Introduction

Definition

Microsporidiosis is infection by eukaryotic unicellular protists of the phylum Microsporidia. They are considered most closely related to the fungi, but customarily are discussed among the protozoa. Several genera of microsporidia have been identified in human infections: *Nosema*, *Brachiola*, *Vittaforma*, *Pleistophora*, *Trachipleistophora*, *Enterocytozoon*, *Encephalitozoon*, *Septata*, and *Annecaliia*. All microsporidia are obligate intracellular parasites, but pathologic changes vary with genus and species. In humans, infection may be latent or subclinical until the immune system is suppressed. Microsporidia are a significant opportunistic pathogen in patients with AIDS. Clinical features vary with the location and extent of infection. Microsporidia may infect virtually any tissue or organ of the body, including muscle, intestine, gallbladder, liver, kidneys, eyes, brain, lungs, skin, and nasal sinuses. Intestinal microsporidiosis is most common, occurring in 30% to 50% of AIDS patients with chronic diarrhea. Untreated intestinal, renal, cerebral, hepatic and disseminated infections are usually fatal.

Synonyms

The phylum Microsporigida was also known as phylum Microspora, formerly subphylum Microspora. Encephalitozoon and Nosema were considered synonymous until 1970, when life cycle features demonstrated that they are separate genera. Nosema corneum is now *Vittaforma corneae*, a new genus having been established based on the ultrastructure of this organism’s developmental stages and inconsistencies with any established genus. Septata intestinalis is also known as *Encephalitozoon intestinalis*. Nosema algerae was reclassified as *Brachiola algerae*, and is now *Annecaliia algerae*. Nosema connori was *Brachiola connori*, and is now *Annecaliia connori*. Microsporidia with diagnostic spores but no identifiable developing stages are collectively called Microsporidium. The terms nosematosis and encephalitozoonosis are occasionally used to describe microsporidiosis caused by Nosema sp or Encephalitozoon sp.

Epidemiology

Microsporidia are ubiquitous in animals (primarily insects, fish, and mammals) in developed and undeveloped, tropical and temperate regions of the world. Human infections have been reported in Africa, Australia, Singapore, Europe, and the United States. Between 1924 and 1985, less than a dozen cases of human microsporidiosis were reported. Since 1985, thousands of cases have been documented, primarily in AIDS patients but also in immunocompetent and organ immunosuppressed patients.
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Infectious Agents

Microsporidia are named for the small, resistant spore stage characteristic of this group. The phylum contains more than 170 genera and approximately 1300 species. These diverse eukaryotes have relic mitochondria, no centrioles, contain 70S ribosomes, similar to prokaryotes, and have the smallest genome of any eukaryote thus far reported. Their ribosomal sequences are more similar to bacteria than to eukaryotes. However, their histochemically identifiable Golgi organelles indicate that they may be parasitically evolved degenerate protists, microtubule gene data, as well as several enzyme processes, place them closest to the fungi.

Spores infecting humans are 1 to 5 \( \mu \text{m} \) in length and \( \sim 1 \mu \text{m} \) in width. All microsporidian spores contain a single long coiled structure called the polar filament, a unique structure attached at the anterior end by a large, mushroom-shaped anchoring disk. Electron microscopy reveals this structure coiled around the single- or double-nucleated sporoplasm inside the thick, resistant and refractile spore coat (Figs 14.1 & 14.2). The straight, anterior portion of the polar filament is surrounded by a polaroplast which may be lamellar, tubular, or both. Light microscopy reveals a PAS-positive granule in the anterior end of the spore (Fig 14.3).

While the spore structures themselves are characteristic of microsporidia, the number of spores produced in sporogony, the manner in which they are produced, and the host-parasite interface (Table 14.1) vary among genera of microsporidia. Host-parasite interface may involve: 1) direct contact with host-cell cytoplasm, 2) indirect contact with host-cell cytoplasm by production of a parasite-secreted envelope (sporophorous vesicle, SPOV), 3) indirect contact by production of a parasite-induced, host-produced envelope (parasitophorous vacuole), or 4) indirect contact by a host-produced envelope (parasitophorous vacuole) and parasite-produced secretions. These characteristics, along with morphologic features of developmental stages, nucleation, site of infection, and serologic and molecular features, help to identify the genus and species.
Table 14.1. Interfacial Relationships of the Microsporidia

