THE MICROSPORIDIA: PATHOLOGY IN MAN AND OCCURRENCE IN NATURE

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Abstract. The microsporidia are a diverse phylum of obligate intracellular parasitic protists. They infect every major group of animals including invertebrates, fish, mammals, and man. While animal infections have long been known, it is only since 1985 that they have been brought to the attention of pathologists as potentially lethal opportunistic parasites in man. In this relatively short time, over a dozen different species of microsporidia have been recognized, resulting in infection of virtually every tissue of the human body. This diversity of both the organisms and their clinical manifestations results in added diagnostic difficulty. As we learn more about the human infectious organisms, their occurrence in non-human hosts and the environment has become clear. The spores of the microsporidia are extremely resistant, surviving years in water, and their small size (1-4 μm) renders them difficult to remove. While some microsporidial genera reported from man have no known animal reservoir hosts, their dissemination via human waste usually provides ample opportunity for access to a water environment. Additionally, there is evidence of other means of environmental dissemination: some, such as *Encephalitozoon cuniculi*, are known to occur in over 30 different mammalian hosts; the newest species described, *Pleistophora ronneafiei*, occurs in a genus whose other hosts are fish; and the most recently identified human infection in skeletal muscle, *Brachiola algerae*, is normally a parasite of mosquitos.

THE PHYLUM MICROSPORIDIA

The phylum Microsporidia contains over 104 genera and about 1,200 species and all are obligate intracellular protistan parasites. There are microsporidia that cause infection in every major group of animals as well as man. They are eukaryotic but their ribosomes have prokaryotic features. They lack mitochondria and centrioles and are thus considered primitive or degenerate, and probably related to fungi (Weiss and Vossbrink, 1999). They have diverse life cycle developmental patterns (Figs 1-2) but they all result in the formation of a very resistant spore that is diagnostic (Fig 3).

The life cycle consists of three phases, the infective/environmental phase, the proliferative phase and the sporogonic phase (Cali and Takvorian, 1999). Both the proliferative and the sporogonic phases are intracellular in the infected hosts and vary tremendously both in their development and host/parasite interface (Cali and Takvorian, 1999). It is the environmental phase, however, that is relatively similar for all microsporidia. This is the phase that contains the diagnostic environmentally resistant spores (Figs 3, 4).

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The spores of microsporidia are generally small, oval or pyriform shaped, resistant structures that vary in length from approximately 1 μm to about 10 μm, 4 μm being the most common size (Whittner and Weiss, 1999).

Those in mammals are generally 1-4 μm in length (Bryan *et al*, 1991, Webber *et al*, 1994) . The presence of a microsporidial infection is most often diagnosed by the detection of the parasite spores. Fresh spores are extremely refractile when viewed by phase contrast microscopy (Figs 3a, 4).

In order for successful transmission of infection to occur, spores must be liberated from the infected host, maintain their viability in the environment, encounter a new susceptible host, and gain entry to host cells that will support the parasite's multiplication.

Microsporidian spores transfer their infective sporoplasm into a susceptible host cell as a result of a series of complex events, which include environmental changes necessary to activate the spore triggering mechanisms. These may be a chemical and/or physical effect that changes the spore's permeability, resulting in the eversion of the polar tube. This is the process that results in the polar tube shooting out of the spore. It is emitted with sufficient force to pierce the plasmalemma of a host cell. The sporoplasm is then transmitted from the spore into the host cell cytoplasm through the everted polar tube, providing for a unique means of inoculation (Figs 4-7).

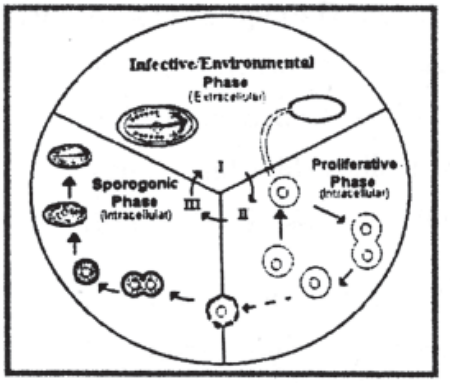


Fig 1- Developmental cycle of the microsporidia (Cali and Takvorian, 1999).

