Simple life cycles are completed in a single host (e.g., for mammalian microsporidia, see Fig. **24**), whereas complex cycles require different hosts with involvement of more than one generation of the parasite with different morphologies. Dimorphism of microsporidia was first suggested by Maddox in his Ph.D. thesis, who suspected that *Nosema necatrix* and *Thelohania diazoma* in the armyworm *Pseudaletia unipunctata* [76] represented two stages of the same parasite [79]. This was later confirmed by Fowler and Reeves, who used for the first time protein profiles created by electrophoresis to differentiate among microsporidian species [170, 171]. These findings later led to the creation of the new genus *Vairimorpha* with the type species *Vairimorpha necatrix* [172]. In the meantime, Hazard and Weiser in 1968 were the first to find that some microsporidia in mosquito hosts (*Amblyospora*, *Parathelohania*) have both *Thelohania*-like and *Nosema*-like sporulation sequences, demonstrating that the two morphologically distinctive spores found in the respective host stages represented a single species [173]. Thereafter, numerous further heterosporous species have been detected in other genera, e.g., *Amblyospora* [174] and *Burenella* [175]. In 1985 Hazard (in cooperation with Sweeney and Graham from Australia) [176] and, independently, Andreadis [177] demonstrated that some *Amblyospora* spp. not only have two sporulation sequences in the mosquito host but a third one in an intermediate, copepod host that is required to complete the life cycle. Hazard's research had important taxonomic implications because to this point, many microsporidian genera and species, particularly in insects, had been described only on the basis of spore type and descriptions of one sporulation sequence, without any information about transmission. But after the descriptions of heterosporous species, the primordial dogma "one spore-one species" was questioned by Hazard and Weiser [173].

Edwin Hazard (Fig. **25**) was with the U.S. Department of Agriculture's (USDA's) Insects Affecting Man and Animal Laboratory at Gainesville, Florida from 1963 to 1981 and thereafter served as research leader of the microsporidia project at the USDA's Gulf Coast Mosquito Research Laboratory at Lake Charles, Louisiana. He was engaged in several biological control studies of vector insects and developed a laboratory method to mass produce *Anncaliia algerae* (then *Nosema algerae*) in larvae of the family Noctuidae (together with J. Weiser based on his experience with *Barathra brassicae* as substitute host [178]) for use in field studies against mosquitoes in lagoons in the Panama Canal Zone [179]. In addition to the significant contributions mentioned above, he published important papers on the taxonomy of microsporidia of the family Thelohaniidae, helping to establish the necessity of using electron microscopy to distinguish microsporidian taxa and the importance of ultrastructural features in microsporidian systematics [27, 174, 180]. Because of his sudden death in 1985, much of his work remains uncompleted, especially his effort to develop a more natural system of classification of the microsporidia [181]. The genera *Hazardia* Weiser, 1977 and *Edhazardia* Becnel, Sprague & Fukuda, 1989 were created to honour him [95, 182].

Finally, in the 1990s it was discovered that some species of the genera *Nosema*, *Vairimorpha*, and *Edhazardia* produced previously unknown binucleate spores that functioned in the spread of infection to other tissues [183-187]. New detection of currently unrecognized sporulation sequences in other genera of microsporidia will have further impact on the taxonomy of the microsporidia.

Fig. (25). Edwin Hazard (1935–1985) (photo courtesy of J Weiser, reprinted with permission from [47]).

TAXONOMY AND PHYLOGENY

Microsporidia are ranked as a separate phylum, Microsporidia, in the kingdom Protista, meaning that they are eukaryotic and unicellular. The unique structure of the microsporidian spore separates the phylum Microsporidia from other protists and indicates that the microsporidia constitute a monophyletic taxon with no evident relationship to other protists.

Originally considered to be ancient organisms evolutionary placed as an early branch leading from prokaryotes to eukaryotes, new evidence favours a more recent origin of the microsporidia, suggesting that they are a part of the crown of eukaryotes that have lost several genes and have undergone considerable gene compaction, probably in response to their growing adaptation to intracellular parasitism [188].

In 1968, Ishihara and Hayashi showed by using sucrose density-gradient centrifugation techniques that the ribosomes and their two subunits of *N. bombycis* have density coefficients like those of the prokaryote *Escherichia coli* and that the RNA content of the *Nosema* ribosomes is 60% like that of *E. coli*, rather than 50% as in other eukaryotes [189]. This startling discovery was later confirmed by Curgy, Vávra and Vivarès, who demonstrated that the two main RNA molecules extracted from the ribosomes of some microsporidia have electrophoretic coefficients identical to those of pro-

karyotes [190]. They pointed out that similar results had been found in Coccidia and speculated that this situation may relate to the endoparasitic way of life. This question was not further investigated until molecular techniques became available, and in microsporidia the molecular approach was pioneered by Charles Vossbrinck. In 1986 Vossbrinck and Woese found by sequencing that ribosomes of the microsporidium *Vairimorpha necatrix* lack the 5.8S RNA subunit that was thought to be a universal eukaryotic characteristic [191]. One year later, Vossbrinck *et al*. used molecular sequencing methods with *V. necatrix* to show the phylogenetic position of microsporidia on the tree of life [192]. After comparing ribosomal sequence data of the microsporidium with already available data from protozoa, plants, animals, and fungi, they found that microsporidia diverged from other eukaryotes before the evolution of the mitochondria. Subsequent analyses on the basis of the SSU rRNA of *Vairimorpha* and several other microsporidia and using different methods of tree construction all supported the view that microsporidia were an early-diverging and therefore presumably extremely old lineage. This hypothesis was also supported by phylogenetic analysis of other proteins of the translational apparatus (translation elongation factors EF-1a and EF-2, glutamyl tRNA synthase) [193].

One additional reason behind the proposal that microsporidia are ancient eukaryotes was their lack of mitochondria [194]. Because microsporidian genomes contain proteins of mitochondrial descent [including mitochondrial heat shock protein (HSP) 70] related to some mitochondrial functions (e.g., iron–sulphur [Fe-S] cluster assembly) [195-198], it was hypothesized that microsporidia have retained a mitochondrial-derived organelle (mitosome), which has been detected by immunohistochemical staining [199]. Vávra recently identified double-membrane vesicles in *Vavraia culicis*, *Amblyospora* sp., *Vairimorpha* sp., and *Marssoniella elegans* and proposed that these polar vesicles were those mitochondrial remnants (mitosomes) [141].

Whereas microsporidian small subunit rRNA gene sequences were found to more closely resemble those of prokaryotes than eukaryotes, suggesting that microsporidia were ancient eukaryotes, phylogenetic analyses of gene sequences for α- and β-tubulin and for the largest subunit of RNA polymerase II, the TATA-boxbinding protein, and mitochondrial HSP70 supported a closer relationship between microsporidia and fungi [196, 200, 201]. This discrepancy may be explained by the long-branch attraction artefact of many phylogenetic methods which leads to erroneously grouping fast-evolving lineages at the base of the tree when they are analyzed together with other slowly evolving lineages [202].