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
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<tr>
<td>Type I. Direct contact</td>
<td>The parasite plasmalemma is in direct contact with the host-cell cytoplasm (hyaloplasm). e.g. Nosema, Brachiola, Annicalia and Enterocytozoon.</td>
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| Type II. Indirect contact by host produced isolation. | A. Parasitophorous vacuole-host formed single membrane surrounding the developing parasite cell cluster. This is present during both the proliferative phase and the sporogonic phase, however the parasite relationship to it changes. The developing parasite cells maintain a very close relationship with this envelope until they develop the thickened sporont plasmalemma, then they appear loose within the vacuole. e.g. Encephalitozoon cuniculi.  
B. Host endoplasmic reticulum (ER) double membrane, surrounds parasite cells throughout development. In the proliferative phase the host ER double membranes follow the plasmalemma of the dividing cells so that no obvious vacuole is formed. In sporogony, the host ER does not divide with the sporonts and instead forms a double membranous parasitophorous vacuole surrounding the cluster of organisms formed in sporogony. e.g. Vittaforma sp, Endoreticulatus sp. |
| Type III. Indirect contact by parasite produced isolation: | A. Parasite secreted envelope, surrounds parasite cells throughout development. It becomes a sporophorous vesicle (SPOV) in sporogony when the parasite plasmalemma pulls away from the secreted envelope and then the plasmalemma thickens. e.g. Pleistophora sp.  
B. The parasite develops in direct contact with the host cell cytoplasm during early development but then a parasite formed membrane isolates the sporonts from host cytoplasmic contact. e.g. Vairimorpha sp. |
| Type IV. Indirect contact by host and parasite produced isolation. | A. Host ER closely abuts the parasite plasmalemma in merogony. Then the parasite produces “blisters” arising from the plasmalemma to form the interfacial envelope. Thus SPOV is formed in sporogony. It may also contain tubules. e.g. Loma sp, and Glugea sp.  
B. The host and parasite contribute to the formation of a thick interfacial envelope that surrounds all stages of parasite cells. e.g. Trachipleistophora sp.  
C. Host formed parasitophorous vacuole surrounds the parasite cluster and parasite secreted material surrounds each parasite cell inside the parasitophorous vacuole. e.g. Septata sp. |
Life Cycle and Transmission

The life cycles of microsporidia infecting humans have 3 phases: infective, proliferative, and sporogonic (Fig 14.4). The infective phase begins when a spore has developed fully in a host cell. Whether it remains within the host or passes to the environment through sputum, urine, or feces, the spore must receive the proper stimulus (pH or specific ions) in order to trigger the eversion of the polar filament resulting in the formation of a long polar tube that emerges with sufficient speed/force to penetrate a new host cell (Fig 14.5). A sporoplasm passes through the tube and is deposited in a new host cell in less than a second, initiating a new infection. Some spores are autoinfective and will evert their polar tubules, releasing sporoplasts within the same host, establishing additional sites of infection.

The proliferative phase begins when a sporoplasm grows and divides producing many organisms within the host-cell cytoplasm. The beginning of the sporogonic phase, sporogony, is usually signaled by one or more changes: secretions deposited on the surface membrane now called a thickened plasmalemma and/or formation of an isolating envelope (SPOV). Sporonts divide one or more times then become sporoblasts, which metamorphose into spores.

Environmental sources of human exposure to microsporidia are not known definitively, but since many human infections are intestinal and cause severe diarrhea, fecal-oral transmission is likely. In disseminating infections of the renal system, spores are passed in urine, providing another possible source of exposure. A strong correlation between soil exposure and microsporidial keratitis in HIV negative patients has been reported. Municipal drinking water chlorination does not inactivate spores, so this is a possible reservoir for microsporidia as well as spores on food consumed raw (Fig 14.6). The use of molecular tools has also led to the identification of human-infecting microsporidia in fecal samples from cats, dogs, primates, cattle, and birds.

Families: Nosematidae & Tublinosematidae

There are over 100 species of Nosema, most of which are parasitic in insects. A few Nosema species have been described from human ocular infections, N. ocularum,
Nosema sp	extsuperscript{,84} and \textit{N. corneum}.\textsuperscript{13,17,29,30,85} The genus \textit{Vittaforma} was created for the organism originally named \textit{Nosema corneum}, after Silveira and Canning suggested that the diplokaryotic arrangement of nuclei was the only characteristic indicative of the genus \textit{Nosema}. \textit{Vittaforma corneae},\textsuperscript{8} as it is now known, is polysporoblastic and thus will probably be omitted from family \textit{Nosematidae} as new molecular sequence data become known. The \textit{Nosema} sp of \textit{Curry}\textsuperscript{84} is accepted in the \textit{Nosema} genus because of the presence and/or absence of features eliminating other possibilities. \textit{Nosema} sp of \textit{Ashton}\textsuperscript{34} lacked definitive generic information and was moved to the “group” \textit{Microsporidium} with a species designation of \textit{M. celonensis}\textsuperscript{55} as was \textit{Nosema} sp of \textit{Pinnolis}\textsuperscript{5} to \textit{M. africanum}.\textsuperscript{55} More recently Loh conducted a four year study that confirmed 124 cases of microsporidial keratitis. While the pathology and therapy were described, the generic designations of the organisms were not demonstrated.\textsuperscript{58}

Three \textit{Brachiola} spp have been described from human infections and recently moved to a new family, based on molecular and morphologic features.\textsuperscript{18} The new family, \textit{Tublinosematidae}, was established in 2005 for organisms with many similarities to \textit{Nosematidae} but with different morphological and molecular features.\textsuperscript{86} While the family was established for microsporidia infecting insects, the human as well as the insect infecting \textit{Brachiola} species were subsequently placed here because of the sequence similarity of \textit{B. algerae} and \textit{Annicalia meligethi}.\textsuperscript{18,87} These organisms possess a thickened plasmalemma throughout development and an elaborate surface tubular network that extends into the host cytoplasm. The genus \textit{Annicalia} was added to this family (2006) and its sequence data so closely resembled \textit{Brachiola algerae} that the genus \textit{Brachiola} became a synonym to the previously described genus \textit{Annicalia}.\textsuperscript{18} However, we retain the genus \textit{Brachiola} in this family, for its type species, \textit{vesicularum}, because of its unique protoplasmic branches that extend into the host cytoplasm, similar to fungal hyphae, not demonstrated in any other microsporidia.\textsuperscript{6}

