CHAPTER 8

Microsporidia

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Abstract: Microsporidia the tiny unicellular eukaryotes are intracellular parasites of almost all animals. The diverged and specialized nature of these organisms, show some similarity to fungi. They cause opportunistic infections in animals and humans ranging between asymptomatic and severe life-threatening infections in immunocompromised individuals. Transmission occurs mainly by oral route, but other methods of transmission include inhalation, sexual contact, ocular mucosa, wounds, and insect bites. Food and water are relevant vehicles of infection. Animals act as reservoirs as they harbor most of the species that can also infect man and might contaminate water and environment with spores expelled in feces and/or urine. Clinical presentation is mainly intestinal with chronic diarrhea, mal-absorption, and loss of weight in immunocompromised persons, and self-limiting diarrhea in the immunocompetent individuals. Dissemination to other organs, may threaten the life of patients. Clinical picture of disseminated infection includes fever, cerebral manifestations or some other unexplained symptoms. Diagnosis of spores in feces, urine, CSF, sputum and in tissue is difficult and necessitates the use of special stains. Other methods of laboratory diagnosis include immunofluorescence, Electron Microscopy, and DNA detection. Treatment with Albendazole is effective for intestinal and other deep infections of various species of microsporidia except *E. bieneusi*, where fumagillin, can be considered. This drug is also used as topical treatment for eye infections by *E. hellem* and other species. Trials to produce vaccine against microsporidia are still under study. The increasing awareness will lead to a better understanding of the epidemiology, clinical relevance and control of microsporidiosis in humans and animals.

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INTRODUCTION

Microsporidia are unicellular, obligate intracellular spore formers of eukaryotic origin. They parasitize almost all animals. Understanding the basic biology of Microsporidia, have taken almost about 150 years of scientific research. The identification of DNA of organisms, created a new era of molecular phylogeny. Microsporidia, which were considered as protozoa (Kingdom Protista), are now considered as highly specialized fungi [1, 2]. There are more than 170 genera and approximately 1300 species of microsporidian organisms that parasitize a wide variety of vertebrates and invertebrates with at least 14 species and 8 genera known to infect humans [3 - 5]. The genera of microsporidia that cause human diseases are: Nosema [6, 7], Brachiola [8, 9], Vittaforma [10], Pleistophora [11, 12], Trachipleistophora [13], Enterocytozoon [14, 15], Encephalitozoon [16 - 18], Septata [19], and AnnCalifornia [20]. Microsporidia have been known to cause seriously damaging diseases in honeybees and silk worms, that consequently led to a serious economic loss [21]. Infection also was detected in different animals as rabbits, laboratory rodents and furred animals [22]. However microsporidia were considered as opportunistic pathogens in humans after the emergence of AIDS pandemic [23], and also have been detected in immunocompetent persons [24]. Infected cases may be asymptomatic or they may suffer severe life threatening disease according to the tissue or organs affected, as microsporidia may infect almost any part of the body. The most common site of infection is the intestine, which may account for up to 50% of all infections, with chronic diarrhea and wasting as the predominant manifestations [23 - 26].

HISTORY

There was an important economic problem in the year 1850, due a decline in the European silk industry as a result of a disease that affected the silk worms. This disease was called the pepper-disease (pébrine). Investigations were carried out in scientific centers in order to identify the microbial causative agents of the disease.
There was some association between the disease and characteristic globular organisms, which were described, later by the Swiss microbiologist Karl Wilhelm von Nägeli in 1857 as the first microsporidium and he gave them the name *Nosema bombycis* [27]. Nägeli described *N. bombycis*, as a yeast-like fungus and included it in the Schizomycetes, which fits into the tree of eukaryotes according to the recent classification [27]. In 1870, Louis Pasteur incriminated microsporidia as a cause of infection of silkworm and cause of decimation of the silk industry. Aided by his colleagues, they could identify the nature of this parasite [28], with subsequent improvement of the European silk industry [29]. Further studies by Edouard-Gérard Balbiani by the year 1882, has created a new group for *Nosema* organisms, gave them the name ‘microsporidies’ and included them in the group of sporozoa within the Kingdom Protozoa [30]. Sporozoa is an old group of pathogens united together in an assemblage based on their similarity as spore formers. Recently, studies showed that they have distant relations and were subgrouped as members of Apicomplexes, haplosporidians and the Cnidosporidia. The last subgroup included Myxosporidia (affecting animals), *Actinomyxidae* (of unknown origin), *Helicosporidia* (green algae) and the Microsporidia [31].

In the year 1976 Sprague created the Phylum *Microspora*, which was later included in the subkingdom Protozoa, a subdivision of the Kingdom Protista created in 1980 by Levine [30 - 33]. Shortly after, Sprague and Bencil changed the name of the phylum to Microsporidia, Balbiani 1882 [34]. This was in honor of Balbiani, who has created the order Microsporidia in 1882 [30].

**Phylogeny and Taxonomy Considerations**

Species of microsporidia have been classified according to studies based on their habitat, morphological and ultrastructural details. However the most important was the recent molecular phylogenetic classification [35, 36]. The most spectacular features of the spores of microsporidia have been explored by electron microscopy [37, 38].

Electron microscopy studies showed that the microsporidial spores lack some important structures of the Eukaryotic cells as mitochondria, peroxomes, Golgi apparatus, flagella and microtubules [39].
Intimate resemblance to fungi was proved by ultrastructural morphological studies based on the spore size, number of coils of the polar tube inside the spore as well as the life cycle and the host parasite relationship [40 - 43]. On the other hand, the resemblance to prokaryotes was revealed by biochemical analysis, after detecting that microsporidia include 70S ribosomes as in case of prokaryotes [44, 45]. Study of the rDNA sequences for the phylogenetic classification of microsporidia, suggested that they were among the deep-branching early eukaryotes [46]. This is based on lacking mitochondria, Golgi bodies and peroxisomes and having the small ribosomes of the prokaryotes as mentioned before [46].

Phylogenetic study of the sequence of the small subunit rRNA gene of Variamphora necatrix, one of the species of microsporidia, showed that there is closer resemblance to prokaryotes than to eukaryotes, suggesting that they have an ancient origin [46]. Furthermore, microsporidia showed that they possess fast evolving genes that make the closer to prokaryotes. At present, microsporidia are considered highly specialized, well-adapted and diverged organisms, that are either belonging to fungi or a near relative to them [47 - 50]. Studies on the genomic molecular sequence of the Encephalitozoon cuniculi, supported its relationship to fungi [51 - 54].

Nevertheless, in most medical textbooks microsporidias are still discussed within the parasite section and also the life cycle still uses the terminology of parasitic pathogens. The first human infection with microsporidia species was detected in a 9 year-old Japanese boy that suffered from fever, vomiting and spastic convulsions due to dissemination of infection with Encephalitozoon [55].

Until 1985, there were only few detected cases with microsporidiosis, when a new species “Enterocytozoon bieneusi” was diagnosed in another case with AIDS from Haiti and subsequently other cases of intestinal microsporidiosis were detected in HIV positive patients in France [56, 14]. A wide range of studies have reported infection with microsporidiosis in non-HIV persons. However there is lacking of data concerned with parasitological detection of spores versus serology [57].

Over the last 25 years, there have been improvements of diagnostic methods and
equipment, which eventually led to the identification of many other species of microsporidia, some of which can disseminate to different organs and show unexplained symptoms [58].

**MORPHOLOGY AND LIFE CYCLE**

Microsporidia are named for their small, resistant spore stage. The spores that infect humans are ovoid in shape, around 1.5 to 5 μm in length and ~1 μm in width. The spore coat is composed of an outer cover of a proteinacious electron dense material, a median endospore made of chitin and protein and a plasmalemma or an inner membrane [59]. The spore has a membrane-bound nucleus and an intra-cytoplasmic membrane system [60]. Nuclear configuration differs among genera of microsporidia. In the spores of some genera, two nuclei are arranged as a tightly joined pair (called a diplokaryon), whereas in others the nucleus is single [22]. In *Enterocytozoon*, diplokarya may occur early in the life cycle [56], while single nuclei occur at later stages. In other genera, the nuclear configuration is constant (either single, *e.g.* *Encephalitozoon*, or double, *e.g.* *Nosema*) throughout the life cycle [57]. When organisms with diplokarya divide, each diplokaryon divides producing “double diplokarya” [9]. The potential link to fungi has been proposed, based on the presence of some important features, mainly the presence of chitin in the wall of the microsporidial spores, identifiable Golgi organelles [58 - 60], the microtubule gene data [61, 62] as well as several enzyme processes [5]. Studies showed that many characteristics of microsporidia are similar to prokaryotes, however their 70S ribosomes make them different from prokaryotes. They also contain 16S and 23S ribosomal ribonucleic acids (RNAs) similar to prokaryotes with the smallest genome of any eukaryote thus far reported [51].

