Microsporidiosis in Humans

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SUMMARY Microsporidia are obligate intracellular pathogens identified ~150 years ago as the cause of pébrine, an economically important infection in silkworms. There are about 220 genera and 1,700 species of microsporidia, which are classified based on their ultrastructural features, developmental cycle, host-parasite relationship, and molecular analysis. Phylogenetic analysis suggests that microsporidia are related to the fungi, being grouped with the Cryptomycota as a basal branch or sister group to the fungi. Microsporidia can be transmitted by food and water and are likely zoonotic, as they parasitize a wide range of invertebrate and vertebrate hosts. Infection in humans occurs in both immunocompetent and immunodeficient hosts, e.g., in patients with organ transplantation, patients with advanced human immunodeficiency virus (HIV) infection, and patients receiving immune modulatory therapy such as anti-tumor necrosis factor alpha antibody. Clusters

of infections due to latent infection in transplanted organs have also been demonstrated. Gastrointestinal infection is the most common manifestation; however, microsporidia can infect virtually any organ system, and infection has resulted in keratitis, myositis, cholecystitis, sinusitis, and encephalitis. Both albendazole and fumagillin have efficacy for the treatment of various species of microsporidia; however, albendazole has limited efficacy for the treatment of *Enterocytozoon bieneusi*. In addition, immune restoration can lead to resolution of infection. While the prevalence rate of microsporidiosis in patients with AIDS has fallen in the United States, due to the widespread use of combination antiretroviral therapy (cART), infection continues to occur throughout the world and is still seen in the United States in the setting of cART if a low CD4 count persists.

KEYWORDS microsporidia, therapy, diagnostics, prevention, *Enterocytozoon*, *Encephalitozoon*, *Anncaliia*, *Vittaforma*, AIDS, albendazole, fumagillin

INTRODUCTION

Microsporidia are a diverse group of unicellular obligate intracellular parasites that were previously believed to be "primitive" early branching protozoa but are now understood to belong to the fungi as either a basal branch or a sister group (1). Phylogenetic studies indicate that the microsporidia should be placed with the Cryptomycota as a basal branch of the fungal kingdom (2). The Cryptomycota have been identified by environmental microbiome studies, and while poorly described at the biological level, they are closely related to the microsporidia (3). Cryptomycota that grow as intranuclear parasites of amoeba have been discovered, and these parasites bear strong morphological similarities to microsporidia (4, 5). These and other microsporidia-like organisms, such as *Nucleophaga terricola*, *Mitosporidium daphniae*, and *Paramicrosporidium*, demonstrate that additional environmental sampling is required to completely ascertain the relationship of fungi and microsporidia and to provide a better understanding of the Cryptomycota and the origin of microsporidia.

The class or order Microsporidia was elevated to the phylum Microspora by Sprague in 1977 (5). In 1998, Sprague and Becnel suggested that the term Microsporidia instead be used for the phylum name (6). Microsporidia produce distinctive unicellular spores that are environmentally resistant (7). The shape and size of these spores vary with the species, but most microsporidia infecting humans have ovoid spores that range from 1 to 4 μm. A defining feature of all microsporidia is the presence of an extrusion apparatus which consists of a polar tube attached to the inside of the anterior end of the spore by an anchoring disk. Depending on the microsporidian species, the polar tube forms 4 to approximately 30 coils around the sporoplasm in the spore. When exposed to specific environmental conditions, such as passage through the host's gastrointestinal tract, germination of the spore occurs. During germination, the polar tube rapidly everts, forming a hollow tube acting as a conduit to deliver the sporoplasm to the host cell. The mechanism by which the polar tube interacts with the host cell membrane is an area of active investigation. If a spore is phagocytosed by a host cell, germination can also occur, enabling the polar tube to pierce the phagocytic vacuole and resulting in the sporoplasm being delivered into the host cell cytoplasm or into an adjacent host cell.

Microsporidia have many typical eukaryotic features and contain an intracytoplasmic membrane system, vesicular Golgi complex, a nucleus with a nuclear envelope, and chromosome separation on mitotic spindles, as well as a mitochondrial remnant organelle called a mitosome (8, 9). Microsporidia have several characteristics that are unusual for eukaryotic organisms. They possess prokaryotic-size ribosomes (10) which lack a 5.8S ribosome subunit; however, in their 23S subunit, there is sequence homologous to the 5.8S region (11). Their genome size varies from 2.18 to 51.35 Mbp, with 2,000 to 5,000 protein coding genes (Table 1) (12, 13). The genomic size of the Encephalitozoonidae is less than 2.5 Mbp, which places them among the smallest eukaryotic nuclear genomes identified to date. In this compact Encephalitozoonidae genome, the gene density is high, almost no

TABLE 1 Microsporidial genomes[a]

Organism	Strain	Genome size (Mbp)	No. of protein coding genes	No. of nonprotein coding genes[b]	No. of pseudogenes	No. of chromosomes
Amphiamblys sp.	WSBS2006	5.62	3,695	48	5	
Anncaliia algerae	PRA109	17.53	5,330	85	0	
Anncaliia algerae	PRA339	12.16	3,661	63	0	
Anncaliia algerae	Undeen	13.82	NA	NA	NA	
Edhazardia aedis	USNM 41457	51.35	4,262	72	2	
Encephalitozoon cuniculi	EC1	2.24	1,898	51	0	12
Encephalitozoon cuniculi	EC2	2.24	1,902	51	0	16
Encephalitozoon cuniculi	EC3	2.24	1,898	51	2	15
Encephalitozoon cuniculi	Ecun III-L	2.29	1,896	51	11	15
Encephalitozoon cuniculi	GB-M1	2.5	2,126	33	0	11
Encephalitozoon hellem	ATCC 50504	2.25	2,006	78	0	12
Encephalitozoon hellem	Swiss	2.18	1,864	51	0	13
Encephalitozoon intestinalis	ATCC 50506	2.22	2,011	72	0	11
Encephalitozoon romaleae	SJ-2008	2.19	1,883	51	1	13
Enterocytozoon bieneusi	H348	3.86	3,806	173	3	
Enterocytozoon hepatopenaei	TH1	3.25	2,581	45	1	
Enterospora canceri	GB1	3.1	2,225	47	9	
Hamiltosporidium tvaerminnensis	OER-3-3	13.27	NA	NA	NA	
Hepatospora eriocheir	canceri	4.83	3,117	59	7	
Hepatospora eriocheir	GB1	4.57	2,770	54	26	
Mitosporidium daphniae	UGP3	5.64	3,428	98	0	
Nematocida ausubeli	ERTm2	4.7	2,831	61	0	
Nematocida ausubeli	ERTm6	4.27	2,544	111	0	
Nematocida displodere	JUm2807	3.05	2,324	46	0	
Nematocida parisii	ERTm1	4.07	2,724	63	0	
Nematocida parisii	ERTm3	4.15	2,788	62	0	
Nosema bombycis	CQ1	15.69	4,643	175	12	
Nosema ceranae	BRL01	7.86	2,678	64	554	
Nosema ceranae	PA08_1199	5.69	3,265	37	19	
Ordospora colligata	OC4	2.29	1,879	59	0	
Pseudoloma neurophilia	MK1	5.25	3,676	31	0	
Spraguea lophii	42_110	4.98	2,596	53	44	
Trachipleistophora hominis	unknown	8.5	3,253	41	0	
Vavraia culicis subsp. *floridensis*	floridensis	6.12	2,875	102	0	
Vittaforma corneae	ATCC 50505	3.12	2,340	101	0	

[a]Data were obtained from MicrosporidiaDB (https://microsporidiadb.org/micro/). NA, not available.
[b]Noncoding genes: rRNA, tRNA, etc.

introns exist, and the encoded proteins are significantly shorter than the orthologous proteins seen in other eukaryotes such as *Saccharomyces cerevisiae* (14, 15).

Analysis suggests that microsporidia may have experienced an early bottleneck in evolution with a massive gene loss resulting in a core set of conserved genes, and this core set has been complemented by expansion of transporters that facilitate the obligate intracellular life cycle of these pathogens (16). Further expansion from this core set of genes has been driven by the acquisition of noncoding DNA (such as transposable elements, etc.), e.g., 9% of the 51.35-Mbp *Edhazardia aedis* genome and 90% of the 2.24-Mbp *Encephalitozoon cuniculi* genome code for proteins (17, 18). Genome data for the microsporidia are available online at MicrosporidiaDB.org (https://microsporidiadb.org/micro/) (19), which is part of the VEuPath database (https://veupathdb.org/veupathdb/) website (Table 1). This website includes the genomes of the human-pathogenic microsporidia *Encephalitozoon cuniculi* (15) (all three strains [20]), *Encephalitozoon hellem* (21), *Encephalitozoon intestinalis* (22), *Enterocytozoon bieneusi* (23, 24), *Anncaliia algerae*, and *Vittaforma corneae*, as well as microsporidia found in other organisms (Table 1).

Microsporidia are classically classified by their ultrastructural features, which include the number of coils of their polar tube, the size, shape, and morphology of their spores, the features of their developmental life cycle, and their host-parasite relationship. Molecular phylogenetic data, such as small subunit rRNA sequences, provides a complementary set
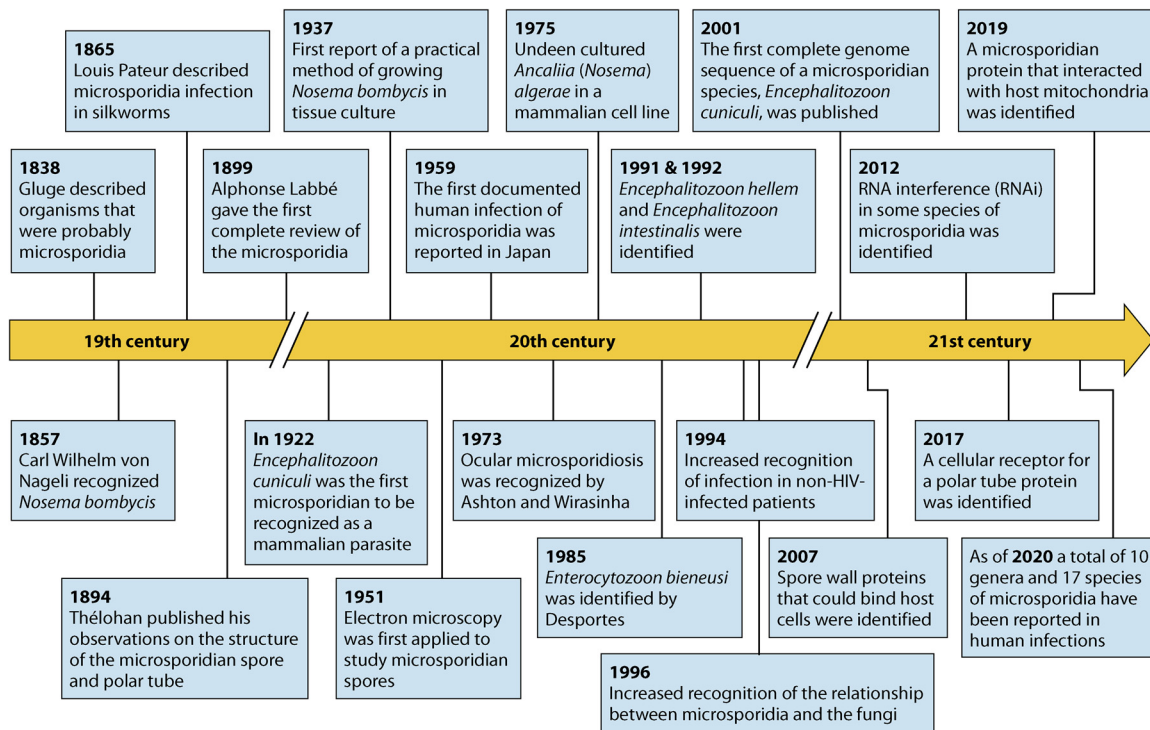
1865
Louis Pateur described microsporidia infection in silkworms

1937
First report of a practical method of growing *Nosema bombycis* in tissue culture

1975
Undeen cultured *Ancaliia (Nosema) algerae* in a mammalian cell line

2001
The first complete genome sequence of a microsporidian species, *Encephalitozoon cuniculi*, was published

2019
A microsporidian protein that interacted with host mitochondria was identified

1838
Gluge described organisms that were probably microsporidia

1899
Alphonse Labbé gave the first complete review of the microsporidia

1959
The first documented human infection of microsporidia was reported in Japan

1991 & 1992
Encephalitozoon hellem and *Encephalitozoon intestinalis* were identified

2012
RNA interference (RNAi) in some species of microsporidia was identified

19th century	20th century	21st century

1857
Carl Wilhelm von Nageli recognized *Nosema bombycis*

In 1922
Encephalitozoon cuniculi was the first microsporidian to be recognized as a mammalian parasite

1973
Ocular microsporidiosis was recognized by Ashton and Wirasinha

1994
Increased recognition of infection in non-HIV-infected patients

2017
A cellular receptor for a polar tube protein was identified

1894
Thélohan published his observations on the structure of the microsporidian spore and polar tube

1951
Electron microscopy was first applied to study microsporidian spores

1985
Enterocytozoon bieneusi was identified by Desportes

2007
Spore wall proteins that could bind host cells were identified

As of **2020** a total of 10 genera and 17 species of microsporidia have been reported in human infections

1996
Increased recognition of the relationship between microsporidia and the fungi

FIG 1 Timeline of investigations on microsporidia.

of data for assigning microsporidia to specific genera as well as distinguishing morphologically similar microsporidia at the species level (25, 26). Examination of molecular data demonstrates the polyphyletic nature of the microsporidia and the problems with current classification systems that use single character states for higher taxonomic groupings. Overviews of the ultra-structural and structural characteristics, life cycle differences, and history of the microsporidian taxa can be found in the work of Tuzet (27), Sprague et al. (5), Larsson (28), Issi (29), Weiser (30), and Sprague et al. (31). Microsporidia are currently separated into three main groups: (i) the "primitive" microsporidia (Metchnikovellidae), which are hyperparasites of gregarines found in annelids and which have a rudimentary polar filament and lack a polaroplast in their spores; (ii) the Burkeidae, Chytridopsidae, and Hesseidsae, which have a short polar filament and minimal development of a polaroplast and endospore; and (iii) the "higher" microsporidia that possess a well-developed polar filament, a polaroplast, and a posterior vacuole. Variations between the current microsporidian classification systems focus primarily on the various characteristics used to divide the "higher" microsporidia into subgroups.

