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Microsporidiosis: current status

Elizabeth S. Didier[a] and **Louis M. Weiss**[b]

[a]Division of Microbiology, Tulane National Primate Research Center, Covington, Louisiana, USA

[b]Departments of Medicine and Pathology, Albert Einstein College of Medicine, Bronx, New York, USA

Abstract

Purpose of review—Microsporidiosis is an emerging and opportunistic infection associated with a wide range of clinical syndromes in humans. This review highlights the research on microsporidiosis in humans during the previous 2 years.

Recent findings—The reduced and compact microsporidian genome has generated much interest for better understanding the evolution of these parasites, and comparative molecular phylogenetic studies continue to support a relationship between the microsporidia and fungi. Through increased awareness and improved diagnostics, microsporidiosis has been identified in a broader range of human populations that, in addition to persons with HIV infection, includes travelers, children, organ transplant recipients, and the elderly.

Summary—Effective commercial therapies for *Enterocytozoon bieneusi*, the most common microsporidian species identified in humans, are still lacking, making the need to develop tissue culture and small animal models increasingly urgent. Environmental transport modeling and disinfection strategies are being addressed for improving water safety. Questions still exist about whether microsporidia infections remain persistent in asymptomatic immune-competent individuals, reactivate during conditions of immune compromise, or may be transmitted to others at risk, such as during pregnancy or through organ donation. Reliable serological diagnostic methods are needed to supplement polymerase chain reaction or histochemistry when spore shedding may be sporadic.

Keywords

diagnostic testing; emerging infection; *Encephalitozoon*; *Enterocytozoon*; microsporidia; opportunistic infection; therapeutics

Introduction

Microsporidia infect animals of virtually all phyla, and are particularly prevalent in fish and insects. Interest in these organisms grew tremendously during the past 20 years after being associated as a cause of persistent diarrhea and systemic disease in persons with AIDS [1]. Increased awareness and improved diagnostics have broadened our knowledge about the wide demographic, geographic, zoonotic, and environmental range of the species of microsporidia that infect humans. Identification of microsporidia in water sources also led to their inclusion on the National Institutes of Health (NIH) Category B list of biodefense pathogens and the Environmental Protection Agency (EPA) microbial contaminant

Correspondence to E.S. Didier, Division of Microbiology, Tulane National Primate Research Center, Covington, LA 70433, USA, Tel: +1 985 871 6249; fax: +1 985 871 6248; esdnda@tulane.edu.

candidates list of concern for waterborne transmission. The fairly recent completion of the *Encephalitozoon cuniculi* genome [2] has led to new insights into the molecular phylogeny and biology of the microsporidia. This review highlights research on microsporidiosis in humans published during the previous 2 years and the questions these findings raise.

Organism

The phylum, Microsporidia, includes approximately 1200 species that infect members of all animal phyla, and 14 of these can infect humans (Table 1) [3•]. *Enterocytozoon bieneusi* and the *Encephalitozoon* spp. currently are the most prevalent microsporidia identified in humans. Microsporidia are single-celled intracellular parasites and infectious stages, or spores, of species that infect humans are small, measuring 1.0–3.0 μM × 1.5–4.0 μM. Spore stages are surrounded by a glycoprotein outer layer and a chitinous inner layer that provide protection from the environment [4]. The cytoplasm of a microsporidian spore consists of a nucleus in a monokaryon or diplokaryon arrangement, an anterior anchoring disk, a membranous lamellar polaroplast that may include an atypical Golgi apparatus, polar vesicles that appear to be reduced mitochondria called mitosomes, endoplasmic reticulum, ribosomes, and a poster vacuole that may function as a peroxisome [5,6,7•,8•]. A coiled polar tube emanates from the anchoring disk and is a structure unique to the microsporidia that functions to facilitate infection of the host cell. A change in osmotic pressure results in swelling of the posterior vacuole and causes the polar tube to evert, followed by transfer of the cytoplasmic contents through the everting polar tube into the host cell (Fig. 1).