All microsporidian spores contain a single long coiled structure called the polar filament, a unique structure attached at the anterior end by a large, mushroom-shaped anchoring disk [63]. Electron microscopy reveals that this structure coils around the single or double-nucleated sporoplasm inside the thick, resistant and refractile spore coat. The host-parasite interface may involve: 1) Direct interaction with the cytoplasm of the host cell, 2) Indirect contact in a parasite-secreted envelope (sporophorous vesicle, SPOV), 3) Indirect contact by production of a
parasite-induced, host-produced envelope “parasitophorous vacuole” [64], or 4) Indirect contact by producing a host-produced “parasitophorous vacuole” and parasite-induced secretions [3, 15, 65].

The life cycle of microsporidia has three phases: infective, proliferative and sporogenic. The majority of microsporidian infections are initiated in the susceptible host via oral ingestion, with the spores gaining access to the digestive tract. This has led to the discovery that spores germinate in response to stimuli such as: pH, ion concentration, osmolarity, digestive enzymes, redox potential, and/or digestive products [66]. The stimulus changes the spore’s permeability, triggering the eversion of the polar filament resulting in the projection of a long hollow polar tubule that jumps out from the anchoring disk coiling several times inside the posterior part of the spore (Fig. 1).

![Fig. (1). Scanning electron micrograph of a microsporidian spore with an extruded polar tubule.](image)

It emerges with sufficient speed and force to penetrate the host cell and transfer the sporoplasmic material directly into the host cell through its 50-500 μM long polar tube [67, 68] initiating a new infection in less than a second [69]. Some spores evert their polar tubules, releasing sporoplasts within the same host, thus establishing a cycle of autoinfection, which leads to chronicity and/or additional sites of infection [4]. The whole process from the beginning of germination, protrusion of the polar tube and the inoculation of the sporoplasmic material into the host cell was described to have a resemblance to a hypodermic needle [70, 71]. In the proliferative phase, extensive multiplication begins when the injected sporoplasm proliferates to meronts. The injected sporoplasm grows and divides either by merogony, by just by simple binary fission or by schizogony, which
occurs by multiple fission producing multinucleate plasmodial forms inside the cytoplasm of the infected host cell [22].

The sporogenic cycle is signaled by one or more changes: secretions deposited on the surface membrane of the meront and/or formation of an isolating envelope called sporophorous vesicle (SPOV). Sporonts divide one or more times then become sporoblasts, which mature into spores [4]. Once the cell becomes full with spores, it bursts into the surroundings releasing the new-formed spores that continue their cycle into new cells (Fig. 2).

![Fig. (2). Scanning electron micrograph showing an infected cell bursting and releasing spores of *Encephalitozoon hellem* into the surroundings.](image)

Multiplication of spores inside the infected cells by both merogony and sporogony produce an enormous number of organisms [72]. While the spore’s structures are characteristic of microsporidia, the number of spores produced in sporogony, the manner in which they are produced, and the host-parasite interface vary among different genera [4].

*Nosema* and *Ann calfia (Brachiola)* spores, members of the families *Nosematidae* and *Tublinomatidae*, are approximately 4 μm with paired abutted nuclei (diplokarya). There are over 100 species of *Nosema*, most of which are parasites of insects; however, few *Nosema* species have been described from human ocular infections [73]. After several nuclear and cellular divisions, large clusters develop. Each sporont produces two sporoplast cells that develop into two spores [3]. *Nosema* form thickened plasmalemma in the sporogenic phase, and *Ancaliia*
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(Brachiola) spp. in all developmental stages [74]. The family Pleistophoridae was named in 1893 by Gurly in relation to the fish parasite Pleistophora typicalis Gurley, 1893 [75]. Species of the parasite have been identified in human muscle infection in immunodeficient persons [11]. Infection has also been diagnosed from an HIV negative case in the USA [12]. Spores of Trachipleistophora hominis are approximately 4 x 2 μm, and develop by multiple fragmentations inside sporophorous vesicles (SPOV). T. anthropophthera is dimorphic. There are 2 types of SPOVs, one contains approximately 8 thick-walled spores, measuring 3.7 x 2.0 μm and the other type contains 2 thin- walled spores with 3-5 polar filament coils and measuring 2.2-5 x 1.8-2.0 μm [76]. Enterocytozoon, family Enterocytozooidae, was the first genus of microsporidia identified from human infections [14, 56]. Spores are 1.3 μm x 0.8 μm, and contain a single nucleated sporoplasm surrounded by approximately 6 polar tubule coils arranged in a double row [15, 77]. Development of the parasite occurs inside the host cell cytoplasm. Finding several polar filaments within a multinucleate plasmodium is diagnostic for Enterocytozoon. The plasmodium divides by multiple fission producing a dozen or more of sporoblasts, which mature into spores [15] (Fig. 3).

Fig. (3). Electron micrograph of Enterocytozoon bieneusi. The arrows are pointing to double rows of polar coils cut in cross section.

E. cuniculi, Family Encephalitozooidae, was first discovered in rabbits in 1924 [78] and was considered as a synonym of the genus Nosema [79]. In 1987, the parasite was reported from over 30 different mammalian hosts including man
Later it was classified as a genus Microsporidia and the family was established in 1989 [80]. This genus is characterized by a phagosome-like parasitophorous vacuole surrounding the 6 developing parasites, isolating them from the host-cell cytoplasm. During development, the parasite may contain one or more separate nuclei and the proliferative cells usually depend on the vacuole membrane, which ruptures to free the spores. Within the parasitophorous vacuole, each sporont elongates, divides and produces spores.

Fig. (4). Electron micrograph of a cell infected with *Encephalitozoon intestinalis* spores developing inside a septated parasitophorous vacuole.

A spore is about 1-1.5 x 0.5 μm, and contains a sporoplasm with a single nucleus, besides approximately 6 polar tubular coils arranged in a single row [81, 57]. *Septata* was the second microsporidial genus created for human infection in 1991, and was considered as a new genus within its family, based on the similarity of some morphological features [19, 65]. *S. intestinalis* species is characterized by parasite secreted material surrounding the developing stages and spores inside the parasitophorous vacuole. The proliferative and sporogenic stages have 1 to 4 nuclei. Cells are rounded at first, but they elongate when containing 2 or 4 nuclei. In sporogony, there is thickening of the plasmalemma and elongation of sporonts. Each sporont divides into 4 single-nucleate sporoblasts. Each develops the polar filament complex and matures into a spore. *S. intestinalis* cells are tightly packed in clusters (Fig. 4) while some cells condense leaving a space between individual
developing forms.

Early and late forms develop asynchronously, with the parasite secretions surrounding individual cells within the parasitophorous vacuole. Spores are 2 x 1.2 μm with a single nucleus and 4-7 (approximately 5) polar tubular coils, in a single row [18, 19]. Based on molecular data, *S. intestinalis* is moved into the genus *Encephalitozoon* and is given the name *E. intestinalis* [4].

**GLOBAL EPIDEMIOLOGY AND RISK FACTORS**

Although microsporidia prevail among almost all members of the animal kingdom from honeybees, silk worms, and mosquitoes to mammals and birds, the prevalence is thought to be underestimated due to difficulty and unreliability in detection [57]. The small size of these organisms makes their identification in specimens very difficult. The epidemiological evaluations mainly depend on the geographical area and the method of diagnosis used. Serological prevalence in different localities reported rates ranging from 0 to 42%. There was a high rate of infection in homosexual males in Sweden as well as in cases infected with other parasitic infections [22, 82, 83]. The prevalence of intestinal microsporidiosis showed no significant seasonal variation among HIV seropositive patients [84].

Until the mid 1980s, microsporidia were not recognized as human pathogens [22, 77]. The identification of the new microsporidian *E. bieneusi*, was reported in 1985 from an AIDS Haitian patient that complained from diarrhea and wasting. *E. bieneusi* is still considered the commonest species associated with HIV positive individuals [14].

Before the introduction of HAART (Highly Active Anti Retoviral Therapy), the identification of intestinal microsporidiosis in HIV-positive cases showed wide variability ranging from 2-50% depending on geographical location, laboratory methods and personal experience in diagnosis [57].

The prevalence of systemic microsporidiosis is difficult to estimate, due to absence of clinical signs or nonspecific symptoms that is why microsporidiosis is usually missed in clinical practice. Chronic diarrhea and wasting were primarily associated with microsporidiosis in HIV-positive cases in the early reports. *E.*
bieneusi and E. intestinalis were the predominant species infecting the small intestine.

In Sub-Saharan Africa, infection with microsporidia in HIV positive cases is a cause of high morbidity and mortality [85, 87]. In other countries, especially in Asia (India, Thailand), the Middle East (Turkey), Europe, Africa (Tunisia, Mali, Uganda, Senegal, Zimbabwe), and Latin America (Brazil, Peru) HIV positive cases account for high number of cases of microsporidiosis [20, 85 - 102]. Although infection prevails among HIV-infected patients, they have also been reported in HIV-negative individuals [72], such as travellers [103, 104], malnourished children [105 - 107], recipients of organ transplantation [108, 109], wearers of contact lens [110] and old individuals [90] among immunocompetent individuals [73].

People having organ transplantation are recently considered a risk group for microsporidiosis [111], since cases recognized in transplantation of solid organs and bone marrow, were all negative for HIV [96, 108, 111 - 124]. In this group of patients, diarrhea was the most common complaint and E. bieneusi was the predominating species of microsporidia [125 - 127]. Transmission of microsporidiosis transplacentally from a mother to the offspring was reported in animals as non-human primates, carnivores, rabbits and rodents [128 - 130]. This finding suggests that the same mode of infection may occur in humans; however, it is not proved till now.