These pathogenic protists were first recognized over 150 years ago with the identification of *Nosema bombycis* by Nageli in 1857 as the cause of the disease pébrine in silkworms, an economically important insect (13) (Fig. 1). In 2008, Franzen (32) published an excellent overview of the history of research on microsporidia, starting with the discovery of *N. bombycis*. Microsporidia cause diseases in a wide range of hosts from invertebrates to vertebrates, including commercially important insects (e.g., silkworms and honeybees), fish, cattle, rabbits, rodents, dogs, and primates. Microsporidia have been developed for use as biologic control agents for several insect pests (13).

The majority of these pathogenic organisms infect their host's gastrointestinal tract; however, there are documented microsporidian infections of almost all organ systems (13, 33). Rare reports of human infection were noted as early as 1959 (34), when microsporidia were identified in a child with encephalitis. In the 1980s, microsporidia were identified as opportunistic pathogens in patients with severe immune deficiency due to HIV infection, and they are now recognized to occur in other immunodeficient patients as well as in

immunocompetent hosts (35, 36). Infection with these pathogens can produce a wide spectrum of clinical diseases, with chronic diarrhea being the most common presentation in patients with immune deficiency. Since the advent of combination antiretroviral therapy (cART), the incidence of microsporidiosis has declined in the HIV-infected population; however, this infection continues to occur in patients who have low CD4 counts despite cART (37–40). Besides gastrointestinal tract involvement, there are many examples in the literature of microsporidian infections causing encephalitis, keratitis, sinusitis, and myositis, as well as disseminated disease (13, 33). The phylum Microsporidia contains at least 1,700 species distributed into approximately 220 genera, of which the following have been demonstrated in human disease (13) (Table 2): *Nosema* (*Nosema corneum*, reclassified as *Vittaforma corneae* [41]; *Nosema algerae*, reclassified initially as *Brachiola algerae* [42] and then *Anncaliia algerae* [43]), *Enterocytozoon* (44), *Septata* (45) (reclassified as *Encephalitozoon* [46]), *Encephalitozoon*, *Pleistophora*, *Trachipleistophora* (47, 48), *Brachiola* (42), *Anncaliia* (43), *Tubulinosema* (49, 50), *Endoreticulatus* (51), and *Microsporidium* (13).

Morphology of the Microsporidian Spore

Microsporidia form distinctive spores (Fig. 2) which are the infectious stage of their life cycle and the only stage that can survive outside of the host cell due to their environmental resistance. The majority of microsporidian infections in humans are thought to be a consequence of the ingestion of these spores in either food or water (52). The spore structure is characteristic of the phylum, and the shape and size of microsporidia vary depending on the species (53, 54). The spores, depending on the species, range from 1 to 12 μm in size, and most spores are either oval or pyriform (5, 55, 56). The general structure of the spore was first observed in 1894 by Thélohan (57). Since those initial observations, studies have defined the internal structure of these spores, including the anchoring disk, polar tube, spore coat, polaroplast, nucleus, and posterior vacuole (58, 59).

The spore coat is composed of three layers, an electron-dense, proteinaceous exospore, an electron-lucent endospore, and an inner membrane or plasmalemma (60). The endospore, composed mainly of chitin, functions as the connection of the exospore and plasma membrane (54, 61). Several proteins found in the spore coat possess adhesion domains that are probably involved in the interaction of microsporidian spores with either the cell surface or mucus of the gastrointestinal tract before germination (62). The anchoring disk is situated at the anterior pole of the spore, where the endospore is thinnest in the anterior region (61, 63). In addition, the anchoring disc is where the polar tube attaches to the spore wall and is released during spore germination (62). The polar tube was first identified more than 125 years ago by Thélohan and is the defining characteristic of the phylum (57). Prior to germination, the polar tube is coiled around the nucleus in the spore (the number of coils depends on the specific microsporidian species) (60, 64, 65). During germination, the polar tube is extruded from the anchoring disk of the spore and forms a hollow tube that acts as a bridge between the microsporidian spore and host cell, allowing transfer of the sporoplasm and nucleus into the host cell cytoplasm, resulting in host cell infection (Fig. 3) (60, 66, 67).

Germination of a spore with extrusion of the polar tube is dependent on environmental conditions and is species specific, probably reflecting microsporidian adaptation to their host and external environment (reviewed by Keohane and Weiss [68]). Conditions that promote spore discharge include various anions and cations, hydrogen peroxide, pH shifts, dehydration followed by rehydration, mucin, polyanions, the calcium ionophore A23187, and UV irradiation. Inhibitors of spore discharge include calcium channel antagonists, low salt concentrations, calmodulin inhibitors, itraconazole, magnesium chloride, ammonium chloride, sodium fluoride, cytochalasin D, demecolcine, and UV light. The exact mechanisms that initiate polar tube extrusion are still poorly understood. It has been suggested that germination is due to increased intrasporal osmotic pressure that results in an influx of water into the spore, which is accompanied by swelling of the polaroplast and posterior vacuole before spore discharge. For *Ancaliia* (*Nosema*) *algerae*, it has been theorized that the increase in osmotic pressure is due to activation, causing trehalose to come into contact with trehalase (69, 70). Another study suggested that calcium displacement from the polaroplast membrane results in polaroplast swelling (71, 72).

TABLE 2 Microsporidia infecting humans

| Microsporidium species	Clinical manifestation(s)	Zoonotic reservoir(s)	In vitro culture (cell lines)	Reference(s)
Anncaliia algerae	Skin infection, keratoconjunctivitis, myositis	Insects (mosquitoes)	E6, HLF, RK13, sf9, GFSK-S1, GFB3C-W1, ZEB2J	182, 350, 351, 355, 456
Anncaliia (Nosema) connori	Disseminated infection	Unknown	Not reported	358, 457
Anncaliia vesicularum	Myositis	Unknown	Not reported	42
Encephalitozoon cuniculi	Keratoconjunctivitis, sinusitis, bronchitis, nephritis, peritonitis, hepatitis, diarrhea, prostatitis, nephritis, cellulitis, disseminated infection, encephalitis	Mammals (rabbits, rodents, dogs, primates)	RCP, E6, HLF, MDCK, MRC-5, RK13, HFF, L929, Vero, SIRC, HeLa	95–97, 99, 169, 170, 458–463
Encephalitozoon hellem	Keratoconjunctivitis, sinusitis, bronchitis, nephritis, cystitis, prostatitis, urethritis, pneumonitis, disseminated infection	Psittacine birds (parrots, lovebirds, parakeets), other birds (ostrich, hummingbirds, finches)	MDCK, E6, HLF, MRC-5, RK13, FBF, HFF, HT-29, Caco2, Vero, SIRC, HeLa	95, 97, 104, 107, 111, 114, 155, 462, 464, 465
Encephalitozoon intestinalis	Diarrhea, intestinal perforation, cholangitis, keratoconjunctivitis, sinusitis, bronchitis, nephritis	Mammals (donkeys, dogs, pigs, cows, goats, primates)	E6, HLF, MDCK, HEL, MDM, RK13, I047, HT-29, Caco-2, HFF, Vero, SIRC, HeLa	97, 116, 118, 120, 462
Enterocytozoon bieneusi	Chronic diarrhea, wasting syndrome, cholangitis, rhinitis, bronchitis	Mammals (pigs, primates, cows, dogs, cats), birds (chickens, waterfowl)	E6, HLF, HT-29, Caco-2	102, 113, 122, 393, 466, 467
Pleistophora ronneafiei	Myositis	Unknown	Not reported	369, 375
Pleistophora sp.	Myositis	Fish	Not reported	368, 373
Trachipleistophora anthropopthera	Disseminated infection, including encephalitis	Unknown	Fibroblasts (derived from mouse brain)	48, 92, 371, 372
Trachipleistophora hominis	Myositis, keratoconjunctivitis, sinusitis	Unknown	MDCK, RK13, COS-1, L6-C10, MM	47, 92, 367, 469
Tubulinosema acridophagus	Myositis, disseminated infection	Insects (Drosophila melanogaster, grasshoppers)	MRC-5, Vero	49, 50, 92
Vittaforma corneae (Nosema corneum)	Keratoconjunctivitis, urinary tract infection, disseminated infection	Unknown	MDCK, MRC-5, XEN, RK13, THP-1, E6, Vero, SIRC, HeLa, HLF	41, 93, 100, 378, 462, 468, 470–472
Nosema ocularum	Keratoconjunctivitis	Unknown	Not reported	377, 473
Endoreticulatus sp.	Myositis	Insects (Lepidopteran)	Not reported	51
Microsporidium africanum	Corneal ulcer	Unknown	Not reported	56, 474
Microsporidium ceylonensis	Corneal ulcer	Unknown	Not reported	56, 475
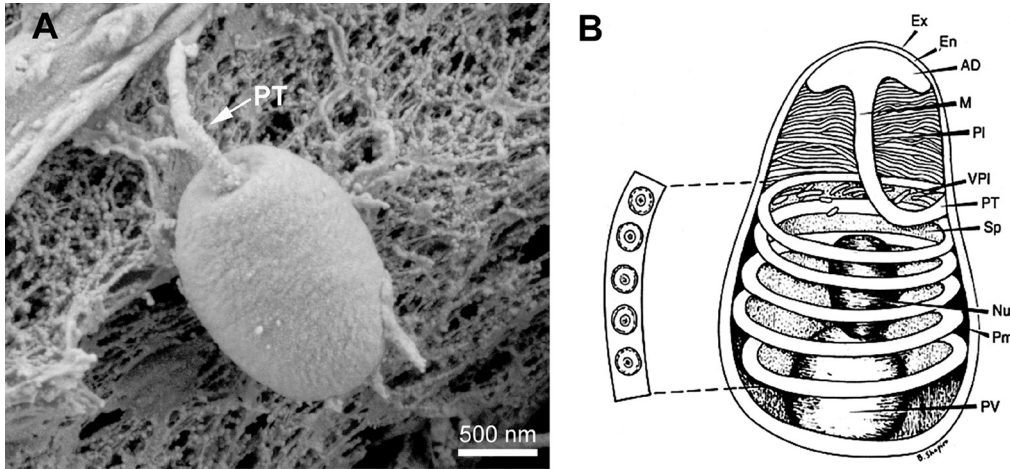
FIG 2 Structure of the microsporidian spore. (A) Scanning electron microscropy (SEM) image of a microsporidian spore. (B) Microsporidian spore diagram. Microsporidian spores range in size from 1 to 10 μm. The spore coat is thinner at the anterior end of the spore and consists of an electron-lucent endospore (En), an electron-dense exospore (Ex), and the plasma membrane (Pm). The sporoplasm (Sp) contains ribosomes, the posterior vacuole (PV), and a single nucleus (Nu). The anchoring disc (AD) at the anterior end of the spore is the site of attachment of the polar tube. It should be noted that the polar tube is often called the polar filament when it is within the spore prior to germination. The anterior or straight region of the polar tube that connects to the anchoring disc is called the manubroid (M), and the posterior region of the tube coils around the sporoplasm. The number of coils and their arrangement (i.e., single row or multiple rows) is used in microsporidian taxonomic classification. The lamellar polaroplast (Pl) and vesicular polaroplast (VPl) surround the manubroid region of the polar tube. The insert depicts the 5 polar tube coils in this figure in a cross section illustrating that the polar tube within the spore (i.e., polar filament) has several layers of different electron densities by electron microscopy. (Reproduced from reference 68 with permission.)

The Microsporidian Life Cycle

The majority of species of microsporidia have simple single-host life cycles; however, a few species have complex life cycles with multiple hosts and can form morphologically distinct spores in different hosts (e.g., *Amblyospora* sp. in mosquitoes) (73, 74). The life cycle pattern for microsporidia can be divided into three stages (Fig. 4): the infective or environmental stage, the proliferative stage, and the sporogonic or spore-forming stage. During development inside of the host cell, microsporidia demonstrate different interfacial relationships depending on the genus of microsporidia (75, 76). These host-parasite interfacial relationships of microsporidia have been divided into four categories (Table 3), which are thought to represent important physiological/nutritional differences among the various microsporidia (77–79).
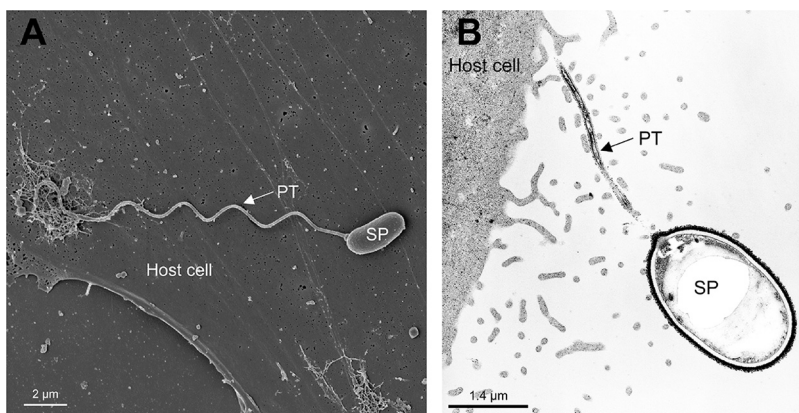

