MICROSPORA IN TETRAPODS (mammals, birds, reptiles, amphibia)

Encephalitozoon, Anncaliia, Nosema, Vittaforma, Enterocytozoon, Trachipleistophora et al.

Overview

Microsporidia are obligate intracellular parasites which lack mitochondria and form small unicellular spores. They were long considered to be a primitive basal group of Protista but molecular phylogenetic studies have revealed many similarities in biochemical pathways and structural components to fungi, where they are now classified. Numerous species have been described in invertebrates (especially insects) and lower (rarely higher) vertebrates. The parasites proliferate in host tissues by merogony (asexual division) followed by sporogony (often involving plasmotomy prior to sporoblastogenesis). Developmental stages may be monokaryotic (single nucleus) or diplokaryotic (paired nuclei) and sporonts may be surrounded by a membranous sporophorous vesicle (pansporoblast) or lie free in the host cell cytoplasm. All spores contain a unique coiled polar tube which can be extruded to inject the infective sporoplasm into host cells. Infections may be disseminated throughout host tissues or they may cause focal lesions and inflammation involving cysts, granulomas or tumour-like xenomas (enlarged host cells). Various species are found in insects (some causing dysentery in honey bees), crustaceans (some denaturing muscles), fish (some forming lesions or deformities) and even humans (some causing diarrhoea, myositis, encephalitis or corneal lesions).

Classification:

Domain:	Eukaryota (membrane-bound nucleus)
Supergroup:	Amorphea (unikonts with single flagellum, or nonflagellated amoebae)
Kingdom:	Fungi (with chitinous walls, includes microsporidia)
Division:	Microsporidia (form unicellular spores, with coiled polar tubes, amitochondriate, all parasitic)
Class:	Microsporea (polar filament well-formed, oval spores)
Order:	Microsporida (polaroplast present)
Suborder:	Pansporoblastina (sporophorous vesicle present)
Family:	Glugeidae (monokaryotic, variable but large number spores produced)
Genus:	*Pleistophora/Trachipleistophora* (parasitic in tissues of mammals)
Suborder:	Apansporoblastina (sporophorous vesicle absent)
Family:	Unikaryonidae (all stages monokaryotic, in cell cytoplasm or in parasitophorous vacuole)
Genus:	*Encephalitozoon* (parasitic in tissues of mammals)
Family:	Nosematidae (diplokaryotic, diplosporoblastic, monomorphic)
Genus:	*Anncaliia/Nosema/Vittaforma* (parasitic in tissues of mammals)
Family:	Enterocytozoonidae (monokaryotic, sporogony with formation of electron-dense discs
Genus:	*Enterocytozoon* (parasitic in tissues of mammals)
Species:	various species cause microsporidiosis in tissues of mammals

Parasite biodiversity and host range: Microsporidia possess a remarkable apomorphic adaptation to life as intracellular parasites, their unicellular spores have polar tubes coiled up inside which can be forcibly everted to penetrate host cells and inject their infective germs (sporoplasm). Microsporidia have reduced cellular complexity, they have small genomes, they are amitochondriate and they have metabolomes partway between 'prokaryotes' (archae- and eu-bacteria) and eukaryotes (nucleated cells). They were long considered to be a primitive basal group of Protista but molecular phylogenetic studies have revealed many similarities in nuclear genes, biochemical pathways and structural components to fungi, where they are now classified.

The systematics of microsporidia has progressed over decades from phenotypic classifications based on spore morphology, developmental cycles and host range to genotypic classifications based on comparative gene sequences. Classical studies divided the microsporidia into those with or without an envelope (sporophorous vesicle) around sporoblasts (Pansporoblastina and Apansporoblastina respectively) and recognized families on the basis of vegetative growth, genera on the process of spore formation and species on the basis of spore morphology. The subsequent inclusion of ultrastructural features led to the recognition of three major assemblages: 'primitive' groups with rudimentary polar tubes and no polaroplasts; 'intermediate' groups with short polar tubes and rudimentary polaroplasts; and 'higher' groups with well-developed polar tubes and polaroplasts. Families continued to be identified on the basis of type of reproduction (merogony and sporogony) and nuclear condition (mono- or diplo-karyotic). More recently, chromosome cycles were used to separate microsporidia into Dihaplophasea (diplokaryon in some phase of life-cycle) and Haplophasea (unpaired nuclei in all life-cycle stages). It is thought that the developmental cycle of Dihaplophasea involves a pairing of gametes which proliferate and undergo haplosis either by meiosis (order Meiodihaplophasida) or by nuclear dissociation (order Dissociodihaplophasida), whereas the development of Haplophasea is entirely haplophasic. Molecular phylogenetic studies, however, have not provided good support for conventional classifications but have separated

representative species into three major clades mostly correlated with host habitat: including the Aquasporidia (in freshwater insects, crustaceans and bryozoans), Marinosporidia (in marine fish and crustaceans) and Terresporidia (in terrestrial insects and vertebrates). Although many taxa have yet to be analysed and classified, most clades are polyphyletic with conventional genera split within and between clades. It is not known whether this reflects an evolutionary history of parasites switching hosts or habitats, or even hosts switching habitats: events quite likely to have occurred considering that many invertebrate hosts have aquatic origins and many have retained aquatic stages in their life-cycles. Further studies are required to reconcile phenotypic and genotypic classifications.

Numerous microsporidian species have been described in invertebrates (especially insects and crustaceans) and lower (rarely higher) vertebrates. Over 1,400 species belonging to ~200 genera have been described; with some 800 species in 109 genera infecting insects, 400 species in 62 genera infecting crustaceans, 120 species in 26 genera infecting fish, 17 species in 11 genera infecting mammals, 5 species in 3 genera infecting birds, reptiles and amphibians, 40 species in 27 genera infecting invertebrates (8 genera in annelids, 7 in arachnids, 4 in molluscs, 4 in bryozoans, 2 in ciliates, one in helminths and one in kinorhynchs) and another 20 species in 14 genera being hyperparasitic in other parasites (8 genera in gregarines, 3 in trematodes, 2 in myxozoans and one in mesozoa). The key characteristics of the microsporidian genera are tabulated below:

Microsporidian genera	**Hosts**	**No. nuclei (meronts, spores)** [1= monokaryotic, 2 = diplokaryotic, 1-2 = both]	**Spore types** [1 = mono-morphic, 2 = di-morphic, 3 = poly-morphic]	**Intracellular location** [CY = cytoplasm, NU = nucleoplasm, PV = parasitophorous vacuole, SP = sporophorocyst, SV = sporophorous vesicle]	**Spores per SV** [n = numerous, na = not applicable]	**Xenoma formation**
Abelspora	decapoda	1,1	1	PV	2	xenoma?
Aedispora	diptera	1-2,1-2	2	PV, SV	2-8	absent
Agglomerata	branchiopoda	1,1	1	SV	8-32	absent
Agmasoma	decapoda	2,1	?	SV	8	absent
Alfvenia	copepoda, maxillopoda	2,1	1	SV	1-2	absent
Alloglugea	anura	1,1	1	PV	na	xenoma
Amazonspora	fish	1,1	1	CY	na	xenoma
Amblyospora	copepoda, amphipoda, maxillopoda, diptera	2,1-2	3	SV	8	absent
Ameson	decapoda, diptera	2,1	1	CY	na	absent
Amphiacantha	hyperparasitic in gregarines in polychaetes	1,1	1	SV	n	absent
Amphiamblys	hyperparasitic in gregarines in polychaetes	1,1	1	SV	32-50	absent
Andreanna	diptera	2,2	1	PV, SV	8	absent
Anisofilariata	diptera	1,1	1	SV	2-16	absent
Anncaliia	diptera, coleoptera, humans	2,2	1	CY	na	absent
Antonospora	hymenoptera, psocoptera	?,2	1	CY	na	absent
Areospora	decapoda	1,1	1	SV	8	xenoma
Auraspora	collembola	2,1-2	2	CY, SV	n	absent
Bacillidium	oligochaeta, thysanura, diptera	2,2	1	CY	na	absent
Baculea	branchiopoda	1,1	1	PV, SV	n	absent
Becnelia	heteroptera	1,1	2	SV	8	absent
Berwaldia	branchiopoda	1,1	1	SV	1	absent
Binucleata	branchiopoda	1-2,1	1	SV	8	absent
Binucleospora	ostracoda	2,2	1	PV	na	absent
Bohuslavia	diptera	2,1	1	SV	8-16	absent
Brachiola	diptera, humans	2,2	1	CY	na	absent
Bryonosema	bryozoa	2,2	1	CY	na	absent
Burkea	oligochaete	1,1	1	PV	na	absent
Burunella	hymenoptera	1-2,1-2	2	PV, SV	8	absent
Buxtehudea	thysanura	1,1	1	PV	na	absent
Campanulospora	diptera	2,2	1	PV	na	absent

Canningia	coleoptera	1,1	1	CY	na	absent
Caudospora	diptera	2,2	1	CY	na	absent
Chapmanium	decapoda, diptera	2,1		SV	8	absent
Chytridiopsis	coleoptera	1,1	1	PV, SV	n	absent
Ciliatosporidium	ciliophora	1,1	1	CY	na	absent
Coccospora	diptera	2,1	1	SV	8	absent
Cougourdella	copepoda, maxillopoda, diptera	1,1	1	SV	4	absent
Crepidulospora	diptera	1,1	1	CY	na	absent
Crispospora	diptera	?,1-2	2	PV	na	absent
Cristulospora	diptera	2,1-2	2	CY, SV	8	absent
Cryptosporina	acari	1,1-2	1	SV	8	absent
Cucumispora	amphipoda	2,2	1	CY	na	absent
Culicospora	diptera	1-2,1-2	2	PV, SV	2-8	absent
Culicosporella	diptera	1-2,1-2	3	PV, SV	2-8	absent
Cylindrospora	diptera	2,1	1	PV, SV	8	absent
Cystosporogenes	lepidoptera	1,1	1	SV?	<60	absent
Dasyatispora	elasmobranch	1,1	1	SV	n	absent
Desmozoon	maxillopoda, copepoda, fish	1-2,1	1	CY	na	absent
Desportesia	hyperparasitic in gregarine in echiurid	1,1	1	CY, SV	32	absent
Dictyocoela	amphipod	2,1-2	1	SV	8	absent
Dimeiospora	diptera	1,1	2	SV	8	absent
Duboscqia	branchiopoda, copepoda, isoptera, diptera	1,1	1	SV	16	absent
Edhazardia	diptera	2,1-2	3	PV, SV	1-8	absent
Encephalitozoon	maxillopoda, orthoptera, acari, birds, mammals (incl. humans)	1,1	1	PV	na	absent
Endoreticulatus	lepidoptera, coleoptera, decapoda	1,1	1	PV	na	absent
Enterocytozoon	mammals (inc. humans), fish	1,1	1	CY	na	absent
Enterospora	decapoda, fish	2,2	1	CY, NU	na	absent
Episeptum	trichoptera	1,1	1	SV	4	absent
Euplotespora	ciliophora	1,1-2	1	SV	1	absent
Evlachovaia	diptera	2,1-2	2	PV, SV	2	absent
Facilispora	maxillopoda, copepoda	1,1	1	CY	na	absent
Fibrillanosema	amphipoda, branchiopoda	1,1	1	CY	na	absent
Flabelliforma	copepoda, ostracoda, cladocera, diptera	1,1	1	CY, SV	n	absent
Geussia	hyperparasitic in gregarine of ephemeroptera	?		SV	6-8	absent
Glugea	amphipoda, fish	1,1	1	SV	n	xenoma
Glugoides	branchiopoda	1,1	1	PV, SV	16	absent
Golbergia	diptera	2,1-2	2	CY	na	absent
Gurleya	branchiopoda, copepoda, decapoda, cladocera, diptera, ephemeroptera, isoptera, lepidoptera, odonata, trichoptera	1,1	1	SV	4	absent
Gurleyides	branchiopoda	?	2	SV	1,4	absent
Hamiltosporidium	branchiopoda	1,2	2	SV	8	absent
Hazardia	diptera	1-2,1-2	2	CY	na	absent
Helmichia	diptera	2,1-2	1	SV?	8	absent
Hepatospora	decapoda	1,1	1	PV	na	absent
Hessea	diptera	2,1-2	1	SV	n	absent
Heterosporis	fish, seasnakes	1,1	3	SP, SV	n	absent

