Microsporidia – Emergent Pathogens in the Global Food Chain

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Keywords (2-6): Zoonotic, Phylogeny, Immune-suppression, Intensive rearing, Aquaculture, Farming,

Abstract

While human population continues to rise, the globalised food chain is under increasing stress from diverse factors relating to farming practice and climatic change. Intensification of food production has potential to drive an array of negative outcomes including increased disease prevalence in food plants and animals, the translocation of exotic pathogens with trade and, enhanced potential for zoonotic transfer of animal pathogens to humans. Microsporidia are diversely distributed, opportunistic and density-dependent parasites infecting hosts from almost all known animal taxa. They are frequent in highly-managed hosts such as farmed fish, crustaceans, honeybees, bumblebees, other mass reared insects and terrestrial livestock, all of which are vulnerable to epizootics, and all of which are critical for stability of the animal-human food chain. Both mass rearing for production purposes and changes in global climate may exacerbate disease by favouring faster reproduction and transmission of parasites in stressed and immune-deficient host animals. Further, human microsporidiosis appears adventitious and primarily associated with an increasing community of immune-deficient individuals. Taken together, there is strong evidence that not only is microsporidian prevalence increasing in animal and human populations but also, human pathogenic forms have origins in diverse animal taxa from terrestrial and aquatic biomes.

Parasites in food chains

In high-income countries around 70% of people die over the age of 70 mainly due to non-communicable, chronic conditions. In low-income countries almost 40% of deaths occur in children under the age of 15 and generally in association with infectious diseases (e.g. HIV/AIDS, malaria, diarrhoea and tuberculosis). Many of these deaths are caused by pathogens transmitted via food and water supplies (**1** Gajadhar et al., 2006). Human food originating from both plants and animals is produced, processed and marketed in intricately linked systems of primary producers (e.g. corn, cattle, fish), input and service providers (i.e. pesticides, water, veterinary drugs), transporters, processors, wholesalers, retailers, consumers and end-users of by-products (e.g. manure). Foodborne diseases comprise a broad range of illnesses caused by ingestion of pathogens, parasites, chemical contaminants and biotoxins that are either naturally present in food or can contaminate food at different points in the production and preparation process (**2** WHO, 2007). Many of the 300 species of helminths and over 70 species of protists known to infect humans are transmitted via food and water (**3** Ellin, 2003). Infectious life stages are acquired by ingesting tissues of infected mammals, fish and invertebrates as well as contaminated food and water supplies or via

contaminated fomites or fingers. Although traditionally associated with tropical outbreaks, perceptions of risk in temperate regions are changing following large outbreaks of parasites such as *Toxoplasma gondii* (**4** CDC, 2011) and cryptosporidiosis (**5** MacKenzie *et al.* 1994). Globalized food trade and travel clearly have the potential to increase the risk of imported parasitoses from tropical countries (**6** Simarro et al. 2012). Microsporidia, although not currently considered as priority foodborne parasites, have the potential to enter the human food chain through waterborne and meat-borne routes and via exposure to the environment. As such, natural hosts of human infective microsporidia can be part of the human food chain (e.g. **7** Slifko *et al.* (2000), **8** Sak *et al.* (2008)). In this review, we consider Microsporidia as agents of emergent disease in hosts from major global biomes and food production sectors (terrestrial, aquatic) and, in human consumers. Further, we combine phylogenetic, ecological and immunological perspectives to propose unifying themes, under a 'One Health' banner which may explain emergence of these opportunists.

Microsporidia - What are they and where did they come from?