FIG 3 Germinated microsporidian spores. SEM (A) and transmission electron microscopy (TEM) (B) images of a germinated spore during infection. Upon exposure to suitable environmental conditions, the polar tube (PT) rapidly discharges out of the spore (SP) and then interacts with the host cell membrane, serving as a conduit for the nucleus and sporoplasm passage into the host cell.
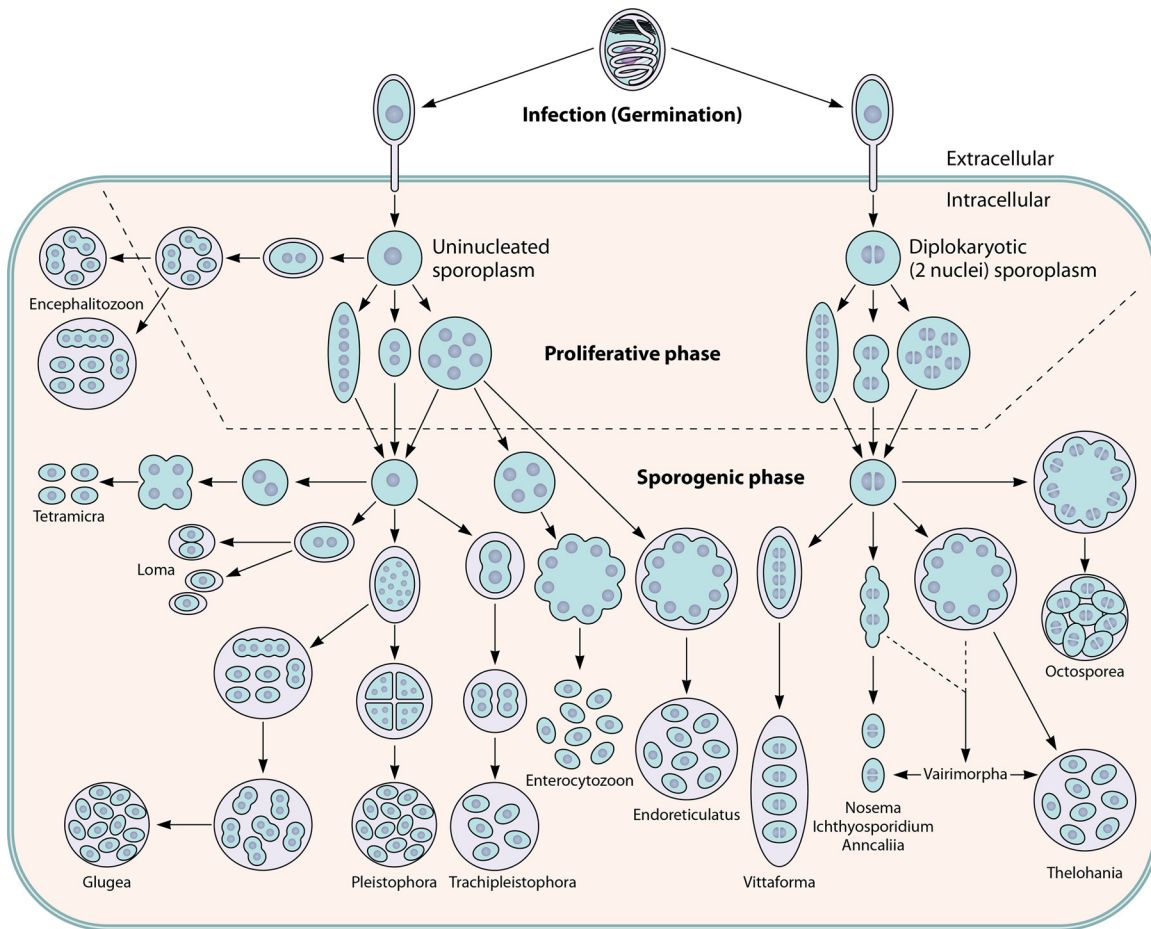
FIG 4 Developmental life cycles of the microsporidia. Spores germinate and the polar tube is extruded when they are exposed to specific environmental conditions, which vary depending on the species of microsporidia. Upon germination, the polar tube is extruded and interacts with the host cell membrane, allowing invasion (see Fig. 6 for a model of this interaction). The sporoplasm traverses the polar tube, is introduced into the host cell cytoplasm, and begins to proliferate. The morphology of the organism during the proliferative phase (which is indicated by the region above the dotted line) is used in classification systems for defining the various microsporidian genera. Relationships of the various microsporidia with their host cells during the proliferative phase are described in Table 3. On the left side of the figure, the sporoplasm is uninucleate and the microsporidian genera that are defined during the development of spores, i.e., the sporogenic phase (which is indicated by the region below the dotted line), are all uninucleate. On the right side of the figure, the sporoplasm is diplokaryotic, and all of the microsporidian genera defined during spore development have diplokaryotic developmental patterns. Regardless of whether they are uninucleate or diplokaryotic, there are three basic types of developmental forms. The first developmental pathway, as illustrated by *Anncaliia* spp., involves cell division by binary fission that immediately follows karyokinesis. The second type of pathway, as illustrated by some *Nosema* spp., involves multiple fission of elongated moniliform multinucleate cells. The third type of developmental pathway, as illustrated by *Endoreticulatus* spp., involves the division by plasmotomy of rounded plasmodial multinucleate cells. During the proliferative phase, cells can have one to several division cycles. Many microsporidia are either in direct contact with the host cell cytoplasm or closely abutted to the host endoplasmic reticulum; however, other microsporidia are found within a parasitophorous vacuole (e.g., *Encephalitozoon* spp. and probably *Tetramicra* spp.) or are separated from the host cell cytoplasm by a thick layer of their own secretions (e.g., *Pleistophora* spp.). In the sporogonic phase, this thick layer of microsporidian secretions becomes the sporophorous vesicle. During sporogony, several of the microsporidian genera, such as *Enterocytozoon*, *Nosema*, *Anncaliia*, *Ichthyosporidium*, and *Tetramicra*, maintain direct contact with the host cell cytoplasm. A sporophorous vesicle, illustrated by a circle around the developing sporogonial stage, is formed by the other microsporidian genera. During the developmental cycle of *Thelohania* and the *Thelohania*-like part of the *Vairimorpha* cycle, it should be noted that the diplokarya separate and continue their development as cells with isolated nuclei. (Adapted from reference 453 with permission.)

The infective stage, i.e., the spores, are the only stage that can survive outside of the host cell. When the spores meet an appropriate environmental condition, germination occurs and the sporoplasm is transported into the host cell via the hollow polar tube (68, 80). As long as the sporoplasm enters the host cell, it will initiate a reproduction cycle, which terminates with the production of mature spores. Merogony follows, during which the injected sporoplasm develops into meronts (the proliferative stage), which multiply, depending on the species, by binary fission or multiple fission. While most microsporidia develop within the host

TABLE 3 Interfacial relationships of the microsporidia

Type of contact	Interfacial relationship
Type I. Direct contact	Direct contact of microsporidian plasmalemma with the host cell cytoplasm. This is seen in *Enterocytozoon bieneusi*, *Anncaliia,* and *Nosema.*
Type II. Indirect contact by host-produced isolation	Subtype A. Microsporidia are in a parasitophorous vacuole (PV)-host-formed single membrane that surrounds the developing organism. The PV is seen in the proliferative and sporogonic phases of development, but the relationship of the developing microsporidia changes during development. Early in development, the organisms have a close association with the PV membrane until the development of a thickened sporont plasmalemma, when they appear loose within the PV. This type of development is seen in *Encephalitozoon hellem* and *Encephalitozoon cuniculi.*
	Subtype B. Throughout development, the microsporidia are surrounded by the host endoplasmic reticulum (ER) that appears as a double membrane surrounding the developing microsporidian cells. In the proliferative phase, these host ER double membranes closely follow the dividing microsporidian plasmalemma and no vacuole is seen. During sporogony, a double-membraned PV is formed by the host ER, which surrounds the cluster of organisms formed in sporogony. This type of development is seen in *Endoreticulatus* and *Vittaforma.*
Type III. Indirect contact by parasite-produced isolation	Subtype A. During development, microsporidia secrete an envelope that surrounds the developing organisms. In sporogony, this envelope becomes a sporophorous vesicle (SPOV) as the microsporidian plasmalemma thickens and pulls away from the secreted envelope. This type of development is seen in *Pleistophora.*
	Subtype B. During early development, the microsporidia are in direct contact with the host cell cytoplasm. As development proceeds, a membrane formed by the microsporidia isolates sporonts from contact with the host cell cytoplasm. This type of development is seen in *Vairimorpha.*
Type IV. Indirect contact by host- and parasite-produced isolation	Subtype A. During merogony, the host ER closely associates with the microsporidian plasmalemma. As development proceeds, the developing microsporidia form an interfacial envelope by producing "blisters" that arise from the plasmalemma, resulting in a SPOV, which can contain tubules, being formed in sporogony. This type of development is seen in *Glugea* and *Loma.*
	Subtype B. During development, both the host and parasite contribute to the formation of a thick interfacial envelope surrounding the developing organisms. This type of development is seen in *Trachipleistophora.*
	Subtype C. During development, a PV formed by the host surrounds the organisms. Within the PV, microsporidian-secreted material surrounds each organism. This type of development is seen in *Encephalitozoon intestinalis.*

cell cytoplasm, some genera of microsporidia will infect the nucleoplasm of the host cell or develop in both the cytoplasm and nucleus (81–84). There are two distinct phases in the development of microsporidia inside of host cells: the proliferative (merogony) stage and the sporogonic stage (Fig. 4 and 5). The merogony stage is responsible for the massive increase in number of infecting organisms (61). At the end of merogony, an electron-dense material is formed on the external face of the merozoite plasma membrane, which is the sign of the switch from merogony to sporogony (85). Sporonts give rise to sporoblasts, which go on to form mature spores without additional multiplication. In most species, the dense material on the plasma membrane in sporogony will become the primordium of the exospore layer of the spore wall (86). The polar tube and Golgi complex will also develop in the sporoplasm of the sporoblast (54, 87, 88). Even though the sporoblast plasma membrane is covered by a continuous layer of dense material, this layer can also form tubular or fibrillar expansions that protrude from the surface of the sporoblast into the parasitophorous vacuole (86). Once a host cell becomes distended with mature spores, the cell ruptures, releasing mature spores into the environment and completing the life cycle. The combination of merogony and sporogony results in a large number of spores being produced from a single infection and underlies the huge reproductive potential of these organisms.

IN VITRO CULTIVATION OF MICROSPORIDIA INFECTING HUMANS

Early studies on the *in vitro* culture of microsporidia focused on economically important species such as *Nosema bombycis* (a pathogen of silkworms), *Nosema apis* (a pathogen of honeybees), *Ameson michaelis* (a pathogen of blue crabs), *Brachiola* (*Nosema*) *algerae* (a pathogen of mosquitoes), and *Vavraia culiceis* (a pathogen of mosquitoes) (6, 89, 90). The first successful *in vitro* cultivation of microsporidia was conducted by Trager in 1934 by inoculating silkworm ovarian tube lining cells with the insect parasite *Nosema bombycis* (91). *Encephalitozoon cuniculi* was the first microsporidian of mammalian origin cultured *in vitro* (89). In order to prevent the growth of bacteria and fungi during the primary isolation
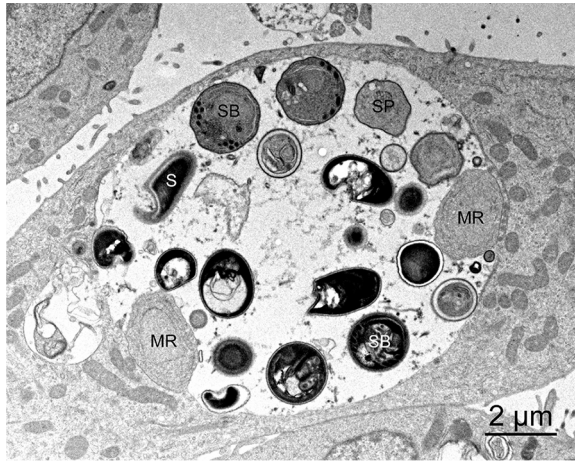
FIG 5 Transmission electron microscopy of an *Encephalitozoon hellem* parasitophorous vacuole. Meronts (MR) can be seen adhering to edge of the vacuole, whereas sporonts (SP), sporoblasts (SB), and mature spores (S) detach from the vacuole membrane and move to the center of the vacuole.

of microsporidia and establishment of *in vitro* cultures, a combination of antibiotics and antifungal agents is usually utilized (61, 89–91).

Since microsporidia were found to be able to infect humans, there have been 10 genera and 17 species of microsporidia reported as etiologic in human infections. Several of these human-infecting microsporidia have been stably cultured in various cell lines (Table 2) (90, 92). The first successful *in vitro* cultivation of a human microsporidium was *Vittaforma corneae,* which was isolated from the corneal biopsy specimen of an immunocompetent patient (93). The corneal samples were either minced or digested by trypsin and collagenase and then inoculated into different cell lines, such as SIRC cells, MDCK cells, or rabbit embryo fibroblasts, and infection could be seen after 30 days postinoculation. More than 13 isolates of *Encephalitozoon cuniculi*, 30 isolates of *Encephalitozoon hellem*, and 30 isolates of *Encephalitozoon intestinalis* have been reported to be successfully cultured (94–121). These parasites were isolated from various tissue or biological samples, including corneal scrapings, conjunctival biopsy specimens, urine, sputum, bronchoalveolar lavage fluid, nasal mucosal biopsy specimens, duodenal biopsy specimens, and stool. *Enterocytozoon bieneusi* is the most frequently identified microsporidian that can cause the diarrhea in AIDS patients. A continuous *in vitro* culture system for *Enterocytozoon bieneusi* has not been established (116, 119). Short-term culture of *Enterocytozoon bieneusi* has been achieved in E6 and HLF cells, but those cultivation systems could not produce significant numbers of spores (119, 122).

EPIDEMIOLOGY

Prevalence in Humans

Microsporidia infect a wide range of animals, including humans, and spores are shed into the environment, increasing the probability for human exposure. Microsporidian spores are often found in surface water, and human-pathogenic microsporidia have been found in municipal water supplies (123), hospital effluent (124), tertiary sewage effluent, and groundwater (125, 126). Human infections due to microsporidia have been identified from all continents except Antarctica, and stool samples from Africa, Asia, Europe, Australia, North America, Central America, and South America have all been shown to contain microsporidia (13, 127).

Before the AIDS pandemic, microsporidia were rarely identified in humans (56, 128). The first authenticated record of a human infected with microsporidia was reported in 1959, when an *Encephalitozoon* sp. was detected in Japan in a boy with convulsions (34). Prior to this, there was a report of a case in 1927 in a newborn baby attributed to *"Encephalitozoon chagasi"* (129), but it is not clear that that organism was correctly

identified. Another early case of microsporidiosis was reported in 1937 in a 4-week-old child who presented with fatal encephalomyelitis and chorioretinitis (130).