**Morphologic Description**

\textit{Nosema} and \textit{Annicalia (Brachiola)} spores are approximately 4 \textmu m in length and have paired abutted nuclei (diplokarya) in every stage of development. The parasite plasmalemma is in direct contact with the host-cell cytoplasm. If there are several nuclear divisions before cytokinesis
takes place, proliferative cells may become elongated. After many cell divisions, large clusters of individual diplokaryotic cells develop. Sporonts produce two sporoblast cells that develop into two spores, all in direct contact with the host-cell cytoplasm.28,52

*Nosema* sp form a thickened plasmalemma only in the sporogonic phase. However, all developmental stages of *Annacalia* (*Brachiola*) sp form a thickened plasmalemma; these stages additionally form elaborate vesiculotubular appendages on the plasmalemmal surface.81 *Brachiola vesicularum* uniquely forms protoplasmic extensions on elongated cells during the proliferative phase and produces spores that contain one to three rows (usually two) of polar filament coils.8

**Clinical and Pathologic Features**

Two *Nosema* infections, subsequently changed to *Microsporidium celonensis* and *M. africanum* have been reported in HIV-negative patients with infections of the corneal stroma that led to perforation or blindness, followed by enucleation.3,4,34,35,85,88 Histologic examination of corneal sections from one patient revealed organisms consistent with microsporidia (Fig 14.7). The central stroma was necrotic and surrounded by acute inflammatory cells. Immediately above Descemet’s membrane were abundant refractile spores measuring 3.5 µm by 1.5 µm, some free and some in macrophages (Fig 14.8). No organisms were found in the exudate. In both infections, the genus *Nosema* was suggested but no specific identification could be made.4,34,88 Two other microsporidia-associated infections of the corneal stroma were reported in the early 1990s in otherwise healthy individuals.3,88 The first patient was a 39-year-old man from Ohio with a corneal ulcer. Examination of Gram-stained biopsy tissue by electron microscopy revealed microsporidia. Spores were 5 µm by 3 µm, binucleated, and contained 9 to 12 polar filament coils (Fig 14.9). The parasite was subsequently named *Nosema ocularum*.3,35 The second patient was a 45-year-old man from South Carolina with no history of prior trauma to the infected eye.88 Biopsy of the stroma revealed a microsporidium parasite that was isolated and grown in cell culture. The spores were 3.7 µm by 1 µm and contained 5 to 6 polar filament coils. This organism, originally named *Nosema corneum*,85,88 is now known as *Vittaforma corneae*.8

From 1990 to the present, hundreds of microsporidian keratoconjunctivitis cases have been reported, in both HIV negative and positive patients, however, only a few reports have identified the organisms beyond being microsporidia. The pathologic changes, however, have been described from the survey of 124 microsporidia positive patients (134 eyes).58 “Common features were follicular papillary conjunctivitis and coarse punctuate epithelial lesions in three patterns—diffuse, peripheral, and paracentral—evolving
into nummular keratitis before resolution"... (99 percent of the cases were resolved with topical fluoroquinolone monotherapy). “New clinical features were diffuse endothelitis (19.4%) with corneal edema and limbitis.”

The first well documented human infection with a microsporidium was in a 4-month-old male with thymic aplasia, severe diarrhea, and malabsorption (Fig 14.10). At autopsy, *Annaliia* (*Brachiola, Nosema*) *connori* spores measuring 4 µm by 2 µm were found in the small and large bowel; no other infectious agent was discovered. Infection had disseminated to the lungs, stomach, kidneys, adrenal glands, myocardium, liver, and diaphragm (Figs 14.11 to 14.18).

A *Brachiola* sp infection was reported in a 31-year-old male AIDS patient who had pain and progressive muscular weakness of the lower extremities of five months duration. It was named *B. vesicularum*. The tissue biopsy contained spores measuring 2.5 µm to 2.9 µm by 1.9 µm to 2 µm. Intramuscular infection and cytolysis were observed by light microscopy. Electron microscopy revealed diplokaryotic microsporidia in all stages of development (Figs 14.19 & 14.20). An elaborate array of vesiculotubular appendages emanated from the plasmalemmal surface of most stages (Fig 14.21), and spores presented with variable polar filament arrangements (Fig 14.22). Additionally, protoplasmic extensions develop on some proliferative stages (Figs 14.23 & 14.24) that are unique to this microsporidium. There was loss of muscle striation in surrounding areas.

*Annaliia* (*Brachiola, Nosema*) *algerae*, a parasite of mosquitoes, has been cultured in mammalian cells at 29° to 37°C and documented by PCR.
techniques in ocular and dermal infections of immunocompetent patients. It has also been documented in deep tissue infections in skeletal muscle from a patient with rheumatoid arthritis being treated with immunosuppressive drugs (Figs 14.24, 14.25a & 14.25b). The infection progressed until the patient died. Infection in both epithelial and connective tissue cells of the false vocal chord area (Figs 14.26 a-e & 14.27) was documented in a terminal cancer patient.

