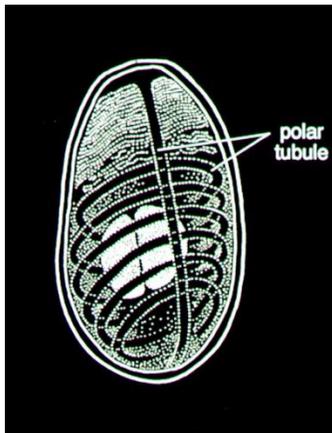


## Microsporidia (*Encephalitozoon intestinalis*)

### Organism:

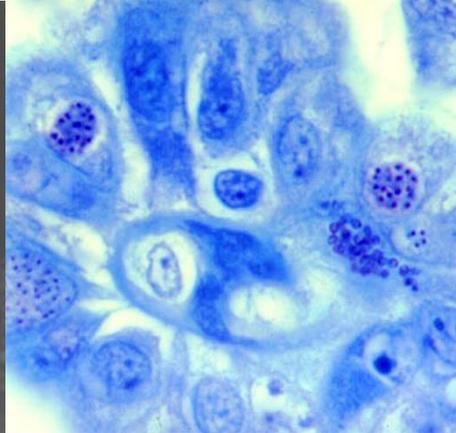
The microsporidia are obligate intracellular parasites that have been recognized in a variety of animals, particularly invertebrates; they have been reclassified with the Fungi in the Phylum Microsporidia. Typical sizes range from 1.5 to 5  $\mu\text{m}$  wide and 2 to 7  $\mu\text{m}$  long; unfortunately, the organisms found in humans tend to be quite small, ranging from 1.5 to 2  $\mu\text{m}$ . Until recently, awareness and understanding of human infections have been marginal; only with increased understanding of AIDS within the immunosuppressed population has attention been focused on these organisms.



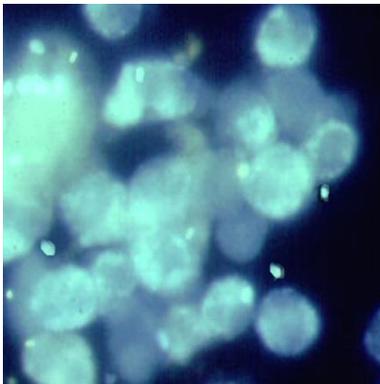
Spore with Polar Tubule



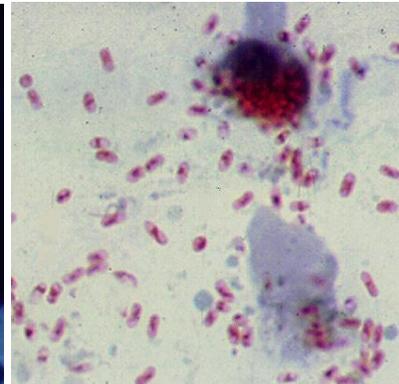
Extruded Polar Tubule



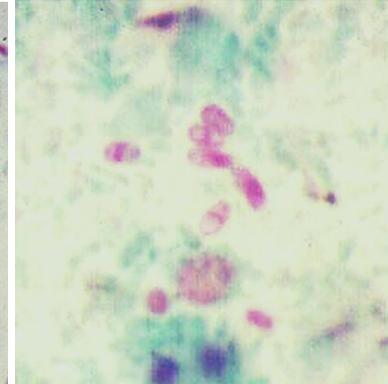
Developing spores in GI tissue



Calcofluor white – urine



Ryan Blue Microsporidial stain



Weber Green Microsporidial stain

### Life Cycle:

Infection occurs with the introduction of infective sporoplasm through the polar tubule into the host cell. The microsporidia multiply extensively within the host cell cytoplasm; the life cycle includes repeated divisions by binary fission (merogony) or multiple fission (schizogony) and spore production (sporogony). Both merogony and sporogony can occur in the same cell at the same time. During sporogony, a thick spore wall is formed, thus providing environmental protection for this infectious stage of the parasite. An example of infection potential is illustrated by *Enterocytozoon bieneusi*, an intestinal pathogen. The spores are released into the intestinal lumen and are passed in the stool. These spores are environmentally resistant and can then be ingested by other hosts. There is also evidence for inhalation of spores and evidence in animals that suggests that human microsporidiosis may also be transmitted via the rectal route.