The Microsporidia are a hyper-diverse phylum of spore forming parasites infecting hosts from all major animal taxa in all global biomes **(BOX1).** The array of hosts is equally diverse, ranging from protists (in some of which Microsporidia are hyperparasites, to infections of vertebrates, including humans. Almost half of the known microsporidian genera infect aquatic hosts, with thousands of taxa remaining undescribed (**9** Stentiford et al, 2013a). Morphological approaches to within-phylum taxonomy have generally been superseded (or at least augmented) by sequence comparisons of the ribosomal rRNA genes (e.g. **10** Vossbrinck, C.R. and Debrunner-Vossbrinck, 2005; **11** Stentiford et al. 2013b). Debate over placement of the Microsporidia within the tree of life have progressed from their initial consideration as early (pre-mitochondriate) eukaryotes (**12** Cavalier-Smith, 1983) to more detailed phylogenies which proposed an affiliation with the fungi (e.g. **13** Keeling et al. 2000). Analysis of the first complete microsporidian genome (*Encephalitozoon cuniculi,* **14** Katinka et al., 2001) confirmed that the previous interpretation of their status as so-called Archezoa was an artifact of long branch attraction (**15** Thomarat et al., 2004); a finding supported by the discovery of highly reduced mitochondria (mitosomes) within the microsporidian cytoplasm (**16** Williams et al. 2002). More recently, although confirmation of a fungal relationship is now accepted by most, their specific relationships, and their branching either within (e.g. **17** Gill and Fast, 2006) or outside the group (**18** Tanabe et al. 2002; **19** Capella-Gutierrez et al. 2012) has been unclear. Although phylogenetic comparison of known taxa from within the Microsporidia or the Fungi has not yet resolved this issue, the recent discovery (and phylogenetic placement) of three novel lineages, the Cryptomycota (**20, 21** Jones et al. 2011a,b), the aphelids (**22, 23** Karpov et al. 2013, 2014) and, the genus *Mitosporidium* (**24**, Haag et al., 2014) as intermediate

between Fungi and the the rest of the eukaryotes has re-ignited the field. The Cryptomycota appear to branch at the base of the Fungi and contain the Microsporidia as well as the aforementioned aphelids and *Mitosporidium*. Discovery of the group is clarifying relationships between the Microsporidia, parasites with intermediate characteristics (such as *Mitosporidium*), and all other eukaryotes (**25** Keeling, 2014).

Opportunistic pathogens in all major biomes

The current checklist of Microsporidia meeting the descriptive criteria defined by the International Committee of Zoological Nomenclature (ICZN), stands at 200 genera (**26** Becnel et al. 2014). Phylogenetic analysis (based upon ssrRNA partial gene sequence) of 70 of these genera reveal 5 apparent clades broadly classified into 3 major groups according to predominant host/environment type, termed the Aquasporidia (Clades 1 and 3), the Terresporidia (Clades 2 and 4) and the Marinosporidia (Clade 5) (**27** Vossbrinck et al. 2014). It is noteworthy that most of these clades contain exceptions, likely associated with either the pathogen or even the host switching to a new habitat. Host-switching may be more likely where the microsporidian parasite is a generalist or where hosts move between habitats (e.g. freshwater to marine, or freshwater to terrestrial). In the case of confirmed human-infecting taxa, representatives are observed across the phylum and include the genera *Enterocytozoon, Encephalitozoon* and *Vittaforma* (Clade 4, Terresporidia), *Anncalia* and *Tubulinosema* (Clade 3, Aquasporidia) and, *Pleistophora* (Clade 5, Marinosporidia) (**27** Vossbrinck et al. 2014). Although not inconceivable that *Homo sapiens* fulfil status as type host for infection with particular microsporidian taxa, given the spread of human-infecting genera across known clades, and the preponderance for infection to occur in immune-compromised patients (see below) it is perhaps more likely that these infections represents zoonotic transfer from hosts inhabiting terrestrial, freshwater and marine environments. The transfer in this case may relate to direct exposure to type host taxa (e.g. via the food chain) but also by contact with extra-host parasite life stages in the environment in which they reside. In this respect, the potential for susceptibility of humans to infection by other Microsporidia across the phylum appears significant.

Microsporidia and immune competence

In nature, Microsporidia typically develop a balanced interaction with their host, leading to long-term sub-clinical infections (**28** Vavra and Lukes, 2013). When the immune condition of the host is compromised, infection can lead to overt signs of clinical disease highlighting the key role of immune competence in mitigating individual- and population-level health effects of microsporidiosis (**29** Didier and Khan, 2014). Human immune-deficiencies can be categorised into primary and secondary types. Primary immune deficiencies (PID) are derived from