**Family: Pleistophoridae**

This family was named from the fish parasite *Pleistophora typicalis* (Gurley 1893). Since that time, approximately two dozen species had been described, all of them in fish until 1985 when a human muscle infection with *Pleistophora* sp was identified. This infection from an immunodeficient, but HIV negative male from the USA, was subsequently studied from the original tissue blocks and named *Pleistophora ronneafiei*. Two additional *Pleistophora* sp cases were identified in HIV+ males, one in Australia and one from Spain. It was not until 1996, that yet another genus, *Trachipleistophora*, was established for human infection from this family of microsporidia. The first species, *T. hominis* was described from muscle and eye infections in an AIDS patient from Australia. A second species, *T. anthropophora*, was described from two AIDS patients. This species disseminated to multiple organs including the brain, heart, kidney, pancreas, thyroid, parathyroid, liver, bone marrow, lymph nodes, and spleen. The most heavily infected cells were epithelia, cardiac myocytes, and astrocytes.

**Morphologic Description**

A thick, parasite-secreted, envelope is produced on the surface of proliferative cells. It separates from the surface and becomes an SPOV in sporogony. *Pleistophora ronneafiei* and *Trachipleistophora* sp develop within the host-cell cytoplasm in this type of parasite produced vesicle. Nuclei are single, even when there are many nuclei (not diplokaryotic) within a plasmodial proliferative cell. In *P. ronneafiei*, proliferative and sporogonic plasmodia divide by multiple fragmentation of large cells. *Pleistophora* is polysporous; 16 to over 100
annulled after the patient presented with myositis and muscle pain showing multiple organisms in the muscle fibers (arrows) with associated cell lysis but little or no inflammation. H&E x100.

Figure 14.22 a,b,c Brachiola vesicularum. a. Mature spore containing a fully developed electron-lucent endospore coat (average thickness 90 nm to 100 nm). The exospore (62 nm) surface has several vesiculotubular structures (T) on it. Note the presence of nine polar filament (Pf) cross sections arranged in two rows. Ribosomes (R) appear in a spiral-like array forming rows around the nuclear area (Nu). x 41,600; b. Spore containing ten polar filament (Pf) cross sections clustered into three rows. (endospore coat = 82 nm, exospore coat = 63 nm) x 36,800; c. Section through a spore revealing the presence of the anterior anchoring disc complex (A) of the polar filament (Pf) and the manubroid (Mpf) portion of it. The cross sections of the polar filament coils arranged in a single row is visible. Multiple rows of ribosomes (R) are also present. Note the presence of vesiculotubular material (T). x26,000.

Figure 14.23 a,b Brachiola vesicularum. Proliferative cells with protoplasmic extensions. a. A very elongated parasite cell (7.1 µm in length and varies between 1.0 µm and 1.2 µm wide) with a vesiculotubular “cap” complex (TvC) at one end and a scalloped thick plasmalemmal surface which contains several channels (arrowheads). Additionally, this cell possesses several protoplasmic extensions (E) of varying lengths, projecting from the cell surface (broad arrows). At the ends of these protoplasmic extensions are vesiculotubular (T) structures with the electron-dense fibrous coating, similar to those previously illustrated on the typical proliferative cells. Note the presence of vesiculotubular (T) structures and myofilaments (F) in the host cytoplasm. x19,300. b. A portion of a parasite cell protoplasmic extension complex measuring 4.80 µm in length and between 0.5 µm to less than 0.3 µm in width. A number of branches of varying lengths, have formed from the cell surface and project into the host cytoplasm. These projections also end in vesiculotubular (T) structures. The surface of the protoplasmic extensions also have some scalloping and shallow indentations present. In the lower third of the figure is a section of a parasite (P) cell with several vesiculotubular (T) structures attached to the cell surface. x 27,000.

Figure 14.24 Annecalia algerae in muscle after the patient presented with myositis and muscle pain showing multiple organisms in the muscle fibers (arrows) with associated cell lysis but little or no inflammation. H&E x100.

Figure 14.25 a,b Annecalia algerae in muscle from patient in Figure 14.24. a. A diplokaryon, a thickened plasmalemma (arrows), and vesiculotubular extensions are evident in proliferative forms. Nu denotes nucleus. x14,000; b. A mature A. algerae spore with a single row of nine polar filaments (arrowheads) in cross section. Mature spores in the biopsy specimen had only single rows of 8 to 12 polar filaments in cross section. x18,300.

spores may be produced from sporonts en- cased in the parasite-secreted SPOV envelope (Figs 14.28 & 14.29).