Microsporidia were previously believed to be among the earliest or deep-branching eukaryotes because they lacked typical mitochondria, Golgi, and peroxisomes, and they possessed small ribosomes like those of prokaryotes [9]. This early divergence hypothesis was questioned on the basis of a long-branch attraction artefact of faster-evolving genes in these phylogenetic analyses, and today, the microsporidia are considered to be highly diverged, well adapted, and specialized parasites that are related or belong to the fungi [10,11••,12,13]. Their exact relationship to the fungi, that is as a sister group, remains to be determined [14••]. Through comparative genome analyses, microsporidia were observed to contain among the smallest genomes of eukaryotes which resulted from gene reduction and compaction [10,11••,15••,16••]. For example, the *Encephalitozoon cuniculi* genome is 2.9 Mb and consists of approximately 2000 genes which are tightly packed having few introns, a shorter gene length and smaller protein size for homologous genes and proteins seen in other eukaryotes, and having overlapping gene-coding regions [6,15••,16••,17•]. Microsporidia have lost many of the genes relating to metabolic and regulatory pathways, and retained those related to transport of energy sources and metabolites, presumably as a consequence of host cell dependence [6,10,11••,12]. Identification of over a dozen genes encoding for mitochondrion-derived proteins and the localization of mitochondrial HSP70 to the mitosome support the likelihood that microsporidia evolved from ancestors that contained mitochondria. Phylogenetic analyses of multiple gene sequences, including those with lower evolution rates, continue to support a relation between the microsporidia and fungi, and more specifically, to the ascomycete and basidiomycete clade [13,14••]. Efforts are under way to obtain sequence data from the *E. bieneusi* genome (S. Tzipori, personal communication) and to continue comparative genome analyses between the microsporidia and other organisms to better understand the forces that impact genome reduction and compaction in relation to phylogenetics and evolution. The fairly recent application of comparative molecular phylogenetic analyses has generated new considerations about the taxonomic classification of many species within the phylum of Microsporidia that was historically based on ultrastructural features, biological and biochemical characters, and habitats [18••,19••].

Clinical features

When the microsporidia were first identified in the setting of HIV-1 infection and diarrhea, there was some debate about whether they were truly pathogenic as these organisms were also detected in persons who did not have diarrhea or other symptoms typically associated with infection. This most likely is a reflection that the immune status of the host plays a role in the expression of clinical signs during infection. AIDS patients with less than 50 CD4+ T cells per mm^3 blood are most likely to experience persistent diarrhea, weight loss, and abdominal pain associated with *E. bieneusi* or *E. intestinalis* infections, whereas HIV-infected individuals receiving antiretroviral therapies, or non-HIV-infected individuals who may be immunologically naive to microsporidia (i.e. children or travelers) may develop diarrhea that subsequently resolves [20•,21•]. Replication of organisms in the villus epithelium of the small intestine, along with reduced villus height and surface area, appear to contribute to malabsorption that leads to the diarrhea [22–25]. *E. bieneusi* infections may spread to the hepatobiliary system to cause cholangitis and a few pulmonary infections have been reported [23,26]. *Encephalitozoon* spp. typically disseminate and infections have been identified in nearly every organ system, including a recently described fatal pulmonary infection in a bone marrow transplant recipient [27,28•]. Of interest are reports of less commonly detected microsporidia species in humans, including a case of *Trachipleistophora anthropopthera* cornea infection in an AIDS patient [29•] and a fatal case of myositis in a women with rheumatoid arthritis, caused by *Brachiola algerae* (recently reclassified as *Anncaliia algerae*), a microsporidian that typically infects mosquitoes [30,31,32•]. This latter case now raises the added potential for vectorborne transmission of microsporidiosis. There are also increasing numbers of case reports suggesting that microsporidia are an emerging cause of ocular infections, including contact lens wearers [33,34•, 35,36,37•,38].