Spores can be excreted in sputum of cases with respiratory infection and also can be expelled in stools or urine indicating a horizontal transmission and cause contamination of the environment. Oral-fecal or oral-oral transmission, inhalation of aerosols or ingestion of food and water contaminated with spores are methods of transmission [57, 131 - 133]. It was reported that mice experimentally infected with E. cuniculi as neonates didn’t show clinical symptoms or developed mortality to experimental infection than adults as they developed cell-mediated and humoral immunity and [134]. Experimental infection of animals orally, intra-rectally or by ocular inoculation with the E. bieneusi, Encephalitozoon species or B. algeriae supported the idea that horizontal transmission might occur between humans [135 - 137].
Intrarectal transmission hints to the possibility of sexual transmission among humans [135]. Since many human species of microsporidia also infect a wide range of animals, zoonotic infection may play a role. Moreover, *E. cuniculi* spores have been recovered from carnivores, rabbits and rodents [22, 129, 133]. The same spores have also been reported from foxes, goats, horses, and from non-human primates [138 - 143]. *Encephalitozoon hellem* spores were identified from birds [133] and *Encephalitozoon* (syn. *Septata*) *intestinalis* were also identified from donkeys, dogs, pigs, cows, goats, and gorillas [144, 145].

Spores of *E. bieneusi* were detected in domestic animals as dogs and cats, farm animals as rabbits, goats, pigs and cattle and in wild animals as llama, raccoons, muskrats, beavers, foxes and otters [90, 146 - 152].

*Nosema* species have been identified from insects [21], and *Pleistophora* species were reported from fish [153]. Microsporidiosis has been reported in a child that showed a sero-conversion after exposure to an animal that was infected with *E. cuniculi* [154]. In 2001, Weitzel and others reported a dual microsporidial infection with *E. cuniculi* and *E. bieneusi* in an HIV-positive patient with a high occupational zoonotic risk (dog hairdresser), giving further evidence for zoonotic transmission [155]. Depression of cell-mediated immunity, as occurring in HIV infection, is considered the main risk factor of human microsporidiosis [156, 157]. In HIV patients infection occurs in high degree of immunedepression with CD4+ counts <100 cells/µL [92, 95, 96, 101, 156 - 158], although it is of importance to consider that seroprevalence is unreliable in HIV cases, due to their lowered immunity [132].

Since the identification of *E. bieneusi* as a human pathogen, it was incriminated as a cause of chronic diarrhea in HIV positive cases and studies were done to recognize the risk factors in humans. HIV/AIDS immunodeficiency, low CD4+ counts (<50 cells/µL), and young age, were the identified as risk factors in cases infected with *E. bieneusi* in their intestine [157 - 162].

Internal transcribed spacer (ITS) could help to identify more than 90 genotypes of *E. bieneusi* in humans and animals. *E. bieneusi* is known to be the only and most common species of the genus *Enterocytozoon* that was reported to infect man
A study carried out on *E. intestinalis* isolates from animals and human showed no molecular differences, which may explain that there is no transmission barrier between different host species [164]. In constrast, antigenic diversity has been demonstrated among isolates of *E. cuniculi* and *E. hellem* from human infections [165] and *E. cuniculi* is considered the species that has the widest distribution within the genus *Encephalitozoon*, among mammals including humans [172].

Water is an important source of transmission of microsporidiosis. Spores of *E. intestinalis* were detected in almost all types of water including surface and ground water as well as sewage treated water [166].

Researchers identified spores of *E. bieneusi* in surface water and were able to identify microsporidial spores in water of swimming pools [167, 168]. *Vittaforma corneae* (syn. *Nosema corneum*) spores were isolated from river Seine [167, 169] and spores of *Nosema* species from ditch water [170] and tertiary effluent [166]. A survey comparing human microsporidiosis in two Mexican villages with different water sources found that people in a village receiving piped, untreated water from a spring had a significantly higher incidence of spores of *Encephalitozoon* in their stool samples than people in a village with a well water supply (40% vs 15%), highlighting the potential of transmission by contaminated water [171]. Water supplies contaminated by feces and urine of animals infected with microsporidia may infect humans, as most of microsporidia lack host specificity [172].

Microsporidian spores are environmentally resistant and live for considerably prolonged durations. Under experimental conditions, some of spores of *E. cuniculi* remained viable and infectious in the medium 199 (M99) of tissue culture system for a duration of 16 days, when incubated at 22°C and 98 days when incubated at 4°C [284]. Their small size allows spores to escape filtration. Besides, the availability in various water sources, favor the role of water as a vehicle for transmission [20, 172]. Microsporidia could live in fresh or salt water, in tissue culture, or after dehydration for prolonged periods at suitable temperatures [174]. *E. cuniculi* were able to survive when incubated in distilled water or freezeed and
thawed for 24 hours after incubation at pH4 and pH9 [173].

Spores of *E. intestinalis* and *E. hellem*, still had the ability to infect cells of tissue culture for weeks to months after incubation in water at 10-30°C [174]. Some epidemiological studies reported that the recreational water as well as hot tubs, occupational water and drinking water constitute a risk factor for human infection [175, 161] and increased rates of infection were in the vicinity of distribution subsystems [176]. In

A study carried out in Peru on HIV/AIDS patients showed that the risk factors for *E. bieneusi* were getting in contact with excreta of ducks and chicken with lack of fresh and clean water, in addition to the flush toilets and collection of garbage [96].

Studies depending on molecular epidemiology have created a better understanding of the geographic, zoonotic, demographic and the environmental outlines of microsporidia that infect man. The role of water in the transmission of infection has led to the consideration of microsporidia into the NIH category B list of Biodefense pathogens, and the important contaminant organisms transmitted by water by EPA “Environmental Protection Agency” [88].

The globalization of food, the increasing travel of consumers and the change in food consumption patterns have created a concern about the role of food in the transmission of microsporidial infections [177, 178]. Investigations showed that eating undercooked beef was associated with microsporidiosis in HIV positive cases, although adequate cooking of infected meat can avoid infection [175]. *Trachiopleistophora hominis*, which is similar to that of fish, was recognized to cause myositis in patients with AIDS [179]. These organisms could grow actively in culture temperature of 32-34°C, which may give in idea that this species is not completely adapted to human infection [180]. Further phylogenetic studies raised the concern that human infection may develop after eating improperly cooked fish or by transmission be mosquito bite [180].

Vector-borne transmission has been studied by trying to inoculate *Brachiola* (syn. *Nosema*) *algerae*, a natural pathogen of mosquitoes, into rats or athymic rats. There was failure in the inoculation of the organisms by oral or intravenous
routes, however local infection occurred after subcutaneous inoculation [181, 182].-Inoculation of the spores of *B. algerae* into the eyes of SCID mice (severely deficient in functional T or B lymphocytes), failed to develop ocular signs, but microorganisms appeared in the liver after 60 days [137].

Additional evidence for vector-borne transmission was explained when infection with *T. hominis* was transmitted to *Anopheles quadrimaculatus* and *Culex quadrimaculatus*. The infection developed in the muscle bundles of the insect’s abdominal segments. Spores of *T. hominis* were later identified in the sugar solution used for feeding mosquitoes, and also from the proventriculi and the rest of the gut of mosquito, indicating that mammalian transmission may occur during blood meals of infected mosquitoes [183].

*Brachiola* (syn. *Nosema*) *algerae* spores were also detected in mosquitoes, and it was suggested that there might be a risk of transmission to HIV infected patients, if they were stung by bees, wasps or hornets [184, 162]. Infection has been traced among asymptomatic apparently healthy individuals.

The indirect immunofluorescence assay (IFA), was used in the Czech Republic for the detection of specific antibodies against microsporidia. The result showed unexpected higher incidence of infection than reported before, raising a doubt of the possibility of reactivation of previous latent infection in immunocompromised cases leading to serious results [185].

**PATHOPHYSIOLOGY**

The pathophysiology of microsporidiosis is not adequately known. The pathogenesis and clinical picture depend mainly on the species of microsporidia causing infection, the site of infection, and most of all on the immune status of the infected host [20, 99, 186]. Although infection is mostly diagnosed in patients with impaired immunity, microsporidia have also been detected from immunocompetent individuals [187, 188]. Infection occurs mainly by ingestion or inhalation of the environmentally resistant spores of microsporidia, in addition to other routes of infection including their passage through injured skin or ocular surface, trauma, sexual route [88], as well as possibility of transmission by insect bites [88, 162]. *E. bieneusi* and *Encephalitozoon spp.* are considered the most
common microsporidia recovered from human infections [88, 132, 189]. They tend to infect the gastrointestinal tract. *E. bieneusi* locates in the apical part of the intestinal villi, while *Encephalitozoon intestinalis* infects not only the intestinal villi, but also the cryptic cells, thus reaching and invading macrophages, fibroblasts, and endothelial cells [20, 23, 190, 191]. The infection of intestinal villi results in flattening of the epithelium and leads to subsequent atrophy of the villi of the brush border with compensatory elongation and hyperplasia of the crypts reducing the absorption surface area up to 40%. There will be lymphoid exocystosis with edema as well as vesiculation and necrosis of the enterocytes [20, 23, 88, 89]. *E. intestinalis* can induce extensive ulcerations with subsequent mucosal atrophy, acute and chronic inflammation, and zones of submucosal macrophage infiltration [24, 192], resulting in malabsorption of lipids, vitamin B12, and D-xylose as well as electrolyte imbalance (especially potassium and magnesium) and decreased level of serum bicarbonate [89, 193, 194]. Infection of the intestinal epithelium with *E. bieneusi* is restricted to the enterocytes at the tip of the villi leading to villous atrophy, cellular degeneration, necrosis and sloughing. The preferred site of infection is the jejunum and the duodenum showed less commonly affected while the large intestine is not included in infection [195, 56];

*E. intestinalis* causes granulomatous interstitial enteritis accompanied by severe diarrhea and may disseminate to lungs and sinuses [19].