In the two species of Trachipleistophora, T. hominis11,98 and T. anthropophthera36, proliferative cells have 2 to 4 nuclei and divide by binary fission. In the sporogony phase, division into sporoblasts is effected by repeated binary fission, producing 2 to 32 spores within the SPOVs, no plasmodial stages are produced (Figs 14.30 to 14.34). Spores of T. hominis are approximately 4 by 2 µm (Fig 14.35). Trachipleistophora anthropophthera is dimorphic,
Figure 14.26 a,b,c,d,e
*Anncaliia algerae*. Tissue biopsied from area of false vocal cords. a. Hematoxylin and eosin-stained tissue presents with a “foamy” appearance in infected cells. b. Brown and Brenn (B&B) Gram stain. c. ZN acid-fast stain. d. B-H stain. Note that these all stain the spore coat, and the elongated oval spores obvious. e. Periodic acid-Schiff (PAS) stains a small granule (arrows) in the anterior end of the spore. These PAS positive granules are diagnostic for microsporidia. The spores are approximately 2 µm by 4 µm. a-e x1000

Figure 14.27
*Anncaliia algerae*. Tissue biopsied from area of false vocal cords illustrating details of the microsporidial developmental stages. Low magnification of infected host cell. Cell is filled with parasites in various stages of development and in direct contact with host cell cytoplasm. Only remaining discernable organelles are the host nucleus (HN) and the plasmalemma; scale bar = 8 µm.
in sporogony, 2 types of SPOVs and spores are formed (Figs 14.36). One type of SPOV contains thick-walled spores (approximately 8, measuring 3.7 by 2.0 µm), each containing 9 polar filament coils. The other type contains 2 thin-walled spores with 3 to 5 polar filament coils and measuring 2.2 µm to 5 µm by 1.8 µm to 2.0 µm.96

Clinical and Pathologic Features

Pleistophora sp primarily infect the muscles of marine and freshwater fish, but three Pleistophora infections in human skeletal muscle have been reported.9,26,94,95 The first, in 1985, was in a 20-year-old immunocompromised man from Florida.9,99,100 Over a 7-month period, the patient experienced progressive wasting and generalized muscle weakness leading to contractures. Biopsies of skeletal muscle contained large clusters of organisms visible by light microscopy with H&E (Fig 14.37), acid-fast (Fig 14.38) and Giemsa stains (Fig 14.39). Electron microscopy of the same biopsy tissue revealed the diagnostic features of Pleistophora.9 Developmental stages from this case were subsequently described (Fig 14.28 & 14.29) and the parasite named P. ronneafiei.10 The two additional cases of myositis caused by Pleistophora in patients with AIDS, in 1993 and in 1996 have not been compared.94,95

Trachipleistophora hominis was described from an AIDS patient in Australia.11,27 The infection was primarily (Fig 14.30) muscular but organisms were also found in corne-
Figure 14.30
*Trachipleistophora hominis*. Spores and larger brown spore precursors forming masses within skeletal muscle fibers. Free spores are visible in the adjacent connective tissues (arrow), and discrete early aggregates of spore precursors are visible in fibers (arrowhead). Warthin-Starry x 400.

Figure 14.31
*Trachipleistophora hominis*. Corneal scraping showing sporophorous vesicles containing spores and spore precursors in epithelial cells (arrows). Note the dispersed spores in the background showing the posterior vacuole (arrowhead). Modified trichrome x1000.

Figure 14.33
*Trachipleistophora hominis*. Two adjacent binucleate meronts with thick outer coats (arrow). x3,000.

Figure 14.34
*Trachipleistophora hominis*. Sporonts undergoing division in a skeletal muscle cell (arrows). x3,000.

Figure 14.35
*Trachipleistophora hominis*. Spore with anchoring disc (arrow), straight portion of polar tube (open short arrow) extending through the polaroplast (open arrow) posterior vacuole, and tangential sections through coils of tube (arrowhead). x12,000.

Figure 14.36
*Trachipleistophora anthropophthera*. Type I mature spore’s polar filament has thicker and inward displaced thinner posterior coils. Bar = 0.5 µm.
al epithelium (Fig 14.31) and nasopharyngeal washings. In experimentally inoculated athymic mice, infection spread to tissue of the bladder and large intestine.11

*Trachipleistophora anthropophthera* was identified in 2 AIDS patients in the United States. Both had disseminated infections involving the heart, kidneys, and brain (Figs 14.40a & 14.40b), manifesting in seizures and impaired cognition, suggestive of toxoplasmosis.24,28,96,97

**Family: Enterocytozoonidae**

*Enterocytozoon* was the first genus of microsporidia created for a human infection.12,30 It has subsequently been found in pigs and cattle.102 This microsporidium has many unique developmental features13 and researchers have had only limited success at growing them in culture.

**Morphologic Description**

*Enterocytozoon* organisms develop in direct contact with the host-cell cytoplasm. As nuclei multiply, plasmodia enlarge. *Enterocytozoon* forms two unique organelles: electron-lucent inclusions and electron-dense disks. They both form in a multinucleate plasmodial cell in direct contact with the host cell cytoplasm (Fig 14.41).13 Electron-lucent inclusions appear very early in the development of the proliferative plasmodia, increase in size and number as the plasmodia grow, and are present throughout the life cycle. Electron-dense disks form at the surface of the electron-lucent inclusions, often in small stacks similar to a stack of red blood cells. Plasmodial cells containing these disks have many rounded nuclei. The disks eventually fuse and form the spores’ polar filament. Finding several polar filaments within a multinucleate plasmodium is diagnostic for *Enterocytozoon* (Fig 14.42).13 The presence of these organelles and the development of multiple polar tubules within a multinucleate parasite cell are all unique features of the developmental cycle of *Enterocytozoon*. The plasmodium divides by multiple fission; producing a dozen or more sporoblasts which mature into spores, all in direct contact with the host-cell cytoplasm. Spores are 1.3 µm by 0.8 µm and contain a single nucleated sporoplasm surrounded by approximately 6 polar tubule coils, arranged in a double row (Fig 14.43).13,24

