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Microsporidia as an entomopathogen: A Review

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Abstract

Microsporidia constitute a substantial portion of the major pathogen of insects and produce sizeable impacts on economically important and harmful insect species. Microsporidia being obligate, intracellular parasites in insects can be divided into two categories based on their life cycles and host-parasite relationships. In Microsporidian pathogens, some genera exhibit uncomplicated life cycles with one spore type likely for oral (horizontal) transmission affecting only one generation of the insects and are not usually host or tissue specific. While some species of microsporidia have complex life cycle, relating to sexual reproduction, vertical and horizontal transmission, two obligate hosts, and three or more morphologically distinct spores. The subject of this review is to understand the effects of microsporidian parasites as an intracellular insect pathogen with a diverse host range, as well as the features that distinguishes insect-parasitic microsporidia with simple life cycles from truly host-specific microsporidian parasites with complex life cycles.

Keywords: *Microsporidia, Intracellular, Genera, Horizontal transmission, Host-specific.*

1. Introduction:

Entomopathogens are significant biological death factors and habitually are a foremost potency in driving the natural cycles of insect populations (Anderson and May, 1981; Maddox, 1987). Presently, almost half of the described genera of microsporidia have an insect as the host (Becnel and Andreadis, 1999). Microsporidia may be the most essential group of entomopathogens of cultivated insects and have been isolated nearly from all insect hosts (Becnel and Undeen, 1992). *Nosema*, an important genus of microsporidia among terrestrial insects, infects more than 150 hosts from 12 insect orders (Inglis *et al.*, 2003). Microsporidian infections in terrestrial hosts have been commonly reported for Lepidoptera, Coleoptera, Orthoptera and Hymenoptera (Inglis *et al.*, 2003; Solter, 2006) but reports on microsporidian infection in Heteropterans are rare.

The spore is the most visible sign of infection characterizing microsporidia by its structure, texture and nature. The spore of microsporidians are small, usually ranging between 3.0-5.0 µm in length and 2.0-3.0 µm in width, oval, ovoid or ovo-cylindrical, shining having high refractive index and exhibiting the characteristic Brownian movement (Becnel and Andreadis,1999).Basically microsporidians are characterized by having unique organelle, the polar capsule involved in the invasion of a host. Some species of microsporidia have complex life cycle, involving sexual reproduction, vertical and horizontal transmission, two obligate hosts, and three or more morphologically distinct spores (Undeen, 1975, 1997). Other microsporidia have simple life cycle with only one kind of spore, formed during the course of a simple asexual reproduction. The microsporidian life cycle can be

separated into three phases; proliferative or merogony, sporogonic (spore maturation) and infective (Cali and Takvorian, 1999, 2004). Proliferation or merogony phase occurs within the host cell cytoplasm after releasing of the sporoplasm, following proliferation they undergo sporogony in which the spore differentiation takes place, terminating in the formation of resistant spores. Microsporidia have complicated life cycles that may use both vertical and horizontal transmission (Undeen, 1997). Also, the majority of microsporidia have mixed transmission cycles and it is unclear whether they are able to change their phenotype according to environmental situations. There is unusually high use of vertical transmission and this has considerable consequences for transmission and pathogenicity. Vertical transmission is connected with low pathogenesis but nevertheless can have significant impact through associated traits such as sex ratio distortion (Smith *et al.,* 1982). All insect-parasitic microsporidia development is restricted to the cytoplasm of the host cell.

Therefore, this review highlights the broad host range of microsporidia in insects undergoing simple as well as complex life cycle and its impact on insect population.