intrinsic and inherited defects in the immune system. Although rare (est. 50,000 PID cases in the USA, **30** Chapel et al., 2014), microsporidian infections have been occasionally been reported in PID patients (**31** Bednarska et al., 2014). More common are secondary immune deficiencies (SID) which are acquired from an array of causes including chemotherapy and/or radiation treatments for malignancies, immunosuppressive therapies to prevent transplant rejection, malnutrition and poor sanitation, aging, and infectious diseases such as HIV/AIDS (**32** Bonilla, 2014). Prior to the HIV/AIDS pandemic in the mid 1980s, microsporidiosis was rarely reported in human patients (**33** Sprague, 1974). The pandemic brought to light the opportunistic capability of microsporidia to infect humans and produce disease in virtually all organs (**34, 35** Desportes et al., 1985, Orenstein et al., 1997). Prior to common use of anti-retroviral therapies, microsporidiosis was reported in at least 15% (and up to 85%) of HIV/AIDS patients (**36** Fayer and Santin-Duran, 2014). However, although prevalence declined with improved therapy, an increase in newly-diagnosed cases of HIV in people over 50 years of age, coupled with an ageing population of patients living with HIV, is leading to so-called HIV-associated non-AIDS (HANA) conditions that accelerate onset of diseases normally observed in the elderly. These patients show accelerated immune senescence, leaving them susceptible to opportunistic infections, including from microsporidia. Reactivation of latent microsporidian infections with age, or with subsequent use of chemotherapy or immunosuppressive treatments, has also been reported (**37** Sak et al. 2011). Although at least 10 microsporidian genera have been associated with human patients (**Table 1, Didier proceedings**), the most frequently detected taxon is the gut-infecting *Enterocytozoon bieneusi* in patients with HIV/AIDS in whom it produces chronic diarrhoea (**34** Desportes et al., 1985).

Age, both young and old, has been associated with elevated burden of microsporidiosis. In very young children (below age 6) immune immaturity coupled with inadequate hygiene practices and malnutrition have revealed surprising levels of infection (e.g. 18.2% of children in one study from Spain) (**38** Lobo et al., 2012). Epidemiological studies of *E. bieneusi* specifically have revealed background prevalence ranging from 4.4% - 22.5% in HIV-negative children (**39** Matos et al., 2012). In the elderly, immune senescence and declining numbers of naïve T cells lead to weakened response to new infections. In one study of HIV-negative individuals with a mean age of 73.5 year, 17% of patients presenting with symptoms of diarrhea were infected with *E. bieneusi* (**40** Lores et al., 2002). Given a growing human global population aged 65 years and over (16% by 2050), immune senescence-associated microsporidiosis is likely to increase (**41** Suzman and Beard, 2011).

Microsporidia in food and water

Ingestion of contaminated food and water, either directly or indirectly (via exposure to the environment) offers the most likely route of transmission of microsporidian spores to humans (**42** Didier & Weiss, 2011). To this end, the majority of human-pathogenic microsporidia detailed in **Table 1** have been detected in water. Comprehensively reviewed by Fayer and Santin-Duran (**36**), it is considered that water, either consumed directly by drinking or indirectly via irrigating or washing foods, bathing, washing, or for recreation provides a critical medium for spore survival and transmission. Excretion of spores from infected humans and animals, via urine and faeces is the primary route of water contamination. Recalcitrance within freshwater and marine environments at a range of temperatures contribute to retention of infectivity and the potential for wide dispersal from point sources (**43** Li et al. 2003). Surveys of surface, drinking, waste and recreational waters have consistently demonstrated the presence of liberated stages of microsporidian parasites. In some cases, filter feeding molluscs have been deployed as sentinels for detection of microsporidia in surface waters; specifically demonstrating the presence of the human pathogens *E. bieneusi*, *Encephalitozoon intestinalis* and *En. hellem* (**44** Graczyk et al. 2004; **45** Lucy et al. 2008). However, given that over 200 genotypes of *E. bieneusi* have so far been identified (some exclusively in human or animal hosts and others infecting both), accurate typing of isolates detected in the water sources used by humans is an important step in understanding the true risk of exposure (**46**). Furthermore, because *E. bieneusi* resides within a family of microsporidia otherwise exclusively infecting fish and crustacean hosts (**47** Stentiford et al. 2011), future studies to investigate the potential for genotypes of *E. bienuesi* (or closely related taxa) to exist in a replicative form within aquatic environments are required (**9** Stentiford et al. 2013).

Microsporidia have also been detected directly in food destined for human consumption. Soft fruits, vegetables and herbs collected from markets in Poland were contaminated with *E. bieneusi* and *En. intestinalis* (**48** Jedrzejewski et al. 2007). Milk contaminated with human pathogenic genotypes of *E. bieneusi* has been reported originating from herds in Korea (**49** Lee, 2008). A foodbourne outbreak of gastrointestinal illness in over 100 people was associated with consumption of *E. bieneusi* contaminated cucumbers in Sweden (**50** Decreane et al. 2012).

