004). In another study in Ireland, surface inland and coastal waters were surveyed for human waterborne enteropathogens by utilizing bivalve mussels (marine mussels, duck mussels, and zebra mussels) as biomonitors at 12 sites located in three river-basin districts with various water quality pressures. Spores of *E. bienersi* were found in different concentrations at eight of the twelve sampling sites, and spores of *E. hellem* and *E. intestinalis* were found at six and three of the twelve sites, respectively. The study concluded that differences in cumulative number of species between sewage outfall sites may reflect the prevalence of these pathogens in the human population discharging to specific sewage-treatment plants (Lucy et al., 2008).

2.3 Persistence

Due to the electron-dense, glycoprotein-composed, and chitinous cell structure, microsporidian spores are relatively temperature insensitive, can survive in fresh and marine waters, and even after dehydration for extended periods of time (Didier et al., 2004)[5]. Sufficient numbers of *E. cuniculi* spores remained viable to cause lethal infections to SCID mice after storage for 2 years at 4 °C or after freezing at -12 and -24 °C for 1 day (Koudela et al., 1999). Spores of *E. cuniculi*, *E. intestinalis*, and *E. hellem* stored in water at temperatures ranging from 10 to 30 °C were still able to infect host cells in tissue culture, from several weeks to as long as one year (Li et al., 2003). The longevity was found to vary with species and decrease with elevated temperature, and *E. intestinalis* was found to be

somewhat harder than *E. hellem* and *E. cuniculi*. Survival of the spores was also observed in a wide range of water salinities and temperatures. Spores of *E. cuniculi* survived more than 9 days in buffer at 37 °C, at least 24 days at 4 and 20 °C, and at least 6 months at -70 °C. Even spores that were dried and then stored at 22 °C at 0 - 2% relative humidity for 28 days remained infectious. Storage in distilled water or freezing and thawing failed to kill all *E. cuniculi* spores, and spores survived at least 1 day after storage at pH 4 and pH 9 (Shadduck and Polley, 1978). Under experimental conditions, at least some *E. cuniculi* spores remained infectious in a tissue culture system after incubation in mild saline solution (M199) for 16 days at 22 °C and after 98 days at 4 °C (Waller, 1979). Spores also survived in seawater but for shorter periods of time. Spores of *E. hellem* appeared the most robust with some remaining infectious in 30 ppt seawater at 10 °C for 12 weeks and in 30 ppt seawater at 20 °C for 2 weeks. Spores of *E. intestinalis* were slightly less robust, remaining infectious in 30 ppt seawater at 10 and 20 °C for 1 and 2 weeks, respectively. Spores of *E. cuniculi* remained infectious in 10 ppt seawater at 10 and 20 °C for 2 weeks but not at higher salinities. Although the spores of the three species of *Encephalitozoon* vary in ability to remain viable in freshwater and over a range of salinities and temperatures found in nature, they can potentially remain infectious long enough to become widely dispersed in various waters (Fayer, 2004). These characteristics of the microsporidian spores also indicate potential health risks due to transmission from the environment to various susceptible hosts, including humans and domestic animals.

3.0 Reductions by Sanitation Management

3.1 Excreta and wastewater treatment

3.1.1 Wastewater Treatment

3.1.1.1 Primary and Secondary Treatment

Conventional and alternative wastewater treatment systems have shown variable efficacy in the removal of microsporidian spores. In northwestern Spain, in eight WWTPs where water treatment processes consisted of a primary treatment (screening, storage and preconditioning) and a secondary treatment (coagulation and flocculation, sedimentation and decantation) without a tertiary treatment (ultraviolet or ozone), microsporidian spores were detected in one sample in the final effluent water from one WWTP, and were seen only in influent water but not in effluent water in the other WWTPs (Izquierdo et al., 2011). In central Spain, microsporidian spores have been detected in 64.3% raw water and in 57.1% finished water in seven WWTPs based on physicochemical and biological treatments with activated sludge (Galvan et al., 2013).

In Tunisia, in 18 WWTPs using different treatment processes (with or without primary decantation, activated sludge, bacterial bed, waste stabilization pond, or ultraviolet light), the reduction in the occurrence of several pathogens was affected by the size of the environmental stage because sedimentation was the key process in all

treatment processes. As a result, a slightly higher reduction in occurrence was achieved for the large *Eimeria* oocysts of $0.62 \log_{10}$ (76%) compared to the small *E. bieneusi* spores which were reduced by $0.24 \log_{10}$ (43%), with the medium-sized *Cryptosporidium* oocysts ($0.47 \log_{10}$ (66%)) and *Giardia* cysts ($0.49 \log_{10}$, 68%) reductions very close to each other. Nevertheless, there were no apparent differences in the reduction of these organisms among different treatment practices. For example, the two plants that were sampled most frequently had a similar reduction in the occurrence of these pathogens, even though the former had no primary treatment and used waste stabilization pond as the secondary treatment, whereas the latter used decantation as the primary treatment and activated sludge as the secondary treatment (Ben Ayed et al., 2012).

In northwestern Ireland, four municipal secondary wastewater treatment plants (plants A - D) were selected to investigate the presence of human-pathogenic microsporidian spores (*E. bieneusi*, *E. intestinalis*, and *E. hellem*) and enterococci during treatment processes, including (a) sludge activation in an oxidation ditch (plant A); (b) sludge activation in extended aeration tanks (plants B and C); and (c) treatment by percolating filter (plant D). After the activated-sludge treatment, settled biosolids were deposited onto sludge dewatering beds. Plants A, B, and D were equipped with sludge dewatering beds, and liquid sewage sludge was collected directly for disposal from a secondary settling tank at plant C. The study showed that the spore removal efficacy of four secondary wastewater treatment systems varied. Although *E. bieneusi* was found at higher concentration than *E. hellem* and *E. intestinalis*, the removal of *E. bieneusi* at all four plants was the most efficient. For *E. intestinalis*, plant A had the least efficient removal of spores ($0.17 \log_{10}$) in wastewater, with $> 2 \log_{10}$ removal at other plants. Nevertheless, some negative removal efficiencies were obtained for *E. bieneusi* with concentrations increasing (at plants C and D, doubling) and for *E. hellem* (at plants A and D, increasing by 90% and 50%). These negative removal efficacy values indicated that some spores of *E. bieneusi* and *E. hellem* were likely delivered to the plants during the treatment processes by visiting avian wildlife, as birds were abundant at wastewater operations. In terms of wastewater processing end-products, high concentrations of microsporidian spores were observed in the dewatered biosolids and the sewage sludge, such as 32 spores/g of *E. hellem* at plant D, 19 spores/g of *E. bieneusi* and 16 spores/g of *E. intestinalis* at plant B, and 1,000 spores/L of *E. bieneusi* at plant C. The increased levels of spores in the biosolids and sludge might also be due to fecal contribution of birds as sludge treatment and storage takes place in open drying beds. These spores in the wastewater processing end-products can be a potential source of human-pathogenic microsporidian contamination to the local environment. Thus further processing is still required to treat these products for inactivation and disintegration of microsporidian spores, such as ultrasonic treatment and quicklime stabilization. In addition, a significant correlation was found between the levels of enterococci and human-pathogenic *E. bieneusi* spores during the wastewater treatment processes, suggesting that enterococci may have