2. History:

This group of microporidian parasites that are pathogenic organisms, first came to light in the 19th century when they were linked with "pebrine disease" of silkworm *Bombyx mori* L. which was caused by *Nosema bombycis* Nageli. *Nosema bombycis*, the contributory agent of pébrine, a disease of the silkworm *Bombyx mori*, was the first microsporidian species to be described (Nageli, 1857). Microsporidian disease, causing chronic early season disease for more than 100 years were variously named as *Nosema* disease, Nosematosis and Nosemosis. Until 1996, the only known causal agent of *Nosema* disease was *N .apis* until when another microsporidian species, *N. ceranae* was described from the Asian honeybee, *Apis cerana*. Among the microsporidian species described so far, at least 200 have been assigned to the genus *Nosema* (Smith and Dunn, 1991) and are often considered the most important and widely distributed group of microsporidia. They infect several commercially important insects and animal species such as salmon and various domesticated mammals. Recently their identification as emerging pathogens of humans has renewed and intensified interest in these parasites. During the last twenty years several microsporidian genera have been identified as being capable of infecting humans, especially individuals with immuno compromised immune systems (Wittner and Weiss, 1999). Based on their cellular structure, life cycle and host specificity (Zelinskaya, 1980), to date, over 1300 species of Microsporidia having almost in 160 genera, have been described. In 1863, Pasteur began investigations to find a technique of managing this disease (Pastuer, 1870). Despite these efforts, pébrine still takes a heavy economic toll on the silk industry today (Liang, 1990).

3. Microsporidia as Natural enemy:

As entomopathogens, there are several reports of Microsporidia found in European *Lymantria dispar* populations (Undeen, 1975; Zelinskaya, 1980, 1981), indicating that Microsporidia are important components of the natural enemy complex of insects. However, inoculation with *E.chrysorrhoea* in *Lymantria dispar* that is infected with *Nosema lymantriae* was not found to be infective (Weiser, 1957). Also, according to Canning (1982) *Nosema pyrausta* has low pathogenicity and is constrained to a single host, the European corn borer. Hence it is not considered pathogenic enough to be used as a microbial pesticide, but, is an important aspect in amending natural populations. Briano and Williams (1997) reported that Microsporidium, the *Lohaniaso lenopsae* is a potential biocontrol agent by studying their virulence to imported fire ants (*Solenopsis richteri*) in a laboratory in USA. The study concluded that the Microsporidium, *T. solenopsae* affected the mortality rate and shortened the longevity of colonies of *S. richteri* reared under laboratory conditions. These laboratory findings are consistent with results of field work promoted by Briano *et al.,* 1995a, 1995b. Though *T. solenopsae* was the first microorganism of South America as a potential biocontrol agent, detailed study is required to establish *T. solenopsae* as a biological control agent of the imported fire ants in the United States. Campbell *et al.* (2007) suggested that the microsporidium, *Nosema fumiferanae*, isolated from Spruce budworm *Choristoneura fumiferana* might play an important role in regulating spruce budworm population. Microsporidia is a pervasive pathogen of insects and infect numerous species of valuable arthropods together with those that are field collected or mass-reared in insectaries for biological pest control in addition to those that provides pest control in nature. These entomopathogens are also common among laboratory-reared beneficial arthropods and frequently cause unceasing disease that reduces host fitness and eventually affects biological control efficacy (Bjornson, 1998, 2008). Also, many published literature have shown that microsporidia can also make accessible itself as

candidate organisms for insect pest control because of their exclusive features of transmission, ability to persist, perpetuate and regulate host populations in nature. Although microsporidia have petite potential as microbial insecticides (Solter, 2006), there is report of *N. locustae* (Canning, 1982) as a registered microbial pest control mediator which can suppress rangeland locusts, grasshoppers and mormon crickets. *N. algerae* and *Vairimorpha necatrix* are being well thought-out as possible candidates for development as microbial insecticides. But Solter and Maddox (1998) reported that they have huge possibility for use as classical biological control agents, fitting more closely the example for parasitoids and predators than that of microbial insecticides.