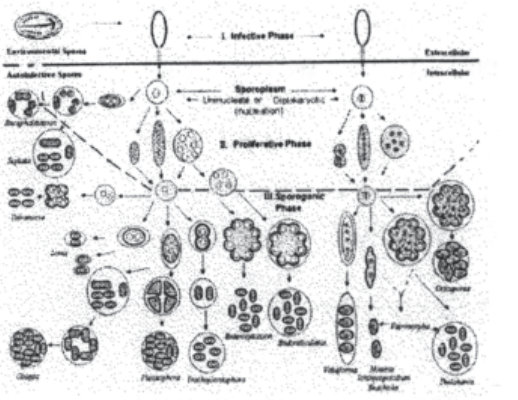
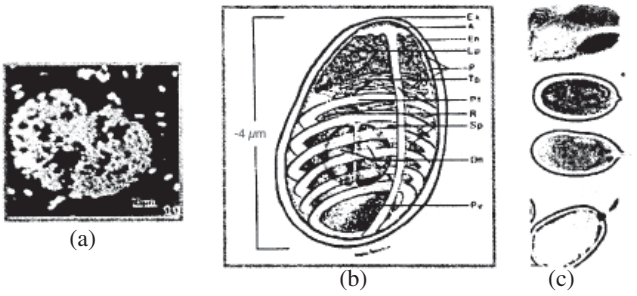


Fig 2- Diversity of developmental patterns in the microsporidia (Cali and Takvorian, 1999).



(a) (b) (c)

Fig 3- Microsporidial spores (Cali and Takvorian, 1999).

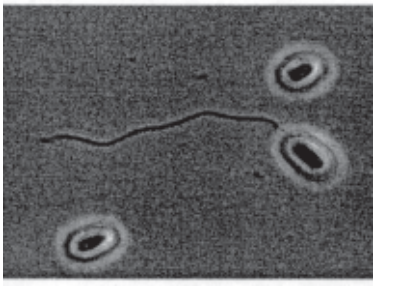


Fig 4- Start of polar tube eversion.

The majority of microsporidian infections are initiated in the susceptible host via oral consumption, with the spores gaining access to the digestive tract. It has been demonstrated that spores respond to one or more stimuli: pH, ion concentration, osmolarity, digestive enzymes, redox potential, and digestive products act as primary germination stimuli (Jaronski, 1979). When the spore is triggered, the polar tubule acts as the transfer vehicle through which the sporoplasm travels. If the polar tubule has pierced a compatible host cell, the proliferative phase begins. This and the sporogonic phase take place within the host cells and are extremely diverse, depending on the microsporidial genus involved. The completion of the developmental cycle is the production of spores within the infected host cell (Cali and Takvorian,1999). The spores eventually enter the environment.

SPORE OCCURRENCE IN THE ENVIRONMENT

Most microsporidian spores spend at least some part of their existence in the environment outside the host. Spores become extracellular by several methods, depending upon the host they are infecting and the site of infection. Infected intestinal epithelial cells generally slough off and degrade within the digestive system, allowing spores to be released with the feces (Orenstein *et al*, 1992). Renal infections produce spores which are excreted with the urine (Orenstein *et al*, 1992), while the spores produced from sinus and respiratory infections can exit the body via sputum (Schwartz *et al*, 1992). The spores of infected animals that die enter the environment by the decomposition of the body, cannibalism, scavenging, or by physical abrasion or tearing due to environmental action.

SPORE SURVIVAL IN THE ENVIRONMENT

Spore survival is variable depending upon the particular species and environmental conditions. *Nosema apis* spores remained viable after 24 hours of exposure to sunlight while *N. necatrix* lost viability after only five hours of exposure (Maddox, 1973). *N. algerae* spores showed no change in infectivity after being exposed to bright sunlight for up to four hours, but eight minutes of exposure to an artificial UV light source decreased infectivity and infection intensity by 99.9% (Kelly and Anthony, 1979). Additional

Fig 5- Germinating spore, most of the polar tube has everted but sporoplasm is still present (TEM) (Cali *et al*, 2002).