potential as an indicator for the possible presence of human-pathogenic microsporidian spores in wastewater (Cheng et al., 2011).

3.1.1.2 Constructed Wetlands

As mentioned, wastewater discharges are worldwide risk factors for the introduction of human pathogens into surface water or groundwater used as drinking and recreational resources. Constructed wetlands of either vertical or horizontal flow are increasingly used worldwide for secondary or tertiary treatment of municipal sewage due to minimum electricity requirements and low maintenance costs (Reinoso et al., 2008; Graczyk and Lucy, 2007). In general, wastewater can be injected under the wetland surface for plug flow hydraulics, i.e., subsurface flow (SSF), or delivered to the wetland surface for free-surface flow (FSF) (Ulrich et al., 2005).

In Keadue, Ireland, two secondary (wetland influent) and three tertiary (wetland effluent) treated wastewater samples were collected in an urban WWTP, which utilized primary treatment by coarse screening, secondary treatment (sludge activation and sedimentation), and the effluent was polished using a constructed small scale free-surface flow horizontal wetland (reed-bed filtration system), with effluent discharging directly to a lake used for recreational activities. By FISH and PCR analyzes, *E. bieneusi* spores were identified in a single influent wetland water sample at a concentration of 10 spores/L and in two of three wetland effluent samples at a mean concentration of 185 spores/L. The presence of a higher concentration of microsporidian spores in tertiary-treated, wetland polished wastewater than in secondary-treated wastewater can be explained that microsporidia might be propagated in the wetland by dogs and livestock or contributed to the wetland by visiting wildlife. Thus the effluents from wetland can substantially contribute to contamination of surface waters used for recreation and drinking water and may represent a serious threat to public health (Graczyk et al., 2009).

In northwestern Ireland, influent water and effluent water samples were collected from four constructed horizontal wetlands, all of which received unchlorinated municipal wastewater subjected to secondary treatment after sewage sludge activation and secondary sedimentation. Wetland A had two components: the first FSF component discharged to SSF component with a final effluent released to groundwater. The remaining three wetlands were small-scale FSF wetlands discharging to surface waters. FISH and PCR analyzes identified two human-pathogenic microsporidia species, *E. hellem* and *E. bieneusi*, in these water samples, however, negative removal efficiencies (increasing concentrations) were observed for *E. hellem* (100% at all wetlands) and for *E. bieneusi* (0 at wetland A, 100% at wetlands B, C, and D). Even the SSF wetland A, with the highest removal rates for *Cryptosporidium* oocysts and *Giardia* cysts, discharged *E.*

*hellem*spores despite the fact that they were absent in the wastewater entering this wetland. This can be also explained by the contribution of wildlife in the wetlands. This study suggested that even with the best pathogen removal rates achieved by SSF wetland, the reduction of pathogens was not enough for a safety reuse of the reclaimed water (Graczyk et al., 2009).

3.1.2 Recreational Water Treatment

As mentioned, occasional detection of microsporidia spores was reported in swimming pools, recreational lakes, and recreational river areas (Dowd et al., 1998; Izquierdo et al., 2011; Fournier et al., 2002; Ye et al., 2012), suggesting that exposure to recreational bathing waters might play a role in waterborne transmission of microsporidiosis. However, diatomaceous earth filters usually used in swimming pools have not been shown efficient in removing small microorganisms such as *Cryptosporidium* oocysts and microsporidian spores, which are likely to escape such filtration systems and further disinfection treatments (ozone or chloramines) must be performed (Hutin et al., 1998). Different from swimming pools, hot springs use different sterilization methods. Chloride-based disinfectants are commonly used for swimming pools. These disinfectants, however, are rarely used for hot springs due to the unpleasant odor. There are other methods that can be used to keep the hot springs clean, including frequent recycling of the spring water through filters, reheating, photoirradiation, and treating with hydrogen peroxide (Fan et al., 2012). Because the microsporidian spores like *V. corneae* were measured at 3.3 by 1.4 μm , recycling of the spring water through filters with pore sizes larger than 2 μm cannot remove the parasite (Fan et al., 2012). *Vittaformacorneae* was also confirmed to exist in wastewater effluent that has undergone tertiary treatment by pressure filtration through mixed-medium filters (Dowd et al., 1998). Therefore, recycled spring water contaminated by microsporidia and treated by passing through filters with too large a pore size can potentially pose a threat to public health (Fan et al., 2012).

3.1.3 Drinking Water Treatment

Water treatment in conventional DWTPs occurs through a series of physical and chemical processes, generally including flocculation, sedimentation, filtration, and disinfection. In the United States, one study evaluated the removal of *E. intestinalis* and a feline calicivirus through conventional treatment in a pilot plant. Several different coliphages with different isoelectric points and *E. coli* were used as controls. The pilot plant included the stages of flocculation, sedimentation, and multimedia filtration with granular activated carbon (GAC), sand, and gravel. During the water treatment processes, *E. intestinalis* was consistently removed with an average 2.47 $\log_{10}$ of the three tests conducted. Following sedimentation, the $\log_{10}$ removal averaged 0.78. An average additional removal of 1.69 $\log_{10}$ was achieved following filtration. Therefore, the greatest removal of the microsporidian spores occurred by the filtration process. The author concluded that the conventional treatment was most effective in the removal of

the larger microorganisms like *E. coli* (2.67 $\log_{10}$) and *E. intestinalis* (2.47 $\log_{10}$), which are similar in size. These were primarily removed by filtration and smaller ones like viruses were removed to the greatest degree by flocculation (Gerba et al., 2003). In central Spain, in four DWTPs where water treatment included preoxidation, precloration, coagulation, flocculation, decantation, and disinfection (ozone and chloramines), microsporidian spores were detected in 35.5% raw water samples and in 18.8% finished water samples (Galvin et al., 2013). In northwestern Spain, in eight DWTPs in Galicia where water treatment included coagulation, flocculation, and clarification through sedimentation, filtration, and disinfection by chlorination without ozonation and ultraviolet treatment, 16 water samples were examined for microsporidia and two samples showed positive results, and the microsporidian spores were detected only in influent water but not in effluent water from these plants (Izquierdo et al., 2011).