Several questions still exist about clinical aspects and consequences of microsporidia infections in humans. Transplacental transmission of *E. cuniculi* has been reported in carnivores and laboratory rodents, and was recently considered to be responsible for the deaths of newborn emperor and cotton-top tamarins in Europe and the Americas [39–41]. Similarities between human and nonhuman primates, as well as the ubiquitous nature of microsporidia, would support the possibility for transplacental transmission in humans, but this has not yet been documented or reported to occur. Questions still persist about why some microsporidia infections do not seem to correlate with expression of clinical signs. Lessons from microsporidia infections in other mammals may offer some answers. In immune-competent laboratory mice experimentally infected with *E. cuniculi*, for example, a mild ascites may develop during the acute phase of infection that subsequently resolves even though the infections remain persistent or chronic for the life of the animals. Rabbits likewise develop persistent infections with *E. cuniculi* and sometimes develop motor paralysis or torticolis (head tilt), but most often, remain asymptomatic [42••]. It seems reasonable that otherwise healthy humans may also develop clinical signs of infection during the early or acute stages of infection, as reported in travelers with diarrhea in which symptoms subsequently resolved even though spore shedding continued [21•]. No formal studies have been reported in humans, however, about whether microsporidia infections routinely persist in a latent state, if they may reactivate during conditions of immune compromise, or if persistently infected individuals can transmit infections to others at risk. An example that supports the latter possibility was a case report of microsporidial keratocon-junctivitis being transmitted by the donor corneal graft [34•]. Microsporidiosis is being reported more frequently in solid organ transplant recipients, but it is not clear if the infections were transferred by the donor or acquired by the host during immunosuppressive therapy [43]. It seems important to determine if asymptomatic and persistent microsporidia infections occur in humans, and if so, improved and reliable diagnostic methods are needed for attempting to prevent transmission to others at risk or to reduce the potential for

reactivation of infection. Many of the species of microsporidia infecting humans tend to disseminate, and since kidney is one of the more common sites of disseminated infection, examination of urine for the presence of microsporidia is likely to improve detection of systemic infections. In addition, if one considers that microsporidia spore shedding in feces or urine may be intermittent or at levels below detection by histochemistry or polymerase chain reaction (PCR), serological approaches may become feasible for diagnosing infections in immune-competent individuals.

Diagnostics

Transmission electron microscopy was used to confirm a diagnosis of microsporidiosis based on detecting the polar filament within spores, and is still important for demonstrating ultrastructural features that, along with newly applied molecular biology approaches, contribute to taxonomic organization of the microsporidia, as evidenced by the recent reclassification of *Brachiola* spp. to *Anncaliia* [19••,32•]. Histochemistry methods were then developed and applied to detecting microsporidia more efficiently in fluids (feces, urine, mucus) and tissues. These included application of fluorescent brighteners (e.g. Calcofluor White, Uvitex 2B, Fungifluor) that target the chitinous spore wall, modified (concentrated) trichrome staining used alone or in combination with Gram stain, and the Warthin-Starry silver stain [44]. Immunofluorescent antibody staining for species-specific identification has been somewhat limited, but the recent production of monoclonal antibodies to *E. bieneusi*, along with earlier reports of monoclonals to *Encephalitozoon* spp., should simplify and improve detection of microsporidia in clinical specimens [45•,46]. PCR-based methods that typically utilized primers for amplification of microsporidial rDNA genes, have been routinely applied in research laboratories for improving both sensitivity and specificity, but are still not routinely applied in diagnostic laboratories [44]. Recently, an oligonucleotide microarray system was reported for simultaneous detection of four species of human pathogenic microsporidia species in clinical specimens that should increase diagnostic throughput, at least in research laboratories [47••].