Heterovesicula	orthoptera	2,1-2	2	SV	8-n	absent
Hirsutosporos	diptera	2,2	1	CY	na	absent
Holobispora	copepoda, maxillopoda	?,1	1	CY	na	absent
Hrabyeia	oligochaeta	2,2	1	CY	na	absent
Hyalinocysta	diptera, copepoda	2,1	1	SV	8	absent
Ichthyosporidium	fish	2,2	1	PV	na	xenoma
Inodosporus	decapoda	2,1	1	SV	8	absent
Intexta	acari	1,1	2	PV	na	absent
Intrapredatorus	diptera	2,1-2	3	SV	8	absent
Issia	trichoptera, diptera	2,2	2	SV?	2	absent
Janacekia	diptera, coleoptera	2,1-2	1	SV	1	absent
Jirovecia	fish, oligochaete	2,2	1	CY, PV?	na	xenoma
Jiroveciana	oligochaete	1,1	1	PV	na	absent
Johenrea	orthoptera	1,1	1	SV	8,16	xenoma
Kabatana	fish	1,1	1	CY	na	absent
Kneallhazia	hymenoptera	1-2,1	1	SV	8	absent
Kinorhynchospora	kinorhyncha	1,1	2	SV	n	absent
Krishtalia	diptera	2,1-2	2	CY	na	absent
Lanatospora	branchiopoda, maxillopoda, copepoda	1,1	1	SV	6-16	absent
Larssonia	branchiopoda	1,1	1	SV	4-32	absent
Larssoniella	lepidoptera	1,1	1	CY	na	absent
Liebermannia	orthoptera	2,2	1	PV	na	absent
Loma	fish	1,1	1	SV	4	xenoma
Marssoniella	maxillopoda	1,1	2	SV	4-8	absent
Merocinta	diptera	2,1-2	2	PV	na	absent
Metchnikovella	hyperparasitic in gregarine in polychaete	1,1	1	PV, SV	8-32	absent
Microfilum	fish	1,1	1	CY	na	xenoma
Microgemma	fish	1,1	1	PV	na	xenoma
Microsporidium (often used for *species inquirenda, incertae sedis*)	branchiopoda, copepoda, cirripedia, isopoda, amphipoda, mollusca, insecta, fish, mammals (incl. humans)	?	?	?	?	?
Mitoplistophora	ephemeroptera	1,1	1	PV	na	absent
Mockfordia	psocoptera	?,1	1	PV	na	absent
Mrazeckia	isopoda	2,2	1	CY	na	absent
Multilamina	isoptera, diptera	1,1	1	SV	1	absent
Myospora	decapoda	2,2	1	CY	na	absent
Nadelspora	decapoda	1,1	1	CY	na	absent
Napamichum	diptera, acari	2,1	1	SV	8	absent
Nelliemelba	copepoda, maxillopoda	1,1	1	SV	1	absent
Nematocida	nematode	1,1	1	PV	na	absent
Neoflabelliforma	oligochaete, hyperparasitic in myxozoa in oligochaete	1,1	1	SV?	?	absent
Neonosemoides	fish	2,1	1	CY	na	xenoma
Neoperezia	diptera	2,1	2	SV	2	absent
Nolleria	siphonoptera	1,1	1	PV, SV	n	absent
Norlevinea	branchiopoda	1,1	1	SV	4	absent
Nosema	branchiopoda, copepoda, decapoda, amphipoda, mollusca, hymenoptera, lepidoptera, acari, fish, mammals (incl. humans); hyperparasitic in myxozoa in fish, trematodes in snails, oysters, fish	2,2	2	CY	na	absent
Nosemoides	branchiopoda, fish,	1,1	1	CY	na	xenoma

	hyperparasitic in gregarine in nemertean					
Novothelohania	diptera	1,1	1	SV	8	absent
Nucleospora	fish	1,1	1	NU	na	absent
Nudispora	odonata	2,1	1	CY	na	absent
Obruspora	fish	1,1	1	CY	na	xenoma
Octosporea	branchiopoda, isopoda, amphipoda, cladocera, diptera, ephemeroptera, hemiptera, lepidoptera, collembola	2,2	1	SV	8	absent
Octotetraspora	diptera	2,1	1	SV	4,8	absent
Oligosporidium	acari, opiliones	1,1	2	CY	na	absent
Ordospora	branchiopoda	1,1	1	PV	na	absent
Ormieresia	decapoda	2,1	1	CY, SV	8	absent
Orthosomella	lepidoptera, coleoptera	1,1	1	CY	na	absent
Ovavesicula	coleoptera	2,1	1	CY, SV	32	absent
Ovipleistophora	fish, hyperparasitic in trematode in fish	1,1	2	CY, SV	na	absent
Pankovaia	ephemeroptera	1,1	11	CY, SV	1	absent
Paradoxium	decapoda	1-2,1	1	CY	na	absent
Paraepiseptum	trichoptera	1,1	1	SV	4	absent
Parahepatospora	decapod	1,1	1	PV	na	absent
Paranosema	coleoptera, orthoptera	1-2,2	1	CY	na	absent
Paranucleospora	maxillopoda, fish	1-2,1-2	2	CY, NU	na	absent
Parapleistophora	diptera	1,1	1	SV	48-64	absent
Parastempellia	diptera	2,1-2	2	SV	4,8,16	absent
Parathelohania	maxillopoda, diptera	2,1-2	2	SV	8	absent
Pegmatheca	diptera, tricoptera	2,1	1	SV	8	absent
Perezia	branchiopoda, decapoda, lepidoptera, coleoptera, hymenoptera, orthoptera, plus hyperparasitic in gregarine of tunicate	2,1	1	CY	na	absent
Pernicivesicula	diptera	2,1	1	SV	24-64	absent
Pilosporella	diptera	2,1-2	2	SV	8	absent
Pleistophora (Plistophora)	branchiopoda, copepoda, decapoda, blattaria, coleoptera, diptera, lepidoptera, orthoptera, mollusca, fish, mammals (incl. humans	1,1	2	SV	n	absent
Pleistophoridium	hyperparasitic in gregarine in ephemeroptera	1,1	1	CY, PV	na	absent
Polydispyrenia	diptera	2,1	2	PV, SV	n	absent
Potaspora	fish, decapod	1,1	1	CY	na	xenoma
Pseudoloma	fish	1,1	1	SV	16	atypical xenoma
Pseudonosema	bryozoa	2,2	1	CY	na	absent
Pseudopleistophora	lepidoptera, polychaete	2,2	1	PV, SV?	n	absent
Pulicispora	siphonoptera	1,1-2	1	SV	8,16,32	absent
Pyrotheca	copepoda, maxillopoda	1,1	1	CY, SV	4	absent
Rectispora	oligochaeta	2,2	1	CY	na	absent
Resiomeria	odonata	2,1	1	SV	8	absent
Ringueletium	diptera	2,2	1	CY	na	absent
Schroedera	bryozoa	1-2,2	1	CY	na	xenoma
Scipionospora	diptera	2,2	1	SV	4	absent
Semenovaia	diptera	?,1-2	2	CY	na	absent
Senoma	diptera	2,2	1	PV	na	absent
Septata	human	1,1	1	PV	na	absent
Simuliospora	diptera	2,1	2	SV	6,32	absent

Spherospora	diptera	2,1-2	2	SV	8-32	absent
Spiroglugea	diptera	?	1	SV?	8	absent
Spraguea	fish	1-2,1-2	2	CY	na	xenoma
Steinhausia	bivalve, gastropod	1,1	1	PV, NU	na	absent
Stempellia	copepoda, amphipoda, opiliones, ephemeroptera, diptera, coleoptera, isoptera	1,1	2	PV, SV	4	absent
Striatospora	diptera	2,1	1	SV	8	absent
Systenostrema	diptera	2,1-2	1?	SV	8	absent
Tabanispora	diptera	1-2,2	2	SV	1-10	absent
Takaokaspora	diptera	1-2,1-2	2	CY, SV	?	absent
Tardivesicula	trichoptera	1,1	1	SV	16-32	absent
Telomyxa	ephemeroptera, diptera, coleoptera	1,1	1	SV	2	absent
Tetramicra	fish	1,1(2?	1	PV	na	xenoma
Thelohania	branchiopoda, copepoda, decapoda, amphipoda, diptera, collembola, ephemeroptera, hemiptera, lepidoptera, hymenoptera, odonata, trichoptera, fish	1-2,1	1	SV	8	absent
Toxoglugea	branchiopoda, diptera, plecoptera, odonata, hemiptera, homoptera	2,1	1	SV	8	absent
Toxospora	diptera	?,1-2	1	SV	8	absent
Trachipleistophora	mammals (incl. human)	1,1	1	PV, SV	2-n	absent
Trichoctosporea	diptera	2,1	2	SV	8	absent
Trichodubosquia	ephemeroptera	2,1	1	SV	16-32	absent
Trichonosema	bryozoa	2,2	1	CY	na	absent
Trichotuzetia	copepoda, maxillopoda	1,1	1	SV	1	absent
Tricornia	diptera	2,1-2	1	SV	8	absent
Triwangia	decapoda	1,1	1	SV	n	xenoma
Tubulinosema	diptera, orthoptera, hymenoptera, coleoptera, mammals (humans)	2,2	1	CY	na	absent
Tuzetia	branchiopoda, copepoda, maxillopoda, ephemeroptera	1,1	1	SV	1	absent
Unikaryon	coleoptera, acari, hyperparasitic in trematode in bivalve	1,1	1	SV	2	absent
Vairimorpha	decapod, lepidoptera, hymenoptera, diptera	2,1-2	2	CY, SV	8	absent
Vavraia	ostracoda, decapoda, diptera, coleoptera, lepidoptera	1,1	1	SV	16-64	absent
Vittaforma	mammals (humans)	2,2	1	PV	na	absent
Weiseria	diptera	2,2	1	CY	na	absent
Wittmania	hyperparasitic in mesozoan in cephalopod	1-2,2	1	CY	na	absent
Zelenkaia	trichoptera	1,1	1	SV	2	absent