Although not directly related to food consumption, the propensity for insect-infecting microsporidia to be vectored to humans either by bite, sting or contamination of the skin by faeces of the insect host, have been demonstrated. Examples include *Anncalia algerae* infections in the eye and musculature (**51** Coyle et al. 2004), *Tubulinosema* sp. infection of the

tongue (**52** Choudary et al. 2011) and *Trachipleistophora* sp. infections of the skeletal muscle and organs (**53** Vavra et al. 1998). Increasing contact with infected insects mass-reared for human consumption poses a future occupational and consumption risk. Similar contact-related risks have been indentified for aquatic animals associated with infections by *Pleistophora* sp. in the musculature of immuno-suppressed patients with and without HIV/AIDS (**54** Cali and Takavorian, 2003; **55** Chupp et al. 1993). Furthermore, microsporidiosis in livestock has been widely reported, including infection of chickens with genotypes of *E. bieneusi* (**56** Reetz et al. 2002) and, *Encephalitozoon* spp. (**57** Fayer et al. 2003); and infection of pigs, cows and humans in close vicinity in China with the same genotypes of *E. bieneusi* (**58** Zhang et al. 2011). Contact between humans and companion animals (pets) has also revealed potential for zoonotic transfer of *E. bieneusi* between guinea pigs and children (**59** Cama et al. 2007) and potentially from a human AIDS patient (infected with *En. intestinalis*) to a cat (**60** Velásquez et al. 2012). Clearly, the environment offers ample potential for food, water and contact-driven transmission of microsporidian parasites from animals to susceptible human hosts. **BOX 2 (A primer on *E. bieneusi*)**

Microsproridia in major food production chains

In addition to the direct risk to humans associated with consuming contaminated water or food, it is appropriate to consider how microsporidia may directly interact with hosts harvested within production chains, or those that provide ecosystem services to enable food production.

Microsporidia infecting terrestrial invertebrates have impacts on natural populations, and can devastate mass-reared colonies of insects used as human or animal food or as biological control agents of agricultural pests. Microsporidia are known to infect more than 30 species of field-collected and mass-reared beneficial insects including parasitoids, predatory insects and mites, phytophagous insects used for weed control, and beneficial nematodes. They decrease food consumption in their host, prolong development, impart physical deformations, reduce fecundity and longevity, and increase mortality (**61** Bjørnson and Oi, 2014). For example, *Muscidifurax raptor,* a parasitoid found naturally on dairy farms where they provide effective house and stable fly control, is mass-reared for inundative release. However, overcrowding and stress in commercial insectaries leads to high prevalence (up to 84%) of *Nosema muscidifuracis*, a vertically transmitted microsporidium that reduces both lifespan and fecundity of the parasitoid and heavily impacts fly control on the farm (**62, 63** Geden et al. 1992, 1995). Because microsporidian infections are typically cryptic, they may overlooked initially in mass-reared colonies (**61** Bjørnson and Oi, 2014). However, with increasing recognition of the potential of insects as a source of protein for the burgeoning global population (**64** van Huis et al. 2013), a requirement for more controlled mass-rearing

conditions, including development of pathogen-free brood lines and appropriate legal frameworks for their trade, are now required.

Insects play pivotal roles in global food production with wild and managed bees providing critical pollination services (**65** Potts et al. 2010). Apparent gaps between global crop pollination needs and the availability of large-scale pollinator populations (e.g. domesticated honeybee colonies), is due (at least in part) to the highly-publicised syndromic condition termed Colony Collapse Disorder (CCD), which has provided focus to research on bee health and disease in recent years (**66** Cornman et al. 2012). Despite the fact that infection by *Nosema apis* and *Nosema ceranae* have specifically been correlated to losses of honeybee colonies (**67, 68** Fries, 1993; Fries et al. 1996), definitively linking microsporidian infections *per se* to colony declines, in either the USA or, in Europe, has not been possible (**69, 70** van Engelsdorp et al. 2009; Dainat et al. 2012). In addition to potential shortfalls in pollination by managed pollinator populations, a global decline in wild pollinator populations has also been reported (**71** Cameron, 2011). 'Spillover' of infectious diseases from domesticated pollinator populations to wild pollinators has been highlighted as a significant potential source of emerging infectious disease (EID) in wildlife (**72** Daszak, 2000). Specifically, the propensity for honeybees to host a wide range of infectious agents (including Microsporidia) (**73** Ratnieks and Carreck 2010) and the detection of parasites such as *N. ceranae* in bumblebees occurring in close proximity to managed honeybee colonies (e.g. **74** Fürst et al. 2014), provides at least some evidence for such spillover. However, lack of historical information, inconsistent application of accurate diagnostics to honeybee and bumblebee infections and, a paucity of well-designed studies to examine possible spillover, make confirmation of this effect difficult (**75** Brown 2011). Recent application of managed bumblebee colonies for greenhouse pollination also has raised questions about the potential for similar spillover effects to surrounding wildlife (**76** Graystock et al. 2014) **(BOX3).**