Since microsporidia infections are increasingly reported in relatively immune-competent individuals such as children, travelers, and the elderly, efforts are growing to develop serological tests using whole organisms or recombinant polar tube protein or spore wall protein as antigens, especially in cases in which the microsporidian species cannot be grown in culture [48,49•,50,51•,52]. Of interest is that the serologic response of humans to the polar tube has been demonstrated to include the glycoepitopes found on this structure [49•, 51•]. The significance of such approaches is to detect subclinical infections in individuals who may transmit microsporidiosis to others at risk (e.g. as transplant donors) or who may develop a risk for reactivation of infection under conditions of immune compromise (e.g. aging). Serology has not been used to routinely detect microsporidiosis in humans due to variable expression of antibodies in immune-deficient individuals.

Generally speaking, however, microsporidiosis is still probably overlooked because organisms are quite small, requiring expertise by microscopists in diagnostics laboratories, and inhibitors of PCR found in many clinical specimens may confound interpretation of results. In addition, microsporidia are often not included in the routine differential diagnoses for diarrhea, and urine specimens are typically not evaluated for microsporidia as a potential cause of systemic infections. As the reports of microsporidiosis continue to increase worldwide and in a wider range of human populations, it is expected that a greater emphasis will need to be placed on recognizing such infections.

Epidemiology and sources of infection

Microsporidiosis in humans occurs worldwide, with prevalence rates ranging between 0 and 50% depending on the geographic region, method of diagnosis, and demographic characteristics of the population being studied [53]. Prior to the application of antiretroviral therapies, prevalence rates for microsporidiosis tended to be highest among HIV-infected individuals with diarrhea and less than 100 CD4+ T cells per mm^3 blood [24,54•]. In regions of South America, Africa, and Asia where antiretroviral therapies are not readily accessible, microsporidiosis has been consistently identified in HIV-infected patients with AIDS and additional risk factors that included poor sanitary conditions and exposure to animals [24,25,55,56•,57,58]. Microsporidiosis continues to be increasingly recognized in non-HIV-infected persons such as travelers, children, the elderly, and organ transplant recipients [20•, 21•,43,59•–61•].

The source of most microsporidia infections is still uncertain, but the genotypes that infect humans have now been identified in domestic, farm, and wild animals, which supports the finding that microsporidiosis is a zoonotic disease [42••]. The associations between the risks for infection with microsporidia through occupational and recreational contact with water sources were recently reviewed [53] and these observations contributed to the inclusion of microsporidia as NIH Category B biodefense pathogens (http://www3.niaid.nih.gov/biodefense/bandc_priority.htm) and EPA microbial contaminant candidates (http://www.epa.gov/safewater/ccl/ccl2_list.html) of concern for waterborne transmission. There also appears to be an association between microsporidia and foodborne transmission as a consequence of contaminated irrigation water, and organisms have been identified on lettuce, parsley, cilantro, and strawberries in Costa Rica [62]. These observations supported the rationale for studies on the transport of microsporidia through sandy porous media for developing mathematical models to assess the potential of microsporidia contamination of potable water supplies [63•]. There is no doubt that continued improvements in diagnostics and molecular epidemiology will improve our understanding about the modes of transmission and risk factors associated with acquiring microsporidiosis and these data can then be employed for the development of rational prevention strategies.

Immunology

The hypothesis that resistance to microsporidiosis depends upon functional T lymphocytes is based on the greater severity of disease in AIDS patients with declining CD4+ T-cell levels and the development of lethal experimental microsporidia infections in mice depleted of CD4+ and CD8+ T cells [64,65]. Recent studies on experimental microsporidiosis in murine models and ex-vivo human studies demonstrated the importance of the proinflammatory (Th1) cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-12, along with a role for nitric oxide, in resistance to *Encephalitozoon* spp. [66•,67]. CD8αα+ intraepithelial lymphocytes were observed to increase rapidly after oral administration of *E. cuniculi* to mice. These cells appeared to participate in proinflammatory responses via IFN-γ production and cytotoxic activity and also contributed to immune regulation via IL-10 secretion [65]. In addition, antibodies were reported to contribute to prolonging survival in severe combined immune deficiency (SCID) mice given *E. cuniculi* per os [68•]. Virtually nothing is known about protective immune responses to *E. bieneusi* infections due to the lack of tissue culture and small animal models. Naturally occurring *E. bieneusi* infections have been reported in rhesus and pigtail macaques and these currently represent the only animal models that simulate infections observed in both immune-competent and immunedeficient humans [69,70].