Although many microsporidian species have been described on the basis of presumed host specificity, recent molecular characterization studies have demonstrated that the host ranges for some species can be very broad, encompassing not only hosts from disparate taxa (e.g. insects and mammals) but also hosts from disparate environments (e.g. marine and terrestrial). While considerable work remains to determine the host ranges and phylogenetic affinities of most microsporidia, the following text considers microsporidia from one of three perspectives associated primarily, but not exclusively, with different host groups; namely, arthropods (mainly insects and crustaceans), fish (bony and cartilaginous), and tetrapods (mammals, birds, reptiles and amphibians). The host range suggests that microsporidia evolved as parasites of aquatic invertebrates, and then fish. A few species, however, have

become established in endothermic birds and mammals despite the temperature barrier and advanced immune defenses. This section considers the microsporidia of tetrapods, mostly mammals (including humans), some birds, several amphibia and a few reptiles.

In mammals, microsporidial infections have been detected worldwide, in rodents, lagomorphs, carnivores, artiodactyls, perissodactyls and primates, including humans. A growing number of clinical infections have been detected in humans, especially in immunocompromised individuals and children. Some infections in humans are similar to species found in animals (possible zoonoses) but some are unique and only found in humans (anthroponoses). To date, 15 species belonging to 9 diverse genera have been detected in human patients. Most species found in humans form monomorphic spores, except some *Nosema* and *Pleistophora* species. Infections involve both monokaryotic genera (pansporoblastic *Pleistophora* and *Trachipleistophora* as well as apansporoblastic *Encephalitozoon* and *Enterocytozoon*) and diplokaryotic genera (apansporoblastic *Nosema*, *Anncaliia*, *Vittaforma* and *Tubulinosema*), all infections being associated with focal lesions in specific tissues and sometimes systemic disseminated infections, but not xenoma formation. Molecular studies have identified several microsporidian clades with representatives infecting tetrapods as well as arthropods. Three clades of predominantly insect microsporidia have been associated with opportunistic infections in immunocompromised humans (clades *Vittaforma/Endoreticulatus/Cystosporogenes, Anncaliia/Tubulinosema* and *Trachipleistophora/Vavraia*) while another specialized clade is widely distributed among animals (*Enterocytozoon/Encephalitozoon*) causing enteric and/or disseminated infections in humans. Molecular characterisation studies have identified over 90 *Enterocytozoon bieneusi* genotypes; some associated with a single host species, others with two or more hosts. Several genotypes have been found to infect only humans, others only particular animal hosts, although a few infect both humans and animals (suggesting their zoonotic potential).

Parasite species	**Spore dimensions**	**Hosts**	**Location**	**Distribution**
Suborder: Pansporoblastina (sporophorous vesicle (SV) present)				
Family: Glugeidae (monokaryotic, variable but large number spores produced)				
Pleistophora ronneafiei	3.2-4 x 2-3 µm (10-12 coils)	Primates (human)	muscle	North America
Trachipleistophora hominis	4.0 x 2.4 µm (11 coils)	Primates (human), experimental infection in Rodentia (mice)	muscles, sinus, eye	Australia, Europe
Trachipleistophora anthropophthera	ma: 3.7 x 2.0 µm (9 coils) mi: 2.2- 2.5 x 1.4-2.0 µm (3-5 coils)	Primates (human)	disseminated	North America, Europe
Pleistophora myotrophica	3.0-6.7 x 1.9-3.0 µm	Anura (common toad, African spotted toad)	muscles	Europe, Africa
Pleistophora bufonis	nr	Anura (toad)	ovary	Africa
Alloglugea bufonis	1.3-1.6 x 0.6-0.8 µm (5-6 coils)	Anura (cane toad)	gut, liver, spleen, kidney	South America
Glugea danilewskyi	3-4 µm	Anura (grass frog), Serpentes (grass snake)	muscles, connective tissue	Europe
Family: Thelohaniidae (meronts usually diplokaryotic, spores monokaryotic, 8 spores in SV)				
Thelohania apodemi	4.2 x 2.3 µm (11 coils)	Rodentia (wood mouse, house mouse)	brain, muscle	Europe
Suborder: Apansporoblastina (sporophorous vesicle (SV) absent)				
Family: Unikaryonidae (monokaryotic, diplo- to poly-sporoblastic sporogony)				
Encephalitozoon cuniculi	2.5 x 1.5 µm (4-7 coils)	Primates (human, chimpanzee, western gorilla, mountain gorilla, squirrel monkey, titi, Goeldi's monkey, bonobo, tamarins, vervet, baboon, ring-tailed lemur), Lagomorpha (rabbit, cottontail, hare), Rodentia (brown rat, house mouse, wood mouse, field mouse, muskrat, guinea pig, hamster, lemming, common vole, water vole, grass vole), Eulipotyphla (shrew), carnivora (mink, dog, wild dog, blue fox, red fox, arctic fox, stone marten, otter, cat, polecat, meerkat, snow leopard, coati), Artiodactyla (sheep, goat, cattle, buffalo, duiker, alpaca, pig), Perissodactyla (horse), birds (Psittaciformes: mealy amazon, Tucuman amazon, red-crowned	brain, eye, kidney, gut, disseminated	cosmopolitan