In terms of aquatic hosts, microsporidia may directly impact the production of animals destined for human consumption, or, may alter prey populations on which animals destined for human consumption (e.g. fish) rely. As mentioned above, aquatic hosts support almost half of the known microsporidian genera (**9** Stentiford et al, 2013a). In terms of wild (fished) populations, microsporidian epizootics have been historically associated with collapse of commercial fisheries (e.g. the North American ocean pout fishery in the 1940s) (**77** Kent et al. 2014) while in aquaculture, numerous microsporidian taxa have impacted production during the hatchery, grow-out (netpen), processing and, marketing phases (see **77** Kent et al. 2014 for context). Recently, an emergent disease condition termed 'emaciative syndrome' was shown to be caused by infection with *Enterospora nucleophila* in farmed seabream (*Sparus aurata*) from

the Mediterranean. Disease associated with infection by this parasite is apparently associated with immune-suppression in its host (**78**, Palenzuela et al. 2014), a feature shared with several other members of the *Enterocytozoon* clade in which this parasite resides. Previously, immune-suppression has been associated with increased severity of microsporidiosis in model fish hosts (e.g. zebrafish infected with *Pseudoloma neurophilia*, **79** Ramsay et al. 2009) while in other scenarios, infection by microsporidian parasites have directly impaired immunity, presumably making their hosts more susceptible to infection by other pathogens (e.g. *Nucleospora salmonis* infection of salmonids, **80** Wongtavatchai et al. 1995). It appears likely that an association between sub-optimal environmental conditions, relative immune-suppression and host proximity in aquaculture settings can encourage microsporidiosis and will lead to further emergence of yield-limiting diseases in farmed fish.

Other high profile examples exist in wild and farmed aquatic invertebrates destined for human consumption. Although parasitism is known to occur across most aquatic invertebrate phyla, it is the aquatic arthropods in particular, hosting over 50 known genera that appear most impacted by microsporidiosis (**9** Stentiford et al, 2013a). In the context of the human food chain, this group, containing the decapod crustaceans (shrimp, crabs, lobsters etc.) support a major economy, amounting to almost $40bn per annum from wild fisheries and aquaculture (**81** Stentiford et al. 2011). Those pathogens that target the edible musculature of crustacean hosts have the potential to render marketable meats inedible (**82** Stentiford et al. 2010), while those infecting connective tissues can blight the visual aesthetics and marketing of high value captured hosts such as king crabs (**83** Stentiford et al. 2014). In aquaculture settings, farmed penaeid shrimp represent one of the highest value traded seafood commodities (see **81** Stentiford et al. 2011). Historic low-level prevalence of microsporidian infections such as *Enterocytozoon hepatopenaeii* have been associated with 'slow growth' syndromes in *Penaeus monodon* (**84** Tourtip et al. 2009). However, increasingly intensive farming of the congeneric penaeid *Penaeus vannamei* in Asia in recent years has led to host-switching of *E. hepatopenaeii* to *P. vannamei*, with accompanying high prevalence and intensity infections being observed in association with the recently emergent and devastating syndromic condition "Early Mortality Syndrome" (EMS) (**85** Tangprasittipap et al, 2013). Once again, the link between microsporidiosis and either sub-optimal environmental conditions experienced within the farm or population immuno-suppression associated with inbreeding may have played a role in recent and rapid emergence across major shrimp farming regions (**86** Doyle, 2014). (**BOX4**).