**Clinical and Pathologic Features**

*Enterocytozoon bieneusi* is one of the most frequently reported microsporidial infection in humans. Incidence in AIDS patients is approximately 7% in Africa,56 20% to 30% in the United States and Australia,43,57,59 and 50% in France.42 The organism infects the apical portion of enterocytes of the small bowel (Figs 14.44 & 14.45). Endoscopically, infection appears as a slight flattening of the epithelium.103 Histologically, the only visible pathologic feature is villus atrophy due to more rapid desquamation of infected enterocytes.12,13,24,30,43,59 The parasite can disseminate to the epithelial lining of the common bile duct, gallbladder epithelium, and biliary and respiratory tracts.19,31,59,104 **Enterocytozoon bieneusi** most commonly infects male AIDS patients caus-
Figure 14.41
*Enterocytozoon bieneusi.* Sporogonial plasmodium containing at least 12 nuclei (N) in a single plane of section. The round dense nuclei are each associated with electron dense disc complexes (arrows) and electron lucent inclusions (*). Electron dense discs fuse into arcs forming polar tube coils. Despite the advanced maturation and organelle separation associated with each nucleus, there is not yet any evidence of cytokinesis or plasmalemmal thickening. x25,600. Bar = 1 µm.

Figure 14.42
*Enterocytozoon bieneusi.* Late sporogonial plasmodium with advanced stages of polar tube formation. Fused electron dense discs are seen in coiled (single arrow), stacked (double arrows) and cross sectional (triple arrows) profiles throughout the cytoplasm. Anterior anchoring discs (A) and associated polaroplast membranes appear at this stage even though individual sporoblast membranes have not yet developed. The electron lucent inclusions are seen in elongated (E) and cross section (C) views. x33,231. Bar = 1 µm. Insert: Connection and arrangement of various structures developing in the plasmodium. Umbrella-shaped anchoring disc (A) and associated polaroplast membranes (P) attached to the manubroid portion of the polar tube (M) which connects with arcs formed by the coiled region of the developing polar tube. This complex of polar tube and associated structures surrounds a single nucleus (N). x28,000. Bar = 1 µm.
Family: Encephalitozoonidae

Encephalitozoon cuniculi was first discovered in 1924 in rabbits.\(^{113}\) It was placed in the microsporidia in 1964 as a junior synonym in the genus Nosema.\(^{51}\) In 1971, it was reclassified as a genus of Microsporidia\(^ {52}\) and described from rabbits, mice and hamsters.\(^ {114}\) The family was established in 1989.\(^ {115}\) This parasite has subsequently been reported from over 30 different mammalian hosts\(^ {51}\) and the first human infection with E. cuniculi was reported in 1987.\(^ {14}\)

From 1989 to 1991, six cases of microsporidian keratoconjunctivitis were reported in patients with AIDS, four from New York, one from Texas, and one from Ohio.\(^ {3,16,35,36,116,117}\) All had conjunctivitis, blurred vision, and photophobia. By 1999 over 20 cases were characterized, reported and reviewed.\(^ {22}\) Organisms were observed in corneal epithelial cell scrapings examined by light and electron microscopy.\(^ {16,118,119}\) The organisms were morphologically similar to E. cuniculi, but a clearly defined parasitophorous vacuole surrounding the organisms was not always visible.\(^ {16}\) Didier et al. reported that the organism was morphologically the same, but serologically different from E. cuniculi and named it Encephalitozoon hellem.\(^ {15}\)

First reported in 1991, Septata was the second microsporidial genus created for a human infection.\(^ {17,29}\) It was placed in this family as a new genus, based on similarity of some of its morphological features with the type species while presenting some unique features including intestinal infection. Infection with S. intestinalis has been reported in the United States,\(^ {17,29,59,120}\) Europe,\(^ {23,59,60}\) and Australia.\(^ {57,59,121,122}\) Subsequent molecular data suggested a closer relationship between the two genera and the species: S. intestinalis was moved into the genus Encephalitozoon.\(^ {53}\)
Morphologic Description

The genus *Encephalitozoon* is characterized by a phagosome-like parasitophorous vacuole that surrounds developing parasites and isolates them from the host-cell cytoplasm. Developing stages (Fig 14.47) may contain one or more nuclei but the nuclei are not attached to each other in diplokaryotic arrangement. Multinucleate cells are long and narrow, not plasmodial (Fig 14.48). Proliﬁerative cells usually abut the vacuole membrane and break free of it in sporogony. Sporonts form a thickened plasmalemma on individual cells. Within the parasitophorous vacuole, each sporont elongates, divides, and produces spores. Spores are 1 µm to 1.5 µm by 0.5 µm and contain a sporoplasm with a single nucleus and approximately 6 polar tubule coils (Fig 14.49), arranged in a single row. *Encephalitozoon hellem* is morphologically the same as *E. cuniculi* except that the parasitophorous vacuole is not always present.