Theoretically all organs could be infected during dissemination of *Encephalitozoon* spesies [196]. Clinically, disseminated microsporidiosis might present as encephalitis, keratoconjunctivitis, sinusitis, pneumonia, myositis, peritonitis, nephritis, and hepatitis [77]. Dissemination of *E. intestinalis* induces inflammatory reactions in infected organs such as the liver, the pancreas, the lungs, and the kidneys [89]. The functional impairment was attributed to be as a result of decline in the enzymatic activities at the basal portion of the intestinal villi [20, 193].

Hepatic infections with *Encephalitozoon spp.* can cause a granulomatous necrosis with the presence of microsporidia disseminated within the hepatic parenchyma or a non-granulomatous inflammatory reaction [23, 20].
Bile duct infection may be complicated by papillary stenosis, alithiasic cholecystitis, bile duct dilatation, and sclerosing cholangitis [20, 23]. Muscle infections with *Pleistophora spp.* lead to muscle atrophy and diffuse degenerative lesions with numerous microsporidia spores infiltrating the muscle fibers [20, 23]. *E. cuniculi* may infect the kidneys and CNS in a big variety of mammals including man. The infected tissue generally exhibits minimal inflammatory reactions, but changes might range from normal tissue architecture to severe degenerative lesions of the epithelium [20, 23].

Kidney lesions are seen as a tubulointerstitial granulomatous nephritis with an inflammatory infiltration composed of macrophages, lymphocytes, plasma cells, and Langhans type multinucleated giant cells [23]. Infection of the ureters can lead to a granulomatous inflammatory reaction. In the bladder, the lesions produce an ulcerated cystitis with lympho-histiocyte infiltration [23]. *Encephalitozoon spp.* infects the genitourinary system in most mammals, including humans [186, 197 - 199].

Granulomatous interstitial nephritis composed of infiltration by plasma cells and lymphocytes are the main pathological finding. Usually it is associated with necrosis of the kidney tubules, with their lumens full of amorphous granular material. Spores of microsporidia are detected in the necrotic tubules and the sloughing tubular cells [105, 109, 116, 119]. Upon their shedding into the urinary bladder, they are able to infect other epithelial cells in their way causing ureteritis, prostatitis, and cystitis [197]. Infection also affects the muscle cells, fibroblasts and macrophages of the affected mucosa and is often associated with the shedding of spores in urine. However sometimes spores of microsporidia may not be detected in urine in cases with renal failure and intestinal microsporidiosis, indicating that dissemination does not always occur [200].

Erosive tracheitis, bronchitis, and bronchiolitis have been reported in microsporidial infection. Typically, organisms are found in intact or sloughed epithelial cells [201, 202].

Biopsies taken from AIDS cases complaining of chronic sinusitis and microsporidiosis showed the presence of spores in the epithelial cells of sinuses as
well as in supporting structures [203 - 205].

Deep inflammatory keratitis may occur in microsporidial ocular infection with inflammatory cellular infiltration and zones of necrosis associated with thickening of the cornea [123, 206]. This reaction is however generally moderate or even absent in the event of superficial keratoconjunctivitis where the inflammatory infiltration is made up of neutrophil polymorphonuclears and mononuclears (punctate keratopathy) [23].

The relation between microsporidiosis and diarrhea in HIV-positive cases is complicated, as there is always the possibility of the effect of other intestinal pathogens in cases with declining immune status plus the direct effect of HIV on the gut. In case of mono-infection with microsporidia, intestinal biopsies showed villus atrophy and crypt hyperplasia where as cases without other intestinal pathogens didn’t show the same changes [77].

IMMUNOLOGY AND IMMUNOPATHOLOGY

The immune response against microsporidia and the receptors involved in recognition by the infected host are not well known. Toll-like receptors (TLR) are receptors that can recognize and bind to certain specific molecules on the surface of pathogens and stimulate a variety of inflammatory reactions [207].

TLR2 can recognize *E. cuniculi* and *E. intestinalis* on primary human infected macrophages which activate nuclear factor, kappa- light-chain-enhancer of activated B cells (NF-κB), releasing some inflammatory cytokines, mainly interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α) [207]. About an hour after infection of macrophages, they develop nuclear translocation of NF-kB with production of TNF-α and IL-8. To test the role of TLR2, small interfering RNA was used to knock down the receptors of the primary human macrophages. After challenge with spores there will be an increased nuclear translocation of NF-14kB and the levels of TNF-α and IL-8 [207]. Infection leads to activation of antibody production by the host. Persistence of these specific antibodies is associated with latent infection and they play an important in resistant to infections. However specific antibodies fail to act as a barrier against infection when acting alone [208]. When hyperimmune serum was injected into athymic mice previously
infected with *E. cuniculi*, it failed to improve their survival rate, which was explained by the possibility of having another ancillary defense mechanism [208]. Macrophage-mediated phagocytosis could be facilitated by an opsonic function of specific antibodies against microsporidia. Sometimes uncontrolled antibody response was associated with disease, in such cases there is hypersensitivity reactions and hypergammaglobulinaemia resulting in the formation of immune-complexes and renal failure. This type of reaction has been reported in some arctic foxes and dogs infected with microsporidiosis [209 - 211].

Immunologically competent animals develop IgG antibody response; two weeks after infection, which peaks at week 5-6 and in most of the cases, it persists lifelong. The long-term presence of high levels of specific antibodies is used in diagnostics to isolate seropositive animals from others [69, 212].

Antibodies against *E. cuniculi* were also detected in immunologically competent people, but the authors did not directly observe microsporidia [83, 213].

Specific antibodies against microsporidia with variable levels; have been detected in HIV positive cases with confirmed microsporidiosis and in HIV negative cases with previous history of microsporidiosis. It is thought that the variability depends mainly on the immune condition of the person at time of microsporidial infection [17, 214].

Macrophages are part of the primary response against pathogens as they reside at the site of their entry, so they are considered a link between innate and adaptive immunity [215]. Some pattern recognition receptors (PRR) on the surface of local macrophages can recognize foreign pathogens resulting in a circle of host defense mediators as cytokines, chemokines, nitric oxide (NO), nitric oxide synthase (iNOS) and radical oxygen species.

Activated T cells secrete IFN that initiate respiratory burst to kill the phagocytized intracellular pathogens [216]. Microsporidia can evade these protective immune responses by using macrophages as their “Trojan horses” to carry them to different organs of the body initiating a disseminated infection [217].

Dissemination is thought to occur in two steps. The first is the initial infection in
the intestine by species like *E. cuniculi* or *E. intestinalis* invading the resident macrophages [218]. These macrophages secrete chemo-attractants in response to infection, to recruit new cells as monocytes to help resolving the infection. In patients with multifocal involvement of the organs with microsporidia, the lesions appear as micro-abscesses and granulomas.

During the second phase, macrophages, which failed to kill the cellular intruder, migrate into the lymphatic system, blood and tissues. Microsporidia gain access to the host cells through eversion of their filaments and penetration of the cell membrane or by phagocytosis of the released spores [218].

Studies showed that microsporidia could inhibit the process of fusion of phagosome and lysosome, thus affecting the ability of the parasite to survive inside the macrophages [219]. This finding can explain the theory that the microsporidia remaining inside the primary phagolysosomes can evade the immune responses of macrophages and continue their life inside the cells. Cell-mediated immunity has its role in the prevention of severe infection with *Encephalitozoon*. The Th-lymphocytes with CD4+ receptors, Te-lymphocytes with CD8+ receptors and a few populations of the TCR-associated with CD3+ trigger the reaction. The adoptive transfer of sensitized T-splenocytes could protect athymic BALB/c and SCID mice infected with microsporidia *E. cuniculi* from death [208, 217, 220].

CD8 T-cells participate in the pro-inflammatory response by the production of cytokines such as interferon gamma (INF-α) and their direct cytotoxic effect [190]. They also contribute to the regulation of the immune response by secreting interleukin-10 (IL10) [23, 85]. Recent studies have identified the significance of pro-inflammatory cytokines as INF-α, tumor necrosis factor and interleukin-12 (IL12) in resistance against *Encephalitozoon* infections [190]. Studies showed that IL-10 blocked the effect of INF-α in controlling the cellular immunity [223]. This implicates IL-10 in preventing early dissemination of microsporidia as observed in SCID mice, which do not produce IL-10 [222]. In immunodeficient mice without T or B-lymphocytes [223], even fully functional macrophages could not produce IL-10 [221]. The higher levels of IL-12 found in this situation stimulates the production of INF-α, which is the main cytokine of macrophages that helps in
the process of phagocytosis and elimination of spores.