Terrestrial farm animals can also be infected with microsporidia. Although no clinical cases of microsporidiosis have been reported in cows or pigs, *E. bieneusi*, including human pathogenic

strains are commonly detected in the feces of dairy and beef herds (**87** Santin and Fayer, 2009) and swine with diarrhea (**88** Jeong et al. 2007). *En. cuniculi* and *En. intestinalis* have also been detected in pigs, again without apparent clinical outcome for the infected host (see **89** Snowden, 2014). Similar associations apparently exist between *En. cuniculi* and goats (**90** Cislakova et al. 2001) and, horses (**91** Goodwin et al. 2006). Human pathogenic strains of *E. bieneusi* have also been detected in feces of goats (**92** Santin et al. 2010). The first case of non-mammalian *E. bieneusi* infection was detected in chickens destined for human consumption (**56** Reetz et al. 2002) and subsequently other avian hosts were shown to be susceptible (in **89** Snowden, 2014). Although published epidemiological studies determining zoonotic transfer of microsporidia from farm animals to humans are rare, evidence for shared genotypes in humans, cows and pigs have been reported from rural communities in China (**58** Zhang et al. 2011). Zoonotic transfer between 'region-specific' food animals and humans have been reported; including guinea pig to human transfer in Peru (**59** Cama et al. 2007) and rabbit to human transfer in New Zealand (**93** Ozcan et al. 2011).

Unifying themes and concluding remarks

Microsporidia are ubiquitous inhabitants of all major biomes. Hyper-diverse opportunists, they exhibit differing degrees of host specificity, life cycle complexity and ability to infect (and cause disease) in almost all known invertebrate and numerous vertebrate phyla, including humans. The presence of free- and host-associated parasite life stages in water, soil and food appear to offer ample opportunity for exposure of humans to animal-infecting forms. Even though the phylogenetic range of human-infecting forms extends to only 10 of the known 200 genera at present, increasingly consistent application of molecular diagnostics to animal and human infections will undoubtedly reveal an increased potential zoonotic range, particularly as new taxa are described from terrestrial and aquatic systems. Conversely, the application of so-called 'environmental DNA' approaches (**94** Bass et al. 2015) will not only uncover hitherto unknown diversity within the Microsporidia but will enable research on identification of reservoirs for human-pathogenic taxa in terrestrial and aquatic wildlife hosts.

Definitive confirmation of 'emergence' or even increased prevalence of microsporidiosis has been difficult to establish for wild populations in absence of long-term monitoring programmes. However, well-publicised cases of emergence, increased pathogenesis and morbidity associated with microsporidian pathogens exist for widely divergent host groups, ranging from farmed shrimp to human patients with underlying infections such as HIV. In all such cases, emergence (including potential for host switching) appears to centre on a common node of altered immune competence in these diverse host groups. In essence, the prevalence of microsporidian infection and, the intensity of the diseases that they cause, provide a living

sentinel of host immune-competence that traverses both host taxonomy and the biome in which these hosts exist. Climate change and other biome-level stressors (e.g. ocean acidification, intensification of farming) may associate to impart greater disease burden on hosts from all biomes and thus increase the contact rate between infected animals and humans. Coupled with an increasing global population of immune-compromised individuals (associated with age, those undergoing treatment for malignancies and, other infectious diseases such as HIV), microsporidiosis may be expected to rise in both prevalence and severity. The major transmission route between host groups is via the food chain. This broader consideration of plant/animal/human diseases with environmental pressures under the 'One Health' agenda will be increasingly required as a means to address the grand challenges associated with global sustainability (http://www.cdc.gov/onehealth) and to manage microsporidian infections in wildlife, food animals and humans (see **Box 5**).

BOX 1 Microsporidia form and function.

Microsporidia are single celled, eukaryotic, spore forming parasites with both generalist and specialist species found in invertebrate and vertebrate hosts. The most common hosts for microsporidia are arthropods and then fish with about 800-900 species of microsporidia described out of between 1,300-1,500 species. There are two main clades of microsporidia, the typical (or advanced) and atypical (or primitive) microsporidia (**95** Vávra and Larsson, 2014). The atypical microsporidia are a small group composed of approximately 13 genera and 42 species (**96** Larsson, 2014). The majority of known microsporidia are of the typical type with about 190 genera and an estimated 1,300 – 1,500 species. This group contains the opportunistic taxa that can have simple to complex developmental sequences and life cycles. Spores of the typical microsporidia contain one or two nuclei (the diplokaryon) and are most commonly oval or pyriform in shape. They are usually in the 2-8 micron range but can be a small as 1 micron or as large as 30 microns in length. The spore has a very complex structure that contains the extrusion apparatus. The spore wall is composed of two layers, an electron-lucent endospore layer that contains chitin and an electron-dense exospore that is often layered. The unique infection apparatus is composed of three main parts: a long, thread-like polar filament, a multilayered polaroplast which is a highly membranous structure that occupies the anterior half of the spore and a posterior vacuole (Fig. I). When the spore is in the appropriate host and environment, the spore germinates and the polar filament is everted to become a hollow tube. The sporoplasm travels through this tube and is inoculated into the cytoplasm of the host cell to begin replication. Generalist species of microsporidia have a broad host range and the ability to infect both invertebrate and vertebrate hosts. Generalists are often responsible for opportunistic infections in higher vertebrates. Some notable genera capable of infecting and developing in arthropod and vertebrate hosts are *Anncaliia*, *Tubulinosema*, *Trachipleistophora* and *Encephalitozoon* although there are other genera that have been implicated by molecular data with species in arthropod and vertebrate hosts (exp. *Entercytozoon*, *Endoreticulatus*). Specialists are restricted to infecting and developing within a narrow range of closely related hosts or some species that require an obligate two host system with a definitive host and intermediate host (exp. *Amblyospora*). Both specialist and generalist species can cause significant problems for all types of beneficial arthropods and vertebrates.