The prevalence data for microsporidiosis in human populations, before the era of AIDS, relied mainly upon serology based on detecting antibodies to *Encephalitozoon cuniculi*. The worldwide seroprevalence rates that were reported ranged from 0% to 42% (9, 56, 131–133). Singh et al. (134) found positive titers in 8.7% of healthy adults in England ($n = 69$), 18.5% of Malaysians with filariasis ($n = 70$), 42% of Nigerians with tuberculosis ($n = 89$), and 36% of Ghanaians with malaria ($n = 92$). In another study, while no nontravelers ($n = 48$) were seropositive, 12% of travelers ($n = 115$) returning from the tropics were seropositive (135). In another study, 33% of HIV-positive men ($n = 30$) were seropositive and of them 100% had traveled to the tropics (132). Antibodies to *Encephalitozoon intestinalis* were seen in 5% of pregnant French women and 8% of Dutch blood donors (136). In a study of HIV-positive Czech patients, 5.3% were seropositive to *Encephalitozoon cuniculi* and 1.3% to *Encephalitozoon hellem* (137). In a study in Slovakia, 5.1% of slaughterhouse workers were seropositive to Encephalitozoonidae (138). Taken as a group, these various serologic studies suggest that exposure to microsporidia is common and that this exposure often results in an asymptomatic infection.

Increased cases of microsporidian infections were reported in humans with the expansion of the AIDS pandemic. The majority of these were due to *Enterocytozoon bieneusi* or *Encephalitozoon intestinalis* (Table 2). Reported prevalence rates in the studies conducted in HIV-infected patients before 1998 and the widespread use of combination antiretroviral therapy (cART) demonstrated a prevalence of gastrointestinal microsporidiosis that varied between 2% and 70%, depending on the symptoms of the population studied and the diagnostic techniques utilized (13, 33, 139). When these early studies were combined, the overall prevalence of microsporidial infection in patients was estimated to be 15% (133, 140–142), and in the setting of chronic diarrhea and advanced AIDS, the prevalence of *Enterocytozoon bieneusi* infection was estimated to be 30%. A more recent meta-analysis in 2018 of 131 studies examining microsporidia, cryptosporidium, and isospora infections in HIV-infected individuals found a pooled prevalence of 11.8% (confidence interval [CI], 10.1% to 13.4%) for human gastrointestinal microsporidiosis using a random effects model (139). A lower prevalence of infection was found in high-income countries and higher prevalence in low-income countries, especially in sub-Saharan Africa (139). In a 10-year study, the prevalence of microsporidia was significantly higher in samples from HIV-positive patients (13.9%) than in samples from HIV- negative patients (8.5%) (143), and the percentage of children that were microsporidia positive (18.8%) was significantly higher than that of adults (10.2%) (143). A study in Mexico in 2019 found that 67% of HIV patients with diarrhea had microsporidiosis. A study in Argentina in 2019 found that 12.6% of HIV patients with chronic diarrhea had microsporidiosis by endoscopy and stool examination (144). A study in Malawi in 2018 in HIV-positive children found a baseline prevalence of microsporidiosis of 37% before starting cART and that this prevalence dropped after cART was started (145). While it is clear from several studies that cART can hasten the resolution of microsporidiosis, the literature contains many reports that microsporidia can still be a problem despite cART, especially if the CD4 count remains low (37–40). In France, a 2012 study found microsporidia in 8% of HIV patients on cART with a low CD4 count who had diarrhea (37). A study in China in 2017 demonstrated an 11.7% prevalence of microsporidia in HIV-positive patients, and half of these patients were on cART (146). A study from Cameroon in 2016 demonstrated a prevalence of microsporidia of 24.5% in HIV patients on cART (147).

Microsporidiosis has been reported in patients without HIV, including travelers to tropical countries (3.3% to 10%), children with and without diarrhea (1.7% to 17.4%), and the elderly (17.2%) (133, 148–151). A study of school children in Malaysia demonstrated a rate of 27% (143, 152). Both *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* infections have been reported in travelers to and residents of tropical countries (26, 153–156). It has also been observed that latent infection with shedding of spores is common in immunocompetent humans (157). In immunocompetent hosts, while the majority of reported cases of microsporidiosis have manifested as self-limited diarrhea, there are also many reports

from Asia of ocular infections with *Vittaforma corneae* that presented as keratoconjunctivitis (158–160). The incidence of microsporidia in healthy individuals indicates that there is high exposure to microsporidia in the environment (151, 161).

Risk Factors

Microsporidia are considered opportunistic pathogens, and the major risk factor, especially in HIV infection, is depressed cell-mediated immunity (162, 163), with individuals with a CD4 T-lymphocyte count below 50 to 100 cells/mm^3 having a high susceptibility to microsporidiosis (164). Other immunosuppressed patients are also at risk, i.e., cases have occurred in patients on chemotherapy, in organ transplant recipients, and in bone marrow graft recipients (165–175). *Enterocytozoon bieneusi* infections have occurred in patients who have had liver, heart-lung, or kidney transplantation; *Encephalitozoon* sp. infections have occurred in patients who have had bone marrow, liver, kidney, or pancreas transplantation; *Tubulinosema acridophagus* has occurred in a patient who had a bone marrow transplantation; and *Anncaliia algerae* has occurred in a patient who had a lung transplantation (49, 168, 169, 171, 176–183). Chronic bilateral keratoconjunctivitis due to *Encephalitozoon* sp. has occurred in a patient taking prednisone (20 mg/day) (184).

There have been several cases of the transmission of microsporidiosis due to transplanted organs (185, 186). Three patients were infected by transplanted organs (kidney, kidney, and bilateral lungs) from an *Encephalitozoon cuniculi*-seropositive donor, and kidney biopsy led to the correct diagnosis in all three cases. The onset of symptoms (fever, renal dysfunction, and encephalopathy) was 7 to 10 weeks posttransplantation, and death in one patient was directly related to failure of the transplanted kidney. The other kidney recipient received 6 months of albendazole therapy and remained healthy afterward (185). In a second cluster, which also involved three patients infected by transplanted organs (kidney, liver, heart/kidney), the index case presented with neurological symptoms, including headache and encephalitis (186). Kidney and urine specimens demonstrated *Encephalitozoon cuniculi* in these patients, and infection responded to albendazole (186).

The consumption of untreated water has been a risk factor for infection among travelers and residents, contact lens use is a strongly associated risk factor in microsporidian keratoconjunctivitis, and both young and advanced ages have been identified as risk factors for infection (148, 154, 187–189). Water contact has been found to be an independent risk factor for microsporidiosis in some studies (190, 191) but not in others (192, 193). Outbreaks of *Vittaforma corneae* infection have been associated with hot springs exposure (194) and exposure to soil (195, 196). *Encephalitozoon cuniculi* spores in water remain viable for 6 days (197).

Modes of Transmission

Microsporidia as an obligate intracellular parasite can infect a wide range of hosts, including all major animal groups and humans; however, the manner of transmission and source of human infection have not been fully elucidated (34, 133, 198). In humans, microsporidiosis is most likely a zoonotic infection. Species of microsporidia that infect humans have been found in wild animals, domestic pets, and food-producing farm animals (140). Transmission routes for infection include ingestion of contaminated food and water, fecal-oral contamination, inhalation of contaminated aerosols, inoculation into open wounds, inoculation into the eye, and sexual transmission (7, 52, 133, 199, 200). *Encephalitozoon cuniculi* was found to be transmissible via rectal infection in rabbits (201). *Encephalitozoon hellem* has been demonstrated in respiratory mucosa, the prostate, and the urogenital tract of patients, indicating that respiratory and sexual transmission are possible in humans (202, 203). While congenital transmission of *Encephalitozoon cuniculi* has not been seen in humans, it has been seen in other mammals, including mice, horses, rabbits, dogs, foxes, alpaca, and squirrel monkeys (204).

Zoonotic Transmission

Many species of microsporidia that can infect humans are also able to infect a wide range of animals, supporting that zoonotic transmission likely occurs. Direct evidence of zoonotic transmission was reported in a child who was infected with *Encephalitozoon*

cuniculi after being exposed to an infected litter of puppies and in a 2-year-old child who was infected with *Enterocytozoon bieneusi* after being exposed to infected guinea pigs (205, 206). The species that infect humans, particularly *Enterocytozoon bieneusi* and *Encephalitozoon* spp., have been reported frequently from a wide range of mammalian hosts, including dogs, pigs, donkeys, cats, goats, rabbits, and cattle (26, 133, 141, 199, 207–210). Molecular epidemiologic studies, based on genotyping of polymorphisms in the ribosomal internal transcriber spacer (ITS) nucleotide sequence, have enabled the identification of *Enterocytozoon bieneusi* isolates that are unique to specific animals or humans as well as isolates seen in both humans and animals (12–14, 26). Additional molecular epidemiologic studies on *Enterocytozoon bieneusi* have been performed using multilocus sequence typing (MLST) based on microsatellites, and this technique supports the ITS data (13). Variations in the ITS region also define isolates of *Encephalitozoon cuniculi* that correlate with their original mammalian hosts and additionally can be used to distinguish isolates of *Encephalitozoon hellem* (12, 13, 97).

Studies performed on urban pigeons revealed that there is no barrier to microsporidian transmission between park pigeons and humans for *Encephalitozoon intestinalis* and *Encephalitozoon hellem,* suggesting the potential zoonotic transmission of microsporidia between birds and humans (211). Visiting urban parks has been identified as a high-risk factor for microsporidiosis in children and the elderly (211). *Encephalitozoon cuniculi* is often identified in rabbits and dogs (97, 212). The Encephalitozoonidae are found in numerous species of mammals and birds, and in several reports, the onset of human infection was associated with exposure to fowl, budgerigars, lovebirds, or livestock (198, 200, 213–215). *Tubulinosema* and *Anncaliia* are pathogens of insects. *Nosema* and *Vittaforma* infections are thought to be due to the inoculation of environmental spores of insect pathogens into the cornea (198). Even though zoonotic transmission has not been proven for all species of microsporidia, there appears to be a risk for infection when there is close contact with infected wild, domesticated, and pet animals and also following exposure to water, food, or aerosols contaminated by microsporidian spores (199, 214, 216, 217).

Waterborne Transmission

Waterborne transmission is an important risk factor for intestinal diseases, causing significant morbidity and mortality worldwide (123, 218). Several characteristics of microsporidian spores indicate a high probability that waterborne transmission is important for microsporidiosis. Microsporidian spores have been demonstrated to survive for extended periods of time in water, the small size of spores make filtering processes less efficient, and the oral infectious dose is relatively low (219). Microsporidian spores are released from feces, urine, and animal carcasses of infected animals and can be dispersed into water sources, contaminating potable and recreational water (220, 221). The presence of microsporidia seen in human infections, e.g., *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Vittaforma corneae*, and *Pleistophora* sp., has been confirmed in tertiary sewage effluent, surface water, and groundwater (123, 125, 222, 223). *Encephalitozoon hellem* and *Encephalitozoon intestinalis* have both been demonstrated to be present in surface waters contaminated by waterfowl feces (224).

Foodborne Transmission

Microsporidia are pathogens of concern for foodborne transmission due to the globalization of food supply, faster transportation of food, modifications in food consumption patterns, and increased consumer travel (225, 226). Transmission of microsporidiosis may occur due to the global food chain industry involving consumption of crustaceans (e.g., clams, lobster, shrimp) and fish (215, 218). Eating raw or insufficiently cooked meat or unwashed vegetables and fruits has been associated with microsporidiosis. People who consumed raw vegetables had a 2-fold increase in infection with microsporidia compared to those who consumed cooked vegetables (150). In a study conducted in agricultural markets in the Central Valley of Cost Rica, microsporidia were detected in cilantro, parsley, lettuce, blackberries, and strawberries (227). A study in 2018 demonstrated the presence of *Enterocytozoon bieneusi* and *Encephalitozoon* spp. in vegetable samples from farms that were irrigated with contaminated

water resources (228). Eating of undercooked meat has also been associated with microsporidian infection in HIV-infected individuals (133, 191). *Encephalitozoon cuniculi* has been identified in fermented meat products that are consumed without cooking (229).

Vertical and Horizontal Transmission

Vertical transmission of microsporidia has been observed in insects, nematodes, fish, crustaceans, rabbits, and carnivores (204, 230–234). Vertical transmission of microsporidia can induce a sex ratio distortion, which makes these the only eukaryotic parasites known to change the sex of their hosts (235, 236). Vertical transmission in humans has not been observed (204, 237–239).

Horizontal transmission is usually characterized by a high parasite burden and accompanying pathogenicity (233). As noted previously, the probable routes of horizontal transmission of microsporidia include ingestion of contaminated food or water, fecal-oral transmission, and inhalation of contaminated aerosols (7, 127, 200, 239). Oral transmission of spores by means of contaminated food and water is probably the major pathway for horizontal transmission (198, 240, 241). An early serologic study of homosexual men in Sweden indicating an increased prevalence of antibodies to *Encephalitozoon cuniculi* in this group suggested that oral-anal contact or anal penetration during sex may contribute to horizontal transmission, and these were subsequently identified as risk factors for intestinal microsporidiosis (132, 191, 242).

HOST-PARASITE INTERACTIONS

Microsporidia possess one of the most fascinating parasite invasion mechanisms, with their environmentally resistant spore and unique polar tube invasion apparatus (243). The polar tube's function is to transmit infection by penetrating the host cell, acting as the conduit for the parasite sporoplasm and nucleus from the spore into a new host cell (13, 66, 243, 244). Due to the size of the spores (1 to 10 μm), diameter of the polar tube (0.1 to 0.2 μm), and rapidity of both polar tube discharge and sporoplasm passage (<2 s), study of this process has been difficult (59, 245, 246). To this end, despite being described over 100 years ago (57), both invasion and the polar tube are poorly characterized. We do not understand how this structure forms during development, how it functions during eversion, or how the polar tube interacts with the host cell to permit penetration of the sporoplasm and nucleus into the host cell (243). Furthermore, we do not understand the full complement of proteins that make up this structure.

Spore Wall Proteins and Host Cell Interaction

There are three layers in the microsporidian spore wall, a proteinaceous exospore, an electron-lucent endospore, and an underlying plasma membrane (Fig. 2) (54, 56, 78). Chitin is a component of the endospore wall (247) and fibrillar system of the exospore wall. To this end, a chitin deacetylase has been identified as a spore wall protein (SWP) (248, 249). It is believed that several of the proteins in the spore wall, especially in the exospore, participate in binding to the host cell or triggering signaling during invasion (62, 250, 251).