Although microsporidia lack some typical eukaryotic characteristics (rRNA of prokaryotic size without separate 5.8S rRNA and ribosomes resemble the ribosomes of prokaryotic organisms; typical mitochondria, peroxisomes, and a classical Golgi apparatus are missing), they are true eukaryotes with a nucleus and an intracytoplasmatic membrane system, and they undergo chromosome separation by mitotic spindles (see Fig. **26**) and even though molecular data indicate fungal affinities, these give no indication of a new systematic position [203]. A new designation of the microsporidia would produce several administrative problems concerning funding programs, institutes in which microsporidia are studied, and decisions about museum and/or collection deposition [47]. However, at the present time there is no fungal taxon in which microsporidia might be ranked, and at a recent Conference in České Budějovice, Czech Republic in July 2004 (*First United Workshop on Microsporidia from Invertebrate and Vertebrate Hosts*), it was the opinion of the gathered experts that the names and type material of new species should follow the rules of the International Code of Zoological Nomenclature [203]. Yet it seems that 150 years after Nägeli's initial placing of *Nosema bombycis* among the fungi, this original taxonomic designation may be borne out [8].

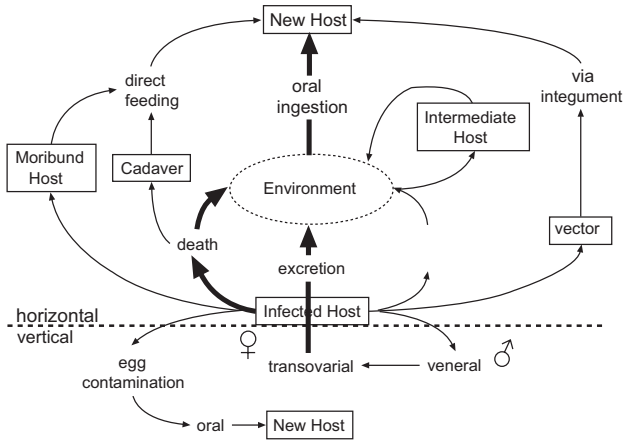

Fig. (26). Meront of *Tubulinosema kingi* undergoing karyogenesis. A mitotic spindle can be seen in the right nucleus. (reprinted with permission from [167]).

Historical classification systems of the microsporidia have been produced on the basis of simple morphologic characters [203]. The modes of spore formation, in particular the number of spores produced by each sporoblast, were used in the first attempts by Thélohan, Doflein, and Pérez to classify the microsporidia [53, 55, 56, 67]. Oddly enough, Thélohan, although working in the laboratory of Balbiani, did not use the term microsporidia, which Balbiani previously proposed. Stempell later concluded that a microsporidian classification system could be produced only when the form and development of vegetative forms, the mode of spore formation, and the form of spores were considered as the characters of family, genus, and species respectively [15]. Léger and Hesse used mainly spore shape and structure as important morphological characters in their classification [46]. Kudo believed that the "system of Léger and Hesse is best fitted to the present state of knowledge on Microsporidia", and he followed this system in his monograph "with slight modifications" [73].

However, the taxonomy of microsporidia remains controversial because all subsequently published classifications differ significantly, and microsporidian taxonomy is cur-

rently under extensive reconstruction. Particularly, the higher-level classification of the microsporidia has been tenuous because the significance of the homology among the morphological characters that could be identified could not be determined. The split of the microsporidia into classes has been based on characters such as whether there is a membrane surrounding the sporoblast (Pansporoblastina versus Apansporoblastina) [26, 204], whether they are diplokaryotic throughout their life cycle or have uninucleate states [95], and the type of nuclear division (Haplophasea versus Dihaplophasea) [27].

Weiser [95] listed 70 characters for the identification of microsporidia, which can be distinguished by light microscopy. After electron microscopy was introduced for taxonomic purposes in the mid 1970s, other important contributions to the identification of microsporidian genera were presented by Issi [117] and Larsson [205, 206]. Issi [117] listed 11 light- and electron-microscopic visible characters for 68 microsporidian genera. The phylogentic tree proposed in her contribution places for the first time *Vairimorpha* close to *Nosema*, which is in perfect agreement with later molecular phylogenetic analysis [207]. Irma Issi, who is head of the research group on microsporidia in St. Petersburg in Russia, studied microsporidia from the early 1960s, when she investigated microsporidiosis of *Pieris brassicae* in the laboratory of Microbiological Control of the All-Russian Institute of Plant Protection in St. Petersburg. She has described many new microsporidian species and identified several new genera, thus contributed significantly to microsporidian taxonomy, and remains engaged in microsporidian research today [208, 209]. The genus *Issia* Weiser, 1977 was named in her honour [95].

Issi's important taxonomic paper was written in the Russian language and therefore for many researchers is not easy to use. Fortunately, a complete English translation of this contribution was made by Lipa for the Division on Microsporidia of the Society for Invertebrate Pathology. Jerzy J. Lipa from the Institute of Plant Protection in Poznan, Poland, who was a postgraduate student of Steinhaus, has been engaged in microsporidian research since the 1950s and in addition to the important translation of Issi's work, he contributed several important papers of his own on microsporidia in insects [112-115, 210].

Larsson [205] presented a similar chart with 11 characters describing 66 microsporidian species from 51 genera and two years later produced a further identification key, including several light and electron microscopical characters for the available microsporidian genera. Ronny Larsson's interest in microsporidia was, among other influences, initiated by Weiser. In 1977 Weiser was invited to give a seminar on insect pathology at Lund University in Sweden, but Larsson, at that time a young zoologist, asked him to speak only on microsporidia [47]. Subsequently, he began his important taxonomic studies on microsporidia during which he described several new genera and species, and he published important papers and monographs [140, 205, 206]. The genera *Larssonia* Vidtman & Sokolova, 1994 and *Larssoniella* Weiser & David, 1997 were created to honour him [211, 212].

There is increasing evidence that, while there are correlations in some cases between the traditionally used characters and phylogenetic relatedness, these taxonomic schemes have almost no relationship to evolutionary history because these characters change relatively rapidly as evolutionary adaptations to host, host environment, and host population parameters occur. As a result, higher-level classifications based on these characters are probably not measures of evolutionary relatedness and indeed vary greatly among classification systems.

Molecular techniques are rapidly becoming an important and integral part of biosystematic studies. Microsporidia are prime candidates for phylogenetic analysis based on DNA sequence data because of the relatively small number of useful morphological characters.