*Encephalitozoon* (Septata) *intestinalis* is characterized by parasite-secreted material surrounding the developing stages and spores (Fig 14.50) inside the parasitophorous vacuole. Proliferative and sporogenic stages have 1 to 4 nuclei. Cells are rounded when single-nucleate and elongated when they contain 2 to 4 nuclei. In sporogony, the plasmalemma thickens and elongated sporonts are produced. Each sporont divides, producing up to 4 single-nucleate sporoblasts. After a ﬁnal cell division, sporoblasts develop the polar ﬁlament complex and metamorphose into spores. *Encephalitozoon intestinalis* cells develop in clusters. During early development, these clusters appear tightly packed. During sporogony and spore formation, some cells condense, leaving a space between individual developing forms. When this happens, the parasite-secreted fibrillar matrix surrounding the different stages is apparent. Early and late forms develop asynchronously, with parasite secretions surrounding individual cells and a parasitophorous vacuole surrounding the cluster (Fig 14.50). Spores are 2 µm by 1.2 µm, with a single nucleus and 4 to 7 (usually 5) polar tubule coils arranged in a single row.

Clinical and Pathologic Features

*Encephalitozoon cuniculi* is probably the most studied microsporidian. Primarily a parasite of animals, it has been reported in over 30 mammalian hosts. In 1984, Bergquist et al reported serologic evidence of *E. cuniculi* in patients with a history of travel to the tropics. In 1987, Terada et al found *E. cuniculi* organisms in the liver of an AIDS patient with hepatitis.
Figure 14.49
Encephalitozoon hellem. Two spores are cut in longitudinal (upper) and cross section. Both have a thick spore coat including thin dense exospores (EX), thick electron-lucent endospore (EN), and the thin inner spore coat membrane (M). In the organism above, a single, large, rounded nucleus (N) is centrally located. Peripheral to and on each side of the nucleus are seven to eight round dense bodies that are cross sections of the polar tubule (PT) coils. In the organism below, the polar tubule is coiled parallel to the plane of the section and appears as a single dense ring situated adjacent to the inner aspect of the spore coat. x32,500.

Figure 14.50
Encephalitozoon (Septata) intestinalis. Deposition of material results in a uniformly thick plasmalemma surrounding the sporont cells. These sporonts continue to secrete the fibrillar lamina. The sporont (ST) is a tetranucleate (n) elongated cell in the process of cytokinesis (arrowhead). This cluster of parasite cells also contains many mature electron-dense spores, proliferative cells (P), and a dense fibrillar lamina separating the individual parasite cells. x9,000

Since then, E. cuniculi has been demonstrated in peritoneal, cerebral, and disseminated infections in AIDS patients (Figs 14.51 & 14.52). In cell culture, E. hellem may develop with or without a surrounding vacuole (Fig 14.48). Encephalitozoon hellem, originally reported from eye infections, has since been associated with disseminated infection and infection of the sinuses, nasal tissue, tongue, respiratory system (Figs 14.53 & 14.54), kidneys (Fig 14.55) and male genital tract (Figs 14.56 & 14.57). Schwartz et al. used immunofluorescence and H&E to demonstrate the organisms in tissues (Figs 14.58).

Encephalitozoon intestinalis is an intestinal epithelial cell parasite, causing diarrhea, malabsorption, and wasting. Because it can also infect macrophage, fibroblastic, and endothelial cells, it can infect the lamina propria below the enterocytes and disseminate to other parts of the body (Figs 14.59 to 14.61). Infections have been reported in the liver, renal system, colon, gallbladder, lungs, sinuses, and conjunctiva. It has been misdiagnosed as Enterocytozoon bieneusi in the intestine and as Encephalitozoon hellem in the eye, sinuses, and urine.

Although size, nucleation, and number of polar tubule coils are similar in Encephalitozoon intestinalis and Enterocytozoon bieneusi, the organisms can be distinguished by the arrangement of polar filaments in the spore stage. In Enterocytozoon bieneusi, the polar filament forms a double row of coils; in Encephalitozoon intestinalis, they form a single row. Another diagnostic feature is observable in tissue sections. Spores and developing stages of Encephalitozoon intestinalis are within individual chambers inside a vacuole in the host-cell cytoplasm. Enterocytozoon bieneusi development is in direct contact with the host-cell cytoplasm (Fig 14.53).

Diagnosis

Identifying the spore stage containing the polar filament is diagnostic for microsporidia. Spores can be seen by light microscopy using stains such as Giemsa.
Neelsen,” Brown & Brenn, Brown & Hopps, Weber’s trichrome,” Warthin-Starry,” and fluorescence." Hematoxylin-eosin is not particularly useful for identifying microsporidia. Spores are birefringent and can be seen with polarized light (Fig 14.8). A PAS-positive granule in the anterior end of the spore is diagnostic for larger microsporidia such as Nosema, Annaliia and Pleistophora (Fig 14.3), but trying to identify the granule in the smaller, more common human-infecting microsporidia, Encephalitozoon and Enterocytozoon, is impractical.