		amazon, red-lored amazon, bronze-winged parrot, scaly-headed parrot, dusky parrot, red-fan parrot, Senegal parrot, African grey parrot, turquoise parrot, eclectus parrot, crimson rosella, green rosella, eastern rosella, budgerigar, Major Mitchell cockatoo, Solomon's cockatoo, monk parakeet, red-crowned parakeet, rose-ringed parakeet, green-cheeked parakeet, cockateil, Australian ringneck, Fischer's lovebird, rosy-faced lovebird, yellow-collared lovebird, blue-streaked lory, chestnut-fronted macaw, red-shouldered macaw; Passeriformes: red-billed firefinch, zebra finch, Brahminy starling, canary; Suliformes: great cormorant; Podicipediformes: crested grebe; Ciconiiformes: white stork; Falconiformes: gyrfalcon)		
Encephalitozoon hellem	1.5-2.5 x 1 µm (4-9 coils)	Primates (human, chimpanzee, western gorilla, moustached monkey, agile mangabey, bonobo), Carnivora (dog, coati), Rodentia (house mouse, wood mouse, field mouse, grass vole), Chiroptera (fruit bat), Lagomorpha (hare), birds (Psittaciformes: yellow-streaked lory, galah, superb parrot, elegant parrot, red-rumped parrot, festive amazon, yellow-crowned amazon, red-lored amazon, rose-ringed parakeet, green-cheeked parakeet, peach-fronted parakeet, peach-faced lovebird, Fischer's lovebird, rosy-faced lovebird, yellow-collared lovebird, African grey parrot, budgerigar, turquoise parrot, blue-fronted parrot, blue-headed parrot, mealy parrot, eclectus parrot, scaly-headed parrot, umbrella cockatoo, white cockatoo, little corella, Australian ringneck, eastern rosella, blue-streaked lory, chestnut-fronted macaw, red-shouldered macaw, blue-and-yellow macaw; Apodiformes: hummingbirds; Passeriformes: Gouldian finch, zebra finch, yellow finch, saffron finch, crow, blue-eared starling, sparrow, canary, double-collared seedeater, blackbird; Anseriformes: mallard duck, greylag goose, mute swan, black-necked swan, black swan, Coscoroba swan; Gruiformes: black-crowned crane; Columbiformes: Nicobar pigeon; Struthioniformes: ostrich), Crocodilia (crocodile)	eye, lungs, disseminated	cosmopolitan
Encephalitozoon intestinalis (syn. *Septata*)	2.0 x 1.2-1.5 µm (4-7 coils)	Primates (humans, bonobo, mountain gorilla, red-ruffed lemur, ring-tailed lemur), Carnivora (dog, cat, red fox, coati), Lagomorpha (hare), Rodentia (house mouse, wood mouse, grass vole), Artiodactyla (pig, cattle, goat), Perissodactyla (donkey), birds (Struthioniformes: ostrich; Passeriformes: pigeon)	gut, kidney, eye, disseminated	cosmopolitan
Encephalitozoon lacertae	2.9 x 1.2 µm	Sauria (wall lizard, African skink)	gut	Europe, Africa
Encephalitozoon pogonae	1-2 µm	Sauria (bearded dragons)	disseminated	Europe, North America, Asia
Family: Tubulinosematidae (diplokaryotic, diplosporoblastic, thickened plasmalemma, surface tubular network)				
Tubulinosema acridophagus	3.3-3.9 µm	Primates (human)	muscles, disseminated	North America
Anncaliia algerae (syn. *Nosema*, *Brachiola*)	4 x 2.7 µm (8-11 coils)	Diptera (mosquito), Primates (human)	eye, skin, muscle, disseminated	North America, Australia
Anncaliia connori (syn. *Nosema,*	4-4.5 x 2-2.5 µm (10-12 coils)	Primates (human)	disseminated	Europe

Brachiola)				
Anncaliia vesicularum (syn. *Brachiola*)	2.9 x 2 µm (8-10 coils)	Primates (human)	muscles, skin, eye	North America
Family: Nosematidae (diplokaryotic, diplosporoblastic, monomorphic)				
Vittaforma corneae (syn. *Nosema corneum*)	3.5-4 x 1-1.5 µm (5-6 coils)	Primates (human)	cornea, bladder	North America, Europe
Nosema ocularum	5 x 3 µm (9-12 coils)	Primates (human)	cornea	North America
Nosema tritoni	nr	Urodela (newt)	skin	Europe
Family: Enterocytozoonidae (monokaryotic, sporogony with formation of electron-dense discs)				
Enterocytozoon bieneusi	1.1-1.6 x 1 µm (5-8 coils)	Primates (humans, gorilla, macaques, marmosets, baboon, langur), Artiodactyla (cattle, goat, deer, llama, kudu, pig), Perissodactyla (horse), Rodentia (muskrat, guinea pig, mice, beaver, squirrel, chipmunk, woodchuck, vole), Lagomorpha (rabbit), Carnivora (dog, fox, cat, raccoon, otter, ermine, coati, black bear), birds (Falconiformes: falcon; Columbiformes: pigeon)	intestines, liver, kidney, eye	tropics
Incertae sedis				
Microsporidium ceylonensis	3.5 x 1.5 µm	Primates (human)	cornea	Sri Lanka
Microsporidium africanum	4 x 1.5 µm (15-16 coils))	Primates (human)	cornea	Africa
Microsporidium buyukmichi	4 x 1.5 µm (15-16 coils)	Carnivora (cat)	cornea	North America
Microsporidium simiae	2-4 µm	Primates (titi monkey)	intestines	Europe
Microsporidium schuetzi	7 x 2 µm	Anura (ranid frogs)	oocytes	North America

Parasite morphology: Microsporidia form three sequential developmental stages: meronts, sporonts and spores. The unique unicellular spores are spherical, ovoid or cylindrical, most ranging in length from 2-8 µm. They are encased within tough chitinous walls comprising a thin electron-dense exospore and a thicker electron-lucent endospore. Mature spores possess an elongate polar tube coiled up inside; most tubes being isofilar (of uniform diameter) although some are anisofilar (tapered, showing a reduction in diameter over length). The polar tubes are attached to an anterior anchoring disc enveloped by a membranous polar sac. For most microsporidian genera, the wall of the polar tube and its central canal are inserted into the polar sac, but for chytridiopsid genera (*Buxtehudea, Chytridiopsis* and *Nolleria*), only the central canal surrounded by a honeycomb layer is inserted into the polar sac. The anterior section of the polar tube is straight and surrounded by the polaroplast, which may be lamellar, tubular or both. Mature spores contain a prominent posterior vacuole (often visible by light microscopy) and an amoeboid nucleated sporoplasm. Spores may be monokaryotic (uninucleate) or diplokaryotic (with two closely-appressed nuclei). Many microsporidia form only one type of spore (monomorphic), while others are heterosporous (dimorphic or polymorphic) forming several different types (usually micro-spores and macro-spores, sometimes meio-spores). Following host cell invasion, parasites undergo asexual merogony (schizogony) and then 1-3 sporulation (sporogony) sequences forming sporoblasts (sporoblastogenesis) which mature to form infective spores (sometimes referred to as germination). Meronts are located either directly within the host cell cytoplasm (often surrounded by host endoplasmic reticulum and sometime host mitochondria) or bound within parasitophorous vacuoles (membranous envelopes of host origin). They appear as clusters of small nucleated intracellular parasites that have divided by binary or multiple fission, although several species form multinucleated plasmodial stages. Sporonts also appear as small clusters of parasitic cells but are characterized by thickened plasmalemmas due to the deposition of parasite secretions on their surface membranes. The exception is *Enterocytozoon* whose sporonts do not immediately secrete an electron-dense coat, but are identified by early (precocious) development of organelle precursors of polar tubes (electron-dense discs that accumulate in stacks). Pansporoblastic species (e.g. *Trachipleistophora*) also form an isolating envelope (membranous sporophorous vesicle) whereas apansporoblastic species lie direct in the host cell cytoplasm (e.g. *Anncaliia*, *Enterocytozoon*) or within parasitophorous vacuoles (e.g. *Encephalitozoon*). Sporonts divide internally one or more times by binary or multiple fission or plasmotomy to form sporoblasts which then mature into spores.