BOX 2 *Enterocytozoon bieneusi* – a zoonotic pathogen of humans

Enterocytozoon bieneusi was originally described in 1985 as a cause of gastrointestinal infection presenting as chronic diarrhoea in humans with advanced HIV-1 infection (i.e. AIDS)

(34 Desportes et al 1985**)**. Spores are smaller (1.0 × 1.5 µm) than those of *Encephalitozoon* spp. (1.2 × 2.2 µm) and more difficult to find in tissue sections. *E. bieneusi* shows interesting intracellular developmental involving multinucleated plasmodia with characteristic electron-lucent, lamellar inclusions. Inclusions associate with the nuclear envelope, the endoplasmic reticulum, or both. *E. bieneusi* appears to be widely distributed in both mammals and birds; it has been reported in pigs, dogs, cows, chickens, pigeons, falcons, and various primates. Family-level relatives exist in fish and other aquatic animals. Zoonotic transmission of *E. bieneusi* has been confirmed (**59** Cama et al 2007). Infection of the epithelium of the gastrointestinal tract is the most frequent presentation of microsporidiosis and over 90% of these infections are caused by *E. bieneusi*, with the remainder mostly caused by *En. intestinalis*. Infection does not produce active enteritis or ulceration, although infection results in variable degrees of villous blunting and crypt hyperplasia. Infection is associated with malabsorption, perhaps as a consequence of increased villous epithelial cell turnover leading to functional immaturity of the villous epithelial cells. In humans with AIDS, *E. bieneusi* infection has also been associated with infection of the biliary track with scerosing cholangitis (**35** Orenstein et al 1992). Hepatitis with infection of the biliary system (including the gallbladder) caused by *E. bieneusi* has also been described in monkeys and pigs and although systemic dissemination is rare, spores have been associated with proliferative serositis (peritonitis) in macaques (*Macaca mulatta*) and in the nasal mucosa of humans (**97** Hartskeerl et al 1995). There are also reports of pulmonary involvement associated with chronic diarrhea, persistent cough, dyspnea, wheezing, and chest radiographs showing interstitial infiltrates with spores being found in stool, bronchoalveolar lavage fluid, and transbronchial biopsy specimens (**98** Del Aguila et al 1997), as well as a reports of this organism being found in the urine in renal transplant patients.

Box 3. *Nosema* disease in bumblebees and honeybees

Nosema species infecting honeybees and bumblebees (Apidae) belong to the phylogenetic clade *Nosema/Vairimorpha*, a microsporidian taxon most frequently isolated from the Lepidoptera. *Nosema bombi*, reported from more than 50 species of bumblebees, is a systemic pathogen that appears to be specific to the genus *Bombus*. Effects of chronic infections on these essential native pollinators include reduced colony size; males with reduced sperm; reduced hibernation survival and colony establishment; and fewer reproductive females with reduced mating capability (e.g. **99** Rutrect and Brown 2009). Some *Bombus* species appear to be more susceptible to *N. bombi* infection than others and, although cause and effect has not been established, higher prevalences have been reported from several North American species with apparently declining populations (**71** Cameron et al. 2011). Concerns that exotic strains of *N. bombi* have been released into North American *Bombus* populations via managed pollination services have not been substantiated, but *Nosema* pressure on susceptible species could potentially lower resistance to other pathogens. The annual value of pollination services and hive products of the western honeybee, *Apis mellifera,* are estimated to exceed $200 billion globally, but anthropogenic, global distribution of this species has resulted in a significant increase of parasites and pathogens that may have host-switched from other hymenopteran species. Among the most invasive is *Nosema ceranae,* thought to have originated from the Asian honey bee, *Apis cerana* (**68** Fries et al. 1996). Similar to *Nosema apis,* which is naturally occurring in *A. mellifera, N. ceranae* infects adult bees and has chronic effects, but this pathogen appears to be dominant and has nearly completely displaced *N. apis,* particularly in honey bee populations below the 50th parallel north in Europe and North America. “Nosemosis” now figures in many reports of colony loss. Unlike *N. bombi* and most other Nosema spp., both *N. apis* and *N. ceranae* are pathogens of the honeybee midgut tissues; however, *N. apis* appears to be specific to *A. mellifera* while *N. ceranae* has been reported from three other *Apis* spp. and at least 14 *Bombus* spp. *N. ceranae* causes energy stress, longer and less frequent foraging flights, and shortens the lifespan of bees (e.g. **100** Mayack and Naug, 2009. It has been reported to synergize the deleterious effects of viruses and a variety of agricultural and