*In vitro*, injection with antibodies against INF-α or IL-12 was able to neutralize the resistance to the parasite [216]. Aged mice infected with *E. cuniculi* developed unusual priming of the T-cells by dendritic cells and could restore their adaptive immunity when injected with dendritic cells (DCs) extracted from younger mice [224]. This observation is in accordance with clinical data from humans, since the elderly are more susceptible to microsporidiosis.

*Encephalitozoon, Trachipleistophora* and *Pleistophora* species could disseminate causing systemic disease with the affection of the sinuses, eyes, liver, muscles, kidneys, peritoneum, CNS and respiratory tract in immunodeficient individuals. *E. cuniculi* can cause serious disease due to development of immune complexes and renal disease in carnivores such as domestic dogs, blue foxes and mink [225, 129, 130].

As a final result, both hyper and hypo-immune responses to microsporidia can be a cause of disease and only the well-regulated immune response in a host can control these pathogens resulting in suclinical infection [225].

**SYMPTOMS**

Clinical manifestations of microsporidiosis mainly depend on both the immunological response of the host and the site of infection, which shows great variability as the organisms can almost infect every tissue and organ of the affected hosts [172]. In immunocompromised patients, microsporidia most likely develop disease, manifesting as severe opportunistic infections, often with fatal outcome [128 - 130]. In AIDS patients with CD4+ counts <100 cells/μL microsporian species *E. bieneusi* and *E. intestinalis* were the most common causes of diarrhea with fever, lack of appetite and loss of weight [77, 226, 227, 132].

Most of the reported cases are confined to the intestine, however there are wide spectrum of diseases ranging from asymptomatic carrier to dissemination to different organs leading to keratoconjunctivitis, hepatitis, cholangitis, ascites, myositis, sinusitis, kidneys and urogenital infections [3, 23, 193]. Infection with *E. intestinalis* and *E. bieneusi* usually manifests as chronic diarrhea of 3-10
motions/day, bloating, lack of appetite and loss of weight not associated with fever [25, 132, 186, 228, 229]. Diarrhea is often associated with mal-absorption, weight loss and wasting [228]. The mortality rate was reported to be more than 50% among cases with wasting and advanced HIV infection [25]. Organ transplant recipients under immunosuppressive therapy were reported to have fever, fatigue, nausea and diarrhea when they develop infection with *E. bieneusi* or *Encephalitozoon spp.* [77, 108, 226]. Children in tropical countries infected by microsporidia, primarily *E. bieneusi*, might suffer from persistent diarrhea, malnutrition and lowered immunity [106, 107, 161]. Recently, it was suggested that the elderly might have decreased immune competency, therefore become more susceptible to infection with microsporidia [90].

The association of microsporidiosis with human disease was discovered in the mid 1980s, when the organism was detected in stool samples of cases with HIV/AIDS with chronic diarrhea [195]. There was controversy on the pathogenicity of microsporidia, as they have been reported in persons without diarrhea [216, 230], until the opportunistic nature of these microorganisms became more clear [88, 216]. In experimental infection of immunocompetent laboratory animals, early acute stage of infection showed clinical signs, followed by asymptomatic shedding, whereas the same infection caused death of immunodeficient athymic and SCID mice [85]. Asymptomatic chronic infection was observed in immunocompetent hosts, which were infected with *E. cuniculi*, naturally or experimentally [128, 129]. In some cases, mild clinical signs are developed early after infections. An example is the formation of ascites in some mice experimentally infected with *E. cuniculi*, which resolved 2 weeks after inoculation. Also the development of motor paralysis, convulsions and torticollis in infected rabbits [128, 129]. Self-limiting traveller's diarrhea, with a duration of about 2-3 weeks may develop in healthy individuals after getting infection with microsporidia [104, 231, 232].

Intestinal and biliary infections: The detected pathogens in these sites in immunocompromised cases were mainly *E. bieneusi* and less frequently *E. intestinalis*.

Infection usually causes severe non-bloody, non-mucoid intermittent diarrhea
with gradual onset and months duration. Cases develop malabsorption of nutrients with progressive weight loss. There is an association between intestinal infection and lactase deficiency, decreased activity of alkaline phosphatase and α-glucosidase at the base of villi with atrophy of villi and reduction in their height. The patients may have nausea and loss of appetite. Reports show that microsporidial spores may be excreted in the diarrheic or normal stool. In patients with chronic diarrhea, without any other known intestinal pathogens, *E. bieneusi* have been detected in 7-50% of the study cases depending upon the methods of diagnosis and the group of study [77, 193]

**Hepatitis and Peritonitis**

*Encephalitozoon spp.* is able to cause hepatitis and/or peritonitis. *E. cuniculi* was identified on the basis of ultrastructure in two HIV-infected patients at autopsy [16, 233]. In these cases, infection was diagnosed on bases of ultrastructural basis without exact species identification. Another case of infection with *Encephalitozoon spp.* in a patient with AIDS that suffered from diarrhea for 2 months before he died from fulminant hepatitis. The autopsy specimen examinations showed disseminated microsporidiosis in the liver, gall bladder and mediastinal lymph node [234]. *T. anthropophthera* have been reported in a 8-year-old HIV-infected girl with disseminated infection in the liver and pancreas [235]. *E. bieneusi* and *E. intestinalis* were detected from the non-parenchymal cells of the liver in some cases with HIV infections without signs of hepatitis.

**Ocular Infections**

Ocular infection is considered the second most common manifestation of microsporidiosis after gastrointestinal infection [73]. Keratoconjunctivitis may be caused by all *Encephalitozoon spp.* (*E. intestinalis, E. cuniculi* and *E. hellem*) in HIV-infected cases. Most cases complained of bilateral conjunctival inflammation and bilateral punctuate keratopathy with subsequent decreased visual acuity. Keratoconjunctivitis is often asymptomatic or moderate, but also could be severe ending in corneal ulcers. Other species of microsporida (*V. cornea, N. ocularum, T. hominis, M. ceylonensis* and *M. africanum*) have also been reported as single case reports [73].
From 1989 to 1991, six cases of microsporidian keratoconjunctivitis were reported in patients with AIDS, four from New York, one from Texas, and one from Ohio [6, 17, 18, 236 - 238]. All had conjunctivitis, blurred vision, and photophobia. By 1999 over 20 cases were characterized, reported and reviewed [73]. Organisms were observed in corneal epithelial cell scrapings examined by light and electron microscopy [18, 239]. The organisms were morphologically similar to *E. cuniculi*, but a clearly defined parasitophorous vacuole surrounding the organisms, was not always visible [81]. *Encephalitozoon hellem* was identified as morphologically identical to *E. cuniculi*, but was serologically different [17]. Topical steroid treatment was thought to promote a localized immunosuppression of the eye with exacerbation of ocular microsporidial infection in some cases [240, 241].

**Sinusitis**

It is one of the common manifestations of microsporidiosis in humans [203]. *Encephalitozoon* species (*E. hellem*, *E. cuniculi* and *E. intestinalis*) have the ability to cause rhinosinusitis in many HIV-infected patients, while other species *E. bieneusi* and *T. hominis* caused less frequent infections in patients with nasal polyps and severe rhinitis [203].

![Fig. (5). Chest X-rays of a female HIV patient with left-sided pneumonia caused by *Enterocytozoon cuniculi.*](image)

**Lower Respiratory Tract Infections**

It is less frequent than other microsporidial infections. It may show asymptomatic infection or could be associated with bronchiolitis. Pneumonia and respiratory failure might be the main manifestation of systemic infection in HIV positive
All species of *Encephalitozoon* have been reported to infect the bronchial epithelial cells in cases with disseminated microsporidiosis in HIV-infected patients. Pulmonary *E. bieneusi* was only detected sporadically [77].

**Urinary Tract Infections**

Urinary tract infection usually occurs with disseminated *Encephalitozoon* infections in HIV positive cases. Infection may be asymptomatic or may present with cystitis or nephritis with dysuria and haematuria, or may be the cause of progressive renal failure [20].

**Myositis**

This type of infection has been described in few immunocompromised cases and infection was diagnosed to be due to *Pleistophora*-like microsporidia and *Trachipleistophora spp.* [8, 11, 179, 242]. Patients presented with fever with generalized muscle weakness. Spores of microsporidia were detected in muscle biopsies.

**Cerebral Infections**

Involvement of the CNS was reported in two HIV-positive children with disseminated *Encephalitozoon* infection [55, 213]. Both patients suffered from signs of intracranial affection *e.g.* headache, vomiting, seizures and spastic convulsions. Diagnosis by immunohistochemistry and molecular analysis, was described in the mentioned cases [243]. Affection of the CNS with *T. anthropophthera* was also diagnosed in cases presented with seizures and cerebral manifestations. Autopsy specimen examination showed disseminated infection including the brain [253].