Touch preps and smears can be made from biopsy materials, eye scrapings, tissue specimens (Fig 14.31) and aspirates stained with Giemsa or Gram’s stain. Spores can be identified in urine, fecal samples (Fig 14.46), or duodenal fluid by light, fluorescence,” or electron microscopy.” Where available, immunologic technology (such as ELISA,” Western blot,” monoclonal and polyclonal an-

Figure 14.51
Encephalitozoon cuniculi (arrows) spores in bronchiole epithelium. B&B x650.

Figure 14.52
Encephalitozoon cuniculi acid-fast spores (arrows) in bronchiole epithelium. (Not all spores are acid-fast) ZN x875

Figure 14.53
Encephalitozoon hellem spores (arrows) in the trachea: Some acid-fast spores ZN x620

Figure 14.54
Encephalitozoon hellem spores in the trachea: Silvered spores in mucosa, GMS x800

Figure 14.55
Cluster of Encephalitozoon hellem spores (arrows) in kidney. H&E x850
Figure 14.56
Cluster of *Encephalitozoon hellem* spores in kidney. Black bodies are Gram-positive spores. B&H x875

Figure 14.57
*Encephalitozoon hellem* spores in bladder exudates. B&H x650

Figure 14.58
Tubular epithelium of the kidney infected with *Encephalitozoon hellem*. Immunofluorescence staining reveals brightly staining clusters of intra-epithelial spores (arrow). x750

Figure 14.59
*Encephalitozoon* (*Septata*) *intestinalis* cells in tightly packed clusters in skin: Silvered 3 µm x 1 µm spores. GMS x790

Microsporidiosis

Fluorescent antibody, fluorescent antibody, and PCR techniques are significant diagnostic aids. Electron microscopy is the preferred method for identifying microsporidia. It can be used to identify the presence of a polar filament in the spore. Additionally, the polar filament arrangement and number of coil cross-sections may aid in identification of organisms. Example: a double row of polar filament coils distinguishes spores of *Enterocytozoon bieneusi* from those of *Encephalitozoon intestinalis* in fecal or other samples. In tissue biopsies, microsporidial families can be identified by the host/parasite interface (Table 14.1). For example: there is no separation from host cytoplasm (Nosematidae, Enterocytozooidae) by vacuole (Encephalitozooidae) or SPOV (Pleistophoridae).

For histologic examination of tissue sections, glutaraldehyde fixation and plastic embedding are highly recommended, even if electron microscopy is unavailable. Parasites are often masked by the host tissue in 6 µm paraffin-embbeded preparations. One-micrometer-thick plastic sections, heat-fixed to a glass slide and stained with toluidine blue, provide better visualization of microsporidia, even by light microscopy, because of the thinness of the section (Fig 14.36). Plastic embedding provides a permanent tissue specimen that can be re-evaluated by electron microscopy if the need or opportunity arises. Additionally, paraffin-embedded tissue can be deparaffinized and reprocessed for plastic embedding.

The most common microsporidia that infect humans are able to disseminate. Therefore, if they are found in a single location, other locations to which they are known to disseminate should be examined/tested.
Treatment

Several drugs have been used to treat microsporidiosis in humans, with varying results. Fumagillin, a compound that inhibits microsporidiosis in honeybees, inhibits replication of *Encephalitozoon cuniculi* in human tissue culture. Athough not recommended or approved for internal use in humans, it has been used topically to treat *Encephalitozoon hellem* infections of the eye with some success. Topical fluoroquinolone monotherapy resulted in resolution of 99% of 124 cases of keratitis.

Albendazole is the most effective treatment for microsporidiosis in humans. The usual regimen is 400 mg administered orally twice a day for 4 to 6 weeks. Albendazole was first used against *Enterocytozoon bieneusi* infection in HIV-positive patients with AIDS, chronic diarrhea, and wasting. After 4 weeks of treatment, most patients had a recurrence of symptoms but responded well to further albendazole treatment. Within a month after stopping treatment, most patients had a recurrence of symptoms but regained weight and wasting. After 4 weeks of treatment with albendazole, most patients had a recurrence of symptoms but regained weight and either gained weight or stopped losing weight. Within a month after stopping treatment, most patients had a recurrence of symptoms but regained weight and wasting. After 4 weeks of treatment with albendazole, most patients had a recurrence of symptoms but regained weight and wasting. After 4 weeks of treatment with albendazole, most patients had a recurrence of symptoms but regained weight and wasting.

Albendazole is more effective against *Encephalitozoon intestinalis* than against *Enterocytozoon bieneusi*. Parasites are cleared from the urine and intestinal tract within 6 weeks, with no recurrence after treatment. Biopsy reveals normal enterocyte morphology with only disintegrating spore remnants in macrophages. Albendazole is also effective against other *Encephalitozoon* sp and *Brachiola* sp, clearing infections with no recurrence. It has proven effective against *Vittaforma corneae* in culture. The effects of albendazole, fumagillin, and TNP-470 on microsporidian replication in culture has also been studied.

References


100. 22


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Figure 14.5. Cali A, Takvorian PM. Brachiola algerae sporoplasms. J Eukaryot Microbiol 2001;Suppl:81S-82S.


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