Phylum Microsporidia: Dangerous Waterborne Pathogens, A Review

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Abstract: Phylum Microsporidia comprises from various species, number of which are pathogenic and capable of infecting humans by the means of water. Important human pathogens Enterocytozoon bieneusi and Encephalitozoon intestinalis were detected in both, ground and surface waters. Other Microsporidia species such as Encephalitozoon cuniculi and Encephalitozoon hellem but also Pleistophora spp. and Vittaforma corneae were also detected in water. Even though disinfection and filtration of drinking water is usually effective against these pathogens, malfunctions in water treatment system can lead to epidemics, such as one that occurred in 1995 in Lyon where 200 people got infected. Therefore pathogens from genus Microsporidia should be routinely monitored in drinking water, but also in water sources and recreational areas.

Keywords: disinfection, Encephalitozoon bieneusi, Enterocytozoon spp., Microsporidia, water,

1. INTRODUCTION

Microsporidiosis belongs to common parasitic diseases worldwide. Parasites from phylum Microsporidia are obligate intracellular sporulating pathogens that were unknown as causes of human disease before HIV pandemics. Human microsporidial infections are detected mainly in the HIV positive immunocompromised patients, rarely in immunocompetent individuals. Prevalence of microsporidiosis in AIDS patients with chronic diarrhea ranges from 7 to 50 % (Bryan, 1995).

Phylum Microsporidia comprises of more than 1300 species and 150 genera, from which 8 genera: Enterocytozoon, Encephalitozoon, Pleistophora, Trachipleistophora, Vittaforma, Brachiola, Nosema and Microsporidium were described in humans. Most common species detected in humans are Enterocytozoon bieneusi, Encephalitozoon intestinalis and Encephalitozoon cuniculi (Valenčáková and Halánová, 2012). Common cause of microsporidial infection is also Encephalitozoon hellem.

There are more ways of transmission. Microsporidia were detected in the water and caused one epidemic in the year 1995 in French town Lyon, where 200, mainly immunocompromised, people had clinical symptoms such as chronic diarrhea, dehydration and significant weight loss. Cause of infection was inadequate treatment and contamination of drinking water, but source of contamination was never determined (Cotte et al., 1999). Spores of phylum Microsporidia are stable in the environment and remain infectious for days to weeks outside their hosts. Their small size (1 ± 5 µm) causes harder detection, but they are susceptible to chlorine disinfection (Wolk et al., 2000).

Another possible way of transmission is by contaminated food, E. intestinalis was found in the water designated for watering of plant products. It is assumed that Microsporidia can be transmitted through the air because Encephalitozoon spp. and Enterocytozoon bieneusi were detected in sputum, liquid obtained by bronchoalveolar lavage and in bronchoalveolar epithelium. Zoonotic transmission is also a possibility as proven by a case of 10 year old girl which was infected with Encephalitozoon cuniculi after a contact with an infected dog (Rinder, 2004). This article will focus on waterborne transmission of Microsporidia that are increasingly recognized as emerging pathogens.