**Rare Manifestations**

**Urethritis**

Microsporidiosis was reported in two AIDS patients suffering from urethritis,
sinusitis and diarrhea. One of the patients had *Encephalitozoon*-like spores in his nasal discharge, stool samples, urine, and urethral pus and the stool samples of the other case [236, 237].

**Cutaneous Microsporidiosis**

A nodular cutaneous lesion has been reported as due to infection with *Encephalitozoon intestinalis* in the leg of an HIV positive patient [4].

**Vocal Cord Infection**

Vocal cord infection with microsporidia has been reported in a patient with lymphocytic leukemia, who had received chemotherapy. The patient complained of hoarseness and shortness of breath. A biopsy of the area of the false vocal cord nodules was examined by Electron Microscopy and confirmed by molecular technique, showed infection with *Annicalila algerae*, that is reported as an insect pathogen [4].

**Systemic Infections**

The first reported human case with microsporidial infection in 1959, was a case of disseminated infection with *Encephalitozoon* in a 9-year-old Japanese child. The patient presented with intermittent fever and signs of CNS affection in the form of: headache, vomiting and spastic convulsions [55]. CSF and urine samples showed the presence of *Encephalitozoon*-like organisms. In the year 1984, a new similar case has been reported in a 2-year-old Colombian boy living in Sweden. The child complained of convulsive seizures and *Encephalitozoon*-like organisms had been recovered from his urine. The patient’s serum samples had IgG and IgM against *E. cuniculi* [213]. Disseminated microsporidiosis with all *Encephalitozoon* species have been reported in immunosuppressed HIV-positive cases [23]. The possible manifestations in such cases include: ocular lesions in the form of keratoconjunctivitis, respiratory tract lesions in the form of bronchiolitis, and pneumonia, urogenital and gastrointestinal lesions. However there were significant distribution pattern for each species of microsporidia [77, 186]. *E. hellem* was identified as a cause of keratoconjunctivitis, sinusitis, bronchial disease and urinary tract infection. *E. intestinalis* was mainly affecting the
gastrointestinal and biliary system. It disseminates to the eyes, nasal sinuses, respiratory tract and kidneys. *E. cuniculi* was identified as a cause of wide dissemination in all organs, with clinical symptoms varying from no symptom to severe disease [20, 77, 186]. There was also a single case report for disseminated microsporidiosis due to infection with other species (*N. connori, V. corneae, T. hominis* and *T. anthropophthera*).

**DIAGNOSIS AND DETECTION METHODS**

Microsporidiosis is probably overlooked as a disease because the detectable elements, “the microsporidian spores”, are very small and microscopical diagnosis therefore requires expertise [88]. The index of suspicion for microsporidiosis should be highest in patients with cellular immunosuppression such as HIV or transplant recipients. Intestinal infection with microsporidia should be included in differential diagnosis in any patient with unexplained chronic diarrhea or hepatobiliary disease [72]. Some authors suggest including microsporidia in the differential diagnosis of travel-associated diarrhea; although in our experience such cases are extremely rare [unpublished data]. Infection with microsporidia should probably be considered in cases of unexplained keratoconjunctivitis or corneal ulcers, in unexplained renal insufficiency or in cases of myositis. Since dissemination can occur, microsporidiosis may affect virtually any organ system, including bone and central nervous system. Therefore the identification of microsporidia in any specimen should prompt a thorough search in all other readily available sources, including stool, urine, sputum, nasal and conjunctival swabs, and possibly cerebrospinal fluid, with consideration of more invasive approaches for other sites of infections e.g. myositis [72]. Since microsporidial spores can occur in virtually any clinical sample, microbiologists and pathologist should be familiar with their appearance. In most stains such as routine Gram or Giemsa stain, they are visible as oval structures resembling yeast cells (Fig. 6 and 7) and microsporidial infection has to be considered in samples with such “yeast-like” cells, which are negative in fungal cultures.

Most methods for the detection of microsporidia were developed to diagnose infections in immunocompromised patients with a higher load of microorganisms. As awareness of microsporidiosis increased and more sensitive (molecular)
techniques became available, more infections in immunocompetent individuals were reported. They might be more frequent than previously expected [185], but if those positive cases represent true infections or only temporary shedding, requires further studies.

Fig. (6). Sample of HIV patient with pneumonia caused by *Encephalitozoon cuniculi*. Routine Gram stain of broncho-alveolar lavage sample showed Gram-positive oval structure (arrow), initially misidentified as yeast cells (A). Tissue gram stain of transbronchial biopsies revealed typical intracellular spores, which were identified with monoclonal antibodies and electron microscopy as *E. cuniculi*.

Fig. (7). Giemsa stain of a broncho-alveolar lavage of an HIV patient with pneumonia caused by *Encephalitozoon cuniculi*.

As soon as microsporidia are detected in a clinical specimen, examination of other body tissues and fluids should be considered. Urine must be examined as a routine in all cases suspected to have microsporidial dissemination. This regimen is thought to have a therapeutic implication because the most common microsporidia causing dissemination, *Encephalitozoon spp.*, is sensitive to albendazole, whereas *E. bieneusi*, which usually does not disseminate, is resistant to this drug [72].
Specimen Collection

Spores of microsporidia can resist environmental conditions for years keeping their infectivity, if they are protected from excessive desiccation. Stool samples sent to the laboratory should be preserved in 5% or 10% formalin or in sodium acetic acid-formalin. In case of suspected dissemination, urine, sputum, bronchoalveolar lavage, nasal secretion, cerebrospinal fluid (CSF), conjunctival smears and corneal scrapings are submitted to the laboratory. They are submitted in formalin for ordinary microscopic examination, fixed in glutaraldehyde for electron microscopy and fresh for cell culture or molecular studies [60].

Stool Examination

Detection of intestinal microsporidiosis by light microscopy in cases with chronic diarrhea requires sufficient experience, as spores have a size similar to bacterial and yeast cells and can easily be missed within the sample’s microflora and debris. In stool specimens (and other samples contaminated by other microorganisms) it is therefore necessary to use special stains to identify microsporidia. The most common stain for stool is Weber’s chromotrope stain (Fig. 8), which is considered practical [228].

Fig. (8). Stool sample of a male HIV patient with chronic diarrhea and wasting stained with Weber’s stain. Microsporidial spores (in this case Enterocytozoon bieneusi) can be identified as multiple red structures with oval shape of 2.3 x 0.8 µm (arrow). The main characteristic is that spores are not homogenously stained but show typical vacuoles (insert).
The use of positive control material is highly recommendable, especially if positive samples are rare. Spore detection requires a total magnification of $\times 1000$ with oil immersion. The spore wall stains with variable degrees of red, while its interior shows a characteristic inhomogeneous pattern “vacuoles” (see Fig. 8).

The counterstain gives the background a blue or green color, according to the type of stain used. At least 100 fields should be examined under oil immersion (1000 x) and the size of spores should be measured. Bacterial spores, as well as other findings of yeast cells and debris could be stained in red color.

Therefore morphological identification has to be performed by an experienced microscopist considering the size and staining pattern. For quality control reasons, positive control smears have to be included [60]. Modifications of Weber’s method have been described and include modified trichrome stain (Ryan’s stain) and the Gram chromotrope method [172, 244]. Optical brightener binds to the chitineous wall of microsporidian spores and can be used to visualize them under UV light. Commonly used agents are Calcofluor white M2R (Fig. 9) and Uvitex 2B (Fungiqual A), others such as fluorescent brightner 28 and Fungi-Flour [245] can also be used in the same manner. Since these reagents bind to chitin, they also stain fungal structures and many fibers.

![Fig. (9). Encephalitozoon intestinalis stained with Calcofluor white.](image)

Therefore these stains can only serve as screening tool and do not allow the differentiation of microsporidia from other structures such as yeast cells. They might increase sensitivity since they allow screening a higher number of microscopical fields. If elements compatible with microsporidial spores are
visualized with these stains, confirmation by specific stains (e.g. Weber’s stain) or other methods has to be performed.

**Direct Immunofluorescence**

Direct immunofluorescence using monoclonal antibodies for the diagnosis of infection and species identification is effective [246, 247]. It is technically simple and rapid, and does not require costly reagents or equipment (Fig. 10).

![Monoclonal antibody-based immunofluorescence identification of Encephalitozoon hellem.](image)

High sensitivity and specificity values have been reported [246]. Some authors find the technique comparable to PCR, which is more complex and costly and is therefore mainly used in research laboratories [247]. Thus, the use of monoclonal antibodies might become the routine method for the specific diagnosis of microsporidiosis [247]. Monoclonal antibodies kits are currently available (e.g. Bordier Laboratories). Still, these kits are not widely available and do not always include positive control slides [1, 246, 247].

**Electron Microscopy**

Before the widespread use of molecular techniques in the diagnosis of microsporidiosis, EM was used as the gold standard for confirmation of diagnosis and for species identification of microsporidia.

Electron microscopy is a very reliable approach in the diagnosis of microsporidia. One of its limitations is the difficulty to have an electron microscope in most
laboratories. Another limitation depends on its restricted ability of morphological identification to the species level, which will need antigenic characterization or molecular identification [60].