3.2 Disinfection

3.2.1 Chemical disinfection (chlorine, ozone)

Microsporidian spores in water can be inactivated by some chemical disinfectants such as chlorine and ozone.

Several studies have documented the effects of chlorine on survival of microsporidian spores in water under experimental conditions. For example, a 3 $\log_{10}$ reduction (99.9% inhibition) in the *E. intestinalis* minimal infective dose was observed at a chlorine concentration of 2 mg/liter after a minimum exposure time of 16 min (pH 7, 25 °C). Multiplying concentration (C) by exposure time (T) results in a CT value of 32 (Wolk et al., 2000). In another study, a CT value of 12 for a 4 $\log_{10}$ reduction of *E. intestinalis* (pH 7, 25 °C) and 16 for a 4 $\log_{10}$ reduction for spores of *E. cuniculi* and *E. hellem* (pH 7, 25 °C) was observed (Johnson et al., 2003). To study the inactivation of *E. intestinalis* by chlorine, another study found that chlorine CT values varied with pH such that 99% (2- $\log_{10}$) CT ranged from 12.8 at pH 6 to 68.8 at pH 8. Compared with other pathogens, *E. intestinalis* is more resistant to chlorine than enteric bacteria and viruses, but not as resistant as *Giardia*. *E. intestinalis* is much less resistant to chlorine than *Cryptosporidium*, which has been found to be essentially unaffected by water treatment chlorination practices (John et al., 2005). Most water utilities provide a median residual chlorine concentration of 1.1 mg/liter after a median exposure time of 45 min, a CT value of 49, by which the level of chlorination required to inactivate spores of *Encephalitozoon* spp. is readily attainable (Wolk et al., 2000; Johnson et al., 2003). Therefore, these results suggested that chlorine treatment may prove to be a reasonable water treatment for inactivation of human-pathogenic microsporidia in municipal water supplies if similar results are obtained in studies performed with natural water, although spores of some fish microsporidia species are highly resistant to chlorine disinfection (Ferguson et al., 2007).

For ozone, the CT values in the inactivation of *E. intestinalis* observed in one study were approximately an

order of magnitude less at 0.59 - 0.84 mg min/L, depending on initial concentration. It also suggested that ozone was at least an order of magnitude more efficient for disinfection of *E. intestinalis* than free chlorine, and *E. intestinalis* is more resistant to ozone than *E. coli* and most viruses but more susceptible than *Cryptosporidium* oocysts (John et al., 2005). Another study evaluated the effect of ozone on the viability of several protist pathogens, and found that microsporidian spores were completely inactivated by using ozonated water after a contact time of nine minutes, suggesting that ozone at an appropriate concentration is capable of inactivating microsporidia in drinking water (Khalifa et al., 2001).

3.2.2 Physical disinfection (Ultraviolet)

Limited studies have been conducted on the effects of physical disinfection on waterborne microsporidian spores. Only two reports have demonstrated the ability of low- and medium-pressure ultraviolet (UV) light to inactivate $> 3.6 \log_{10}$ of *E. intestinalis* spores in water at a dose of 6 mJ/cm^2 or higher as determined using a cell culture approach (Huffman et al., 2002) and a $3 \log_{10}$ at 8.43 mW s/cm^2 (John et al., 2003). The results indicated that UV light at dosages utilized for drinking water treatment is capable of achieving high levels of inactivation of *E. intestinalis* spores (Huffman et al., 2002). In one study focusing on the effect of solar disinfection on the viability of intestinal pathogens, the best results were tubes exposure to sun for 24 hr in summer, where *C. parvum*, *G. duodenalis*, and *Cyclospora cayentanensis* wer