The first complete genome sequence of a microsporidian species, *Encephalitozoon cuniculi*, was published in 2001 [198] (http://www.genoscope.cns.fr/externe/English/Projets/Projet_AD/AD.html). This important work was initiated by Christian Vivarès of the Laboratoire de Protistologie Moléculaire et Cellulaire des Parasites Opportunistes of the Université Blaise Pascal in Aubière, France, who has been studying microsporidia since the 1970s, and was carried out by Genoscope (the French National Sequencing Center). *E. cuniculi* has a very compact genome (one of the smallest eukaryotic genomes known to date) of 2.9 Mbases organized into 11 chromosomes. A genome sequence survey on a second microsporidian species, *Paranosema locustae* (called *Antonospora locustae* by Patrick Keeling and his team, who have done this research [213]), has been completed recently [214, 215] http://www.botany.ubc.ca/keeling/AntonosporaGSS.html). Several further genome sequence surveys are currently under way, including those on *Anncaliia algerae* (http://www. genoscope.cns.fr/externe/English/Projets/ Projet_KI/KI.html), *Spraguea lophii* [216] (http://jbpc.mbl.edu/Spraguea-HTML/), *N. bombycis*, *Vittaforma corneae* [217], *Enterocytozoon bieneusi*, *Encephalitozoon hellem*, and *Edhazardia aedis* (203) and an expressed sequence tag survey for *P. locustae* (http://amoebidia.bcm.umontreal.ca/public/pepdb/agrm.php) [203, 214].

Phylogenies based on molecular sequence data are helping to resolve a large number of questions about the evolution of characters in the microsporidia. Most of these studies use sequence data from the small subunit rDNA. This is because a large number of sequences have become available for this gene and it has become somewhat of a standard; however, other genes will have to be sequenced to confirm and further clarify microsporidial relationships. Again Vossbrinck *et al*. was the first who used comparative analysis of rRNA sequence data to present an unrooted tree of five microsporidian species from a phylogenetic analysis. [216a] Another early study published by Baker (a graduate student of Vossbrinck) *et al*. showed that *N. bombycis* and the *Vairimorpha* spp. were closely related [207]. In traditional classification schemes of that time, these species were divided into distantly related taxa (with the exception of Issi's system from 1986 [117]) because *N. bombycis* is diplokaryotic throughout its life cycle whereas *Vairimorpha* spp. produces both isolated diplokaryotic spores and uninucleate octospores in packets surrounded by a pansporoblastic mem-

brane. As both microsporidia are parasites of Lepidoptera, this analysis highlights the importance of the host as an important taxonomic character that, however, may not be valid for all genera [208]. This analysis and other studies [131] further indicated that some *Nosema* and *Vairimorpha* spp. are consistently grouped together with the type species *N. bombycis*, whereas other *Nosema* spp. form clades of their own containing, for example, *Nosema algerae*, *Nosema locustae*, *Nosema kingi*, and *Nosema acridophagus* [131, 168, 207]. It has been concluded that some characteristics of the genus *Nosema* (e.g., being diplokaryotic throughout the life cycle) have evolved more than once and that the genus *Nosema* is a polyphyletic collection of microsporidia [26, 207]. Subsequently, several of these unrelated *Nosema* spp., including *Nosema algerae* (=*Anncaliia algerae*), *Nosema connori* (=*A. connori*) [208], *Nosema corneum* (=*Vittaforma corneae*) [217a], *Nosema locustae* (=*Paranosema locustae*), *Nosema grylli* (=*P. grylli*), *Nosema whitei* (=*P. whitei*) [121, 209], *Nosema cristatellae* (=*Pseudonosema cristatellae*) [125], *Nosema kingi* (=*Tubulinosema kingi*), and *Nosema acridophagus* (=*T. acridophagus*) [156] have been given new generic designations and other new genera (*Trichonosema*, *Bryonosema*, *Fibrillanosema*) [125, 218], and even two new families, Pseudonosematidae and Tubulinosematidae, have been created [125, 156].

In a recent analysis of 125 microsporidian species (sequences obtained from GenBank), Vossbrinck and Debrunner-Vossbrinck showed that groups or clades are formed based largely on habitat and host [219]. They stated that structural and ultrastuctural characters are unreliable for distinguishing among higher-level microsporidian taxa and, based on their SSU-rRNA analysis, proposed three classes that reflect the habitat of each group: the Aquasporidia, which are found primarily in freshwater habitats; the Marinosporidia, which are found in hosts of marine origin; and the Terresporidia, which are primarily from terrestrial environments. Larsson completely disagrees with Vossbrinck and Debrunner-Vossbrinck about the usefulness of cytological characters for microsporidian systematics [220]. He argues that none of the distinguished clades of microsporidia of marine, freshwater, and terrestrial origin is strictly confined to the particular habit. Another problem with molecular data (especially from public databases) is that morphological evidence for the identification of species is lacking in most molecular studies and nobody really knows the origin of all these sequences in the databases. There is an urgent need for a microsporidian database that contains only DNA sequences together with morphological data of properly defined species so that in the future, only sequences from confirmed species will be used in phylogentic analyses. However, as the new classes (Aquasporidia, Marinosporidia, Terresporidia) have not been described as is required by the relevant taxonomic code (The International Code of Zoological Nomenclature 1999), they are currently nomina nuda that are not valid and that can be made available at a later time [220].

Microsporidian taxonomy has obviously reached a breaking point where classical morphology-based methods are losing importance and will be supplemented or even replaced by molecular-based methods. Without a doubt, molecular techniques present an excellent means of identifying species and also provide excellent data for proposing evolutionary relatedness through phylogenetic analysis. Because sequence data are characters unique to an organism, species descriptions should whenever possible contain sequence data (rRNA). On the other hand, morphological data (primarily obtained by electron microscopy) also provide information that is often unique to a genus or even species, which could be expected since the morphology is determined by the genome. Nevertheless, all published classification systems demonstrate that morphology alone does not provide enough information for producing accurate phylogenies. Several morphological characters of microsporidia, such as the number of nuclei, the number of spores or sporonts, the length, arrangement and structure of the polar tube, and other details of the life cycle may change very rapidly during adaptation to different hosts or tissues, and some characters (e.g., being diplokaryotic, development in direct contact with the host cell cytoplasm) seem to have evolved several times simultaneously in different lineages of microsporidia. Thus, as morphology is the visual expression of the genome, careful evaluation of both molecular and morphological data should lead to phylogenies that are based on two sets of characters and that correspond and are consistent with each other.

EPIDEMIOLOGY AND TRANSMISSION

Microsporidia have a worldwide distribution and can be found in almost any group of animals including protozoa and helminthes [221]. Since their discovery, they have been well known as the agents of disease in invertebrate hosts (mainly insects) and as the cause of several diseases and mortality in wild, farmed, and aquarium fish. But most research on microsporidia was initially done by entomologists and insect pathologists.