2. Disinfection of Microsporidia in the water

Microsporidia can be shed from hosts in urine and feces (Cali and Takvorian, 1999) and can contaminate drinking water by waste water or animal sources. There are various studies (Wolk et al., 2000; Huffman et al., 2002; John et al., 2003; Johnson et al., 2003;
John et al., 2005; Gaafar, 2007) concerning effects of disinfectants on waterborne *Microsporidia* spores. The most common measure for describing a disinfectant’s efficacy for selected organism is $C \times t$, i.e. concentration of disinfectant (mg/L) x disinfectant contact time (min.) (John et al., 2005). Ultraviolet disinfection system is capable to transfer electromagnetic energy into genetic material (DNA or RNA) of an organism, where it destroys cell’s ability to reproduce (EPA, 1999a). Huffman et al. (2002) demonstrated that UV light at dosages utilized for drinking water treatment is capable of achieving high levels of inactivation of *Microsporidia*. For a low- and medium-pressure UV light, $C \times t$ values were found to be 6 mWs/cm$^2$ for a 3.6-log$_{10}$ reduction of *E. intestinalis* (Huffman et al., 2002), and 8.4 mWs/cm$^2$ for a 3-log$_{10}$ reduction, i.e. 99.9 % reduction of *E. intestinalis* with low pressure UV at 25 °C (John et al., 2003). Even though exact mechanism by which chlorine destroys pathogens has not been elucidated, it can cause oxidation of enzymes and amino acids, inhibition of protein synthesis, decreased oxygen and nutrient uptake and DNA destruction (CDC, 2008). Wolk et al. (2000) described that chlorine treatment of water is reasonable for inactivation of spores in municipal water supplies. For chlorine, $C \times t$ values were found to be 32 for a 3-log$_{10}$ (99.9%) reduction of *E. intestinalis* spores (pH 7 and 25 °C) in one study (Wolk et al., 2000), and in another at least 12 for a 4-log$_{10}$ reduction of *E. intestinalis* (pH 7, 25 °C) and 16 for a 4-log$_{10}$ reduction (in both cases 100 % inactivation) for spores of *E. cuniculi* and *E. hellem* (pH 7, 25 °C) (Johnson et al., 2003). Ozone is a very strong oxidant which is capable of destruction of cell walls and can cause damage to nucleic acids (EPA, 1999b). John et al. (2005) reported that for disinfection of *E. intestinalis* ozone is more efficient than chlorine. Ozone disinfection $C \times t$ values (mg-min./L) from two concentrations (pH 7, 5 °C) were 0.84 at 1mg/L seeded ozone (0.7mg/L initial residual) and 0.59 at 0.5mg/L seeded ozone (0.3mg/L initial residual) for 2-log$_{10}$ decline (90%), and 1.40 (1mg/L) and 1.08 (0.5mg/L) for 3-log decline (99.9%). Gaafar (2007) experimented with solar disinfection of protozoan pathogens and *Microsporidia* sp.. This type of disinfection was less effective on *Microsporidia* than on other monitored pathogens.

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3. *Enterocytozoon bieneusi*

There is only one species of genus *Enterocytozoon, E. bieneusi*, which belongs to common causes of microsporidiosis in immunocompromised and immunocompetent humans (Sulaiman et al., 2003).

Number of hosts of *E. bieneusi* genotypes based on ITS nucleotide sequence grows rapidly. Currently, there are 93 genotypes, from which 34 was detected only in humans, 11 in human and animals, 12 only in cattle, 11 solely in pigs, one in cattle and pigs and one in cattle and birds. Five genotypes were described in cats, two in horses and one in dogs. There are also genotypes that infect raccoons, muskrats, beavers, otters... Because of this wide host spectrum *E. bieneusi* belongs to dangerous pathogens (Sulaiman et al., 2003).

In various studies (Sparfel et al., 1997; Dowd et al., 1998; Hutin et al., 1998) were spores of this parasite detected in ground and surface waters. Dowd et al. (1998) identified *E. bieneusi*, along with *Encephalitozoon intestinalis* in the surface water. In the year 1998, Hutin et al. identified swimming pools as one of the most dangerous places concerning infection of immunocompromised human. Fournier et al. (2002) also detected *E. bieneusi* in a swimming pool. Other risk factors include water used for recreational purposes, hot tubs, and drinking water (Hutin et al., 1998). *E. bieneusi* was also identified in seawater, lakes and rivers and is common in waste water (Sparfel et al., 1997). Graczyk et al. (2007c) detected this pathogen in samples from recreational bathing area. In another study of surface and coastal waters in Ireland were surveyed *E. bieneusi*, along with *Encephalitozoon intestinalis* and

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**Table 1: Disinfectants and their efficacy**