SWP1, a glycine- and serine-rich protein, is developmentally regulated and is present in the exospore wall (67, 86, 252). SWP2, a 150-kDa spore wall glycoprotein, is found in mature spores of *Encephalitozoon intestinalis* (253). A third spore wall protein (SWP3/Enp2) was identified by proteomic studies and is found in all microsporidia (254, 255). A fourth spore wall protein localized in the exospore wall (SWP4/Enp1) appears to be involved in the adhesion of spores to various carbohydrates (62, 251, 255). Other putative SWPs have been identified in *Nosema bombycis* (256–260) but have not been confirmed to occur in other microsporidia. Several of these various SWPs are modified by posttranslational glycosylation involving mannosylation (261–263). These modifications are probably important for spore adherence to mucin or host cells during passage of spores in the gastrointestinal tract, facilitating invasion; in support of this concept, exogenous glycosaminoglycans decreased adherence of spores to host cells (62, 251, 255). Using proteomic techniques, SWP5 from *Encephalitozoon cuniculi* (EcSWP5) and *Encephalitozoon hellem* (EhSWP5) (264) were also identified. Antibody
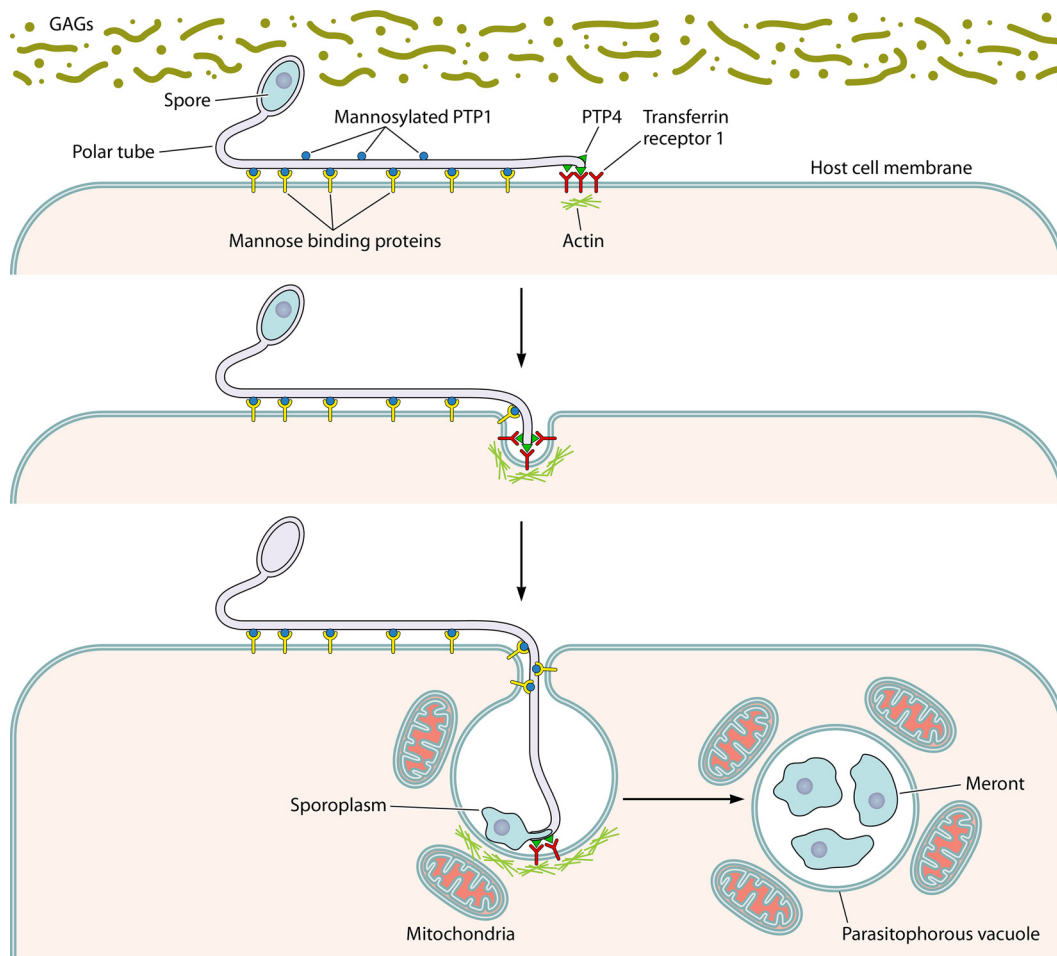
FIG 6 Model of microsporidian (*Encephalitozoon*) host cell invasion. The spore wall contains spore wall proteins (SWPs) that can interact with glycosaminoglycans (GAGs) and other substances in the mucin layer (green) of the gastrointestinal track. These interactions are probably involved in germination. As the polar tube germinates, the polar tube adheres to the host surface by interactions of polar tube protein 1 (PTP1) with host cell surface mannose binding proteins (MBP). This allows the polar tube to form an invasion synapse by pushing into the host cell membrane. In the formation of the invasion synapse, interactions of PTP1 (and possibly PTP4) with the host cell membrane result in the establishment of a protected microenvironment for the extruded microsporidian sporoplasm which excludes the external environment. Within the invasion synapse, epitopes of polar tube protein 4 (PTP4) that are exposed at the tip of the polar tube interact with transferrin receptor 1 (TfR1), and possibly other host cell-interacting proteins (HCIPs), at the host cell plasma membrane, triggering signaling events. During the final steps of invasion, these various interactions lead to the formation of the invasion vacuole, which can include clathrin-mediated endocytosis as well as the involvement of host cell actin. The sporoplasm (and meront) possesses surface proteins, such as sporoplasm protein 1 (SSP1), which interact with various host cell surface proteins, tethering the sporoplasm to the plasma membrane during invasion and facilitating development of the invasion vacuole. At this early stage of infection, host mitochondria are already located around the invasion vacuole. As the vacuole completes its internalization, the sporoplasm becomes a meront and starts replicating. The meront surface interacts with the invasion vacuole membrane, forming electron-dense membrane structures that allow meront SSP1 to interact with voltage-dependent anion selective channels (VDAC) located on the outer membrane of the mitochondria. The interaction of SSP1 and VDAC appears to play a crucial role in association of host cell mitochondria with the invasion vacuole, facilitating energy acquisition from the host cell by the replicating meronts.

to EcSWP5 stained parasites that were undergoing cytokinesis but did not stain mature spores, indicating that SWP5 is developmentally regulated (264).

Several SWPs identified from *Antonospora locustae*, *Nosema bombycis*, and *Encephalitozoon* spp. are able to interact with host cells through their ability to bind to host cell surface sulfated glycosaminoglycans (GAGs) as well as heparin-binding motifs (HBM) (62, 251, 253, 257, 265, 266). *Encephalitozoon cuniculi* EnP1 was the first spore wall protein reported to bind host cell GAGs (Fig. 6) (62). Six spore wall proteins of *Nosema bombycis* (NbSWP7, NbSWP9, NbSWP11, NbSWP12, NbSWP16, and NbSWP26) have been shown to have heparin binding domains and have been demonstrated to be important for host cell binding and invasion (265, 267–269).

The spore wall protein EhSWP1 of *Enterocytozoon hepatopenaei* also contains a heparin binding domain and can bind to GAGs of host cells during invasion (270). In addition to binding to GAGs, *Nosema bombycis* spore wall protein NbSWP26 interacts with *Bombyx mori* turtle-like protein (BmTLP), which is a receptor involved in antigen presentation to the immune system (271). Incubation of host cells with recombinant alpha 5 beta 1 and alpha 3 beta 1 human integrin proteins inhibited host cell infection and spore adherence, indicating that integrins on the host cell surface are involved in invasion and spore adhesion (272). Genomic analysis of *Encephalitozoon intestinalis* indicates that numerous proteins in this organism possess integrin binding motifs that could participate in interactions of the spore wall and host cell surface integrins (272).

Polar Tube Proteins and Host Cell Interaction

Microsporidia possess a unique, highly specialized invasion apparatus termed the polar tube (Fig. 3). During infection, the polar tube is rapidly extruded from the spore under appropriate environmental stimulation and transports the nucleus and sporoplasm into the host cell (59, 245, 246). There are six polar tube proteins (PTP1 to PTP6) that have been identified from microsporidia, and several of these PTPs are involved in host cell interactions (Fig. 6) (243, 273). PTP1 is a key component of the polar tube (274–276) and is a heavily mannosylated protein with a substantial number of O-linked mannosylation modification sites (262). Mannose pretreatment of host cells decreased *Encephalitozoon hellem* infection, consistent with an interaction between the glycoepitopes of PTP1 and host cell mannose binding molecules such as dectin-2, mannose binding lectin (MBL), and C-type lectin receptors (262, 277). Proteomic and immunologic approaches resulted in the identification of PTP2 and PTP3 (276, 278). PTP1, PTP2, and PTP3 can be purified as a complex using cross-linking agents (278, 279). Yeast two-hybrid methods confirmed an interaction of PTP1, PTP2, and PTP3 and allowed mapping of PTP1 subdomain interactions (279). Interestingly, PTP1 interacts with itself and other PTPs at both its C- and N-terminal subdomains, but its central repetitive core does not interact with other PTPs (279), consistent with the hypothesis that the PTP1 central region is not involved in protein-protein interactions that are needed to form the polar tube. A unique epitope of *Encephalitozoon hellem* PTP4 was found to specifically localize to the tip of the polar tube and was shown to bind to the host transferrin receptor 1 (TfR1), which is one of the key receptor proteins in the clathrin-mediated endocytosis pathway (280). Blocking this interaction with recombinant proteins or antibodies specific to either protein reduced host cell invasion, indicating that PTP4 and TfR1 are crucial for microsporidian invasion (280). PTP4 and PTP5 homologs are present in the genomes of other microsporidia such as *Anncaliia algerae*, *Encephalitozoon intestinalis*, and *Encephalitozoon cuniculi*. Like the *ptp1* and *ptp2* genes (276) that occur in close proximity to each other in the genome, the *ptp4* and *ptp5* genes are also found in close proximity to each other within the genome, suggesting that they may have been linked in either evolution or expression (13, 60). More recently, a sixth polar tube protein (PTP6) was identified from the insect microsporidian *Nosema bombycis*. NbPTP6 is rich in histidine and serine and has multiple glycosylation sites (273). This protein has also been identified by proteomic studies of the composition of the polar tube in *Encephalitozoon hellem* (L. M. Weiss, unpublished data). NbPTP6 has been shown to bind to the host cell surface, suggesting a potential role in the process of polar tube interaction with host cell (273).

Sporoplasm Proteins and Host Cell Interaction

It had been postulated that the polar tube functions like a hypodermic needle in penetration of the host cell (13, 66, 243); however, electron micrographs of the polar tube interaction with its host cell demonstrated that an invagination occurs at the site of this interaction (13, 243). If spores are germinated in medium and the sporoplasm emerges from the polar tube tip, it is very delicate and swells and breaks, suggesting that survival of the emerging sporoplasm probably requires a protected environment (13). It is thought that the polar tube invaginates the host cell membrane, forming a microenvironment

(the invasion synapse) in which final penetration occurs (Fig. 6). During infection, the sporoplasm is discharged from the spore through the polar tube into this invasion synapse, which is formed by the polar tube and host cell plasma membrane (243). An ultrastructural study of *Anncaliia algerae* demonstrated that the extracellular discharged sporoplasm tightly abutted the host plasmalemma and appeared to be in the process of being incorporated into the host cytoplasm by phagocytosis and/or endocytosis (281).

A sporoplasm surface protein (SSP1) was identified from *Encephalitozoon hellem,* and EhSSP1 was shown to bind to the host cell surface at the site where the polar tube invaginated the host cell membrane during invasion (282). Upon invasion, the sporoplasm enters the proliferative phase of the microsporidian life cycle, which is marked by extensive multiplication via merogony (243, 283). Host cell voltage-dependent anion channels (VDAC1, VDAC2, and VDAC3) interact with EhSSP1 and EhSSP1 colocalized with host mitochondria and the microsporidian parasitophorous vacuoles in infected cells. (282). Electron microscopy demonstrated that the outer mitochondrial membrane interacted with meronts and the parasitophorous vacuole membrane, that mitochondria clustered around meronts, and that VDACs were concentrated at the interface of the mitochondria and parasites (282). RNA interference (RNAi) knockdown of VDAC1, VDAC2, and VDAC3 in host cells resulted in a significant decrease in the number and size of the parasitophorous vacuoles and a decrease in mitochondrial parasitophorous vacuole association (282). Microsporidia lack mitochondria and are reliant on the host for ATP generation, suggesting that the interaction between the microsporidian intracellular sporoplasm and the parasitophorous vacuole is crucial for the energy transportation from the host (8, 284, 285). The interaction of EhSSP1 with VDAC probably plays an important part in energy acquisition by microsporidia via its role in the association of mitochondria with the parasitophorous vacuole (Fig. 6).

An ATP-binding cassette (ABC) transporter subfamily protein NoboABCG1.1 identified in *Nosema bombycis* was found on the sporoplasm, meront, and mature spore. This ABC transporter has been shown to be important for microsporidian nutrient acquisition from its host cells (286). The family of nucleotide transporters (NTTs) which localize to the surface of the sporoplasm membrane of *Encephalitozoon cuniculi* and *Trachipleistophora hominis* has been demonstrated to transport ATP from the host cytoplasm (287). The major facilitator superfamily protein, which localizes to the proliferative meronts of microsporidia, can also uptake ATP and GTP (288). In *Encephalitozoon* spp., host mitochondria are in direct contact with the parasitophorous vacuole, which is thought to facilitate the energy transport into meronts (289, 290).