In 1838, the first microsporidium recorded from a vertebrate host was reported by Gluge [20]. This parasite, later determined to be *Glugea anomala* [21], causes subcutaneous cysts resulting in spectacular deformations in sticklebacks. *Encephalitozoon cuniculi* was the first microsporidian to be recognized as a mammalian parasite. They were first seen as Gram positive microorganisms measuring "never more than 4 x 1.5 μm" in the brain, spinal cord, and kidney of laboratory rabbits that were used in experiments on transmission of poliomyelitis by Wright and Craighead in 1922 in Boston [222]. The parasite was named by Levaditi, Nicolau and Schoen [223, 224], who observed similar organisms also in the brain of rabbits. The same authors also suggested that these organisms were microsporidia [224, 225], which was not generally accepted because the presence of the polar filament was not demonstrated [226]. The reports of Levaditi *et al.* and Wright and Craighead were also neither cited in Kudo's monograph [73] nor by Jírovec [94], and the true nature of *Encephalitozoon* remained obscure until in the 1960s, when Nelson [227] and Lainson *et al.* [228] independently rediscovered *E. cuniculi* in laboratory rodents and substantiated the previously rejected claim of the original authors that this parasite is a microsporidium. A persistent infection of laboratory animals was observed at the Rockefeller Institute Farm in Princeton, New Jersey, and Nelson [227], extruding the polar filaments from spores obtained from peritoneal macrophages of infected mice, showed that the responsible organism was indeed a microsporidium. Aggregations of small organisms in mammalian brains had also

been noted for 50 years in laboratory animals at the Winches Farm Laboratories (a field station of the London School of Hygiene Tropical Medicine in St Albans, Hertfordshire, UK) when Lainson *et al*. [228] used transmission electron microscopy to demonstrate the presence of the polar filament in spores obtained from rats. These authors and Weiser [229] transferred the species *E. cuniculi* to the genus *Nosema* (*N. cuniculi*) because they concluded that *Encephalitozoon* was a junior synonym. Ann Cali later differentiated the genera *Nosema* and *Encephalitozoon* on the basis of diplokarya in the former and isolated nuclei in the latter and consequently reinstated the genus *Encephalitozoon* [166]. The genus *Anncaliia* Issi, Krylova & Nicolaeva, 1993 was created to honour this important work [230]. *Thelohania apodemi* was the second microsporidium found in a warm-blooded host [231]. It was identified in the brain of voles collected by J. M. Doby on an excursion together with J. Weiser in the Bretagne, France in an old oak grove at a castle near Ploermal [47].

The first sufficiently documented human case of microsporidial infection was a case of disseminated *Encephalitozoon* infection in a 9-year-old Japanese boy, who suffered from recurrent fever, headache, vomiting, and spastic convulsions, reported by Matsubayashi *et al*. in 1959 [232]. The parasite was isolated by inoculating cerebrospinal fluid and urine into mice, and in this case 30 control mice remained negative (this parasite was later identified as *Anncaliia (Nosema) connori* by Weiser [233]). It was 25 years later that a similar illness in a 2-year-old Columbian child living in Sweden was found to be of microsporidian origin [234]. Another fully substantiated report was published by Margileth *et al*. in 1973 [234a], and unidentified microsporidia caused corneal infection in two further cases [235, 236]. Thus, microsporidia became important in human and veterinary medicine. This increase in profile stimulated research on microsporidia in mammals, resulting in better funding for the field and producing a new research discipline [47].

Fewer than 10 well-documented human microsporidia infections had been reported up to 1985 when the new species *Enterocytozoon bieneusi* was described in a 29-year-old Haitian AIDS patient from France [237, 238]. Similar organisms (probably *E. bieneusi*) had already been observed in intestinal biopsy specimens from several HIV-infected patients since August 1982, but for the pathologists their identification as protozoal parasites of the phylum Microsporidia did not come easily [239]. William Gourley, a pathologist at the University of Texas at Galveston, probably was the first to detect *E. bieneusi* in a duodenal biopsy from a homosexual patient with chronic diarrhoea. Using electron microscopy, he observed spores of a "yeast or protozoan" that was "not recognized by light microscopy". Seven months later he showed the electron micrographs to participants of the annual Binford-Dammin Society of Infectious Disease Pathology dinner at the International Academy of Pathology, U.S. and Canadian Annual Meeting. In attendance were Ronald Neafie and Daniel Connor from the Armed Forces Institute of Pathology, who were familiar with microsporidian ultrastructure from a case of disseminated microsporidian infection in a child [234a], and they recognized the coiled polar filament in the spores. In May 1984 an abstract written by Dobbins and Weinstein describing an unknown organism in a gastrointestinal biopsy from a patient with AIDS [240] came to Gourley's attention. Comparison of both organisms showed that they were identical microsporidia. At about the same time, a similar case was studied by Robert Owen, a gastroenterologist at San Francisco VA Medical Center, and Ann Cali [241]. In August 1993 and March 1994 two similar cases were also observed by Jan Marc Orenstein, pathologist at George Washington University, Washington, D.C., who showed electron micrographs to participants of the First International Conference on AIDS in Atlanta in spring 1995 without success; the identification of these two organisms as microsporidia did not come until Orenstein saw Dobbins and Weinstein's paper that was published in Gastroenterology in

Fig. (27). Geographic distribution of human microsporidiosis. Dark regions indicate areas with published human cases, grey regions indicate areas with cases that have not been published (personal communications). Modified from [245]. Based on a literature review as of September 2005.

March 1985 [239, 242]. Unaware of this research, R. Modigliani, a French gastroenterologist from Paris, together with Isabelle Desportes observed the same microsporidium in small bowel biopsies of a heterosexual Haitian HIV-infected patient with chronic diarrhoea. They correctly identified the microsporidium as the first member of a new genus that they named *Enterocytozoon*, and the species was named *Enterocytozoon bieneusi* after the patient [237, 238].

Only about 40 additional cases occurred in the next five years [243], but as of August 1991, 104 cases of intestinal microsporidiosis attributable to *E. bieneusi* had been reported [239]. Since then, several hundred patients with intestinal *E. bieneusi* infection have been reported from all continents except Antarctica (Fig. **27**); in 1994 the first case in a non HIV-infected patient was reported [244], followed by several other cases in immunocompetent and immunocompromised persons. The occurrence of microsporidiosis as common opportunistic diseases in immunocompromised patients further stimulated research in this field during the last decade of the 20th century.

Two pathogenic species of *Encephalitozoon* that infect humans, *Encephalitozoon cuniculi* and *Encephalitozoon hellem*, are morphologically similar by light and electron microscopy, and can only be distinguished by antigenic, biochemical, or nucleic acid analysis. Several cases of *Encephalitozoon* infection were reported to occur in patients with and without AIDS prior to 1991 [246, 247]. It was assumed that these infections were due to *E. cuniculi* following light and/or electron microscopic analysis. However, in 1991 Didier *et al*. used biochemical and antigenic methods to describe a new species of *Encephalitozoon*, termed *Encephalitozoon hellem*, found in three patients with AIDS [248]. This new species was morphologically indistinguishable from *E. cuniculi* and required special methods to differentiate from it. Following that report, all published cases of *Encephalitozoon* infections in humans appeared to be caused by *E. hellem* [249]. There was some doubt as to whether *E. cuniculi* did in fact cause human infection until 1995, when De Groote *et al*. and Franzen *et al.* described two homosexual men with AIDS and disseminated *E. cuniculi* infection, confirmed by an immunofluorescent assay and by DNA identification [250, 251]. To date, *E. cuniculi* has been detected in at least 20 and *E. hellem* in about 50 HIV-infected or otherwise immuno-compromised patients [252].