apicultural pesticides, including fumagillin, which is used to treat nosemosis (**101** Huang et al. 2013).

BOX 4 *Enterocytozoon hepatopenaeii* – emergent pathogen in farmed shrimp

Enterocytozoon hepatopenaei was described infecting the hepatopancreas (gut) of farmed tiger shrimp (*Penaeus monodon*) in 2009 (**84** Tourtip et al. 2009). Phylogenetic analysis placed the parasite within the *Enterocytozoon* clade, closest to the human gut pathogen *E. bieneusi* and, another intranuclear pathogen, infecting the hepatopancreas of European edible crab (*Cancer pagurus*), *Enterospora canceri* (**102** Stentiford et al. 2007). At the time, *E. hepatopenaei* was considered a low prevalence/low intensity pathogen of tiger shrimp and was partly implicated in a condition termed 'monodon slow growth syndrome'. Over the past decade, shrimp farming in Asia has increasingly focused on production of the sister taxon *Penaeus vannamei*, an intensively reared species which now dominates the global market with first sale values exceeding $10bn per annum. In 2012, an emergent condition termed 'Early Mortality Syndrome' (EMS) began to devastate *P. vannamei* production across Asia. The syndrome, was later primarily associated with infection of the shrimp gut by a strain of *Vibrio parahaemolyticus* containing a plasmid-derived toxin capable of causing significant pathology in the hepatopancreas, and death to the host. The disease was renamed as Acute Hepatopancreatic Necrosis Disease (AHPND) (**103** Lee et al. 2015). Significant surveillance efforts at the time of emergence of EMS (and AHPND) revealed other pathologies and agents potentially implicated in the regional outbreak. Of these, the most significant was an apparent host switching event in which *E. hepatopenaeii* emerged as a significant disease causing agent in *P. vannamei*, appearing at both high prevalence and intensity in farmed stocks either undergoing outbreaks of EMS/AHPND or in ponds not undergoing outbreaks (**85** Tangprasittipap et al, 2013). The rapid emergence of this microsporidian has prompted high profile warnings to industry from regional bodies such as the Network of Aquaculture Centres in the Asia Pacific (NACA) (http://www.enaca.org) advising that E. *hepatopenaei* should be added to list of pathogens screened for during production of post-larvae for eventual stocking to commercial farms. In addition, NACA advised against the use of live animals (e.g. polychaetes, clams etc) for feeding of shrimp broodstock due to the potential for such taxa to vector viable spores of *E. hepatopenaei* to naïve shrimp. Sensitive diagnostic tests developed for such are now available (**104** Suebsing et al. 2013).

BOX5 Outstanding Questions

1. Do appropriate phylogenetic tools exist for known and novel members of the phylum Microsporidia in order to allow for detailed molecular epidemiology?
2. Can humans be infected with a broader range of microsporidian taxa than currently recognised?
3. What are the conditions that allow microsporidia to cross species and what allows emergent infections in this phylum
4. Is *Enterocytozoon bieneusi* able to replicate in aquatic vertebrate or invertebrate host taxa?
5. Is there a common node of interaction (immunological, biochemical) among microsporidia from across the phylum and their broad range of hosts?
6. Can common nodes of interaction be exploited to evade infection or, to mitigate pathogenic outcomes in infected hosts?
7. Can techniques be developed to allow genetic manipulation of the microsporidia that would facilitate experiments aimed at understanding the biology of these organisms?

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