

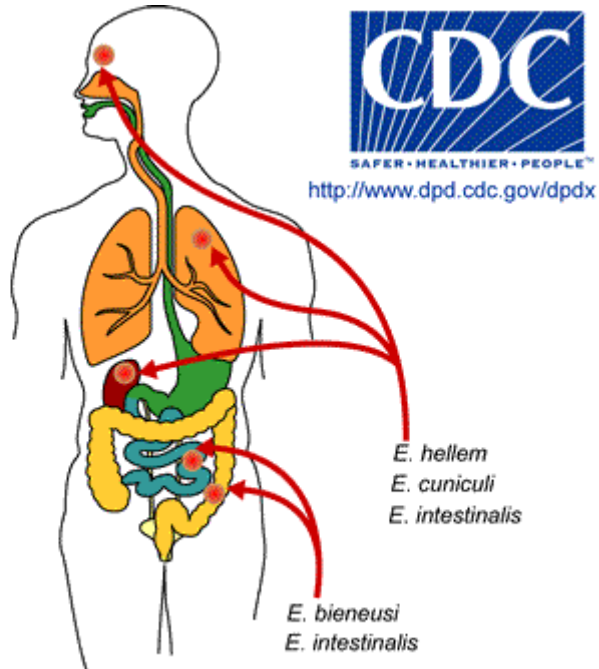
# Microsporidiosis

## Causal Agents:

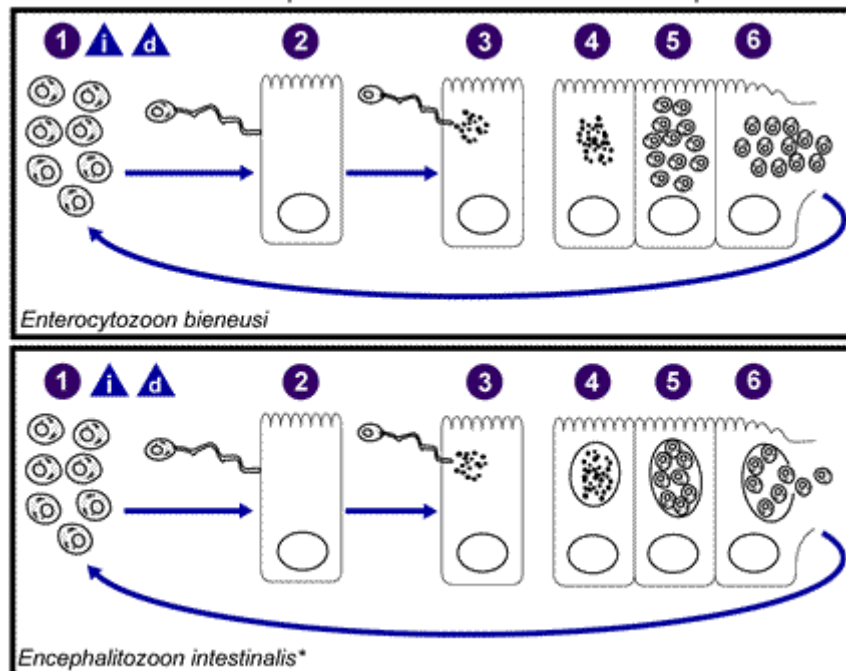
The term microsporidia is also used as a general nomenclature for the obligate intracellular protozoan parasites belonging to the phylum Microsporidia. To date, more than 1,200 species belonging to 143 genera have been described as parasites infecting a wide range of vertebrate and invertebrate hosts. Microsporidia, are characterized by the production of resistant spores that vary in size, depending on the species. They possess a unique organelle, the polar tubule or polar filament, which is coiled inside the spore as demonstrated by its ultrastructure. The microsporidia spores of species associated with human infection measure from 1 to 4  $\mu\text{m}$  and that is a useful diagnostic feature. There are at least 14 microsporidian species that have been identified as human pathogens: *Brachiola algerae*, *B. connori*, *B. vesicularum*, *Encephalitozoon cuniculi*, *E. hellem*, *E. intestinalis*, *Enterocytozoon bieneusi*, *Microsporidium ceylonensis*, *M. africanum*, *Nosema ocularum*, *Pleistophora* sp., *Trachipleistophora hominis*, *T. anthropophthera*, *Vittaforma corneae*. *Encephalitozoon intestinalis* was previously named *Septata intestinalis*, but it was reclassified as *Encephalitozoon intestinalis* based on its similarity at the morphologic, antigenic, and molecular levels to other species of this genus. Based on recent data it is now known that some domestic and wild animals may be naturally infected with the following microsporidian species: *E. cuniculi*, *E. intestinalis*, *E. bieneusi*. Birds, especially parrots (parakeets, love birds, budgies) are naturally infected with *E. hellem*. *E. bieneusi* and *V. corneae* have been identified in surface waters, and spores of *Nosema* sp. (likely *B. algerae*) have been identified in ditch water.

## Life Cycle:

▲ i = Infective Stage  
▲ d = Diagnostic Stage



Intracellular development of *E. bienersi* and *E. intestinalis* spores.



\*Development inside parasitophorous vacuole also occurs in *E. hellem* and *E. cuniculi*.