References

- Ardila-Garcia, A.M., Raghuram, N., Sihota, P. and Fast, N.M. (2013). Microsporidian Diversity in Soil, Sand, and Compost of the Pacific Northwest. *Journal of Eukaryotic Microbiology*.
- Asmuth, D.M., DeGirolami, P.C., Federman, M., Ezratty, C.R., Pleskow, D.K., Desai, G. *et al.* (1994). Clinical features of microsporidiosis in patients with AIDS. *Clinical Infectious Diseases*. 18(5), pp. 819-825.
- Barratt, J.L.N., Harkness, J., Marriott, D., Ellis, J.T. and Stark, D. (2010). Importance of nonenteric protozoan infections in immunocompromised people. *Clinical Microbiology Reviews*. 23, pp. 795-836.
- Behera, B., Mirdha, B.R., Makharia, G.K., Bhatnagar, S., Dattagupta, S. and Samantaray, J.C. (2008). Parasites in patients with malabsorption syndrome: a clinical study in children and adults. *Digestive Diseases and Sciences*. 53, pp. 672-679.
- Ben Ayed, L., Yang, W., Widmer, G., Cama, V., Ortega, Y. and Xiao, L. (2012). Survey and genetic characterization of wastewater in Tunisia for *Cryptosporidium* spp., *Giardia duodenalis*, *Enterocytozoon bieneusi*, *Cyclospora cayetanensis* and *Eimeria* spp. *Journal of Water and Health*. 10, pp. 431-444.
- Ben Ayed, L., Yang, W., Widmer, G., Cama, V., Ortega, Y. and Xiao, L. (2012). Survey and genetic characterization of wastewater in Tunisia for *Cryptosporidium* spp., *Giardia duodenalis*, *Enterocytozoon bieneusi*, *Cyclospora cayetanensis* and *Eimeria* spp. *Journal of Water and Health*. 10, pp. 431-444.
- Bern, C., Kawai, V., Vargas, D., Rabke-Verani, J., Williamson, J., Chavez-Valdez, R. *et al.* (2005). The epidemiology of intestinal microsporidiosis in patients with HIV/AIDS in Lima, Peru. *The Journal of Infectious Diseases*. 191(10), pp. 1658-1664.
- Borchardt, M.A. and Spencer, S.K. (2002). Concentration of *Cryptosporidium*, *microsporidia* and other water-borne pathogens by continuous separation channel centrifugation. *Journal of Applied Microbiology*. 92, pp. 649-656.
- Bouzahzah, B. and Weiss, L.M. (2010). Glycosylation of the major polar tube protein of *Encephalitozoon cuniculi*. *Parasitology Research*. 107, pp. 761-764.
- Cali, A., Neafie, R., Weiss, L.M., Ghosh, K., Vergara, R.B., Gupta, R. *et al.* (2010). Human vocal cord infection with the *Microsporidium Anncalia algerae*. *Journal of Eukaryotic Microbiology*. 57, pp. 562-567.
- Calvo, M., Carazo, M., Arias, M.L., Chaves, C., Monges, R. and Chinchilla, M. (2004). Prevalencia de *Cyclospora* sp., *Cryptosporidium* sp., microsporidiosis y determinación de coliformes fecales en frutas y vegetales frescos de consumo crudo en Costa Rica. *Archivos Latinoamericanos de Nutrición*. 54, pp. 428-432.
- Cama, V.A., Pearson, J., Cabrera, L., Pacheco, C., Gilma, R., Meyer, S. *et al.* (2007). Transmission of *Enterocytozoon bieneusi* between a child and guinea pigs. *Journal of Clinical Microbiology*. 45, pp. 2708-2710.
- Carr, A., Marriott, D., Field, A., Vasak, E. and Cooper, D.A. (1998). Treatment of HIV-1-associated microsporidiosis and cryptosporidiosis with combination antiretroviral therapy. *Lancet*. 351, pp. 256-261.
- Chacin-Bonilla, L., Panunzio, A.P., F Castillo, M., Cepeda, I.E., Parra and Martinez, R. (2006). Microsporidiosis in Venezuela. Prevalence of intestinal microsporidiosis and its contribution to diarrhea in groups of human immunodeficiency virus infected patients from Zulia State. *The American Journal of Tropical Medicine and Hygiene*. 74, pp. 482-486.
- Champion, L., Durrbach, A., Lang, P., Delahousse, M., Chauvet, C., Sarfati, C. *et al.* (2010). Fumagillin for treatment of intestinal microsporidiosis in renal transplant recipients. *American Journal of Transplantation*. 10, pp. 1925-1930.
- Chan, C.M., Theng, J.T., Li, L. and Tan, D.T. (2003). Microsporidial keratoconjunctivitis in healthy individuals: a case series. *Ophthalmology*. 110, pp. 1420-1425.
- Cheng, H.W., Lucy, F.E., Graczyk, T.K., Broaders, M.A. and Mastitsky, S.E. (2011). Municipal wastewater treatment plants as removal systems and environmental sources of human-virulent microsporidian spores. *Parasitology Research*. 109, pp.

595-603.

Conteas, C.N., Berlin, O.G., Ash, L.R. and Pruthi, J.S. (2000). Therapy for human gastrointestinal microsporidiosis. The American Journal of Tropical Medicine and Hygiene. 63, pp. 121-127.

Cotte, L., Rabodonirina, M., Chapuis, F., Bailly, F., Bissuel, F., Raynal, C. *et al.* (1999). Waterborne outbreak of intestinal microsporidiosis in persons with and without human immunodeficiency virus infection. The Journal of Infectious Diseases. 180, pp. 2003-2008.

Coupe, S., Delabre, K., Pouillot, R., Houdart, S., Santillana-Hayat, M. and Derouin, F. (2006). Detection of *Cryptosporidium*, *Giardia* and *Enterocytozoon bienersi* in surface water, including recreational areas: a one-year prospective study. FEMS Immunology and Medical Microbiology. 47, pp. 351-359.

Coyle, C., Kent, M., Tanowitz, H.B., Wittner, M. and Weiss, L.M. (1998). TNP-470 is an effective antimicrosporidial agent. The Journal of Infectious Diseases. 177, pp. 515-518.

Coyle, C.M., Weiss, L.M., Rhodes, LV.3rd, Cali, A., Takvorian, P.M., Brown, D.F. *et al.* (2004). Fatal myositis due to the microsporidian *Brachiola algerae*, a mosquito pathogen. The New England Journal of Medicine. 351, pp. 42-47.

Dado, D., Izquierdo, F., Vera, O., Montoya, A., Mateo, M., Fenoy, S. *et al.* (2012). Detection of zoonotic intestinal parasites in public parks of Spain. Potential epidemiological role of *microsporidia*. Zoonoses Public Health. 59, pp. 23-28.

Dascomb, K., Clark, R., Aberg, J., Pulvirenti, J., Hewitt, R.G., Kissinger, P. *et al.* (1999). Natural history of intestinal microsporidiosis among patients infected with human immunodeficiency virus. Journal of Clinical Microbiology. 37, pp. 3421-3422.

Decraene, V., Lebbad, M., Botero-Kleiven, S., Gustavsson, A.M. and Löfdahl, M. (2012). First reported foodborne outbreak associated with *microsporidia*, Sweden, October 2009. Epidemiology and Infection. 140, pp. 519-527.

De Roever, C. (1998). Microbiological safety evaluations and recommendations on fresh produce. Food Control. 9, pp. 21-347.

Didier, E.S. (2005). Microsporidiosis: an emerging and opportunistic infection in humans and animals. Acta Tropica. 94, pp. 61-76.

Didier, E.S. (1997). Effects of albendazole, fumagillin, and TNP-470 on microsporidial replication *in vitro*. Antimicrob Agents Chemother. 41, pp. 1541-1546.

Didier, E.S., Stovall, M.E., Green, L.C., Brindley, P.J., Sestak, K. and Didier, P.J. (2004). Epidemiology of microsporidiosis: sources and modes of transmission. Veterinary Parasitology. 126, pp. 145-166.