Therapy and disinfection

Immune reconstitution with antiretroviral therapies has greatly reduced the occurrence of microsporidiosis-associated clinical symptoms in persons with HIV infection [24,25,71], and a recent study suggested that aspartyl protease inhibitors of HIV also directly inhibited growth of *E. intestinalis* in tissue culture [72•]. Albendazole, a benzimidazole that inhibits microtubule assembly, was effective against several microsporidia, including the *Encephalitozoon* spp. but was less effective against *E. bieneusi* [73,74]. Fumagillin, an antibiotic and antiangiogenic compound produced by *Aspergillus fumigatus*, was more broadly effective against *Encephalitozoon* spp. and *E. bieneusi* but was toxic when administered systemically [75]. Recent therapeutic development studies have focused on compounds that target microsporidian polyamines (e.g. polyamine analogues), methionine aminopeptidase 2 (e.g. fumagillin-related compounds and analogues), chitin (e.g. nikkomycins), and topoisomerases (e.g. fluoroquinolones) [71,76,77,78•,79]. These studies utilized *Encephalitozoon* spp. as the lack of tissue culture and small animal models for *E. bieneusi* have limited studies to directly identify effective compounds for this organism.

There are significant concerns about the potential of waterborne and foodborne transmission of microsporidia. Recent studies demonstrated successful disinfection of *E. intestinalis* in water using chlorine and ozone disinfection, successful disinfection of *E. cuniculi* in food by high-pressure processing, and that exposure of *E. cuniculi* to bleach, ethanol, HiTor, or Roccal was effective at reducing infectivity of these organisms in a tissue culture model system [80–82].

Conclusion

The tremendous growth in research on the microsporidia since their recognition as causes of opportunistic infections in AIDS patients has led to a greater appreciation for their ability to adapt and infect a wide range of animals, including humans. New information is challenging current paradigms about the biology of microsporidia infections and should result in a better definition of the consequences of infection and the development of effective preventive and therapeutic strategies.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 513–514).