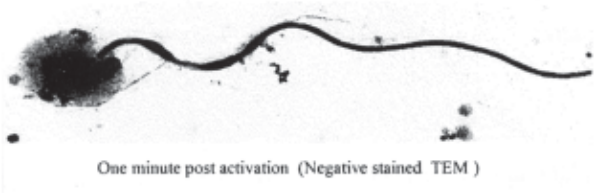


Fig 7- *Brachiola algerae* sporoplasm attached to polar tube (Cali *et al*, 2002).

Fig 6- Germinated spores (Cali *et al*, 2002).

SPORE SIZES FOR MICROSPORIDIA ASSOCIATED WITH HUMAN INFECTION

• *Enterocytozoon*	*1 x .7 µm*	• *Nosema*	*4 x 2 µm*
• *Encephalitozoon*	*2 x 1 µm*	• *Vittaforma*	*4 x 1 µm*
• *pleistophora*	*4 x 2 µm*	• *Brachiola*	*3 x 2 µm*
• *Trachipleistophora*	*4 x 2 µm*	• *Microsporidium*	

Fig 8- Spore sizes for microsporidia associated with human infection.

experiments showed that infectivity was reduced by 48% after one minute of UV exposure and 76 % after two minutes (Kelly and Anthony, 1979).

Spores from insects, placed on dry surfaces at room temperature, remain infective for several weeks or up to one year. Spores in fecal pellets or dried cadavers lasted less than 6 months under the same conditions, but could still produce infections after a year, if maintained in a cold aqueous media (Kramer, 1970). *Octosporea muscaedomesticae* spores survived 16 months in dried feces when maintained at 5˚C with 50% humidity, but only 8 months at room temperature (Kramer, 1970). *Nosema destrutor* retained viability after a 20-minute exposure at 55˚C but were inactivated at 70˚C (Maddox, 1973). Similar effects were observed in *N. necatrix*, which lost viability after 30 minutes at 60˚C, 90 minutes at 55˚C, or 5 hours at 50˚C, but remained infective for three weeks at 40˚C (Maddox, 1973). These reports indicate that elevated temperatures severely reduce infectivity while cold seems to lengthen spore survival. In addition to temperature, exposure to water also has a lengthening effect. Spores which only retain infectivity for 6 months when dry, were still infective for as long as 10 years when stored in cold distilled water (Kramer, 1970).

MICROSPORIDIA IN HUMANS

Eight genera and over a dozen species of microsporidia have been reported. These genera include: *Enterocytozoon, Encephalitozoon, Nosema, Brachiola, Vittaforma, Trachipleistophora*, *Pleistophora,* and *Microsporidium* (Fig 8). They are most commonly gastrointestinal pathogens, however, they have been demonstrated in association with pathology from every tissue and organ including respiratory, muscle, excretory, and nervous systems and their spores are common in the environment (Wittner and Weiss, 1999; Cali and Takvorian, 2003; Cali *et al*, 2003).

OCCURRENCE OF HUMAN INFECTING MICROSPORIDIA IN THE ENVIRONMENT

The majority of microsporidial infections are initiated by oral ingestion and cause diarrhea. In a 1995 study of AIDS associated diarrheas from US patients, up to 40% of patients were shedding microsporidial spores (Kotler, 1995). A survey of human stools for the presence of *Encephalitozoon* spores, identified by monoclonal antibody, in two rural villages in Mexico, resulted in a finding of 7.84% (20 of 255) positive subjects and 21.4% (15 of 70) households had at least one member who was positive suggesting that *Encephalitozoon* may be commonly present in community settings (Enriquez *et al*, 1998). Another

survey of human stools for microsporidial baseline data is from Lyon, France. Stool samples from1,454 persons were assessed for microsporidia from 1993 to 1996. Microsporidia were identified from 338 persons. Of these, 261 persons were HIV-positive, 16 persons were transplant patients, and 61 were grouped as other. Thus, microsporidiosis appears to be endemic in HIV-positive persons (prevalence = 0.1%) and sporadic (prevalence <1/1 million) in HIV-negative persons (Cotte *et al*, 1999).

Other sources of human microsporidial shedding include urine from renal infections and sputum or other respiratory fluids. These reports suggest that human infecting microsporidial spores are continuously being shed into the environment by humans.