A third species of *Encephalitozoon*, *Encephalitozoon intestinalis*, was first described in 1992 by Orenstein *et al*. as a microsporidium with ultrastructural similarities to the genus *Encephalitozoon* [253]. Nevertheless, it has been placed as a new species in a new genus, *Septata intestinalis* by Cali *et al*. [254], but on the basis of DNA sequence data it later was placed in the genus *Encephalitozoon* and renamed *Encephalitozoon intestinalis* [255]. *E. intestinalis* is one of the most common microsporidial species in humans (beside *E. bieneusi*) and has been reported in at least 200 patients [252].

Several other new species (*Trachipleistophora hominis* [256], *Trachipleistophora anthropophthera* [135], *Nosema ocularum* [257], *Pleistophora ronneafiei* [258], *Vittaforma corneae* [217, 259], *Anncaliia* (formerly *Brachiola/Nosema*) *vesicularum* [260], *Anncaliia* (formerly *Brachiola/Nosema*) *connori* [261], *Microsporidium africanum* [236], *Microsporidium ceylonensis* [235]) or species new in humans (*Anncaliia* (formerly *Brachiola/Nosema*) *algerae* [262, 263]) have been detected during the last decades, but infections with these parasites are reported only as sporadic cases (q.v. Diseases).

Data on epidemiological characteristics of microsporidiosis are rapidly increasing, but many questions have yet to be answered. Reliable estimates of prevalence are missing because nearly all published studies are not based on truly random samples but refer to highly selected patient populations, such as HIV-infected patients with chronic diarrhoea. Different diagnostic approaches, noncontrolled and nonrandom study designs, and multiple species with diverse clinical

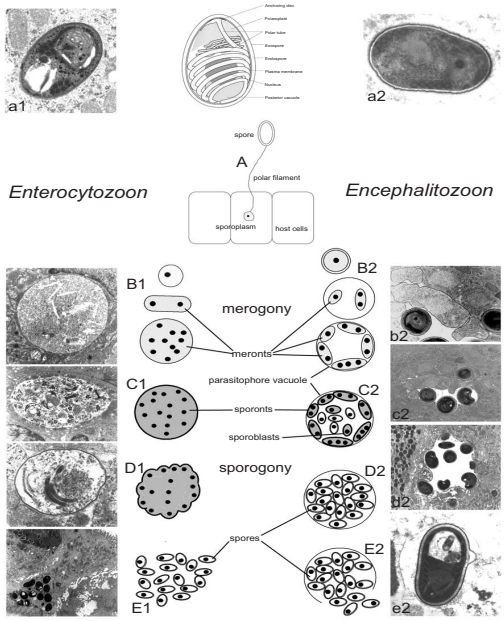

Fig. (28). Major and minor pathways of transmission for insect parasitic microsporidia (from [264] with permission).

manifestations make it very difficult to understand the true burden of microsporidiosis. Studies on *Enterocytozoon bieneusi* in AIDS patients with chronic diarrhoea have reported prevalences between 4% and 50%, depending on the study group and method of diagnosis. Combined, these studies have evaluated over 2,500 patients and confirmed about 500 cases of *E. bieneusi* infection (20%) and the total number of reported cases exceeds 1,000. Epidemiological estimates of *Encephalitozoon* infections have not been done so far [252].

Transmission of microsporidia in insects has been studied for a long time and both vertical (transovarial) as well as horizontal transmission occur regularly among insects (for review see Fig. **28** and [264, 265]).

Routes and sources of human infection have been difficult to ascertain, but animal reservoirs of microsporidia infecting humans have been confirmed recently (Table **1**) [252, 266]. For *E. cuniculi* several animal hosts were already known long before it was described as a pathogen in humans, and this species is commonly found in several mammals [267, 268]. Before the description of *E. hellem* in 1991, *Encephalitozoon*-like microsporidia were described on several occasions from psittacine birds (parrots) [118]. Later, molecular analyses confirmed that cases of *E. hellem* in psittacine birds derived from budgerigar chicks (*Melopsittacus undulatus*), two eclectus parrots (*Eclectus roratus*), and peach-faced lovebirds (*Agapornis roseicollis*). The first detection of *E. hellem* in a wild psittacine bird was a yellow-streaked lory (*Chalopsitta scintillata*) captured on Aru Island (Indonesia), and infections have been reported from nonpsittacine birds as well [horned puffin (*Fratercula corniculata*), ostrich (*Struthio camelus*), several species of hummingbirds, and Gouldian finch (reviewed by [252, 266, 269]). *E. intestinalis* was detected by an immuno-fluorescence test using a monoclonal antibody in fecal samples from several domestic animals from a rural area in central Mexico [270]. Transmission electron microscopy on these specimens revealed microsporidian-like structures. PCR was done several months later, and fecal samples from goat, pig, cow, dog, and donkey were positive for *E. intestinalis* but negative for *E. cuniculi* and *E. hellem* [271]. Other studies have not confirmed these high-prevalence data, and the situation regarding *E. intestinalis* is still unclear. Eleven years after its discovery as a human pathogen, *E. bieneusi* was for the first time detected in animals (pigs) [272], and subsequent studies have confirmed the occurrence of *E. bieneusi* in pigs and calves with high prevalence [273]. The parasite has also been detected in cats, dogs, a goat, a llama, a variety of species of wild mammals (beavers, foxes, muskrats, otters, and raccoons), hedgehogs, and, recently, nonmammalian hosts (chickens and pigeons). Natural infections with *E. bieneusi* have been documented in captive monkeys, namely, rhesus macaques (reviewed in [252]).

In 1993, Lom suggested that microsporidian isolates of the same species from different hosts may differ and that this may have bearing on the varying degree of susceptibility in different hosts and thus on epidemiology [274]. Molecular analysis of different human *E. cuniculi* isolates and isolates from rabbits, dogs, mice, and blue foxes later showed that there are indeed differences [275] and that all isolates from humans were of the same subtype as the isolates from rabbits (strain I) and dogs (strain III) (Table **1**) [276]. Strain II, that is found in mice and rats, has never been identifierd in humans. However, no direct proof of transmission from animals to humans has been documented, with the exception of one case in which a 10-year-old girl seroconverted after close contact with a dog infected with *E. cuniculi* [277]. Thus, firm evidence for transmission of microsporidia from animals to humans is currently lacking, but clues are emerging as to possible animal links with human microsporidial disease. As with *E. cuniculi*, different genotypes have been identified for *E. hellem*, and one genotype was determined for isolates from several patients from the U.S. and Europe and for one isolate each from a parrot caught in the wild in Indonesia and from a psittacine bird from the U.S. Other genotypes consist of human-derived *E. hellem* isolates from Switzerland (three isolates) and Tanzania (one isolate). In contrast to the situation with other human-infecting *Encephalitozoon* spp., *E. intestinalis* seems to be a very homogeneous species, and no genetic differences have been detected among different isolates so far. Seventeen different ITS genotypes of *E. bieneusi* infecting humans have been confirmed, but limited information is available on the geographic distribution of human-derived genotypes, and only four seem to have a zoonotic potential, having also been discovered in vertebrate hosts with what appears to be some degree of host specifity. Further characterization of both animal and human microsporidia will certainly produce firm evidence for the zoonotic origin of human microsporidiosis and as several studies have found microsporidia in animals that are associated with or farmed by humans, then such infections may be zoonotic.