Although electron microscopy was used in the diagnosis of microsporidiosis in body fluid specimens with success, it showed difficulty to differentiate species in the tissue biopsy specimens due to absence of proliferative stages and the small amount of sample to be examined renders this technique of low sensitivity [193]. It is advised to always place the body fluid sediments or the tissue biopsies in case of suspected infection in the fixatives used for Electron Microscopy for further examinations [60]. EM is important for the diagnosis of the details of the spores of microsporia. Slides should be ultrathin (1 micron) to identify the internal structures. Preparation and examination of the specimens are also time consuming and need trained personnel.

**Molecular Methods**

The molecular analysis of microsporidia can provide a highly sensitive and specific tool for detecting and differentiating species in biological samples. It could also explain a wide range of geographic distribution of microsporidia that may infect man, as well as their demographic data, zoonotic relationship and their survival in the environment [146, 193, 248].

Conventional PCR is a sensitive, specific and reproducible method that is considered an alternative to electron microscopy. The detection threshold for microsporidia is 102 spores/g fecal samples, much lower than detected by light microscopy, where the cutoff is around 104-106 spores/g [20, 156, 172, 193, 249]. Conventional PCR has however several drawbacks. First, it is a long, expensive technique performed by specialized laboratories. There is also risk of contamination and the parasite load cannot be quantified [249]. Since small number of spores might be ingested with food or water, false positive results are possible. Quantitative PCR: Over the last few years, quantitative PCR, particularly with the advent of real-time procedures, has revolutionized the diagnosis of certain infectious diseases, including microsporidiosis.

The Quantitative PCR is considered one of the most reliable methods of detection
and identification of microsporidia in stools up to <40 spores/ml suspensions [248]. Real-time PCR offers the advantage of eliminating all post-PCR manipulation, reducing the reaction time and limiting the risk of contamination, and consequently of false positives [249, 250].

Multiplex PCR: This technique is designed to use two couples of probes specific for two species or more simultaneously. Multiplex PCR is a sensitive specific technique for the diagnosis of E. bieneusi and Encephalitozoon spp. [251, 252]. Also multiplex PCR could be used, if the specimen is thought to be containing more than one pathogen [253]. Cell culture: The first attempt to culture microsporidia was made in 1937 by Trager, who was partially successful in establishing Nosema bombycis infection of a cell culture developed from the ovarian tube lining cells of the silkworm (Bombyx mori) [254].

Culture

In vitro culture has also been implicated to determine the species as well as the antimicrosporidial effect of several drugs on E. cuniculi, E. hellem and E. intestinalis [255, 256]. Microsporidia couldn’t be grown well in axenic cultures. However cell culture were used to e.g. monkey and rabbit kidney cell lines (Vero and RK-13), human fetal lung fibrobalsts cell line (MRC-5) and Madin-Darby canine kidney cell line (MDCK) were used to grow Encephalitozoon spp., Trachipleistophora hominis, V. corneae and B. algerae. E. bieneusi which is an important human pathogen, grew only in short term cultures [255, 256].

Only a few attempts have been made to isolate microsporidia into culture from feces because enteric bacteria and yeasts usually overgrow the rich culture medium that is used, which impedes isolation of the fastidious microsporidia, especially E. bieneusi [257].

Nucleospora salmonis, a fish microsporidia was successfully cultured on salmonid leukocytes for a relatively long duration [258]. This cell line is promising for growing E. bieneusi in culture. Little success was achieved in growing Pseudoloma neutriphilia on different cell lines of fish [258]. Although in vitro culture of microsporidia has a limited diagnostic value, however it may provide important diagnostic information [255].
Serological Methods

A variety of serological methods have been used to detect the antibodies IgG and IgM against microsporidial antigens, particularly *E. cuniculi* in experimental animals. Antibodies against *Encephalitozoon* species (*E. cuniculi* and *E. hellem*) have been identified in both HIV and non-HIV positive cases. The presence of these antibodies does not explain if there is a true infection or not, due to possibility of cross reaction with other species or non-specific reactions. The lack of long-term culture made it difficult to prepare suitable antigens for proper serological studies [60].

TREATMENT

Many drugs have been tried for the management of intestinal microsporidiosis. Efficacy has been variable depending on the causal species. The criteria of therapeutic success are the resolution of the clinical manifestations and the absence of spores from samples [191, 259]. At the present time, albendazole and fumagillin are considered the most effective compounds against *Encephalitozoon intestinalis* and *E. bieneusi*, respectively. Other therapeutic alternatives are either less effective or under trial [172, 260 - 263]. Albendazole, is a benzimidazole derivate used for the treatment of a variety of helminthic infestations and giardiasis, that has been tested against microsporidia *in vitro* and *in vivo* with a considerably a good therapeutic effect against *Encephalitozoon spp.* especially *E. hellem* [88, 172, 191, 259, 264 - 267].

The mechanism of action of albendazole consists in the inhibition of microsporidial division by blocking the synthesis of tubulin, a major constituent of the mitosis spindle [89, 191, 260]. Thus, it causes inhibition of the microtubule assembly of microsporidia, including the *Encephalitozoon spp* [268, 269]. Electron microscopical study showed that albendazole affects mainly the developmental stages leading to partial inhibition of the reproduction of the microsporidia affecting the small intestine [270] and was suggested as a suitable treatment for systemic parasitic disease [271]. Clinical studies have demonstrated the efficacy of albendazole against species of the *Encephalitozoon* genus in HIV-infected patients for whom it is the treatment of choice for intestinal, ocular and
disseminated microsporidiosis [172, 259]. On the other hand, it exhibits a less effective action against *E. bieneusi* since it yields only a decline in the parasite load and degenerative alterations of the spores. Clinically, diarrhea might become less severe and the body weight might stabilize. Relapse is however common after treatment withdrawal [279, 260]. Consequently, albendazole has a parasitostatic effect on *E. bieneusi* by incomplete inhibition of replication; stool and duodenal biopsy samples remain positive [172, 189, 191, 194, 259, 260, 269, 278]. Albendazole is absorbed well after oral intake when associated with a fat-rich meal. It is metabolized in the liver where a sulfoxide metabolite is formed and is more active and less toxic than albendazole itself [191, 194, 261, 269]. The oral dose of albendazole is 400 mg b.i.d. for adults and 7.5 mg/kg b.i.d for children with total daily dose of 15 mg/kg for 2-4 weeks [89, 194, 260]. Severely immunosuppressed individuals might require longer periods of treatment or maintenance therapy. Albendazole is well tolerated and does not require special surveillance. Rare adverse effects have been described in the form of abdominal pain and diarrhea. There also may be minor elevation of the serum transaminases, which is reversible at withdrawal as well as proteinuria and/or neurological manifestations. Exceptional cases of reversible alopecia, malaise with vertigo, skin rash, fever, pruritus, and less commonly, hematological disorders (neutropenia, pancytopenia) have been reported [194, 260, 262]. It is contraindicated for patients with known hypersensitivity to albendazole as well as pregnant or lactating women [194, 262]. Drug interactions exist with cimetidine, dexamethasone and praziquantel, leading to increased serum levels of albendazole [194, 262].

**Other Benzimidazole Derivates**

Certain benzimidazole derivatives have been studied in terms of efficacy for the treatment of microsporidiosis. Mebendazole has been found to be active against *E. intestinalis in vitro* but is poorly absorbed after oral administration. Nocodazole and parbendazole also showed anti microsporidial effect, but presented toxic effects limiting their use. Thiabendazole is well absorbed but poorly active [191, 262]. Fenbendazole may be of potential interest because of its rapid absorption after oral intake and its metabolism into oxfendazole. Fenbendazole and oxfendazole are very active against *E. intestinalis* and are non-toxic *in vitro*.
These compounds appear to be promising for the treatment of microsporidiosis [191, 260].