IMMUNOLOGY

There are limited data for humans on the immune response to microsporidiosis. A significant humoral response clearly occurs during infection, and antibodies are formed that react with both the spore wall and polar tube. Immune defects in cell-mediated immunity are associated with microsporidiosis, as demonstrated by the prevalence of this infection in patients with AIDS or transplantation. *Encephalitozoon cuniculi* infection in mammals results in chronic infection with persistently high antibody titers and ongoing inflammation, as demonstrated by the occurrence of persistent encephalitis and chronic renal disease in rabbits. Latent infection occurs, allowing relapse, and albendazole treatment cannot eliminate latency in immunodeficient mice (291, 292). The protective immune response to microsporidia is dependent on cell-mediated immunity. Adoptive transfer of sensitized syngeneic T-enriched spleen cells protects athymic or SCID mice against lethal *Encephalitozoon cuniculi* infection but not naive lymphocytes or hyperimmune serum (293, 294). Studies using macrophages have demonstrated that reactive nitrogen and oxygen species or iron sequestration can contribute to the ability of these cells to control *Encephalitozoon cuniculi* infection (295). Maternal antibodies protect newborn rabbits from infection with *Encephalitozoon cuniculi* during the first 2 weeks of life (296). IgM antibodies against PTP1 in normal human serum may play a role in preventing infection with *E. cuniculi* (297). These data indicate that antibodies almost certainly have a role in limiting infection in the host, although they are not sufficient by themselves to prevent mortality or

cure infection. Studies with both *Encephalitozoon intestinalis* and *Encephalitozoon cuniculi* have shown that gamma interferon (IFN-γ) knockout mice cannot clear infection (298). Treatment of mice infected with *Encephalitozoon cuniculi* with neutralizing antibody to IFN-γ or interleukin 12 (IL-12) results in increased mortality (299). In p40 knockout mice, which are unable to produce IL-12 and have impaired CD8$^+$ cell function, *Encephalitozoon cuniculi* infection results in death (300, 301).

The role of individual T-cell subtypes in disseminated microsporidiosis following intraperitoneal infection has been examined using murine models (301). The cytotoxic T-cell response is a key factor in the immune response to *Encephalitozoon cuniculi*. Mice deficient in CD8$^+$ cells succumb to infection, but mice deficient in CD4$^+$ cells are similar to wild-type mice. The protective effect of CD8$^+$ T cells is mediated by their ability to produce cytokines and to reduce microsporidian tissue burden. Studies have demonstrated that the protection provided by CD8$^+$ T cells is through the perforin pathway, and mice lacking perforin die when infected with *Encephalitozoon cuniculi* (301). The route of infection is important in cases where immune cells are involved in a protective response. For example, if mice are infected orally with microsporidia, both the CD4$^+$ and CD8$^+$ T cells are involved in the protective immune response generated by the gastrointestinal tract (302, 303). The CD8$^+$ $\alpha\beta$ population is a critical cell subset for protective immunity (304). IFN-γ production by dendritic cells is important for priming the gut intraepithelial lymphocyte response following oral infection with *Encephalitozoon cuniculi* (304). Dendritic cells play a key role in the immune response to microsporidia (305) by facilitating T-cell responses through recognition of the pattern recognition receptors expressed by these pathogens (e.g., TLR4 [306] and TLR2 [307]). Observations indicate that aging is a risk factor for microsporidiosis; consistent with this observation, plasmacytoid dendritic cells in older mice infected with *Encephalitozoon cuniculi* have been shown to downregulate CD8$^+$ T-cell responses by inhibiting the maturation of normal dendritic cells (308).

CLINICAL MANIFESTATIONS IN HUMAN INFECTIONS

Microsporidia can cause localized or disseminated infection in humans (309). The microsporidian genera associated with human infection and related clinical manifestations are summarized in Table 2. Human microsporidiosis represents a significant emerging opportunistic disease: once thought to be restricted to immunocompromised patients, infections in immunocompetent individuals are also known to occur (161, 310). The clinical manifestations of microsporidiosis are diverse, varying according to the mode of transmission, the causal species, and the underlying host immune status and response (Fig. 7 and 8) (1, 309). *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the most commonly detected microsporidia in human infection, and chronic diarrhea is the most frequent clinical syndrome. Although most reported cases of microsporidiosis involve diarrhea, the gamut of diseases caused by these organisms includes myositis, kidney and urogenital infection, cholangitis, ascites, sinusitis, hepatitis, keratoconjunctivitis, and disseminated infection, as well as asymptomatic infection (1, 7, 13, 162, 311) (Fig. 7).

Enterocytozoon bieneusi

In 1985, the first case of *Enterocytozoon bieneusi* infection was reported in a Haitian AIDS patient with diarrhea and wasting (44). *Enterocytozoon bieneusi* infection in non-HIV-infected individuals was first reported in travelers to the tropics, and the microsporidium-associated diarrhea in those immunocompetent patients tended to be self-limiting (154). *Enterocytozoon bieneusi* is the most commonly seen microsporidium causing human infection, being etiologic in 90% of cases of chronic diarrhea in AIDS patients (33, 161). Infection does not produce active enteritis or ulceration, although it does result in various degrees of crypt hyperplasia and villous blunting (Fig. 8B). *Enterocytozoon bieneusi* is found at the apical surface of small intestinal enterocytes, biliary tract epithelial cells, and pancreatic epithelial cells. Spores are essentially not seen on the basal surface of these cells or in the lamina propria (1, 33, 128, 219, 312) (Fig. 7F and 9A). Infection with *Enterocytozoon bieneusi* usually presents with chronic diarrhea (which can last for years [313]), anorexia, weight loss, and bloating without associated fever. Infection frequently occurs in AIDS patients with CD4$^+$ counts lower than 50 cells/μl. On a
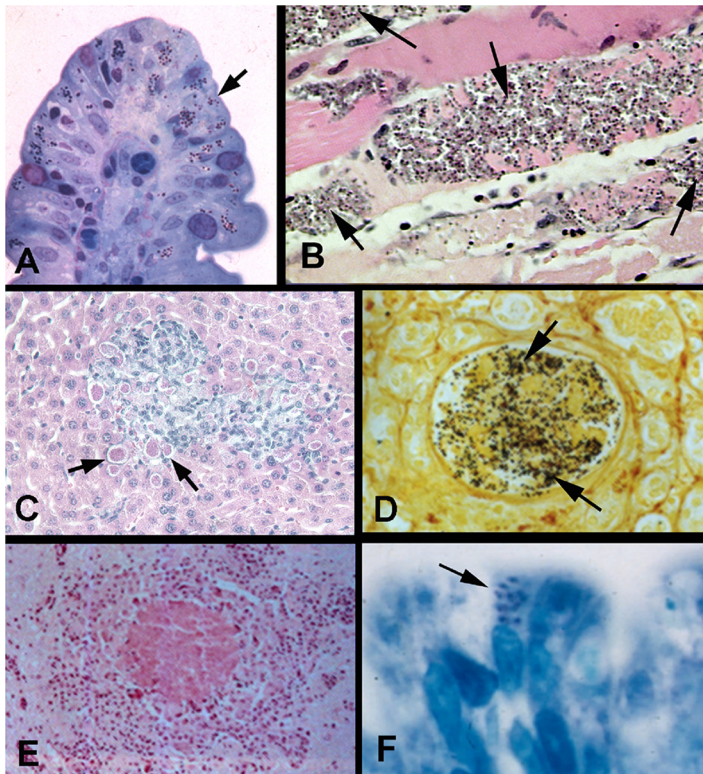
FIG 7 Light microscopy of biopsy specimens from patients with microsporidiosis. (A) Methylene blue-azure II-fuchsin stain of an intestinal biopsy specimen from a patient with *Encephalitozoon intestinalis* (arrow) infection. (Reproduced from reference 454 with permission.) (B) Hematoxylin and eosin stain of muscle tissue from a patient with rheumatoid arthritis treated with antibody to TNF-α demonstrating *Anncaliia algerae* (arrow) myositis. (C) Tissue chromotrope stain of liver biopsy specimen from an immunodeficient mouse infected with *Encephalitozoon cuniculi* (arrows). (D) Steiner stain of a renal biopsy specimen demonstrating *Encephalitozoon hellem* (black spores) within the lumen of the renal tubule. (Reproduced from reference 386 with permission.) (E) Hematoxylin and eosin stain of brain tissue from a rabbit with torticollis, demonstrating a microgranuloma with central necrosis due to *Encephalitozoon cuniculi* infection. No spores are seen in this image. (Reproduced from reference 386 with permission.) (F) Light micrograph of intestinal villus biopsy specimen (plastic section) stained with methylene blue-azure II-fuchsin from a patient with AIDS and *Enterocytozoon bieneusi* infection. The arrow points to microsporidia within cells. These spores were present only on the apical epithelial surface; no spores were present on the basal surface or in the lamina propria. (Reproduced from reference 454 with permission.)

daily basis, 3 to 10 bowel movements which are loose to watery and do not contain blood or fecal leukocytes occur (314). Malabsorption is usually evident, is often worsened by food ingestion, and can result in weight loss and a wasting syndrome. This pathogen can also invade the cholangioepithelium (315), leading to sclerosing cholangitis (316). When this occurs, imaging studies such as endoscopic retrograde cholangiopancreatography (ERCP), computerized axial tomography, abdominal ultrasonography, or endoscopic ultrasonography can demonstrate irregularities of the bile duct wall (Fig. 8C), dilated biliary ducts, and gallbladder abnormalities, such as distention, thickening, or the presence of sludge. *Encephalitozoon intestinalis* can also cause biliary infection (316). Reports of systemic dissemination of *Enterocytozoon bieneusi* are rare; respiratory involvement associated with chronic diarrhea, wheezing, dyspnea, persistent cough, and chest radiographs showing interstitial infiltrates have been reported (317, 318).

Encephalitozoonidae

The *Encephalitozoon* species, including *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Encephalitozoon cuniculi,* are widely distributed in mammals and have been identified in both immunocompetent and immunocompromised humans (128, 310). These microsporidia have the capacity to disseminate widely in their hosts and can involve almost any organ system during infection (Fig. 7A, C, D, and E) (7, 13, 33, 319). The
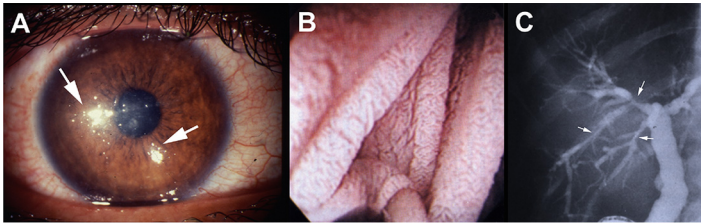
FIG 8 Clinical images from patients with microsporidiosis. (A) *Encephalitozoon hellem* keratoconjunctivitis demonstrating punctate corneal lesions (white arrows). (B) Endoscopy of jejunal mucosa of a patient with gastrointestinal microsporidiosis due to *Enterocytozoon bieneusi* demonstrating fusion of the villi. (Reproduced from reference 454 with permission.) (C) Endoscopic retrograde cholangiogram (ERCP) of a patient with HIV infection (AIDS) and sclerosing cholangitis due to *Enterocytozoon bieneusi* infection. The ERCP demonstrates diffuse dilations of the common bile duct with irregular walls, plus areas of narrowing and dilation (arrows) of the intrahepatic bile ducts. (Reproduced from reference 454 with permission.)

Encephalitozoonidae have been described as etiologic in cases of sinusitis, bronchiolitis, nephritis, keratitis, peritonitis, hepatitis, fulminant hepatic failure, gastroenteritis, cerebritis, cystitis, urethritis, prostatitis, nodular skin lesions, and disseminated infection (7, 13, 33, 99, 320–327). Infection is most commonly seen in immunocompromised patients (e.g., patients with HIV infection, especially in patients with CD4 counts of <50/mm^3, transplant recipients, and those receiving immunomodulatory therapy) (49, 169–171, 219, 328–330). Interstitial nephritis due to microsporidiosis, similar to what has been described in rabbits, occurs in AIDS patients and has also been seen in patients with renal transplants (169, 171, 178, 331). *Encephalitozoon* species have also been identified in immunocompetent travelers with a symptom of self-limiting diarrhea (154).

About 10% of cases of diarrhea and wasting in immunodeficient patients with AIDS has been due to *Encephalitozoon intestinalis* (116) (Fig. 7A and 9B). Infection can result in areas of bowel necrosis and a presentation similar to that of an acute abdomen (322) with perforation of the intestine and peritonitis (332). This pathogen has also been reported to cause cholangitis (333), keratoconjunctivitis, osteomyelitis of the mandible (334), renal failure, upper respiratory infection, and disseminated infection (118, 335).

Encephalitozoon cuniculi infection has been reported to cause hepatitis (336), peritonitis (323), hepatic failure (320), renal insufficiency, disseminated disease with fever (324), intractable cough (94), and endocarditis (337) (Fig. 7C). *Encephalitozoon cuniculi* has been found in the periprosthetic tissue of several patients in Poland who presented with hip implant loosening, requiring hip replacement, despite an absence of symptoms associated with infection (338). There are many reports of disseminated infections in transplant recipients. The diagnosis is often delayed, perhaps because of the lack of specificity of symptoms and a low index of suspicion. Granulomatous encephalitis due to this pathogen has been reported in mammals (rabbits) since 1922, and infection in humans has been reported to cause encephalitis and seizures in AIDS patients (99, 324) (Fig. 7E). *Encephalitozoon* infection was reported in a 3-year-old boy with seizures and hepatomegaly, as confirmed by the presence of IgG and IgM antibodies that reacted with *Encephalitozoon cuniculi* (339). Infection with *Encephalitozoon* sp. with recurrent fever has also been reported in a 9-year-old Japanese boy with spastic convulsions, headache, and vomiting (34). *Encephalitozoon cuniculi* and *Streptococcus intermedius* were isolated together from a brain abscess in a patient with diabetes (340).