RESERVOIR HOSTS

Knowledge of reservoir hosts of human infections is variable according to the specific microsporidial parasite and is changing rapidly, as we uncover new information about both human and animal hosts. While the genus *Enterocytozoon* was established for a human infection and later found in pigs (Deplazes *et al*, 1996) and primates (macaques) (Mansfield *et al*, 1997), *Encephalitozoon* was first described in rabbits (Wright and Craighead, 1922) and subsequently reported from over 30 other warm blooded vertebrate hosts (Cali and Owen, 1989) before the first description of infection in a human (Terada *et al*, 1987). The genus *Nosema* is most noted for infection in invertebrate hosts (over 200 species described), especially insects. The genus *Pleistophora*, was originally described from fish (Gurley, 1893), is not known to infect warm blooded hosts except for the species *ronneafiei* recently described in human infection (Cali and Takvorian, 2003). Most recently, *Brachiola algerae*, a parasite of mosquito larvae, has been demonstrated to cause a severe myositis in a patient who had been immune suppressed for rheumatoid arthritis treatment (Cali *et al*, 2003; Coyle *et al*, 2004).

REPORTED PRESENCE OF HUMAN INFECTING MICROSPORIDIA IN WATER

A number of recent reports demonstrate the presence of human infecting microsporidia in water for both crops and human consumption.

Irrigation water

Microsporidia have been reported in irrigation water (Thurston-Enriquez *et al*, 2002). Twenty-eight percent of water samples (100-400 l) collected in the US and Central America were positive for microsporidia. Two of the positive samples were sequenced and demonstrated to be *Encephalitozoon intestinalis* and *Pleistophora* spp, two human pathogenic species. These water samples came from rivers, lakes, and agricultural canals.

"The presence of human pathogenic parasites in irrigation waters used in the production of crops traditionally consumed raw, suggests that there may be a risk to consumers who come in contact with or eat these products" (Thurston-Enriquez *et al*, 2002).

Surface water

Sequential water samples (300-600 l) of the Seine river, in France, were collected during a one year period. The water filters were tested by PCR for the presence of microsporidia, of the 25 samples, 16 were positive (64%). Eight samples were positive for *Vittaforma corneae* or *Pleistophora* sp, and one was positive for *Enterocytozoon bieneusi* (Fournier *et al*, 2002).

Tertiary sewage effluent , surface and ground water

Dowd *et al*, 1998 reported the occurrence of human pathogenic microsporidia in tertiary sewage effluent , surface and ground water. This study reports a species level confirmation of human-pathogenic microsporidia in water, indicating that they may be water-borne pathogens. A total of 14 water concentrates were screened; seven (50%) of these contained human pathogenic microsporidia. Confirmation of human-pathogenic microsporidia, *Enterocytozoon bieneusi, Encephalitozoon intestinalis,* and *Vittaforma corneae* in water was demonstrated. *Encephalitozoon intestinalis* was confirmed in tertiary sewage effluent, surface water, and ground water. *Enterocytozoon bieneusi* was confirmed in surface water. *Vittaforma corneae* was confirmed in tertiary sewage effluent (Dowd *et al*, 1998).

Tap water

In two rural Mexican villages, microsporidial surveys were compared to their water sources. The village that received piped in untreated water from a spring, had a significantly higher incidence of *Encephalitozoon* positive stool samples as compared to the village in which the residents used well water, 40% *vs* 15% (Enriquez *et al*, 1998).

Microsporidial outbreak

In the summer of 1995, a water-borne outbreak of microsporidiosis occurred in Lyon, France with approximately 200 cases, (primarily in immuno-