**Fumagillin**: (Fumidil B, Fumadil, Fugillin, Fumagillin, Flesint), a known antibiotic, anti-angiogenic substance and a product of Aspergillus fumigatus, has demonstrated a good anti-*E. bieneusi* activity [191, 272], although various adverse side effects have been reported [272, 273]. Fumagillin was identified in 1949 and was used in 1953 by beekeepers against encephalitozoonosis in bees caused by *Nosema apis* and as a human drug for the treatment of amoebiasis prior to the development of more effective amoebicidal agents [88, 89, 191, 260, 272]. The target of fumagillin is a cellular metalloprotease, methionine aminopeptidase-2 (MetAP2). This enzyme is indispensable for microsporidia metabolism and survival. It is essential for eliminating methionine on the terminal end of proteins, necessary for post-translational and functional modifications [88, 273]. The fumagillin acts by inhibition of the replication of microsporidia by blockage of the site of action of MetAP2 and by the inhibition of RNA synthesis and consequent death of the organism [88, 191, 171, 260, 273]. Fumagillin has been used successfully against species 28 of the *Encephalitozoon* genus and against *Vittaforma cornea in vitro* and in humans for the treatment of ocular infections caused by *E. hellem* and intestinal infection by *E. bieneusi* [88, 273]. Topical fumagillin is effective as eye drops in a concentration of 70 µg/mL in saline. Microsporidial eye infections might require long-term and maybe lifelong therapy [263, 274]. Systemic side effects are negligible. Since in such cases systemic spread is possible, a combination with albendazole should be considered. The drug is prescribed for oral intake 20 mg t.i.d for a total dose of 60 mg/ d for 14 days. The efficacy of systemic fumagillin is counter balanced by its adverse effects. When administered orally, the drug exhibits bone marrow toxicity by its direct effect on the megakaryocytic line and myeloid progenitors [272]. Thrombocytopenia and neutropenia are the most common adverse effects requiring regular medical follow up for the entire duration of treatment [88, 89, 189, 191, 194, 271, 273]. In addition, abdominal pain, diarrhea, vomiting, and hyperlipasemia have been noted during the use of fumagillin. This drug is contraindicated in the event of hypersensitivity [194, 261]. A fumagilin analog, TNP-470, with fewer side effects could replace fumagillin for the systemic
treatment of \textit{E. bieneusi} infection in the future [274]. Other drugs used: \textbf{Nitazoxanide} (Cryptaz): Is a broad-spectrum oral anti-parasite agent against protozoa such as amoeba, nematodes, cestodes, and trematodes [260, 275]. It is also used for the treatment of cryptosporidiosis [260, 262, 275]. The drug inhibits the action of pyruvate ferredoxine oxidoreductase of the electron transport system [262]. It is prescribed at the dose of 1g b.i.d for 60 days [260, 262]. Nitazoxanide has proven efficacy \textit{in vivo} on cell cultures of \textit{E. intestinalis} and \textit{Vittaforma cornea}. A clinical effect has been reported in a single case report of an AIDS patient with \textit{E. bieneusi} infection [275].

\textbf{Antiretroviral Therapy (HAART):} These combination therapies aim to suppress viral replication and to restore cell-mediated immunity. As immunodeficiency is the main factor promoting microsporidiosis, HAART is the most effective treatment for microsporidiosis in HIV patients [265]. Countries where HAART has been used showed a dramatic decline in the microsporidial incidence among HIV cases.

Apart from the restoration of cell-mediated immunity, some antiretroviral drugs might also exhibit direct antiparasitic activity (\textit{i.e.}, protease inhibitors) [276]. In situations where there is difficulty to get HAART, there will be no improvement in the incidence of microsporidiosis [57].

With antiretroviral therapy, HIV-infected patients have a lower viral load and improved CD4 counts with reconstitution of their immune defense. Consequently, anti-retroviral therapy reduces the prevalence of opportunistic infections, including microsporidiosis, and reduces the morbidity and mortality related to HIV infection [84, 95, 172, 189, 259, 277]. It also enables the eradication of microsporidia infection without use of a specific treatment. It considerably reduces the risk of recurrent microsporidiosis observed after treatment withdrawal in subjects with severe immune deficiency [277].

\textbf{PERSPECTIVES OF CONTROL}

In the management system for the control of microsporidiosis, it is of great importance to view the epidemiological extent of the infection. The potential role of water and food in the transmission of microsporidia is of importance and the
usual measures and precautions should be taken to prevent their contaminations with the urine or feces of infected animals or humans.

Unfortunately, in many countries there is insufficient epidemiological data on the clinical and environmental magnitude of infection and contamination with these organisms.

Microsporidial spores are able to survive for a considerably long durations and remain infective in the environment [278]. Spores of *E. cuniculi* could be affected by exposure to bleach, ethanol, and other disinfectants leading to decrease in the infectivity of the organisms in tissue culture models [21, 279 - 281]. Physicians should contribute in diagnosis and treatment of infection by including microsporidiosis in the differential diagnosis of chronic diarrhea, fever of unknown origin, myositis, renal disease, biliary-hepatic disease, and other unusual symptoms in HIV patients, organ transplant recipients, and other patients at risk. Laboratories should also include the methods for the detection of microsporidia e.g. staining and DNA identification of these parasites. Besides, the laboratory staff should be trained for different techniques and the morphological features of microsporidia in specific and unspecific stains.

The Center of Disease Control and the HIV Medicine Association of the Infectious Diseases, provide a comprehensive guideline for the treatment and control of the opportunistic infections detected in HIV cases including microsporidiosis [282]. There are new guidelines are available in: http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oil.pdf [282]. For immuno-compromised patients, as the organ transplant recipients or AIDS, microsporidiosis could be life threatening. The recommendations are similar to other opportunistic food- or water-borne and zoonotic infections. They include proper hand hygiene and drinking bottled or at least boiled water [57]. Meat, fish, and seafood should be properly cooked and fruits and vegetables washed before consuming. Avoiding exposure to animals suspected to be carriers of microsporidia or handing them with great care. Boiling of water for not less than 5 can kill spores of *E. cuniculi*. The application of some disinfectants as Quaternary Ammonium, ethyl alcohol (70%), Formaldehyde (0.3-1%), phenol, Hydrogen peroxide (1%), chloramine or Sodium hydroxide for 30 minutes completely
destroyed *E. cuniculi* spores [173, 276]. Treatment with Ozone, ultraviolet, gamma rays and chlorination at pH 7 reduced the infectivity of *Encephalitozoon spp.* [277, 283 - 286]. Antiparasitic prophylactic agent has not be mentioned, however the improvement of immunity in the immunocompromised patients, as in case of using HAART is effective in remission of microsporidiosis. [287 - 289].

Ocular infection, which may occur as a result of conjunctival inoculation with fingers contaminated with organisms from body secretions, could be prevented by hand washing. Some instructions should be given to infected people to avoid dispersal of spores in their sputum or respiratory secretions.

In hospitals, spores of *E. cuniculi* have the ability to survive and keep their infectivity to a period of about one month, however they can be rendered noninfectious by a 30-minute exposure to the most common disinfectants and by the methods employed for sterilization. Therefore, it is of utmost importance to limit infection in hospital rooms by sufficient cleaning with proper disinfectants.

In experiments with the *E. intestinalis*, a 16-minute exposure to a 2.0 mg/l chlorine treatment was needed to achieve a three-log reduction (99.9%) of viable spores as determined by infection of cell cultures [290]. Treatment with chlorine at concentrations of approximately 2.5 mg/l for a minimum of 4 minutes produced a 3.3 log inactivation of *E. cuniculi*, but only a 0.70 log reduction of *E. hellem* [285]. Scientists developed a very efficient and rapid extraction free, filter-based method to prepare DNA template for the use in PCR, to identify microorganisms including microsporidia spores. The method could be adapted for detection of parasites from clinical and environmental samples using multiplex PCR with great sensitivity and minimal preparation [291].

The United States Environmental Protection Agency prepared some instructions 1622 and 1623 which have been modified in 2005, These instructions were given in response to the Safe water Amendments of 1999 to identify and determine the water-borne parasites: *Cryptosporidium parvum* and *Giardia intestinalis*. The recommended methods are also applied to detect and identify microsporidia (http://www.epa.gov/nerlcwww/). The main steps of these approaches depend on filtration followed by separation of spores by an immune-magnetic bead
separation (IMS) assay. Immunofluorescence antibody (FA) staining is used to identify the separated microsporidia. Another variation in the methods of detection uses the separation of spores on an immune-magnetic separation assay (IMS) followed by PCR [167] or water filtration followed by PCR [169].

VACCINES

Little has been done and published in the field of preparation and use of vaccines against microsporidia. Some of the main difficulties are establishing and maintaining microsporidial culture, development of resistance associated with cellular immune response and the choice of the biological product that can serve as a vaccine. However the main few successful trials carried out were directed towards the protection of farm fish from serious infections with microsporidia. Spores of a low virulence strain of the microsporidian Loma salmonae were used to vaccinate trout against the gill disease by intraperitoneal route and showed success in protecting fish against infection with the virulent strain [292]. In an experiment concerned with Loma salmonae, Rodriguez-Tavor et al., reported that the main argument that favors the production of a vaccine was the development of resistance associated to cellular immune response [293]. In another study, the juvenile rainbow trout were vaccinated against microsporidiosis using whole viable spores of the microsporidian Glugea anomala and Glugea hertwigi by intraperitoneal route. The results showed reduction in the numbers of branchial xenomas by 80% and 91%, respectively, after a standard experimental infection with the microsporidian Loma salmonae. Significant protection was obtained when killed-spore preparations were used [294]. Although these studies are limited, yet they may direct the attention towards the success that may be obtained with vaccination in animals or humans.

CONCLUSIONS

Microsporidia are tiny unicellular organisms that parasitize cells of almost all living creatures particularly the immunocompromised subjects. Infection occurs mainly by oral route, however inhalation, sexual relations, direct inoculation into ocular mucosa and wounds and insect bites are also described. Animals are important reservoirs of infection to humans. Food and water are relevant vehicles
of infection and could be contaminated by animal and human excreta. Most human infections affect the intestine, however dissemination to other organs may occur with serious clinical manifestations. Proper diagnosis is needed for proper management of cases. The increased awareness of the modes of infection, pathogenesis and diagnosis can improve the understanding of the epidemiology and management of microsporidiosis in humans and animals.

CONFLICT OF INTEREST

The authors confirm that they have no conflict of interest to declare for this publication.

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