The infective form of microsporidia is the resistant spore and it can survive for a long time in the environment **1**. The spore extrudes its polar tubule and infects the host cell **2**. The spore injects the infective sporoplasm into the eukaryotic host cell through the polar tubule **3**. Inside the cell, the sporoplasm undergoes extensive multiplication either by merogony (binary fission) or schizogony (multiple fission) **4**. This development can occur either in direct contact with the host cell cytoplasm (e.g., *E. bienersi*) 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 <sup>5</sup>. 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 <sup>6</sup>. These free mature spores can infect new cells thus continuing the cycle.

### Geographic Distribution:

Microsporidia are being increasingly recognized as opportunistic infectious agents worldwide. Cases of microsporidiosis have been reported\* in developed as well as in developing countries, including: Argentina, Australia, Botswana, Brazil, Canada, Czech Republic, France, Germany, India, Italy, Japan, The Netherlands, New Zealand, Spain, Sri Lanka, Sweden, Switzerland, Thailand, Uganda, United Kingdom, United States of America, and Zambia.

\*These data account for infections caused by at least one of the microsporidian species listed in the causal agent section.

### Clinical Features:

Human microsporidiosis represents an important and rapidly emerging opportunistic disease, occurring mainly, but not exclusively, in severely immunocompromised patients with AIDS. Additionally, cases of microsporidiosis in immunocompromised persons not infected with HIV as well as in immunocompetent persons also have been reported. The clinical manifestations of microsporidiosis are very diverse, varying according to the causal species with diarrhea being the most common.

Microsporidian species	Clinical manifestation
<i>Brachiola algerae</i>	Keratoconjunctivitis, skin and deep muscle infection
<i>Enterocytozoon bieneusi</i> *	Diarrhea, acalculous cholecystitis
<i>Encephalitozoon cuniculi</i> and <i>Encephalitozoon hellem</i>	Keratoconjunctivitis, infection of respiratory and genitourinary tract, disseminated infection
<i>Encephalitozoon intestinalis</i> (syn. <i>Septata intestinalis</i> )	Infection of the GI tract causing diarrhea, and dissemination to ocular, genitourinary and respiratory tracts
<i>Microsporidium</i> ( <i>M. ceylonensis</i> and <i>M. africanum</i> )	Infection of the cornea
<i>Noesma</i> sp. ( <i>N. ocularum</i> ), <i>Brachiola connori</i>	Ocular infection
<i>Pleistophora</i> sp.	Muscular infection
<i>Trachipleistophora anthropophthera</i>	Disseminated infection
<i>Trachipleistophora hominis</i>	Muscular infection, stromal keratitis, (probably disseminated infection)
<i>Vittaforma corneae</i> (syn. <i>Noesma corneum</i> )	Ocular infection, urinary tract infection

\*Two reports of *E. bieneusi* in respiratory samples have also been published, one in 1992 and the other in 1997.

## Laboratory Diagnosis:

There are several methods for diagnosing microsporidia:

- Light microscopic examination of the stained clinical smears, especially the fecal samples, is an inexpensive method of diagnosing microsporidial infections 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. 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. This technique, however, is lengthy and time consuming and requires about 90 min. A recently developed "Quick-Hot Gram Chromotrope technique" however, cuts down the staining time to less than 10 min and provides a good differentiation from the lightly stained background fecal materials so that the spores stand out for easy visualization. The spores stain dark violet and the belt-like stripe is enhanced. In some cases dark staining Gram positive granules are also clearly seen. Chemofluorescent agents such as Calcofluor white are also useful in the quick identification of spores in fecal smears. The spores measure from 0.8 to 1.4  $\mu\text{m}$  in the case of *Enterocytozoon bieneusi*, and 1.5 to 4  $\mu\text{m}$  in *Brachiola algerae*, *Encephalitozoon* spp., *Vittaforma corneae*, and *Nosema* spp.
- Transmission electron microscopy (TEM) is still the gold standard and is necessary for the identification of the microsporidian species. However, TEM is expensive, time consuming, and not feasible for routine diagnosis.
- Immunofluorescence assays (IFA) using monoclonal and/or polyclonal antibodies are being developed for the identification of microsporidia in clinical samples.
- Molecular methods (mainly Polymerase Chain Reaction, PCR) is an alternative method for the laboratory diagnosis of microsporidiosis. PCR is available only in research laboratories and has been successfully used for the identification of *Brachiola algerae*, *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Encephalitozoon cuniculi*. The principal drawback is that it does not work well on formalin-fixed samples stored for long term.

## Treatment:

The treatment of choice for ocular microsporidiosis (*Brachiola algerae*, *Encephalitozoon hellem*, *E. cuniculi*, *Vittaforma corneae*) is oral albendazole\* plus topical fumagillin. Corneal infections with *V. corneae* often do not respond to chemotherapy and may require keratoplasty. Oral fumagillin has been effective to treat *Enterocytozoon bieneusi* infections, but it has been associated with thrombocytopenia. Albendazole\* is the drug of choice to treat gastroenteritis caused by *Encephalitozoon intestinalis* and to treat disseminated microsporidiosis (*E. hellem*, *E. cuniculi*, *E. intestinalis*, *Pleistophora* sp., *Trachipleistophora* sp., *Brachiola vesicularum*) and skin and deep muscle infection (*Brachiola algerae*).

\* This drug is approved by the FDA, but considered investigational for this purpose.