Arthropods are the most common hosts of microsporidia, and experimental infections of mice by *Anncaliia* (formerly *Brachiola/Nosema*) *algerae* - a mosquito parasite that molecular biology indicates is phylogenetically distant from typical members of the genus *Nosema* - have been achieved [278, 279]. Human cases of keratitis and myositis caused by *A. algerae* have been reported [262, 263], and the ultrastructural features of *Anncaliia vesicularum*, which have been identified in muscle biopsies of an HIV-infected patient, are most closely aligned with *A. algerae* as well [260]. But whether other insect microsporidia might infect vertebrates or even humans is unknown and currently under investigation [156].

Common environmental sources of microsporidia include ditch and other surface waters, and several species of microsporidia can be isolated from such sources [280]. Detection of human microsporidia (*E. bieneusi, E. intestinalis*, and *V. corneae*) in environmental water samples has been reported [281, 282], and there is one report of a presumed waterborne outbreak [283]. Risk factors for intestinal microsporidiosis also suggest water as the source of infection [284], but whether microsporidiosis is a really a waterborne disease is unknown [285].

There is considerable serologic evidence that humans without clinical signs of infection have been exposed to microsporidia (studies have focused exclusively on human exposure to *Encephalitozoon* spp.) [286-289], but whether these persons are chronically infected or active infections are newly acquired is unknown; neither are the sources of

Table 1.

Species	Discovery			No. of reported cases[a]		Site(s) of infection[a]	Animal host(s)[a]	Environmental sources[a]	Therapy[a]
	Year	Host	References	Immuno compromised[b]	Immuno competent				
Encephalitozoon cuniculi[c]	1923	Rabbit	[223]	~20	-	Systemic infection, eye, brain, repiratory tract, urinary tract, peritoneum	Several mammals	Unknown	Albendazole Fumagillin
Encephalitozoon hellem[c]	1991	Human	[248]	~50	3	Systemic infection, eye, respiratory tract, urinary tract	Birds	Unknown	Albendazole Fumagillin
Encephalitozoon intestinalis (originally named *Septata intestinalis*)	1993	Human	[254, 255]	~200	2	Systemic infection, intestine, biliary tract, urinary tract, repiratory tract bone, skin	Several mammals	Water	Albendazole Fumagillin
Enterocytozoon bieneusi[c]	1985	Human	[237, 238]	~2,000	~20	Intestine, biliary tract, respiratory tract	Several Mammals birds	Water	Fumagillin HAART[d]
Vittaforma corneae (originally named *Nosema corneum*)	1990	Human	[217, 259, 321]	1	3	Eye, urinary tract	Unknown	Water	Fumagillin
Pleistophora ronneafiei (originally named *Pleistophora* sp.)	1985	Human	[258, 324]	1	-	Muscle	Fish?[e]	Water	Not reported
Pleistophora spp.[f]	1993	Human	[330, 331]	2	-	Muscle	Fish?[e]	Water	Not reported
Trachipleistophora hominis	1996	Human	[256, 325]	2	1	Muscle, eye	Unknown	Unknown	Albendazole
Trachipleistophora anthropophtera	1998	Human	[135, 327]	3	1	Systemic Infection, eye	Unknown	Unknown	Not reported
Anncaliia (Nosema/Brachiola) algerae (originally named *Nosema algera*, later transferred to the genus *Brachiola*, recently transferred to the genus *Anncaliia*)	1970	Mosquito	[78, 208]	1	1	Muscle, eye	Mosquitos	Ditch water?[g]	Not reported
Anncaliia (Nosema/Brachiola) connori (originally named *Nosema connori*, later transferred to the genus *Brachiola*, recently transferred to the genus *Anncaliia*)	1974	Human	[208, 261, 232[h], 233[h]]	2	-	Systemic infection	Unknown	Unknown	Not reported

(Table 1). Contd…..

Anncaliia (Brachiola) vesicularum (originally named *Brachiola vesicularum* recently transferred to the genus *Anncaliia)*	1998	Human	[208, 260]	1	-	Muscle	Unknown	Unknown	Not reported
Nosema ocularum	1991	Human	[257]	-	1	Eye	Unknown	Unknown	Fumagillin
Microsporidium ceylonensis	1973	Human	[118, 235]	1	-	Eye	Unknown	Unknown	Not reported
Microsporidium africanum	1981	Human	[118, 236]	1	-	Eye	Unknown	Unknown	Not reported

[a]adepted from several references.
[b]HIV-infected patients or otherwise immunocompromised patients including organ transplant recipients.
[c]different genotypes have been reported.
[d]highly active antiretroviral therapy leading to immunreconstitution in HIV-infected patients.
[e]species of the genus *Pleistophora* are known to infect fish, but wether *P. ronneafiei* infect fish is unknown.
[f]re-examination suggested that both cases involved an infection due to *Trachipleistophora* [328].
[g]*Nosema* sp. that was morphological identical at the light microscopic level to *A. algerae* [280].
[h]suspected to be *Anncaliia* (*Nosema/Brachiola*) *connori* by Weiser [233].

human infections really known. Spread within the human population and acquisition of parasites from animals or water are obvious possibilities that can be neither confirmed nor denied at this time. If persistence of antibodies is indicative of latent infections, reactivation is likely to occur under immunosuppression.

DIAGNOSIS

Serological methods such as ELISA and western blot assays have been useful for diagnosing microsporidiosis in immunologically competent laboratory animals and humans [286-289]. However, reliance on these *E. cuniculi*-based serology assays became complicated by the emergence of new species of microsporidia found to infect humans and animals and by concerns that microsporidia infections often occur in immunodeficient individuals who may not express significant or specific antibody responses [276].

Microsporidia are difficult to detect, and usually special techniques are necessary to diagnose microsporidia infections [290]. Identification of microsporidia has been based primarily on ultrastructural studies using transmission electron microscopy. Microsporidial ultrastructure is unique and pathognomonic for the phylum, and electron microscopy allows one to distinguish among all microsporidial genera [140]. However, during the last decade, diagnostic procedures to detect microsporidia have been changed remarkably and have been reviewed several times (e.g. [290-292]). New staining methods, suitable for light microscopy, have been developed, and modified trichrome stains (concentrated chromotrope 2R) [293] and fluorescent staining techniques using optical brighteners (e.g. Uvitex 2B, Calcofluor White, or Fungifluor) [294, 295] were major breakthroughs. Originally used for stool and body fluids, these stains were later adopted for the staining of histological sections as well [296, 297]. Microsporidia stain poorly with H & E in tissue sections, but tissue Gram stains (Brown-Brenn and Brown-Hopps) are useful for detecting microsporidia in tissue biopsies, and silver stains (e.g. Warthin-Starry) also have been applied for detecting microsporidia in tissue sections [292]. Another excellent method for revealing microsporidia is Goodpasture's carbol-fuchsin stain. Immunofluorescent staining techniques using poly- and/or monoclonal antibodies are established in several research labs, but none of these antibody detection procedures has been commercialized so far. Nucleotide sequencing of microsporidian genes offers new opportunities to perform highly sensitive molecular-based techniques, such as polymerase chain reaction, with many advantages in diagnosis and species differentiation of human micro-sporidial infections [290, 298, 299].