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Encephalitozoon hellem using molluscan shellfish as biomonitor (Lucy et al., 2008). This pathogen was also detected in storm water samples collected from basins and watershed (Guo et al., 2014) and in samples from wetlands (Graczyk et al., 2009a). E. bieneusi, together with Encephalitozoon cuniculi and Encephalitozoon intestinalis were detected in drinking water treatment plants and wastewater treatment plants in Spain (Galvan, 2013). E. bieneusi was also identified in samples of sewage sludge from wastewater treatment plant (Graczyk et al., 2007a). Cheng et al. (2011) and Graczyk et al. (2009b) determined that waste water, even after treatment, still contains a certain number of spores that can cause infection of humans. Li et al. (2012) proved that in waste water are found mainly genotypes that can infect wide range of hosts, including human.

Source of water contamination is usually hard to identify and usually remains unknown. It can be feces of fur bearing mammals living close to the water (Sulaiman et al., 2003) or carcasses of animals that float in the water (Hu et al., 2014). Also birds have been confirmed as a possible source of water contamination (Graczyk et al., 2007b).

E. bieneusi is detected mainly in the people with lowered immunity. In North America, Europe and Australia were HIV positive patients with diarrhea infected in 2 - 78% of cases with this parasite (Lobo et al., 2012). In developing countries such as African countries, South American counties and India prevalence in HIV positive patients with diarrhea ranges from 2.5 to 51% (Samie et al., 2007; Mohandas et al., 2002; Sulaiman et al., 2003). In these countries is prevalence in HIV negative 5.4 - 58% (Bretagne et al., 1993). In Czech Republic Sak et al. (2011) detected prevalence of infection caused by this pathogen around 2% in immunocompetent humans. In Slovakia prevalence of E. bieneusi in children living in Roma settlements was 4.2% (Halánová et al., 2013).

Most common symptoms of E. bieneusi infection in immunocompromised humans are diarrhea accompanied with fever, loss of appetite, weight loss and wasting disease. Infection is usually localized in small intestine, but in severe cases was described cholangitis and cholecystitis. Malabsorption, decreased mucosal surface area and immaturity of villus epithelial cells are believed to contribute to the diarrhea caused by E. bieneusi (Weber et al., 2000).

4. Encephalitozoon spp.

There are three different species form genus Encephalitozoon that can cause human infections - Encephalitozoon cuniculi, Encephalitozoon intestinalis and Encephalitozoon hellem. E. intestinalis is the second most prevalent microsporidian species infecting humans (Weber et al., 1994). E. intestinalis is, similar to other species from phylum Microsporidia, a pathogen with wide host range. Originally thought to infect only human, there is a wide variety of mammals in which it was described, such as donkeys, pigs, cattle and dogs (Bornay-Lilinares et al., 1998).

E. cuniculi is one of the most studied species from phylum Microsporidia. It has been reported in over thirty hosts (Canning et al., 1986). There are four known genotypes that are not strictly host specific (Didier et al., 2005; Talabani et al., 2010). E. cuniculi infect wide range of hosts among mammals, mainly rabbits, carnivores, non-human primates and human (Mathis et al., 2005).

E. hellem was first described as a cause of human keratoconjunctivitis (Didier et al., 1991). It is common human pathogen, but it was also described in animals, such as birds. E. hellem is especially prevalent in aquatic birds that are in contact with surface water, like waterfowl (Slodkowicz-Kowalska et al., 2006; Malčeková et al., 2013). There are seven genotypes of this pathogen based on ITS and PTP gene regions (Mathis et al., 1999; Xiao et al., 2001).

From genus Encephalitozoon E. intestinalis is commonly identified as a waterborne pathogen. It was found in both, ground and surface water and in sewage effluent as described by Dowd et al. (1998). Thurston-Enriquez et al. (2002) discovered human-pathogenic E. intestinalis in irrigation water. Graczyk et al. (2007c) found spores of this pathogen in water from recreational bathing area in USA. It was also detected along with E. hellem in molluscan shellfish from Ireland (Lucy et al., 2008). Izquierdo et al. (2011) identified this pathogen from one sample collected in recreational river area in Spain. E. intestinalis and E. cuniculi were detected in samples from drinking water treatment plants and waste water treatment plants (Galvan et al., 2013). E. intestinalis was also detected in sewage sludge (Graczyk et al., 2007a).