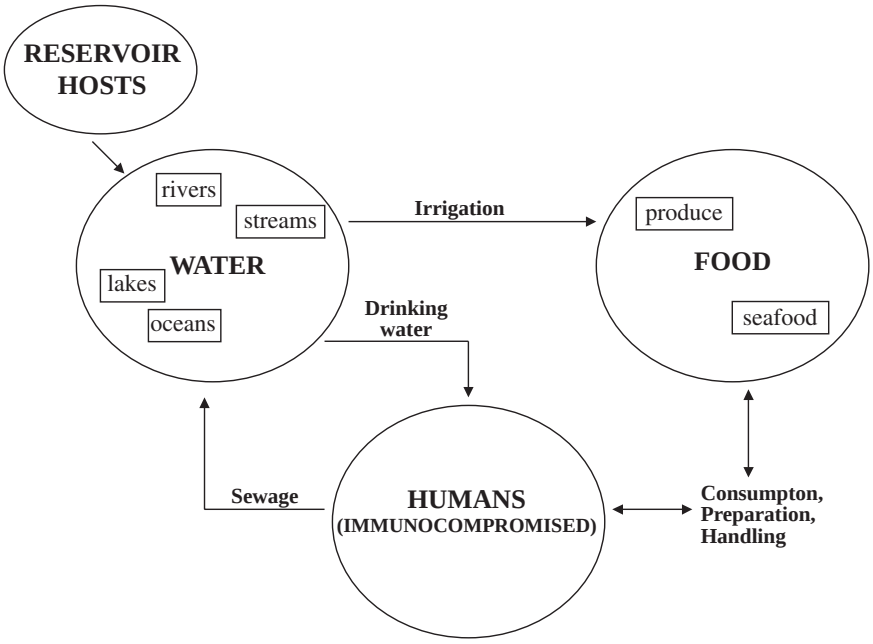


Fig 9- The food-water connection between microsporidia and human infection.

compromised people). Drinking water contamination from a nearby recreational lake was suspected because the majority of cases clustered in an area whose water was provided from a pumping station with a water source from the suspected lake. The primary water supply for Lyon is two pumping stations, which obtain their water from the Rhone river where it is naturally filtered by alluvia. The third water source (the suspected lake) is independent of the Rhone and mainly utilized in the summer (Cotte *et al*, 1999).

REMOVAL OF SPORES FROM WATER

The removal or inactivation of microsporidial spores from water by chemical inactivation (too preliminary to draw conclusions other than the variable nature of the microsporidia)

A baseline chlorination of 1.1mg/l residual concentration was used for drinking water as a standard. In experiments with the microsporidium, *Encephalitozoon intestinalis*, a 16-minute exposure to a 2.0 mg/l chlorine treatment was needed to achieve a three log reduction (99.9%) of viable spores as determined by infection of cell cultures (Wolk *et al*, 2000).

Another report testing multiple *Encephalitozoon* species, suggests that they are variable in their resistance to disinfection. Experiments using cell culture as an assay method suggest that treatment with chlorine at concentrations of approximately 2.5 mg/l for a minimum of 4 minutes produced a 3.3 log inactivation of *E. cuniculi*, but only a 0.70 log reduction of *E. hellem.* (Johnson *et al*, 2003).

SURVIVAL AT DIFFERENT ENVIRONMENTAL TEMPERATURES

Culture-derived spores of *Encephalitozoon cuniculi, E. hellem*, and *E. intestinalis* were stored in water at various temperatures to determine the longevity of spores in a water environment. At 10 ˚C, spores of *E. intestinalis* were still infective after 12 months, spores of *E. hellem* 9 months, and *E cuniculi* 3 months. At 20 ˚C, spores of *E. intestinalis* were still infective after 7 months, spores of *E. hellem* 5 months, and *E. cuniculi* 1 month. At 30 ˚C, spores of *E. intestinalis* were still infective after 3weeks, spores of *E. hellem*1 month, and *E. cuniculi* 1week.

The study concludes that while the three species vary in their survivability, they "have the potential to remain infective in the environment long enough to become widely dispersed" (Li *et al*, 2003).

WHAT WE KNOW ABOUT HUMAN INFECTING MICROSPORIDIA

There are many species that infect humans.

The majority of microsporidian infections are initiated by oral inoculation.

Human infecting spores enter the environment from both human and animal sources.

These spores are in the range of 1-4 μm and are most highly resistant in cold water.

They have been identified in a wide variety of aquatic environments (ditches, rivers, and lakes). The food-water connection between human health and the environment is well illustrated by a diagram from Slifko *et al* (2000). We have modified for Microsporidia (Fig 9).

The combination of the continuous dispersal of spores from humans and animal hosts, the small size of the spores, their resistance, and their long term survivability in water, make them a potential threat to drinking water safety.

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