### Acquired:

Fecal-oral transmission via infective spores; contaminated food and water

### Epidemiology:

Worldwide, primarily human-to-human transmission

### Clinical Features:

Chronic intractable diarrhea, fever, malaise, and weight loss are symptoms with *E. intestinalis* infections, symptoms that are similar to those seen with cryptosporidiosis or isosporiasis. AIDS patients tend to have four to eight watery, nonbloody stools which can be accompanied by nausea and anorexia. There may be dehydration with mild

hypokalemia and hypomagnesemia, as well as d-xylose and fat malabsorption. The patients tend to be severely immunodeficient, with a CD4 count always below 200 and often below 100. A dual infection with *E. bienersi* and *E. intestinalis* has also been reported. Once the primary infection has been established, the immune status of the host plays a very important role in determining the length and severity of the illness. Patients infected with *Cryptosporidium* spp. may also have concurrent infections with microsporidia, and this number may approach 30%. These findings emphasize the importance of considering both organisms as potential causative agents of diarrhea in compromised patients, particularly those with HIV.

These infections can also be found in the immunocompetent host; however, most patients will self-cure within several weeks.

#### **Clinical Specimen:**

Intestinal: Stool, examination of mucosal surface (biopsy); dissemination to kidneys, lower airways and biliary tract appears to occur via infected macrophages.

Extraintestinal: Fluids, biopsy specimens

#### **Laboratory Diagnosis: Note: ID to the species level is not possible from special stains.**

Intestinal: Ova and Parasite examination (concentration ONLY); from concentrate sediment, (500 x g for 10 min) modified trichrome stains are performed. Some fecal immunoassays are available in Europe, but are not FDA approved for use within the United States. Multiple fecal examinations may be required to recover the organisms, particularly if the stools are formed; there is a direct relationship between the stool consistency and the number of spores present (diarrhea = more spores).

Stool preparations must be very thin, the staining time is 90 min, and the slide must be examined at x 1,000 (or higher) magnification. Unfortunately, there are many objects within stool material that are oval, stain pinkish with trichrome, and measure approximately 1.5 to 3 µm. If this stain is used for the identification of microsporidia in stool, positive control material should be available for comparison. Additional modifications of this method include the use of heat and a shorter staining time. There is also some evidence to indicate that pretreatment of fecal specimens (1:1) with 10% KOH may provide a better quality smear to examine when using the modified trichrome stains.

Another approach involves the use of chemofluorescent agents (optical brightening agents) such as Calcofluor, Fungi-Fluor, or Uvitex 2B. These reagents are sensitive, but nonspecific; objects other than microsporidial spores will also fluoresce. This is a particular problem when examining stool specimens; both false positive and false negative results have been seen.

Extraintestinal: Modified trichrome stains

Tissue: Tissue stains such as PAS, Silver, tissue Gram stains and others are specifically recommended for the microsporidial spores. Microsporidial infections can be misdiagnosed in tissues and can be confused with *Cryptococcus neoformans* infections. Mucus granules in goblet cells can take up stain and can be very confusing. Good preservation and thin tissue sections (1 µm) that have been resin-embedded enhance the resolution of cellular detail. Demonstration of the coiled polar tube within spores is diagnostic for microsporidial infection. *Encephalitozoon intestinalis* is not confined to epithelial cells, but is seen in macrophages in the lamina propria. Although the primary site appears to be the small bowel, these organisms can disseminate to other sites, including duodenum, jejunum, ileum, colon, kidney, liver, and gallbladder.

#### **Organism Description:**

Spore: Oval spores, containing a coiled polar tubule. However, the polar tubule is not visible in every spore; when seen, it appears as a horizontal or diagonal line across the spore. Without seeing the polar tubule, it is not possible to definitively ID the structures as polar tubules/microsporidial spores.

Tissue: Developing spores (groups) can be seen within the tissue cells.

#### **Laboratory Report:**

Microsporidial spores present; if in stool, the two most likely species are *Enterocytozoon bienersi* and *Encephalitozoon intestinalis*.

#### **Treatment:**

Garcia, L.S. 2007. Diagnostic Medical Parasitology, 5<sup>th</sup> ed., ASM Press, Washington, D.C.

Although a number of drugs have been tested, few are totally effective. This species responds well to albendazole, whereas *Enterocytozoon bienersi* does not.

#### **Control:**

Improved hygiene, adequate disposal of fecal waste, adequate washing of contaminated fruits and vegetables