Didier, E.S., Vossbrinck, C.R., Baker, M.D., Rogers, L.B., Bertucci, D.C. and Shadduck, J.A. (1995). Identification and characterization of three *Encephalitozoon cuniculi* strains. Parasitology. 111(Pt 4), pp. 411-421.

Didier, E.S. and Weiss, L.M. (2011). Microsporidiosis: not just in AIDS patients. Current Opinion in Infectious Diseases. 24, pp. 490-495.

Didier, E.S. and Weiss, L.M. (2006). Microsporidiosis: current status. Current Opinion in Infectious Diseases. 19, pp. 485-492.

Didier, P.J., Phillips, J.N., Kuebler, D.J., Nasr, M., Brindley, P.J., Stovall, M.E. *et al.* (2006). Antimicrosporidial activities of fumagillin, TNP-470, ovalicin, and ovalicin derivatives *in vitro* and *in vivo*. Antimicrob Agents Chemother. 50, pp. 2146-2155.

Dowd, S.E., Gerba, C.P., Kamper, M. and Pepper, I.L. (1999). Evaluation of methodologies including immunofluorescent assay (IFA) and the polymerase chain reaction (PCR) for detection of human pathogenic *microsporidia* in water. Journal of Microbiological Methods. 35, pp. 43-52.

- Dowd, S.E., Gerba, C.P. and Pepper, I.L. (1998). Confirmation of the human-pathogenic *microsporidia* *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *Vittaforma corneae* in water. *Applied and Environmental Microbiology*. 64, pp. 3332-3335.
- Dowd, S.E., John, D., Eliopolus, J., Gerba, C.P., Naranjo, J., Klein, R. *et al.* (2003). Confirmed detection of *Cyclospora cayetanensis*, *Encephalitozoon intestinalis* and *Cryptosporidium parvum* in water used for drinking. *Journal of Water and Health*. 1, pp. 117-123.
- Fan, N.W., Wu, C.C., Chen, T.L., Yu, W.K., Chen, C.P., Lee, S.M. *et al.* (2012). Microsporidial keratitis in patients with hot springs exposure. *Journal of Clinical Microbiology*. 50, pp. 414-418.
- Fayer, R. (2004). Infectivity of *microsporidia* spores stored in seawater at environmental temperatures. *Journal of Parasitology*. 90, pp. 654-657.
- Fayer, R. and Santin, M. (2014). Epidemiology of *Microsporidia* in Human Infections, in *Microsporidia: Pathogens of Opportunity*. (Weiss, L.M. and Becnel, J.J., ed.). John Wiley and Sons, Inc.
- Feng, Y., Li, N., Duan, L. and Xiao, L. (2009). *Cryptosporidium* genotype and subtype distribution in raw wastewater in Shanghai, China: evidence for possible unique *Cryptosporidium hominis* transmission. *Journal of Clinical Microbiology*. 47, pp. 153-157.
- Ferguson, J.A., Watral, V., Schwindt, A.R. and Kent, M.L. (2007). Spores of two fish *microsporidia* (*Pseudoloma neurophilia* and *Glugea anomala*) are highly resistant to chlorine. *Diseases of Aquatic Organisms*. 76, pp. 205-214.
- Fournier, S., Dubrou, S., Liguory, O., Gaussin, F., Santillana-Hayat, M., Sarfati, C. *et al.* (2002). Detection of *Microsporidia*, cryptosporidia and *Giardia* in swimming pools: a one-year prospective study. *FEMS Immunology and Medical Microbiology*. 33, pp. 209-213.
- Fournier, S., Liguory, O., Santillana-Hayat, M., Guillot, E., Sarfati, C., Dumoutier, N. *et al.* (2000). Detection of *microsporidia* in surface water: a one-year follow-up study. *FEMS Immunology and Medical Microbiology*. 29, pp. 95-100.
- Franzen, C. and Muller, A. (2001). Microsporidiosis: human diseases and diagnosis. *Microbes and Infection*. 3, pp. 389-400.
- Franzen, C. and Muller, A. (1999). Molecular techniques for detection, species differentiation, and phylogenetic analysis of *microsporidia*. *Clinical Microbiology Reviews*. 12, pp. 243-285.
- Franzen, C. and Muller, A. (1999). Cryptosporidia and *microsporidia*-waterborne diseases in the immunocompromised host. *Diagnostic Microbiology and Infectious Disease*. 34, pp. 245-262.
- Galván, A.L., Magnet, A., Izquierdo, F., Fenoy, S., Rueda, C., C Vadillo, F. *et al.* (2013). Molecular characterization of human-pathogenic *microsporidia* and *Cyclospora cayetanensis* isolated from various water sources in Spain: a year-long longitudinal study. *Applied and Environmental Microbiology*. 79, pp. 449-459.
- Gerba, C.P., Riley, K.R., Nwachuku, N., Ryu, H. and Abbaszadegan, M. (2003). Removal of *Encephalitozoon intestinalis*, calicivirus, and coliphages by conventional drinking water treatment. *Journal of Environmental Science and Health - Part A Toxic/Hazardous*. 38, pp. 1259-1268.
- Ghosh, K. and Weiss, L.M. (2009). Molecular diagnostic tests for *microsporidia*. *Interdiscip Perspect Infect Dis*. 2009, pp. 926521.
- Giacometti, A., Cirioni, O., Fortuna, M., Osimani, P., Antonicelli, L., Del Prete, M.S. *et al.* (2000). Environmental and serological evidence for the presence of toxocariasis in the urban area of Ancona, Italy. *European Journal of Epidemiology*. 16, pp. 1023-1026.
- Graczyk, T.K., Conn, D.B., Lucy, F., Minchin, D., Tamang, L., Moura, L.N. *et al.* (2004). Human waterborne parasites in zebra mussels (*Dreissena polymorpha*) from the Shannon River drainage area, Ireland. *Parasitology Research*. 93, pp. 385-391.