Encephalitozoon hellem infections have been reported in cases of keratoconjunctivitis, nephritis, pneumonia, bronchitis, and disseminated disease with renal failure (112, 203, 341). *Encephalitozoon hellem* can also infect the nasal epithelium, causing sinusitis in AIDS patients (342). The majority of reported ocular infections presenting as punctate keratoconjunctivitis are caused by Encephalitozoonidae (160, 343–345), and of these, the majority are due to *Encephalitozoon hellem*, including three cases originally classified as *Encephalitozoon cuniculi* (346, 347) (Fig. 8A and 9E). The few reported cases not due to *Encephalitozoon hellem* were caused by *Encephalitozoon* sp. or *Encephalitozoon intestinalis*. Infection presents as bilateral coarse punctate epithelial keratopathy limited to the superficial epithelium of the cornea
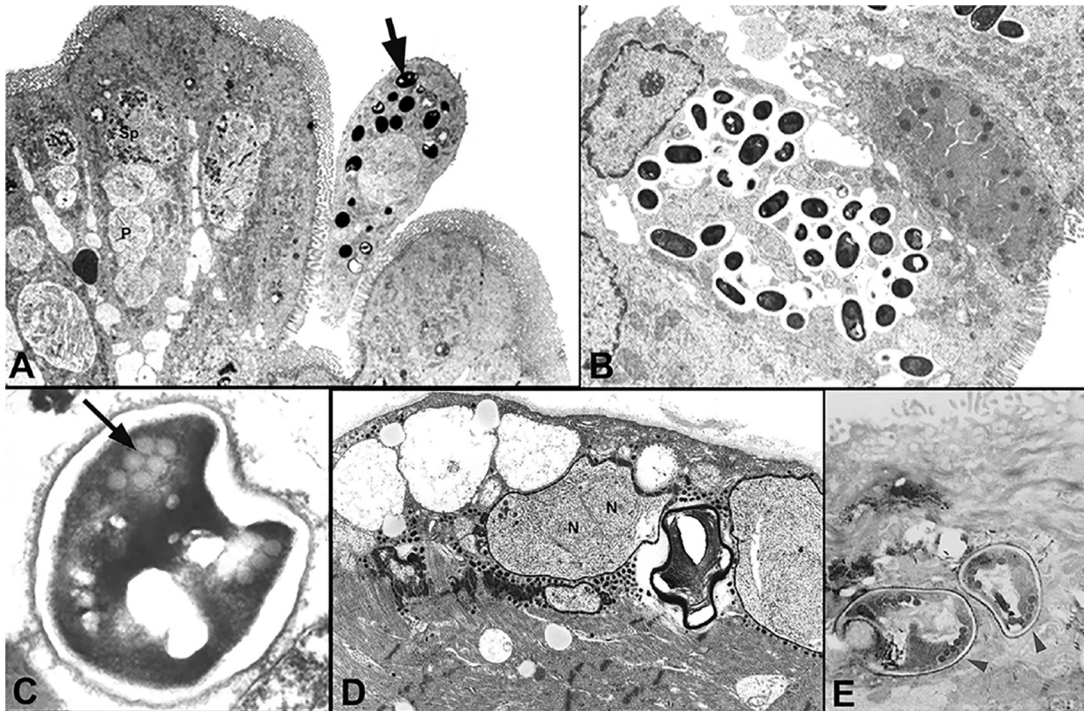
FIG 9 Transmission electron micrographs of biopsy specimens from patients with microsporidiosis. (A) Duodenal epithelium from a patient with AIDS and *Enterocytozoon bieneusi* infection demonstrating proliferating stages (P) and late sporogonial plasmodia (Sp). The arrow points to a sloughing enterocyte containing mature spores. (Reproduced from reference 386 with permission.) (B) Intestinal biopsy specimen from a patient with AIDS and *Encephalitozoon intestinalis* infection demonstrating spores within vacuoles containing a fibrillar matrix. (Reproduced from reference 386 with permission.) (C) *Enterocytozoon bieneusi* spore demonstrating the characteristic finding of two rows of three cross sections of the polar tube (indicated by an arrow). (Reproduced from reference 386 with permission.) (D) Muscle biopsy specimen from a patient with rheumatoid arthritis on an anti-TNF-α antibody who presented with myositis. Proliferative forms are seen in the biopsy specimen with a characteristic diplokaryotic nucleus (N). (E) Conjunctival biopsy specimen from a patient with keratoconjunctivitis due to *Encephalitozoon hellem* demonstrating characteristic spores (arrowheads) with a single row of six cross sections of the polar tube.

and conjunctival inflammation. Clinically, infection may be bilateral or unilateral and can present with redness, foreign body sensation, photophobia, excessive tearing, blurred vision, and changes in visual acuity (13, 347). Disseminated disease is often present in patients with microsporidian keratoconjunctivitis; therefore, microsporidian spores can be found in urine samples in patients with this ocular infection.

Anncaliia spp.

There are three species in the *Anncaliia* genus that have been reported in humans: *Anncaliia algerae*, *Anncaliia connori*, and *Anncaliia vesicularum* (Table 2). *Anncaliia algerae* was first known as *Nosema algerae* and then as *Brachiola algerae* before being moved to the genus *Anncaliia* (43). *Anncaliia algerae* was originally recognized as a pathogen of insects but has now been seen in human infections (Fig. 7B and 9D) (348–350). Infection with *Anncaliia algerae* has been reported to cause severe myositis in patients getting immunosuppressive medication for solid-organ transplantation and for rheumatoid arthritis and in a patient with graft-versus-host disease after hemopoietic stem cell transplantation (182, 350–354). *Anncaliia algerae* has also been reported to cause skin abscesses and infection of the false vocal cord in patients receiving chemotherapy for hematologic malignancies as well as myositis and keratitis in a man with no significant medical history (182, 350–354). *Anncaliia algerae* infection of the cornea has also been reported (355, 356), and this infection has been associated with endophthalmitis (356). *Anncaliia vesicularum* (previously called *Brachiola vesicularum*) has been reported to cause myositis in a patient with AIDS, and *Anncaliia connori* (previously called *Nosema connori*) was reported in a case of disseminated infection in an infant who had thymic dysplasia and malabsorption syndrome (42, 357, 358).

Vittaforma corneae

Vittaforma corneae was first described as *Nosema corneum* in a stromal biopsy specimen from a patient without HIV, and it is now recognized to be responsible for keratoconjunctivitis in healthy persons (with over 300 cases being reported associated with water or soil exposure) and HIV-infected patients (41, 100, 359–363). An outbreak of *Vittaforma corneae* has been associated with exposure to soil during a rugby tournament in Singapore (159, 196). *Vittaforma corneae* has also been seen in the urine in a patient with AIDS, indicating that it is capable of dissemination in immunocompromised hosts (100). This pathogen has also been reported as a cause of enteritis in both HIV-positive and HIV-negative patients from Portugal (364). Stromal keratitis due to microsporidia occurs less often than keratoconjunctivitis, is typically identified in immunocompetent individuals, and has been caused by infection with *Vittaforma corneae*, *Nosema ocularum*, and *Trachipleistophora* species (365). A case of urinary tract infection with prostatitis due to *Vittaforma corneae* has been reported in a patient with AIDS (366).

Other Species of Microsporidia

The genus *Trachipleistophora* was established for a microsporidium isolated from the skeletal muscle of an AIDS patient, and subsequently, more cases of myositis caused by *Trachipleistophora* spp. have been reported (47, 367–370). Disseminated infection due to *Trachipleistophora hominis* has been described in several patients with AIDS (47), and infection has been reported to cause myositis, keratoconjunctivitis, and sinusitis. *Trachipleistophora anthropopthera* infection has presented with myositis, encephalitis, and keratoconjunctivitis (48, 371, 372). *Pleistophora* sp. and *Pleistophora ronneafiei* infections presenting as myositis have occurred in patients with AIDS (368, 373–375). The presentation of *Pleistophora* myositis included myalgias, elevated serum creatine phosphokinase (CPK) and aldolase levels, weakness, and abnormal electromyograms consistent with an inflammatory myopathy. *Pleistophora* spp. have also been reported as a cause of myositis in patients who are HIV negative (368, 373). There are several cases of *Tubulinosema acridophagus* being reported to cause myositis and disseminated disease in immunosuppressed patients (49, 50, 376). *Endoreticulatus* spp. were reported from a patient with myositis with difficulty swallowing, muscle pain, and necrotizing granulomatous inflammation in the muscle tissue (1). *Microsporidium ceylonensis* and *Microsporidium africanum* were identified from an 11-year-old Tamil boy and a woman from Botswana, respectively, causing corneal ulcers and deep corneal stroma infection (the genus *Microsporidium* is used for unclassified microsporidia) (349). *Nosema ocularum* (377), *Nosema corneum* (now *V. corneae*) and *Trachipleistophora hominis* have been described as the causes of corneal stromal microsporidiosis in humans (41, 92, 362, 377, 378).

DIAGNOSIS

During the past 20 years, with the recognition of microsporidia as opportunistic pathogens, the diagnostic procedures for their detection has dramatically improved (379). With these improved techniques, there has been increasing recognition of microsporidial infection in immunocompetent humans (151, 161, 343, 351). Species-level identification of microsporidia requires ultrastructural examination (e.g., electron microscopy) or molecular techniques (e.g., a species-specific PCR assay). Serologic tests for microsporidiosis have been developed for research purposes and have been used to estimate prevalence of infection in epidemiologic studies. Serologic tests, however, are not useful for the diagnosis of active microsporidiosis. Isolation of microsporidia using tissue culture has not been useful for routine clinical diagnosis; however, it should be pursued if a novel microsporidium is identified in a clinical sample.

Light Microscopy

Examination of stool specimens by light microscopy is the standard technique for diagnosing gastrointestinal microsporidiosis. Since renal involvement with shedding of spores in the urine is common in microsporidia that disseminate, urine specimens should be obtained whenever the diagnosis of microsporidiosis is being considered. This has therapeutic implications, as microsporidia that disseminate (e.g., Encephalitozoonidae)
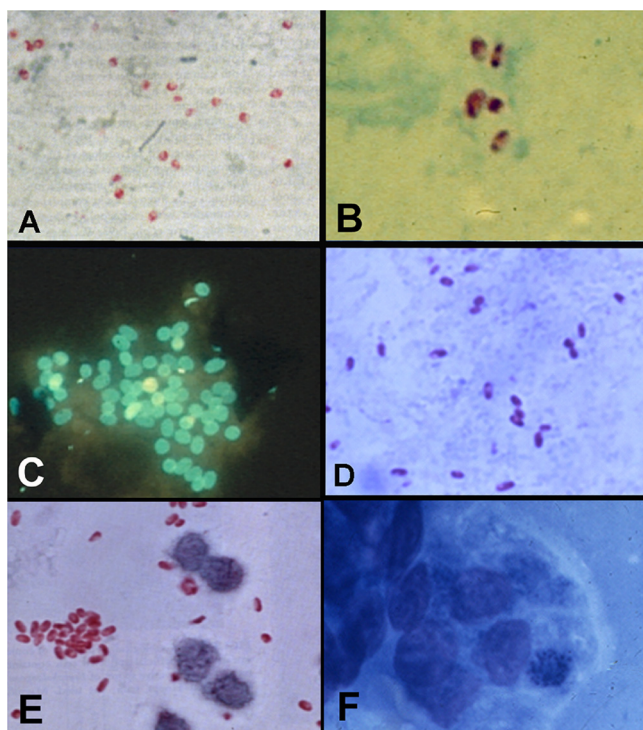
FIG 10 Demonstration of microsporidia in stool, urine, and other clinical specimens. (A and B) Chromotrope stains of stool specimens from patients with AIDS and diarrhea demonstrating spores of *Enterocytozoon bieneusi* measuring 0.7 to 1.0 by 1.1 to 1.6 μm. Spores have a pink to reddish hue with this stain. Stained spores can have a safety pin appearance as well. (Reproduced from reference 386 with permission.) (C) Conjunctival scraping from a patient with *Encephalitozoon hellem* keratoconjunctivitis stained with a chemifluorescent brightening agent (that stains chitin) demonstrating microsporidian spores. (D) Stool specimen from a patient with AIDS and *Encephalitozoon intestinalis* infection stained with a quick hot Gram-chromotrope stain demonstrating spores of 1.0 to 1.2 by 2.0 to 2.5 μm. The spores have a violet hue with this stain. (Reproduced from reference 455 with permission.) (E) Chromotrope stain of urine sediment from a patient with HIV infection who had a disseminated *Encephalitozoon cuniculi* infection demonstrating pink- to red-colored spores that are 1.0 to 1.5 by 2.0 to 3.0 μm. (Courtesy of Elizabeth Didier; reproduced with permission.) (F) Methylene blue-azure II-fuchsin stain of a touch preparation of an intestinal biopsy specimen (obtained by endoscopy) from a patient with intestinal *Enterocytozoon bieneusi* infection demonstrating intracellular spores.

are sensitive to albendazole, whereas those that do not disseminate (e.g., *Enterocytozoon bieneusi*) are resistant.

There are several methods available for the detection of microsporidia (219, 380) (Fig. 10). Examination of body fluids (e.g., urine, respiratory specimens, duodenal aspirates, cerebrospinal fluid) or histologic examination of biopsy specimens by light microscopy is one of the most common ways for the diagnosis of microsporidial infection, but this method cannot confirm the species of microsporidia. In contrast to stool, it is easier to identify microsporidian spores in body fluids because of the absence of bacteria and debris that could be confused with microsporidian spores. Demonstration of microsporidia by light microscopy is accomplished by the use of stains that produce differential contrast between the spores of microsporidia and the cells and debris in clinical samples. As spores are 1 to 3 μm in size magnification, a $\times$60 to $\times$100 objective is required for their visualization. The proteinaceous exospore and the chitinous endospore of microsporidia make it possible to stain spores by different methods, such as Giemsa, Gram, and trichrome stains and fluorescent dyes (i.e., Uvitex 2B and calcofluor white). Giemsa and Gram stains, however, while useful in histology, are not useful for stool examination, as these methods cannot distinguish between microsporidia and other similarly sized elements present in stool (381). Chromotrope 2R stains and fluorescent stains have markedly improved detection of microsporidia in body fluids and stool samples (219) (Fig. 10). The chromotrope 2R staining technique described by Weber et al. is based on a modification of routine trichrome staining using a 10-fold-higher concentration of chromotrope 2R

and a longer staining time (382) (Fig. 10A, B, and E). Modifications of this staining method, which are preferred by some laboratories, have been described by Ryan and coworkers (383) (using aniline blue in place of fast green) and by Kokoskin and colleagues (384) (using a higher temperature). Spores stained by chromotrope 2R, against a green (Weber stain) or blue (Ryan stain) background, appear as ovoid, light pink structures that are usually 1 to 3 μm in size, and these spores display a belt-like stripe girding them diagonally and equatorially (382) (Fig. 10B). The chromotrope 2R stains are time-consuming procedures, and the hot Gram-chromotrope technique which stains microsporidian spores dark violet in a much shorter period time is preferred by some laboratories (385) (Fig. 10D). Fluorescent stains that bind to chitin in the spore wall of microsporidia, such as calcofluor white M2R, Uvitex 2B, Fungi-Fluor, Fungiqual A, Cellufluor, and other chemofluorescent optical brighteners, have also been useful for visualizing microsporidian spores in various clinical samples (386) (Fig. 10C). These stains are easy and quick, but these stains also stain fungi and other fecal elements (387). Microsporidia can be distinguished from yeast cells by their size and lack of budding. Both chromotrope 2R and chemifluorescent brightening stains have demonstrated excellent sensitivity (100%) and specificity (100% for chromotrope 2R and 90 to 100% for chemifluorescent stains) (387–389). The limit of detecting microsporidia by these techniques appears to be 50,000 organisms/ml. Microsporidian spores in food can give a false-positive result; however, despite the fact that microsporidia are common in the environment, this has not been a significant problem in diagnosis. Neither the chromotrope nor chemifluorescent stain provide microsporidian species-specific information.