Establishment of an *in vitro* propagation system for microsporidia enables pathobiological studies of the parasites and the production of large numbers of spores for many different types of *in vitro* and *in vivo* studies, including the generation of monoclonal and polyclonal antibodies, and immunologic, molecular, biochemical, physiologic, and animal inoculation studies [300]. Initially, microsporidia were mostly cultivated in insect cell lines. Trager in 1937 achieved partial success in culturing *N. bombycis* [301], but Ishihara and Sohi in 1966 were the first to publish a truly successful and practical method of growing *N. bombycis* in ovarian tissue culture of *Bombyx mori* [302]. Ron Ishihara began to study microsporidia in Tokyo, Japan, in the 1960s and later contributed significant findings to the microsporidia literature. He moved to Ontario, Canada, where he established the *in vitro* culture of *N. bombycis* [301] and elucidated the prokaryotic nature of microsporidian ribosomes [189]. Back in Japan, he discovered the previously unknown binucleate spores of some *Nosema* spp. that function in the spread of infection to other tissues [183, 184].

Undeen in 1975 established the first microsporidian species in a mammalian cell line by growing *Anncaliia algerae* in pig kidney cells at 26 °C [302a]. This parasite, which also causes human infections [262, 263], has later been successfully grown in many insect cell cultures [303, 304] and other mammalian cells as well [263, 305, 306].

Interest in the *in vitro* cultivation of certain microsporidia that cause human disease intensified in recent years after several genera were identified as opportunistic pathogens of humans, especially in AIDS patients, and this approach was pioneered by Govinda S. Visvesvara. Murine isolates of *E.*

cuniculi had already been cultivated in cell cultures by Shadduck in 1969 [307] and later by other researchers [308], when in 1991, Visvesvara *et al.* and independently Didier *et al.* isolated *E. hellem* from HIV-infected patients [248, 311]. Hence, a number of isolates of *E. cuniculi*, *E. hellem*, and *E. intestinalis*, two isolates each of *Vittaforma corneae*, *Anncaliia* (formerly *Brachiola/Nosema*) *algerae*, and *Trachipleistophora hominis* and one isolate of *Trachipleistophora anthropophthera* have been established in culture, many of them in Visvesvara's lab in Atlanta, Georgia [300]. However, the most common microsporidial species infecting humans, *E. bieneusi*, has not yet been cultured in long-term cultures [310], which has inhibited progress in finding an effective antimicrosporidial agent against this important infection.

DISEASES

Microsporidia are widespread pathogens of invertebrates and fish, and almost half of the described microsporidian genera have an insect as type host. There is a wide variation in the types of pathologies that can occur in insects as a result of infection with microsporidia. Infections are usually chronic, slow-acting processes and are rarely acute. The various signs and symptoms associated with microsporidiosis in insects range from obvious tissue manifestations to abnormal developmental and behavioural changes [264]. Economically important diseases caused by insect microsporidia have been mentioned above: Pébrine disease in silkworms is caused by *N. bombycis*, and severe dysentery in honeybees is caused by *N. apis*. *N. bombi* infects bumblebees that are used to pollinate plants in greenhouses, and several other diseases in economically important insects occur (for review see [264, 311].

About 150 microsporidia species from 14 genera have been described from fish, and several cause severe diseases [312]. Fish microsporidia can be divided into those associated with xenoma formation (e.g. *Glugea, Ichthyosporidium, Jirovecia, Loma, Microfilum, Microgemma, Nosemoides, Spraguea*, and *Tetramicra*) and non–xenoma-forming species (*Nucleospora, Heterosporis, Pleistophora,* and *Thelohania*) [313]. Some are host specific, at least at the genus level, but a few are widely distributed among different hosts. Comprehensive lists of fish hosts and their microsporidia can be found in Canning and Lom (1986) and Lom (2002) [118, 312]. Microsporidiosis in fish are of important economic impact because diseases not only occur in wild and cultured food fish but also in aquarium fish [118]. In wild fish, declines of entire commercial fisheries have been attributed to microsporidiosis: In 1946 *Pleistophora macrozoarcides* was responsible for the collapse of the North American ocean pout fishery [314], and the decline in the rainbow smelt fishery of New Hampshire was caused by infections from *Glugea hertwigi* [315]. Cultured fish are particularly susceptible to microsporidia, and high mortality has occurred in farmed fish from infections of *Heterosporis anguillarum*, *Loma salmonae*, or *Nucleospora salmonis* and in ornamental fish by *Glugea* spp. *Pleistophora hyphessobryconis* is well known to fish hobbyists as the cause of the extremely common neon tetra disease [118] (for review see [313]).

Microsporidial infections have been reported in various wild and domestic mammalian animals and also from avian, amphibian, and reptilian hosts [267, 269], and the history of mammalian microsporidiosis has already been described above. Species occurring in vertebrate hosts were summarized for the first time by Canning and Lom in 1986 [118]. As already mentioned earlier, *E. cuniculi* occurs regularly in rabbits, causing encephalitis and chronic renal disease and in several wild and laboratory rodents. *E. hellem* has been reported in different birds with and without symptoms (enteritis). Several other species have been described in vertebrates, but most of these only as single reports (for review see [267]).

Microsporidia in humans are considered opportunistic pathogens because they are most likely to cause disease if the immune status of a host is suppressed so that the infection cannot be controlled. Clinical symptoms and diseases associated with microsporidiosis vary with the species causing the infection and the status of the host's immune system. During the last decade of the 20th century, the clinical pictures of human microsporidiosis have been described in hundreds of case reports and research papers and have been reviewed several times (e.g. [276, 295, 316, 317]).

The most common clinical manifestation of human microsporidiosis is diarrhoea resulting from infection with *E. bieneusi* or the less-common *E. intestinalis*. HIV-infected patients with fewer than 100 CD4 cells per microliter are most susceptible, but otherwise immunocompromised or even healthy individuals, such as travellers, may be affected as well [318-320]. Whereas *E. bieneusi* causes relatively localized disease mainly of the gastrointestinal tract and less commonly of the respiratory tract, all three *Encephalitozoon* spp. typically disseminate causing clinical syndromes that include sinusitis, keratoconjunctivitis, encephalitis, tracheobronchitis, interstitial nephritis, hepatitis, or myositis, and microsporidiosis should be considered in the differential diagnosis of HIV-related symptomatic disease of virtually all organ systems (Table **1**). However, there are clear differences in the typical distribution pattern for each microsporidian species. *E. hellem* mainly parasitizes the keratoconjunctivals, urinary tract, nasal sinuses, and bronchial system. On the other hand, *E. intestinalis* appears to be mainly confined to the gastrointestinal and biliary tracts with dissemination to the kidneys, eyes, nasal sinuses, and sometimes the respiratory tract. *E. cuniculi* causes widely disseminated infections involving nearly all organ systems, but clinical manifestations vary substantially, ranging from no symptoms to severe disease with the gastrointestinal tract only sporadically involved.