- Graczyk, T.K., Kacprzak, M., Neczaj, E., Tamang, L., Graczyk, H., Lucy, F.E. *et al.* (2007). Human-virulent microsporidian spores in solid waste landfill leachate and sewage sludge, and effects of sanitization treatments on their inactivation. *Parasitology Research*. 101, pp. 569-575.
- Graczyk, T.K. and Lucy, F.E. (2007). Quality of reclaimed waters: a public health need for source tracking of wastewater-derived protozoan enteropathogens in engineered wetlands. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 101, pp. 532-533.
- Graczyk, T.K., Lucy, F.E., Mashinsky, Y., Thompson, R.C., Andrew, Koru, O. and Dasilva, A.J. (2009). Human zoonotic enteropathogens in a constructed free-surface flow wetland. *Parasitology Research*. 105, pp. 423-428.
- Graczyk, T.K., Lucy, F.E., Tamang, L., Mashinski, Y., Broaders, M.A., Connolly, M. *et al.* (2009). Propagation of human enteropathogens in constructed horizontal wetlands used for tertiary wastewater treatment. *Applied and Environmental Microbiology*. 75, pp. 4531-4538.
- Graczyk, T.K., Lucy, F.E., Tamang, L., Mashinski, Y., Broaders, M.A., Connolly, M. *et al.* (2009). Propagation of human enteropathogens in constructed horizontal wetlands used for tertiary wastewater treatment. *Applied and Environmental Microbiology*. 75, pp. 4531-4538.
- Graczyk, T.K., Lucy, F.E., Tamang, L. and Miraflor, A. (2007). Human enteropathogen load in activated sewage sludge and corresponding sewage sludge end products. *Applied and Environmental Microbiology*. 73, pp. 2013-2015.
- Graczyk, T.K., Lucy, F.E., Tamang, L. and Miraflor, A. (2007). Human enteropathogen load in activated sewage sludge and corresponding sewage sludge end products. *Applied and Environmental Microbiology*. 73, pp. 2013-2015.
- Graczyk, T.K., Sunderland, D., Awantang, G.N., Mashinski, Y., Lucy, F.E., Graczyk, Z. *et al.* (2010). Relationships among bather density, levels of human waterborne pathogens, and fecal coliform counts in marine recreational beach water. *Parasitology Research*. 106, pp. 1103-1108.
- Graczyk, T.K., Sunderland, D., Tamang, L., Lucy, F.E. and Breysse, P.N. (2007). Bather density and levels of *Cryptosporidium*, *Giardia*, and pathogenic microsporidian spores in recreational bathing water. *Parasitology Research*. 101, pp. 1729-1731.
- Gross, U. (2003). Treatment of microsporidiosis including albendazole. *Parasitology Research*. 90 Supp 1, pp. S14-18.
- Gumbo, T., Hobbs, R.E., Carlyn, C., Hall, G. and Isada, C.M. (1999). *Microsporidia* infection in transplant patients. *Transplantation*. 67, pp. 482-484.
- Guo, Y., Alderisio, K.A., Yang, W., Cama, V., Feng, Y. and Xiao, L. (2014). Host Specificity and Source of *Enterocytozoon bieneusi* Genotypes in a Drinking Source Watershed. *Applied and Environmental Microbiology*. 80, pp. 218-225.
- Huffman, D.E., Gennaccaro, A., Rose, J.B. and Dussert, B.W. (2002). Low- and medium-pressure UV inactivation of *microsporidia* *Encephalitozoon intestinalis*. *Water Research*. 36, pp. 3161-3164.
- Hutin, Y.J., Sombardier, M.N., Liguory, O., Sarfati, C., Derouin, F., Modaï, J. *et al.* (1998). Risk factors for intestinal microsporidiosis in patients with human immunodeficiency virus infection: a case-control study. *The Journal of Infectious Diseases*. 178, pp. 904-907.
- Hu, Y., Feng, Y., Huang, C. and Xiao, L. (2014). Occurrence, Source, and Human Infection Potential of *Cryptosporidium* and *Enterocytozoon bieneusi* in Drinking Source Water in Shanghai, China during a Pig Carcass Disposal Incident. *Environmental Science and Technology*.
- Hynds, P.Dylan, M. Thomas, K. and Pintar, K.Dorothy Mi (2014). Contamination of Groundwater Systems in the US and Canada by Enteric Pathogens, 1990-2013: A Review and Pooled-Analysis. *PLoS ONE*. 9, (Kirk, M., ed.). pp. e93301. doi: 10.1371/journal.pone.0093301.
- Izquierdo, F., Hermida, J.A., Castro, Fenoy, S., Mezo, M., González-Warleta, M. and del Aguila, C. (2011). Detection of *microsporidia* in drinking water, wastewater and recreational rivers. *Water Research*. 45, pp. 4837-4843.