Prevalence of Encephalitozoon infections in humans is unknown because it is often asymptomatic. Prevalence data based on serological tests utilizing E. cuniculi as an antigen ranged from 0 to 42% (Singh et al., 1982). In Mexico, prevalence of encephalitozoonosis was almost 8% (Enriquez et al., 1998). Serological studies on Dutch blood donors and French pregnant women revealed prevalence of Encephalitozoon spp. to be 8 and 5% (Van Gool et al., 1997). Highest seroprevalence was detected in homosexual men from Sweden and in population presenting other parasitic diseases (Bergquist et al., 1984). Sak et al. (2011) identified prevalence of infections caused by Encephalitozoon spp. in...
immunocompetent humans to be around 36%. In Slovakia seroprevalence of *E. cuniculi* and *E. intestinalis* ranges from 6% in healthy humans (Malčeková et al., 2010; Halánová et al., 2003) to 37.5% in immunodeficient patients (Halánová et al., 2003).

*E. intestinalis* is an intestinal epithelial cell parasite that can cause diarrhea, malabsorption and wasting in infected humans. It can also disseminate to other parts of the body such as liver, renal system, gallbladder, lungs, sinuses and conjunctiva. *E. cuniculi* can cause hepatitis, peritonitis or disseminated infection, mainly in immunosuppressed patients. *E. hellem* was associated with eye infections, disseminated infections, infections of nasal tissue, sinuses, tongue, respiratory tract and kidney (Anane and Attouchi, 2010).

5. Other reported *Microsporidia* species

Other pathogens from phylum *Microsporidia* reported in water include *Noosema* species, *Pleistophora* species and *Vittaforma cornea*. Genus *Noosema* comprises of insect pathogens and can cause keratoconjunctivitis in human. Only one study (Avery and Undeen, 1987) found this pathogen in water. *Pleistophora* spp. is a genus that causes infections in fish, amphibians and reptiles. In human they can cause myositis (Anane and Attouchi, 2010). *Pleistophora* spp. was identified by Avery and Undeen (1987) in ditch water, Thurston-Enriquez et al. (2002) in water used for irrigation of crops and by Fournier et al. (2000) and Sparfel et al. (1997) in rivers Seine and Loire. *Vittaforma cornea* is a less common pathogen that can cause keratitis and systematic infections in human (Mittleider et al., 2002). It was identified in a tertiary effluent (Dowd et al., 1998) and in river Seine (Fournier et al., 2000).

6. CONCLUSION

For these reasons were *Microsporidia* included by United States Environmental Protection Agency on the list of microbial contaminants of drinking water Safe Drinking Water Act in the years 1998 and 2005 (EPA). They were also included in the category B priority pathogens by National Institute of Allergy and Infectious Diseases (NIAID). In Europe, the regulation is related to the quality of drinking water, adapted from Directive 98/83/EC which specifies the need for detection of fecal bacterial indicators, some microorganisms and parasites. However, microsporidia are not specifically monitored (The Council of the European Union, 1998). The management of the bathing water quality is done in accordance with the bathing water directive 2006/7/EC (The European Parliament and the Council of the European Union, 2006) while the use of the sewage sludge is governed by Directive 86/278/EEC (The Council of European Communities, 1986). However, these laws do not direct routine monitoring of *Microsporidia* in the water which can be therefore transmitted in the water and act as a threat to public health. We assume that monitoring of phylum *Microsporidia* is insufficient and can lead to possible future outbreaks. Even though they are susceptible to commonly used disinfectants, their small size makes them difficult to remove by conventional water filtration techniques.

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