Additional species have caused diseases in humans with less frequency. Corneal infection was caused by *Microsporidium africanum* in a young woman [236] and by *Microsporidium ceylonensis* in a young boy [235]. *Nosema ocularum* caused a corneal ulcer in a 39-year-old man [257], and *Vittaforma corneae* caused stromal keratitis in three immune-competent individuals [217, 259, 321, 322], and urinary tract infection in a patient with AIDS [323]. *Anncaliia (Brachiola/Nosema) algerae*, a microsporidium commonly found in mosquitoes, has been found as the cause of keratitis and fatal myositis in humans [262, 263]; *Anncaliia* (formerly

Brachiola) vesicularum caused myositis in a patient with AIDS [260]; and a disseminated infection due to *Anncaliia* (formerly *Brachiola/Nosema) connori* was responsible for diarrhoea and malabsorption in an athymic infant [234]. *Pleistophora ronneafiei* caused myositis in an immune-competent man [258, 326]. *Trachipleistophora hominis* was isolated from two AIDS patients with myositis and an immunocompetent man with stromal keratitis [256, 322, 325, 326], and *Trachipleistophora anthropophthera* was detected in several organs at autopsy of two patient with AIDS and in the cornea of a third HIV-infected patient [135, 327-329]. Re-examination of two other cases of myositis, where the responsible organisms were reported as *Pleistophora* spp. [330, 331], suggested that both cases involved an infection of *Trachipleistophora*, as well [326].

The "new" microsporidian species, *E. bieneusi, E. hellem, E. intestinalis, T. hominis, T. anthropophthera*, and *A. vesicularum* were first identified in HIV-infected patients, but recent cases have indicated that most of these microsporidian species are not confined to HIV-infected patients. *E. bieneusi* and *E. intestinalis* have been detected as a cause of self-limiting diarrhoea in immunocompetent persons [244, 320], and microsporidia have emerged recently as cause of disease in patients who were immunosuppressed secondary to organ transplantation [332-334]. Intestinal microsporidiosis seems to be an underappreciated cause of travelers' diarrhoea, as well [318-320].

THERAPY

Many different chemicals have been tested for control of nosematosis in honeybees, but only Fumagillin (Fumidil-B®, Nosem-X™), an acyclic antibiotic produced by some strains of *Aspergillus fumigatus* and originally isolated because of its antibiotic properties, especially against protozoa such as *Entamoeba histolytica* and *Plasmodium* spp., has proven effective [335]. Since fumagillin does not affect spores, treatment with this drug does not completely eliminate the disease from the bee colony, and the infection will continue after all the medication has been stopped. Fumagillin is also very effective against human microsporidia, and efficacy against *E. cuniculi* was already demonstrated by Shadduck in 1980, who suggested that the drug may be useful for the treatment of encephalitozoonosis [336]. Although Fumagillin was really used for the first time to treat microsporidial keratoconjunctivits in three HIV-infected patients as topical treatment by Diesenhouse *et al*. and by Rosberger *et al*. in 1993 [337, 338], several other successfully treated patients have been reported since (for review see [339]). Used systemically, Fumagillin is also an effective treatment for chronic *E. bieneusi* infection in immunocompromised patients, but severe side effects (neutropenia and thrombocytopenia) were observed in 50% of patients under therapy [340]. The synthetic fumagillin analog TNP-470 was as effective as fumagillin against microsporidia in *in vitro* studies [341] and was well tolerated systemically in AIDS patients with Kaposi's sarcoma, but other human studies with microsporidiosis are lacking [342].

Initial reports about successful treatment of intestinal microsporidiosis with metronidazole were not supported by later studies, and metronidazole has no effect on microsporidia in *in vitro* cultures [343]. Several other agents were tested without success until albendazole, a benzonidazole that binds to tubulin, was found to be highly effective against at least some insect and human microsporidian species [343, 344]. Human infections with *Encephalitozoon* spp. that were treated with albendazole showed remarkable improvement of symptoms and clearance of the parasite after therapy [345]. Initial reports described some clinical efficacy of albendazole against *E. bieneusi* as well [346, 347], but subsequent studies failed to show any effect [348].

Since the introduction of highly active antiretroviral therapy in HIV-infected patients, the prevalence rates for microsporidiosis in these patients have fallen dramatically, and several reports have shown remission of microsporidia-associated symptoms under therapy [349-352] and even clearance of the parasites has been described [353].

CONCLUSION

Since the naming of *Nosema bombycis* 150 years ago, there has been a lot of research on the microsporidia, and because microsporidia infect a wide range of host species, diverse disciplines were engaged in this research with limited opportunities for interaction. Historically, researchers dealing with microsporidia from various hosts have discussed these organisms within their segregated scientific communities [203]. Insect microsporidiologists interacted separately from people dealing with microsporidia infecting fish and from parasitologists, pathologists, and infectious disease specialists dealing with mammalian and human microsporidia. While the insect microsporidiologists had their annual meeting of the *Society for Invertebrate Pathology*, and people dealing with fish microsporidia attended the *International Symposia on Fish Parasites*, other researchers met in symposia as part of international protozoology meetings [e.g., the *International Congresses of Protozoology* (ICOP)] or at specialised meetings dealing with microsporidia as opportunistic pathogens [e.g. the *International Workshops on Opportunistic Protists* (IWOP 1-8), the *Workshop on Intestinal Microsporidia in HIV* infection organized in Paris, France in 1992, or the *Workshops on Microsporidiosis and Cryptosporidiosis in Immunodeficient Patients* in České Budějovice, Czech Republic in 1993 and 1997 (Fig. **29**)].

However, in 2004 a NATO advanced research workshop (ARW) was held from July 12 to 15, 2004, at the Institute of Parasitology of the Academy of Sciences of the Czech Republic in České Budějovice, Czech Republic. This meeting entitled *Emergent Pathogens in the 21st Century: First United Workshop on Microsporidia from Invertebrate and Vertebrate Hosts*, brought together experts in insect, fish, veterinary, and human microsporidiosis to discuss the state of knowledge of microsporidia (Fig. **30**). Important contributions of this meeting were presented as articles in a special issue of *Folia Parasitologica*, edited by Jiří Lom, Jiří Vávra, Louis M. Weiss, and Vladimír Bukva (Vol. 52, Number1/2, May 2005), which also contained all 55 abstracts of this important meeting.

In 2007 a *Second United Workshop on Microsporidia from Invertebrate and Vertebrate Hosts* was held during the *V European Congress of Protistology* from July 23 to 27,

Fig. (29). Participants at the *Second Workshop on Microsporidiosis and Cryptosporidiosis in Immunodeficient Patients*, June 30 to July 3, 1997, in České Budějovice, Czech Republic.

Fig. (30). Participants of the *First United Workshop on Microsporidia from Invertebrate and Vertebrate Hosts* held in České Budějovice, Czech Republic, in July 2004.

2007 in St. Petersburg in Russia and the attendees agreed to meet every three or four years for such a meeting.

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