Site of infection: All microsporidian species in tetrapods are histozoic parasites with obligative intracellular development within host cells; either being in direct contact with the host cell cytoplasm or being enclosed within parasitophorous vacuoles (membrane of host origin) or sporophorous vesicles (envelopes of parasite origin). Many infections have exhibited some tissue tropism with development occurring preferentially within particular tissues: including the gut (*Enterocytozoon bieneusi, Encephalitozoon intestinalis*), muscles (*Pleistophora ronneafiei, Trachipleistophora hominis*, *Anncaliia algerae*, *Anncaliia vesicularum,*

Tubulinosema acridophagus), central nervous system (*Encephalitozoon cuniculi*, *Trachipleistophora anthropophthera*, *Encephalitozoon hellem*) and eye (*Nosema ocularum*, *Vittaforma corneae*, *Anncaliia connori, Microsporidium ceylonensis, Microsporidium africanum*). However, more extensive studies have since found most infections to be more widely disseminated throughout host tissues, involving a variety of organ systems.

Pathogenesis: During development, microsporidia are metabolically dependent on host cells and are able to mobilize host cell organelles to meet their demands. Infected cells become swollen and the host cell nucleus becomes enlarged or fragmented. The parasites ultimately cause lysis of infected host cells (subacute presentation), contributing progressively to both structural and functional deficits (with acute and/or chronic presentation). Infections in tissues may be diffuse, spreading from cell to cell, or localized within cysts. Parasites are responsible for tissue displacement/replacement, inflammation and space-occupying lesions. Infections in humans involving pansporoblastic (*Pleistophora* and *Trachipleistophora*) and apansporoblastic genera (*Nosema, Enterocytozoon, Encephalitozoon, Anncaliia, Vittaforma* and *Tubulinosema*) have variously been associated with neurologic (convulsions, vomiting, headaches, fever, coma), ocular (keratoconjunctivitis, chronic sinusitis), muscular (atrophy, muscle fibre degeneration), enteric (diarrhoea, fever, malaise, weight loss) and pulmonary (respiratory) signs. Most patients have been immuno-compromised due to HIV-AIDS, immunosuppressive chemotherapy or organ transplantation, but some infections have also been detected in immunocompetent individuals. Infections are typically chronic and subacute in immunocompetent hosts but severe and even fatal in immunocompromised hosts.

In animals and humans, *Encephalitozoon cuniculi* has been associated predominantly with three clinical syndromes: encephalitis (granulomatous and nonsuppurative lesions with torticollis, ataxia, circling, paresis, paralysis), renal dysfunction (interstitial nephritis), and ocular lesions (keratoconjunctivitis, cataract formation, phacoclastic uveitis). Disseminated infections have also involved the digestive (enteritis, peritonitis, hepatitis), urinary (urethritis, cystitis) and respiratory tracts (rhinitis, sinusitis). Young animals (esp. rabbits and puppies) often develop a mixed encephalitis-nephritis syndrome with anorexia, weight loss, behavioural changes, weakness, loss of awareness, incoordination, ataxia, tremors, convulsions and blindness. In birds, *Encephalitozoon* spp. infections have been associated with illthrift, diarrhoea, dehydration, weight loss and respiratory difficulties. *Encephalitozoon hellem* has been associated with ocular (keratoconjunctivitis), respiratory (sinusitis, rhinitis, pneumonitis, bronchiolitis) and urinary signs (nephritis, ureteritis, cystitis, urethritis, prostatitis) in immunocompromised humans, while *Encephalitozoon intestinalis* has been associated mostly with gastrointestinal perturbations (chronic diarrhoea, enteritis, small bowel perforation, cholangitis, cholecystitis) but sometimes extending to urinary (nephritis, urethritis, cystitis), respiratory (sinusitis, rhinitis, bronchitis) and ocular signs (keratoconjunctivitis). *Enterocytozoon bieneusi* most often causes intestinal signs (enteritis, villous atrophy, malabsorption, watery diarrhoea), sometimes with biliary tract involvement (cholangitis, cholecystitis), all contributing to general malaise, dehydration, malnutrition and weight loss in (almost exclusively) immuno-compromised patients. Rarely, it has been associated with respiratory symptoms involving chronic cough and dyspnoea. Microsporidial infections of the eye are common in both immuno-compromised and immuno-competent patients, possibly because the eye is an immuno-privileged site at a lower temperature and thus more susceptible to opportunistic infections. Several microsporidial species infect the corneal stroma and keratocytes causing keratoconjunctivitis involving follicular papillary conjunctivitis and coarse punctate epithelial lesions in three patterns (diffuse, peripheral, paracentral) progressing to nummular keratitis. Symptoms include ocular redness, eye pain, foreign body sensation, light sensitivity, excessive tearing, astigmatism and blurred vision due to corneal ulceration and opacification due to scarring. Infections in the brain have resulted in encephalitis and seizures due to lesions in the hippocampus and cerebrum, while infections in the heart and skeletal muscles have been associated with necrotizing myositis, cellular infiltrates, weakness, myalgia, fever and progressive weigh loss despite maintaining regular food intake.

Developmental cycle and mode of transmission: Microsporidia infecting humans and other tetrapod animals have simple asexual monoxenous life-cycles, involving sequential multiplication and sporulation within host tissues. Most infections are thought to be initiated by the ingestion of infective spores contaminating food and/or water supplies. Mature spores have tough resistant walls which allow them to survive in the external environment under suitable climatic conditions (excluding temperature extremes, desiccation and direct solar radiation). Each spore contains an infective sporoplasm which is injected into a host cell through the polar tube when it is forcibly everted by swelling of the posterior vacuole and polaroplast (triggered by changes in calcium influx, osmotic pressure, pH, mechanical compression, etc.). The parasites then undergo vegetative reproduction by merogony (schizogony) by binary or multiple fission, although cytokinesis is sometimes delayed thus forming multinucleate plasmodia which divide by plasmotomy to form more plasmodia or segment into uninucleate meronts again. Meronts may lie direct in the host cell cytoplasm or be contained within membranous parasitophorous vacuoles. The parasites then form thickened cell membranes and/or enveloping sporophorous vesicles and undergo further division called sporogony. Sporonts divide by binary or repeated fission or plasmotomy to form sporoblasts (sporoblastogenesis) which subsequently mature to form spores (sporulation). The developmental stages of many microsporans have single isolated nuclei throughout their development, ultimately giving rise to uninucleate sporoblasts and spores (e.g. *Pleistophora, Encephalitozoon, Enterocytozoon*). Others have paired nuclei which divide synchronously and remain closely appressed throughout their development into diplokaryotic sporoblasts (e.g. *Anncaliia, Nosema, Vittaforma, Tubuliosema*). The number of spores formed by each sporont (di-, tetra-, octo-, poly-sporous) can be a defining characteristic for many genera, particularly those developing within sporophorous vesicles. Merogony and sporogony may take place within the same or different tissues of individual hosts. Most tetrapod microsporidia form monomorphic spores, although some *Pleistophora* and *Nosema*