Histologically, microsporidian spores can be identified in routine fixed tissue by using several standard stains, including a modified tissue chromotrope 2R, tissue Gram stain (Brown-Hopps or Brown-Brenn), or Luna stain (390) (Fig. 7). If microsporidia are suspected, then a sample of the biopsy or autopsy material should also be put into electron microscopy fixative, as the definitive classification of a microsporidian species depends on ultrastructural information. Since microsporidian infections often involve mucosa or epithelium, cytological preparations are especially useful for diagnosis. In cases of microsporidian keratitis, microscopic examination material obtained by gently rubbing the conjunctiva and cornea with a tissue swab will demonstrate microsporidia within the epithelial cells (Fig. 10C). Monoclonal antibodies to *Encephalitozoon hellem* (121) *Encephalitozoon intestinalis* (380), and *Enterocytozoon bieneusi* (391, 392) have been developed and used for immunofluorescence or other immunostaining techniques to detect microsporidia in tissue sections, stool, and other body fluids. Commercial kits for the detection of microsporidia in environmental samples that utilize antibodies to Encephalitozoonidae and *Enterocytozoon bieneusi* are available.

Electron Microscopy

Electron microscopy is the "gold standard" for diagnostic confirmation and species identification of microsporidia (386). While mature spores seen in body fluids or stool samples can provide important ultrastructural data for species identification, definitive identification requires examination of the developmental life cycle seen in tissue samples (219) (Fig. 9). In general, it is not used for routine diagnosis, as electron microscopy is less sensitive than routine microscopy or molecular techniques, requires specialized equipment, is very labor-intensive, and is time-consuming (393). Molecular techniques can also be used to identify the exact species of microsporidia that are known to cause human infections and are much more sensitive for diagnosis (394). Electron microscopy is, however, very important when a novel microsporidium is suspected as causing an infection, as along with sequencing the rRNA gene of such organisms, the ultrastructural data provided by electron microscopy enables classification of such novel microsporidia.

Molecular Biology-Based Techniques

The successfully sequencing of various microsporidial genomes has led to the application of molecular biology-based techniques for the diagnosis of microsporidial infections in humans (219). Weiss and Vossbrinck (25) and Ghosh and Weiss (395) have reviewed molecular diagnostic tests for microsporidiosis. The detection of microsporidia using molecular-based methods such as PCR and *in situ* hybridization provides sensitive and specific

methods for species identification (219, 396). PCR-based tests using single and nested primer pairs of the small subunit rRNA (SSU rRNA), large subunit rRNA (LSU rRNA), and the intergenic spacer region have been described for the detection of different species of microsporidia in humans (219, 397). PCR-based tests have been used with both tissue biopsy specimens and body fluids, including stool samples (219, 395, 398–400). Nucleic acids are isolated from clinical specimens by mechanical disruption of the microsporidial spores in clinical specimens combined with the use of lyticase and/or chitinase to increase the efficiency of DNA extraction (219, 401). PCR-based methods have been shown to be able to detect 100 to 1,000 spores/ml in clinical samples (402, 403). In a study comparing real-time PCR and traditional microscopy for three species of microsporidia, the real-time PCR method was found to be three times more likely to detect the presence of spores in samples (402). *In situ* hybridization using rRNA probes has also been used to identify microsporidia to the species level in clinical samples and can be useful in examining tissue specimens (396, 404, 405). However, *in situ* hybridization is not widely used for routine clinical diagnosis, as it is less sensitive than PCR, is time-consuming, and requires significant expertise to perform (395).

THERAPY

Microsporidiosis is often seen in immunocompromised hosts, especially in patients with advanced AIDS and CD4$^+$ cell counts lower than 50/μl. Improved immune function can result in a clinical response in patients with gastrointestinal microsporidiosis leading to elimination of the organism and normalization of the intestinal architecture (406–410) (Table 4). Based on these observations, the institution of effective cART is important as part of the primary treatment of microsporidiosis in the setting of AIDS. Immune reconstitution syndrome (IRIS), while reported, has not generally been a problem in the setting of microsporidiosis (411). Interestingly, relapse of microsporidiosis can occur if there is a decline in immune function and falling CD4$^+$ counts, suggesting that latent infection is occurring. To this end, latent infection has been observed in immunodeficient mice, where albendazole treatment has not been able to eliminate microsporidia and relapse is seen. Costa and Weiss (412) have reviewed the drugs used for the treatment of microsporidiosis in humans and animals.

Based on studies *in vitro* and *in vivo* in animal models as well as clinical data on microsporidiosis in humans, albendazole (which inhibits β-tubulin) and fumagillin (which inhibits methionine aminopeptidase type 2) have the most consistent activity against the microsporidia that cause human infections (118, 312, 335, 357, 413–418) (Table 4). Albendazole is highly active *in vitro* against *Encephalitozoon hellem*, *Encephalitozoon cuniculi*, and *Encephalitozoon intestinalis* (357, 419), their β-tubulins demonstrate an amino acid sequence that is known to be associated with sensitivity to benzimidazoles (420), and numerous reports demonstrate the efficacy of 2 to 4 weeks of albendazole at 400 mg twice daily for these infections (118, 335, 417, 418). Other benzimidazole derivatives can also inhibit the growth of Encephalitozoonidae *in vitro* (421). The sequences of both *Enterocytozoon* (422) and *Vittaforma* β-tubulin contain amino acid residues associated with albendazole resistance consistent with the observation that albendazole is less effective for the treatment of *Enterocytozoon bieneusi* infection (423). Albendazole has been effective in patients with microsporidiosis due to *Trachipleistophora hominis* and in patients with infections due to *Anncaliia* spp. (42, 47, 350, 354).

Fumagillin has been used for the treatment of microsporidiosis in various types of fish as well as the treatment of honeybees infected with *Nosema apis* (412, 424). Microsporidia, unlike most eukaryotes, are dependent on methionine aminopeptidase type 2, as they lack aminopeptidase type 1. Fumagillin and its semisynthetic analogues have *in vitro* and *in vivo* activity against the Encephalitozoonidae and *Vittaforma corneae* and have been used to treat ocular microsporidiosis (312, 357, 413–416). Fumagillin (20 mg three times a day) has been effective for the treatment of *Enterocytozoon bieneusi* gastrointestinal infections in patients with AIDS and in patients with various organ transplants (413, 424–427). The main limiting toxicity of fumagillin has been reversible thrombocytopenia.

There are several other medications that have been reported as treatments for microsporidiosis. Atovaquone has shown variable results against microsporidia (350,

TABLE 4 Therapy of microsporidiosis

Organism	Therapy	Dosage[a]
All microsporidia	Immune reconstitution (cART in HIV with a goal of a CD4 count greater than 100 cells/μl) is associated with resolution of symptoms of enteric microsporidiosis. Severe dehydration, malnutrition, and wasting syndrome should be managed by fluid support and nutritional supplementation. Diarrhea can be controlled by antimotility agents as needed.	
Enterocytozoon bieneusi	No current effective commercial therapy. Fumagillin[b] (oral form has been effective in a clinical trial and is available on expanded access). Alternatives: Nitazoxanide at 1,000 mg BID with food for 60 days has shown efficacy in anecdotal cases. Albendazole has some limited efficacy; however, the sequence of *Enterocytozoon bieneusi* contains amino acids associated with resistance to this class of drugs. In general, medications have also been less effective in patients with low CD4 counts.	20 mg TID (i.e., 60 mg/day)
Encephalitozoonidae (systemic infections); *E. cuniculi*, *E. hellem*, and *E. intestinalis*	Albendazole	400 mg BID
Encephalitozoonidae keratoconjunctivitis	Fumagillin solution (70 μg/ml)[c]. Patients may also need albendazole if systemic infection is present.	2 drops every 2 h for 4 days and then 2 drops 4 times a day
Vittaforma corneae keratoconjunctivitis	No defined therapy. Fumagillin solution has had activity. Topical fluoroquinolones and itraconazole have been used in case reports. The sequence of *V. cornea* tubulin contains amino acids associated with resistance to albendazole and related agents in this class of drugs.	
Trachipleistophora hominis	Albendazole	400 mg BID
Anncaliia spp.	Albendazole In a reported case of *Anncaliia vesicularum*, itraconazole (400 mg BID) was added to albendazole	400 mg BID
Tubulinosema spp.	No defined therapy. There was no response to albendazole in published cases. Fumagillin is a reasonable therapeutic choice.	
Enoreticulatus spp.	Albendazole	400 mg BID

[a]The duration of treatment for microsporidiosis has not been established. Relapse of infection has occurred on stopping therapy. Patients should be maintained on treatment for at least 4 weeks. If immunosuppression persists (e.g., CD4 count less than 200 cells/μl), then consideration should be given to continuing therapy. TID, three times a day; BID, twice a day.
[b]Fumagillin is available from Sanofi in France as Flisint 20-mg capsules and has been obtained for patients in the United States (contact https://app.smartsheet.com/b/form/4dea2fbff8c449f095ce5f335b9c7881).
[c]Topical fumagillin solution must made by a compounding pharmacy, as it is not commercially available in the United States. This solution can be made by using fumagillin bicylohexylammonium (Fumidil B) at 3 mg/ml in sterile saline (fumagillin, 70 μg/ml).

414, 428). Furazolidone has been shown to have transient efficacy for *Enterocytozoon bieneusi* (429). Itraconazole has been used in the treatment of microsporidial stromal keratitis, sinusitis, and keratoconjunctivitis in both immunocompetent and immuno-compromised patients (72, 430–432). While some early case reports suggested that metronidazole was effective for *Enterocytozoon bieneusi,* the majority of data have shown that this drug is not effective (71, 312, 433). Nitazoxanide has been effective in a few cases of *Enterocytozoon bieneusi* infection in patients with AIDS (434–436), and unpublished anecdotal experience exists for its successful use in the treatment of this infection in transplant patients. Thalidomide, an anti-tumor necrosis factor alpha (TNF-α) drug, has been effective in decreasing diarrhea in 50% of patients with gastrointestinal microsporidiosis, but on biopsy, the organisms were still present in responding patients (437).

Several potential new therapeutic approaches for the treatment of microsporidiosis have been published. Novel synthetic polyamines have been shown to be effective in the treatment of experimental microsporidiosis (438). The process of spore germination is cal-cium dependent; to this end, calmodulin inhibitors (trifluoperazine and chlorpromazine) and calcium antagonists (verapamil and lanthanum) both prevented spore germination in *Spraguea lophii* (439). Nifedipine has been demonstrated to inhibit the spore germination of *Encephalitozoon intestinalis* and *Encephalitozoon hellem* (71, 72). The endospore of micro-sporidia contains mainly chitin and proteins, which is important for extracellular survival and infection of microsporidia (419). Chitin synthesis inhibitors such as polyoxins, nikkomycins, and lufenuron have shown some efficacy against microsporidia (440–442). A topoisomerase

IV subunit (part C) was identified in the *Vittaforma corneae* genome, and fluoroquinolones have been shown *in vitro* to inhibit both *Vittaforma corneae* and *Encephalitozoon intestinalis* (443). Triosephosphate isomerase (TIM) plays a crucial role in glycolysis of *Encephalitozoon intestinalis*, and its inhibition by three different sulfhydryl reagents {MTSES [methanethiosulfonate ethylsulfonate], MMTS [methyl methanethiosulfonate], and DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)]} inhibited microsporidian energy use (444).

PREVENTION

The presence of infective spores in bodily fluids indicates that safety measures when handling body fluids in clinical settings and personal hygiene measures should help prevent infection. Common-sense approaches include drinking boiled water, washing hands, eating thoroughly cooked meat, and carefully washing vegetables and fruits before ingestion (127, 133, 140, 200, 445). Spores can be killed in the environment by exposure to 70% ethanol, phenolic derivatives, formaldehyde, 1% hydrogen peroxide, sodium hydroxide, chloramine, or amphoteric surfactants for 30 min at 22°C and autoclaving at 120°C for 10 min or boiling for 5 min (197, 446). No prophylactic agents that prevent microsporidiosis have been identified. Patients have developed microsporidiosis while on trimethoprim-sulfamethoxazole prophylaxis (447), and infection has been reported in patients receiving dapsone, atovaquone, azithromycin, itraconazole, or pyrimethamine. The most effective preventative measure is restoration of immune function in immunocompromised hosts. Based on the epidemiology of these infections, screening close contacts of index cases for infection and/or carriage is rational.

CONCLUSIONS

The first clearly defined case of human infection with microsporidia was a 9-year-old Japanese boy with disseminated *Encephalitozoon* infection who suffered from recurrent fever, vomiting, headache, and spastic convulsions in 1959 (34, 448). Microsporidiosis was rarely reported until the advent of the AIDS epidemic and the subsequent identification of *Enterocytozoon bieneusi* gastrointestinal infections in patients with AIDS, chronic diarrhea, and wasting syndrome (44, 449). Since that initial description, microsporidia have been recognized as opportunistic pathogens in patients with various type of immune suppression as well as in immunocompetent hosts (136, 151, 161, 450–452). There are about 10 genera and 17 species of microsporidia associated with human infection, and infections have occurred in almost all organ systems. There are data to suggest that latent infection can occur in humans, and the importance of this during immune suppression remains to be elucidated. Observations indicate that environmental exposure to microsporidia is high and that asymptomatic infection is common. Many of the microsporidia that are human pathogens are probably zoonotic infections that are either water- or foodborne. More research is needed on the natural reservoirs and environmental sources of microsporidia as well as improved therapeutic agents and prevention strategies to avoid transmission. Furthermore, additional studies are needed to more fully understand the molecular pathogenesis of microsporidiosis.

ACKNOWLEDGMENTS

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