- Jedrzejewski, S., Graczyk, T.K., Slodkiewicz-Kowalska, A., Tamang, L. and Majewska, A.C. (2007). Quantitative assessment of contamination of fresh food produce of various retail types by human-virulent microsporidian spores. *Applied and Environmental Microbiology*. 73, pp. 4071-4073.
- John, D.E., Haas, C.N., Nwachuku, N. and Gerba, C.P. (2005). Chlorine and ozone disinfection of *Encephalitozoon intestinalis* spores. *Water Research*. 39, pp. 2369-2375.
- John, D.E., Nwachuku, N., Pepper, I.L. and Gerba, C.P. (2003). Development and optimization of a quantitative cell culture infectivity assay for the microsporidium *Encephalitozoon intestinalis* and application to ultraviolet light inactivation. *Journal of Microbiological Methods*. 52, pp. 183-196.
- Johnson, C.H., Marshall, M.M., DeMaria, L.A., Moffet, J.M. and Korich, D.G. (2003). Chlorine inactivation of spores of *Encephalitozoon* spp. *Applied and Environmental Microbiology*. 69, pp. 1325-1326.
- Kahler, A.M. and Thurston-Enriquez, J.A. (2007). Human pathogenic *microsporidia* detection in agricultural samples: method development and assessment. *Parasitology Research*. 100, pp. 529-538.
- Kaplan, J.E., Benson, C., Holmes, K.K., Brooks, J.T., Pau, A., Masur, H. *et al.* (2009). Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recommendations and Reports*. 58, pp. 1-207.
- Khalifa, A.M., Tamsahy, MM.El and Naga, IF.Abou el (2001). Effect of ozone on the viability of some protozoa in drinking water. *Journal of the Egyptian Society of Parasitology*. 31, pp. 603-616.
- Kim, K., Yoon, S., Cheun, H.I., Kim, J.H., Sim, S. and, (2015). Detection of *Encephalitozoon* spp. from Human Diarrheal Stool and Farm Soil Samples in Korea. *Journal of Korean Medical Science*. 30, pp. 227-232.
- Kotler, D.P. and Orenstein, J.M. (1998). Clinical syndromes associated with microsporidiosis. *Advances in Parasitology*. 40, pp. 321-349.
- Koudela, B., Kucerova, S. and Hudcovic, T. (1999). Effect of low and high temperatures on infectivity of *Encephalitozoon cuniculi* spores suspended in water. *Folia Parasitologica*. 46, pp. 171-174.
- Kwakye-Nuako, G., Borketey, P., Mensah-Attipoe, I., Asmah, R. and Ayeh-Kumi, P. (2007). Sachet drinking water in Accra: the potential threats of transmission of enteric pathogenic protozoan organisms. *Ghana Medical Journal*. 41, pp. 62-67.
- Kwok, A.K., Tong, J.M., Tang, B.S., Poon, R.W., Li, W.W. and Yuen, K.Y. (2013). Outbreak of microsporidial keratoconjunctivitis with rugby sport due to soil exposure. *Eye*. 27, pp. 747-754.
- Liguory, O., Fournier, S., Sarfati, C., Derouin, F. and Molina, J. (2000). Genetic homology among thirteen *Encephalitozoon intestinalis* isolates obtained from human immunodeficiency virus-infected patients with intestinal microsporidiosis. *Journal of Clinical Microbiology*. 38, pp. 2389-2391.
- Li, N., Xiao, L., Wang, L., Zhao, S., Zhao, X., Duan, L. *et al.* (2012). Molecular Surveillance of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bienersi* by Genotyping and Subtyping Parasites in Wastewater. *PLOS Neglected Tropical Diseases*. 6, pp. e1809.
- Li, X., Palmer, R., Trout, J.M. and Fayer, R. (2003). Infectivity of *microsporidia* spores stored in water at environmental temperatures. *Journal of Parasitology*. 89, pp. 185-188.
- Lobo, M.L., Xiao, L., Antunes, F. and Matos, O. (2012). *Microsporidia* as emerging pathogens and the implication for public health: A 10-year study on HIV-positive and - negative patients. *International Journal for Parasitology*.
- Loh, R.S., Chan, C.M., Ti, S.E., Lim, L., Chan, K.S. and Tan, D.T. (2009). Emerging prevalence of microsporidial keratitis in Singapore: epidemiology, clinical features, and management. *Ophthalmology*. 116, pp. 2348-2353.
- Lores, B., López-Miragaya, I., Arias, C., Fenoy, S., Torres, J. and del Aguila, C. (2002). Intestinal microsporidiosis due to

Enterocytozoon bieneusi in elderly human immunodeficiency virus-negative patients from Vigo, Spain. *Clinical Infectious Diseases*. 34, pp. 918-921.

Lucy, F.E., Graczyk, T.K., Tamang, L., Miraflor, A. and Minchin, D. (2008). Biomonitoring of surface and coastal water for *Cryptosporidium*, *Giardia*, and human-virulent *microsporidia* using molluscan shellfish. *Parasitology Research*. 103, pp. 1369-1375.

Marshall, M.M., Naumovitz, D., Ortega, Y. and Sterling, C.R. (1997). Waterborne protozoan pathogens. *Clinical Microbiology Reviews*. 10, pp. 67-85.

Martínez-Moreno, F.J., Hernández, S., López-Cobos, E., Becerra, C., Acosta, I. and Martínez-Moreno, A. (2007). Estimation of canine intestinal parasites in Cordoba (Spain) and their risk to public health. *Veterinary Parasitology*. 143, pp. 7-13.

Mathis, A., Tanner, I., Weber, R. and Deplazes, P. (1999). Genetic and phenotypic intraspecific variation in the microsporidian *Encephalitozoon hellem*. *International Journal for Parasitology*. 38, pp. 2389-2391.

Matos, O., Lobo, M.L. and Xiao, L. (2012). Epidemiology of *Enterocytozoon bieneusi* Infection in Humans. *Journal of Parasitology Research*. 2012, pp. 981424.

Metge, S., Van Nhieu, J.T., Dahmane, D., Grimbert, P., Foulet, F., Sarfati, C. *et al.* (2000). A case of *Enterocytozoon bieneusi* infection in an HIV-negative renal transplant recipient. *European Journal of Clinical Microbiology and Infectious Diseases*. 19, pp. 221-223.

Mofenson, L.M., Brady, M.T., Danner, S.P., Dominguez, K.L., Hazra, R., Handelsman, E. *et al.* (2009). Guidelines for the Prevention and Treatment of Opportunistic Infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recommendations and Reports*. 58, pp. 1-166.

Mohammed, H., Endeshaw, T., Kebede, A. and Defera, M. (2009). Comparison of Chromotrope 2R and Uvitex 2B for the detection of intestinal microsporidial spores in stool specimens of HIV patients attending Nekempt Hospital, West Ethiopia. *Ethiopian Medical Journal*. 47, pp. 233-237.

Molina, J.M., Tourneur, M., Sarfati, C., Chevret, S., de Gouvello, A., Gobert, J.G. *et al.* (2002). Fumagillin treatment of intestinal microsporidiosis. *The New England Journal of Medicine*. 346, pp. 1963-1969.

Mossallam, S.F. (2010). Detection of some intestinal protozoa in commercial fresh juices. *Journal of the Egyptian Society of Parasitology*. 40, pp. 135-149.

Mungthin, M., Subrungruang, I., Naaglor, T., Aimpun, P., Areekul, W. and Leelayoova, S. (2005). Spore shedding pattern of *Enterocytozoon bieneusi* in asymptomatic children. *Journal of Medical Microbiology*. 54, pp. 473-476.

Pagornrat, W., Leelayoova, S., Rangsin, R., Tan-Ariya, P., Naaglor, T. and Mungthin, M. (2009). Importance of nonenteric protozoan infections in immunocompromised people. *Journal of Clinical Microbiology*. 47, pp. 3739-3741.

Reinoso, R., Torres, L.A. and Becares, E. (2008). Efficiency of natural systems for removal of bacteria and pathogenic parasites from wastewater. *Science of the Total Environment*. 395, pp. 80-86.

Santin, M. and Fayer, R. (2011). Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. *Research in Veterinary Science*. 90, pp. 363-371.

Santin, M. (2015). *Enterocytozoon bieneusi* in *Biology of Foodborne Parasites*. (Xiao, L., Ryan, U. and Feng, Y., ed.). CRC Press.