species form two types of spores during their development. Mature spores are released by host cell lysis and may infect neighbouring cells (auto-infection and dissemination) or be voided from the host digestive, urinary or respiratory tract to contaminate the environment. Spores have been detected in drinking, recreational and irrigation waters as well as in wastewater from treatment plants (including biosolids). Contaminated foods have included strawberries, raspberries, mung bean sprouts, parsley, curly lettuce, celery, cilantro, cucumbers, mangoes and milk. Spores are thought to access the eye via contaminated contact lenses, fingers or aerosols. There is some epidemiological evidence to also suggest transmission through the skin via insect bites and rarely by sexual transmission. Vertical transmission has not been documented for infections in humans, but transplacental transmission has been observed in other mammalian hosts.

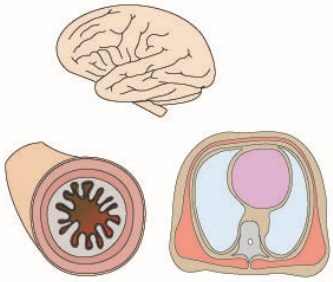
Differential diagnosis: Infections are best diagnosed by the detection of parasites within host tissues, by conventional light microscopy to detect spores or contemporary molecular techniques to detect parasite DNA. Microscopic examination of wet mounts (faecal concentrates, urinary sediments, gut or respiratory aspirates, cerebrospinal fluid) and squash preparations, impression smears or histological sections (of tissue biopsies or autopsy samples) may reveal the presence of characteristic microsporidian spores. Unstained samples are best examined at medium to high power (400X magnification) by bright-field microscopy with a suboptimal illumination system (introduce diffraction/contrast through specimen by racking down condenser and/or partially closing diaphragm), by phase-contrast or differential interference-contrast microscopy, or by fluorescence microscopy with ultraviolet illumination (the chitinous spores are often autofluorescent or can be stained with calcofluor white or Uvitex 2B). Mature spores are highly refractile, phase-bright, Gram-positive, acid-fast, have a PAS-positive polar granule, and exhibit autofluorescence. Treatment of spores with dilute hydrogen peroxide can induce mature spores to evert their polar tubes. Histological sections are best stained using Ziehl-Neelson acid-fast, Periodic acid Schiff, Giemsa, chromotrope or silver stains to highlight merogonous and sporogonous stages. Given the small spore size and their homogenous appearance, transmission electron microscopy is often conducted to reveal the presence of the coiled polar tube within spores and/or the presence of a sporophorous vesicle. Some microsporidian species have also been successfully established in tissue culture monolayers using several mammalian cell lines. A range of immunodiagnostic procedures have been developed to detect host antibodies against microsporidia, including India-ink immunoreaction assays (CIA), complement fixation tests (CFT), indirect fluorescent-antibody tests (IFAT), direct agglutination tests (DAT), enzyme-linked immunosorbent assays (ELISA) and intradermal skin test. Test sensitivity and specificity has been variable and most tests cannot differentiate between current infection and previous exposure. Several polyclonal and monoclonal antibody preparations have been applied to the detection of parasite antigens in host and environmental samples using fluorescent-labels, enzyme-substrate interactions or immunomagnetic bead separation techniques. Pulsed-field gel electrophoresis (PFGE) has also been used for karyotype profiling of several microsporidian species. More recently, considerable success has been achieved in detecting parasite DNA by polymerase chain reaction (PCR) amplification of specific gene sequences (small subunit (SSU) ribosomal RNA (rRNA), internal transcribed spacers (ITS) and polar tubule protein (PTP) genes). Molecular characterization techniques are facilitating not only more sensitive and specific diagnoses but also allowing more comprehensive phylogenetic analyses of relationships between taxa.

Treatment and control: No drug treatments have proven totally effective against microsporidial infections in tetrapods, but symptomatic improvement and/or partial clearance have been reported when treating human infections with albendazole, fumagillin (ocular, intestinal and disseminated infections), nitzaoxanide, metronidazole (intestinal infections) and trimethoprim-sulfamethoxazole (disseminated infections). Albendazole and fumagillin are most effective against *Encephalitozoon* spp. and fenbendazole has also been used to treat *E. cuniculi* infections in rabbits. Nitazoxanide is used for the primary treatment of *Enterocytozoon bieneusi* in humans but side effects are common, including abdominal pain, vomiting, nausea and diarrhoea. The oral administration of fumagillin can also produce thrombocytopenia (reduced platelet counts). The controversial drug thalidomide has helped alleviate diarrhoea and improved weight gain in patients with *E. bieneusi* but it is highly toxic and should only be considered when all other treatments have failed. Enteric infections in many HIV-AIDS patients showed clinical improvement following highly-active anti-retroviral therapy (HAART), although relapses were observed when treatment was discontinued. Symptomatic relief for persistent enteric infections is also afforded by fluid replacement therapy, either orally or parenterally. Myalgias caused by *Trachipleistophora* spp. have responded well to combination therapy with albendazole, pyrimethamine, sulfadiazine and folinic acid. Infections of the eye have been ssuccessfully treated with topical fluoroquinolone monotherapy or oral albendazole and topical fumagillin. Keratoconjunctivitis has also improved using topical prednisolone and chloramphenicol. Permanent damage to the eye or deep stromal infections may require corneal transplantation, preferably using full-thickness penetrating grafts over lamellar grafts to avoid recurrent infection. The prevention of infections in human and animal populations is dependent on good hygienic practices not only to minimize environmental contamination by spores but also restrict their uptake by susceptible hosts. The small size of microsporidian spores and their hardy resistance to adverse environmental conditions means they may survive some routine sewage and water treatment procedures. Comprehensive experimental studies are required to assess the efficacies of various decontamination (sedimentation, coagulation, filtration) and disinfection (heat, chlorination, ozonation, irradiation) processes. Immuno-compromised individuals at risk of developing life-threatening infections are recommended to drink bottled or boiled water, cook all meat products and wash fruit and vegetables prior to consumption, and practice elevated levels of sanitation and hygiene (especially with respect to toilet habits and hand-washing).