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Abbreviation

PCR polymerase chain reaction

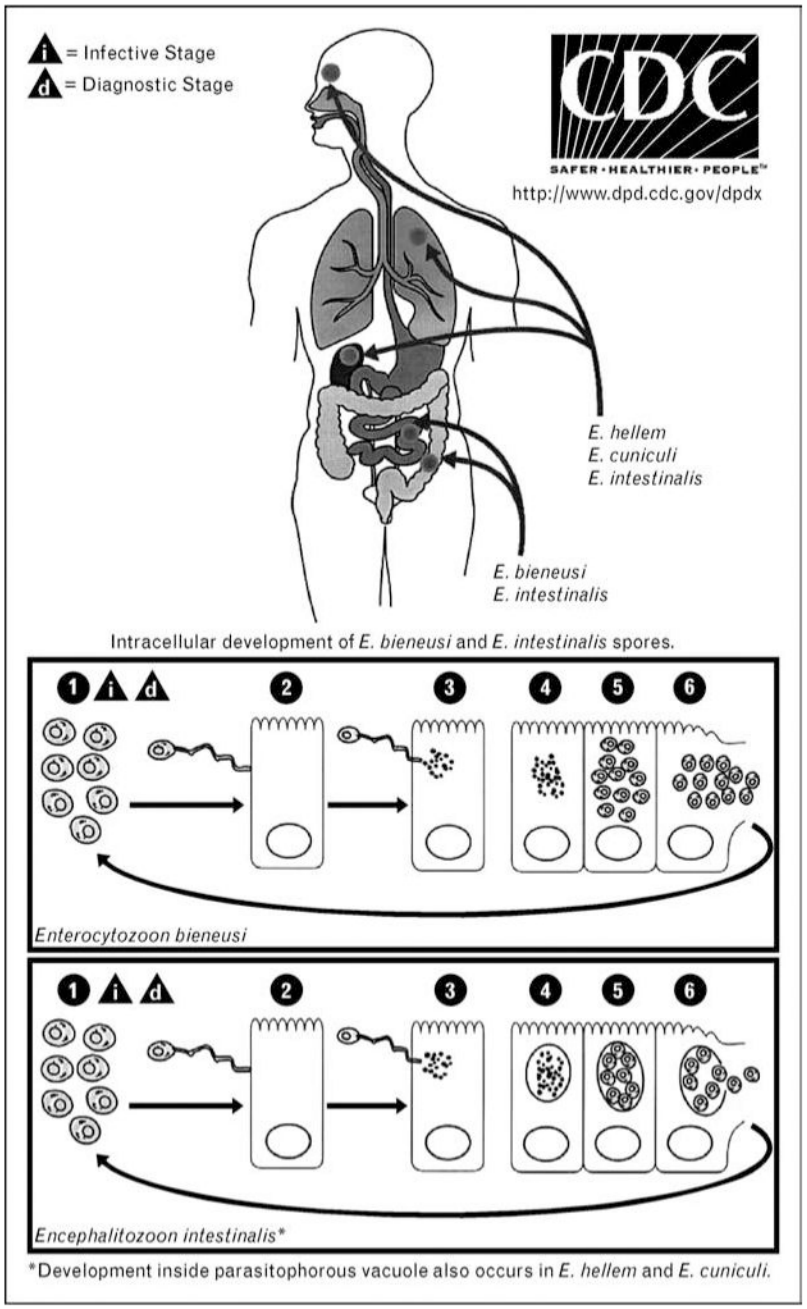

Figure 1. Life cycle of the four most prevalent species of microsporidia that infect humans

Most infections are believed to occur through ingestion or inhalation of spores that are the mature stages of the microsporidia. *Encephalitozoon* species are shown to typically cause disseminated infections. *Enterocytozoon bieneusi* primarily infects the gastrointestinal tract, but recent reports suggest that extraintestinal infections may also occur. Organisms are typically shed with feces, urine, or respiratory secretions to transmit infections. This figure was reprinted with permission from the DPDx: CDC's website for parasite identification; http://www.dpd.cdc.gov/dpdx/.

Table 1
Species of microsporidia infecting humans

Microsporidia species	Sites of infection
Anncaliia (syns. *Nosema* and *Brachiola*) *algerae*[a]	Eye, muscle
Anncaliia (syns. *Nosema* and *Brachiola*) *connori*	Systemic
Anncaliia (syns. *Nosema*-like and *Brachiola*) *vesicularum*	Muscle
Encephalitozoon (syn. *Nosema*) *cuniculi*[a]	Systemic, eye, respiratory tract, urinary tract, liver, peritoneum, brain
Encephalitozoon hellem[a]	Eye, respiratory tract, urinary tract, systemic
Encephalitozoon (syn. *Septata*) *intestinalis*[a]	Intestine, biliary tract, respiratory tract, bone, skin, systemic
Enterocytozoon bieneusi	Intestine, biliary tract, respiratory tract
Microsporidium africanum (syn. *Nosema* sp.)	Eye
Microsporidium ceylonensis (syn. *Nosema* sp.)	Eye
Nosema ocularum	Eye
Pleistophora ronneafiei (syn. *Pleistophora* sp.)	Muscle
Trachipleistophora anthropopthera[a]	Systemic, eye
Trachipleistophora hominis[a]	Muscle, eye
Vittaforma corneae (syn. *Nosema corneum*)[a]	Eye, urinary tract

[a]Species that can be grown in long-term culture for harvesting organisms.