Shadduck, J.A., Bendele, R. and Robinson, G.T. (1978). Isolation of the causative organism of canine encephalitozoonosis. *Veterinary Pathology*. 15, pp. 449-460.

Shadduck, J.A. and Polley, M.B. (1978). Some factors influencing the *in vitro* infectivity and replication of *Encephalitozoon cuniculi*. *Journal of Protozoology*. 25, pp. 491-496.

- Sorel, N., Guillot, E., Thellier, M., Accoceberry, I., Datry, A., Mesnard-Rouiller, L. *et al.* (2003). Sorel N1, Guillot E, Thellier M, Accoceberry I, Datry A, Mesnard-Rouiller L, Miégevillle M. Journal of Applied Microbiology. 94, pp. 273-279.
- Sparfel, J.M., Sarfati, C., Liguory, O., Caroff, B., Dumoutier, N., Gueglio, B. *et al.* (1997). Detection of *microsporidia* and identification of *Enterocytozoon bieneusi* in surface water by filtration followed by specific PCR. Journal of Eukaryotic Microbiology. 44, pp. 78S.
- Stark, D., Barratt, J.L., van Hal, S., Marriott, D., Harkness, J. and Ellis, J.T. (2009). Clinical significance of enteric protozoa in the immunosuppressed human population. Clinical Microbiology Reviews.
- Stewart, M.H., Yates, M.V., Anderson, M.A., Gerba, C.P., Rose, J.B., De Leon, R. *et al.* (2002). Predicted public health consequences of body-contact recreation on a potable water reservoir. Journal American Water Works Association. 94, pp. 84-97.
- Sulaiman, I.M., Fayer, R., Lal, A.A., Trout, J.M., Schaefer, F.W.3rd and Xiao, L. (2003). Molecular characterization of *microsporidia* indicates that wild mammals harbor host-adapted *Enterocytozoon* spp. as well as human-pathogenic *Enterocytozoon bieneusi*. Applied and Environmental Microbiology. 69, pp. 4495-4501.
- Talabani, H., Sarfati, C., Pillebout, E., van Gool, T., Derouin, F. and Menotti, J. (2010). Disseminated infection with a new genovar of *Encephalitozoon cuniculi* in a renal transplant recipient. Journal of Clinical Microbiology. 48, pp. 2651-2653.
- Talebi, M., Rahimi, F., Katouli, M., Möllby, R. and Pourshafie, M.R. (2008). Epidemiological link between wastewater and human vancomycin-resistant *Enterococcus faecium* isolates. Current Microbiology. 56, pp. 468-473.
- Tan, J., Lee, P., Lai, Y., Hishamuddin, P., Tay, J., Tan, A.L. *et al.* (2013). Microsporidial keratoconjunctivitis after rugby tournament, Singapore. Emerging Infectious Diseases. 19, pp. 1484-1486.
- Thurston-Enriquez, J.A., Watt, P., Dowd, S.E., Enriquez, R., Pepper, I.L. and Gerba, C.P. (2002). Detection of protozoan parasites and *microsporidia* in irrigation waters used for crop production. Journal of Food Protection. 65, pp. 378-382.
- Tumwine, J.K., Kekitiinwa, A., Nabukeera, N., Akiyoshi, D.E., Buckholt, M.A. and Tzipori, S. (2002). *Enterocytozoon bieneusi* among children with diarrhea attending Mulago Hospital in Uganda. The American Journal of Tropical Medicine and Hygiene. 67, pp. 299-303.
- Ulrich, H., Klaus, D., Irmgard, F., Annette, H., Juan, L.P. and Regine, S. (2005). Microbiological investigations for sanitary assessment of wastewater treated in constructed wetlands. Water Research. 39, pp. 4849-4858.
- van Hal, S.J., Muthiah, K., Matthews, G., Harkness, J., Stark, D., Cooper, D. *et al.* (2007). Declining incidence of intestinal microsporidiosis and reduction in AIDS-related mortality following introduction of HAART in Sydney, Australia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 101, pp. 1096-1100.
- Visvesvara, G.S., Belloso, M., Moura, H., da Silva, A.J., Moura, I.N., Leitch, G.J. *et al.* (1999). Isolation of *Nosema algerae* from the cornea of an immunocompetent patient. Journal of Eukaryotic Microbiology. 46, pp. 10S.
- Waller, T. (1979). Sensitivity of *Encephalitozoon cuniculi* to various temperatures, disinfectants and drugs. Laboratory Animals. 13, pp. 227-230.
- Weber, R. and Bryan, R.T. (1994). Microsporidial infections in immunodeficient and immunocompetent patients. Clinical Infectious Diseases. 19, pp. 517-521.
- Weber, R., Deplazes, P. and Schwartz, D. (2000). Diagnosis and clinical aspects of human microsporidiosis. Contributions to Microbiology. 6, pp. 166-192.
- Weber, R., Ledergerber, B., Zbinden, R., Altwegg, M., Pfyffer, G.E., Spycher, M.A. *et al.* (1999). Enteric infections and diarrhea in human immunodeficiency virus-infected persons: prospective community-based cohort study. Swiss HIV Cohort Study. JAMA Internal Medicine. 159, pp. 1473-1480.
- Wichro, E., Hoelzl, D., Krause, R., Bertha, G., Reinthaler, F. and Wenisch, C. (2005). Microsporidiosis in travel-associated

chronic diarrhea in immune-competent patients. The American Journal of Tropical Medicine and Hygiene. 73, pp. 285-287.

Wolk, D.M., Johnson, C.H., Rice, E.W., Marshall, M.M., Grahn, K.F., Plummer, C.B. *et al.* (2000). A spore counting method and cell culture model for chlorine disinfection studies of *Encephalitozoon* (syn. *Septata*) *intestinalis*. Applied and Environmental Microbiology. 66, pp. 1266-1273.

Ye, J., Xiao, L., Ma, J., Guo, M., Liu, L. and Feng, Y. (2012). Anthroponotic enteric parasites in monkeys in public park, China. Emerging Infectious Diseases. 18, pp. 1640-1643.

Zychowski, J. and Bryndal, T. (2015). Impact of cemeteries on groundwater contamination by bacteria and viruses - a review. Journal of Water and Health. 13, pp. 285-301.