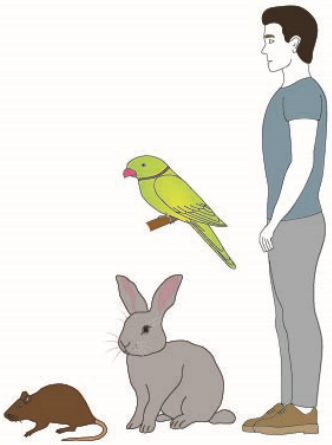
Microspora (tetrapod hosts)
e.g. *Encephalitozoon*

most with simple monoxenous cycles
most monokaryotic

form unicellular spores
with unique polar tubes

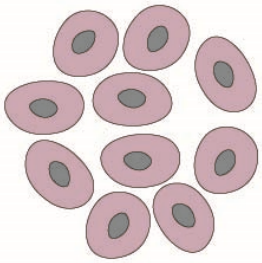


histozoic (gut, viscera, brain)
(lesions/cysts, inflammation,
dysfunction, mortalities)

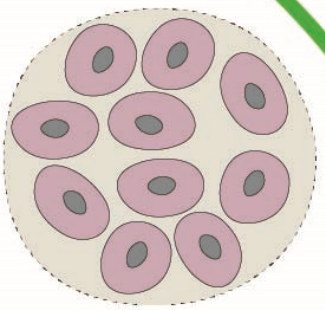


Vertebrate Hosts
(mammals, birds)

development may occur direct in host cell cytoplasm
or in parasitophorous vacuole (membrane of host origin)
or in sporophorous vesicle (envelope of parasite origin)

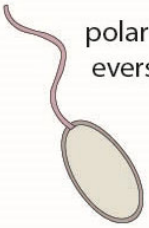


multiplication by merogony
(binary or multiple fission, although
several form multinucleated plasmodia)

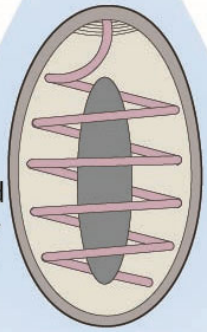


spore formation by sporogony
(binary or multiple fission,
a few by plasmotomy)

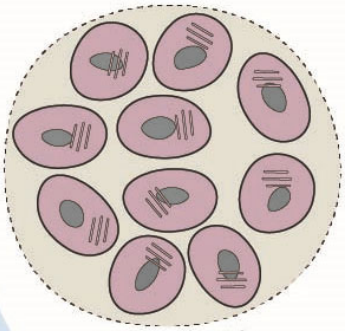
sporoplasm injected
into host cell



polar tube
eversion



coiled
polar
tube



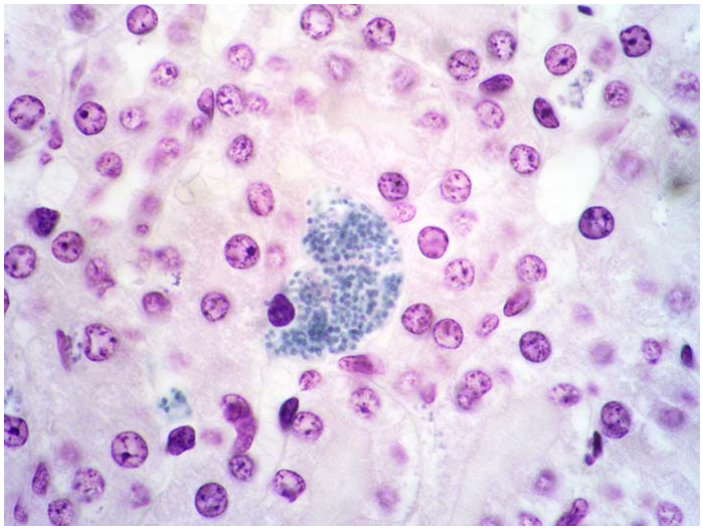
sporont with
sporoblasts

infective spores
ingested/inhaled

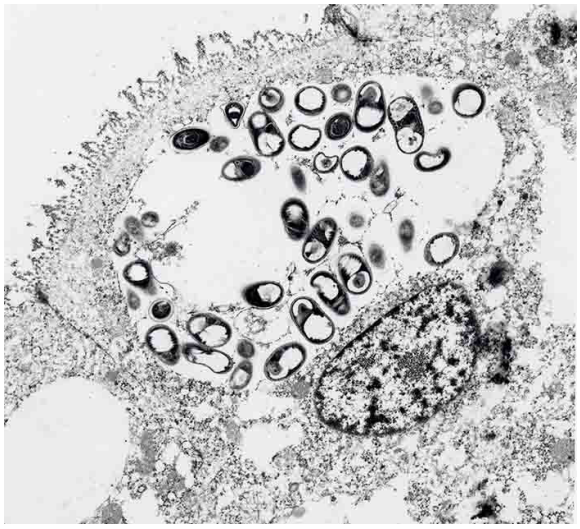
microspore
(2-8 µm)

mature spores
released

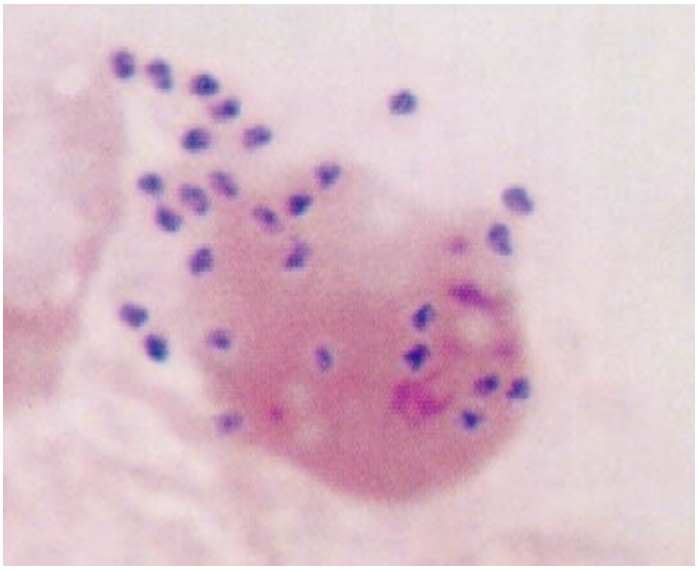
most transmission between hosts direct via spores
contaminating external environment (soil, water, food)



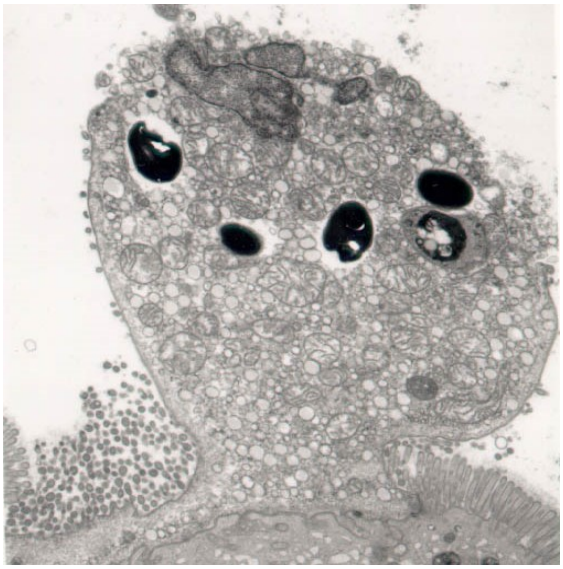
Encephalitozoon cyst in rabbit brain



Encephalitozoon cyst in koala (tEM)



Enterocytozoon spores in human gut



Enterocytozoon spores in human gut (tEM)