4. Invasion and transmission of Microsporidia:

Development of *Nosema* spp. was studied by Fujiwara (1980, 1984) and Iwano and Ishihara (1988, 1991) in silkworm and other Lepidopteran species. Canning (1990) had described the complex and diverse types of sporogony observed in different Microsporidian genera. Sprague *et al.* (1992) introduced and described a classification system on, diplokaryotic state of some species at some point of time in the life cycle (dihaplophasea) or uninucleate stage throughout its life cycle (haplophasea). Similarly, Iwano and Kurtti (1995) had observed variable spores that are dimorphic spores of *Nosema furnacalis* when it was cultured in *Helicoverpa zea* cell line. Accordingly, Sprague and Becnel (1999) descibed that, the schizont to be restricted to haplophasic (uninucleate) individuals whereas, the meronts are considered to be diplokaryotic (two closely appressed nuclei). Kleespies *et al.* (2003) had also described Microsporidia, *Cystosporogenes* sp., lifecycle that is isolated from European grape vine moth *Lobesia botrana* and observed that the most basic noticeable stage is the uninucleate schizont. Canning and Curry (2004) conducted life cycle study on *Cystosporogenes*, that is isolated from *Operphtera brumata* and they further investigated that the division of meronts and sporonts were chiefly by binary fission. Hyliš *et al.* (2006) had studied the life cycle of *Nosema chrysorrhoea* from Browntail moth and described that their life cycle consisted of primary and secondary developmental cycles, which vary in time and tissue specificity in the host organisms and the different types of spores produced. Microsporidia have developed a specific process to infect their hosts and spread their pathogenesis in vitro (Vossbrinck *et al.*, 1987). It involves spore activation, germination and polar tube discharge, by which the infective sporoplasm is inocculated into the host cell cytoplasm. This state of spore germination is followed by instant breakdown of sporoplasm, compartmentalization, due to which there is an increase in osmotic pressure which triggers polar tube eversion and finally the injection of the sporoplasm into the host cell (Bigliardi and Sacchi, 2001). Spread of infection is a key factor in pathogen-host interface that can influence the population dynamics of the host (Anderson and May, 1981). Most common mode of transmission of Microsporidia is via horizontal transmissions, when a susceptible host ingests spores and are released into the environment in the feces of infected individuals or via decomposed cadavers (Becnel and Undeen, 1992). Microsporidia were far more host specific as demonstrated by the horizontal transmission experiments that was based on exposure of uninfected *L.dispar* larvae to infected *L.dispar* larvae than direct feeding experiments (Solter *et al.*, 1997). According to Solter (2006) pathogen may be vertically transmitted by one or more of other various mechanisms together with transovum, transovarial (or transovarian) and venereal transfer, or may involve intermediate hosts, sex ratio distortion (eg. host-feminizing and male sterilization or killing), and vectoring the pathogens. Transmission of a microorganism via the egg, either in the embryo or yolk, or adhered to the surface of the egg chorion is a broad term for Transovum transmission. Transmission exclusively refers to transovum transmission in which the pathogen is transmitted to the host embryo by incursion of the egg or yolk via the ovarioles of the infected female host (Becnel and Undeen, 1992; Solter and Maddox, 1998b).The cross infectivity study is a most important step towards predicting the ecological range (host range in the field) of the species. Cross infectivity of *Nosema Lymantriae* isolated from *Lymantria dispar* to the closely related species *Euproctis chrysorrhoea* was tested by Weiser (1957). Similarly, experiments were conducted by Solter *et al.* (1997) in that they inoculated five biotype of Microsporidia isolated from *Lymantria dispar* from the field population of Europe to the forest Lepidoptera that were inhabitant to the Northeastern United States

5. Changes in host behavior resulting from microsporidia infection:

In addition to modulating host activities on a cellular level, microsporidia such as the *Nosema ceranae, (*infecting honey bees) can also change host behavior (Naug and Gibbs , 2009; Ceper *et al.*, 2014). European honey bee

populations have been decimated recently due to a phenomenon called 'colony collapse disorder' that may be at least partially due to infection by *Nosema ceranae*. A recent report suggests that, a measure of the bees' ability to return to the hive after kidnapping and being released from a far-away location (homing success), was significantly reduced in *N. ceranae* infected bees as compared to control animals (Wolf *et al.*, 2014). This dissimilarity was largely due to decreased flight times and increased rest intervals of infected bees, rather than differences in navigation or other flight characteristics. The authors note that although this inability to return home can reduce colony size, it also can alleviate spread of infection throughout the colony, highlighting the complexity of factors at play in host response to microsporidia infections (Shi *et al.*, 2014). The pathogen load in infected tissues is critical during the development and passing of the disease (Ding *et al.*, 2016).

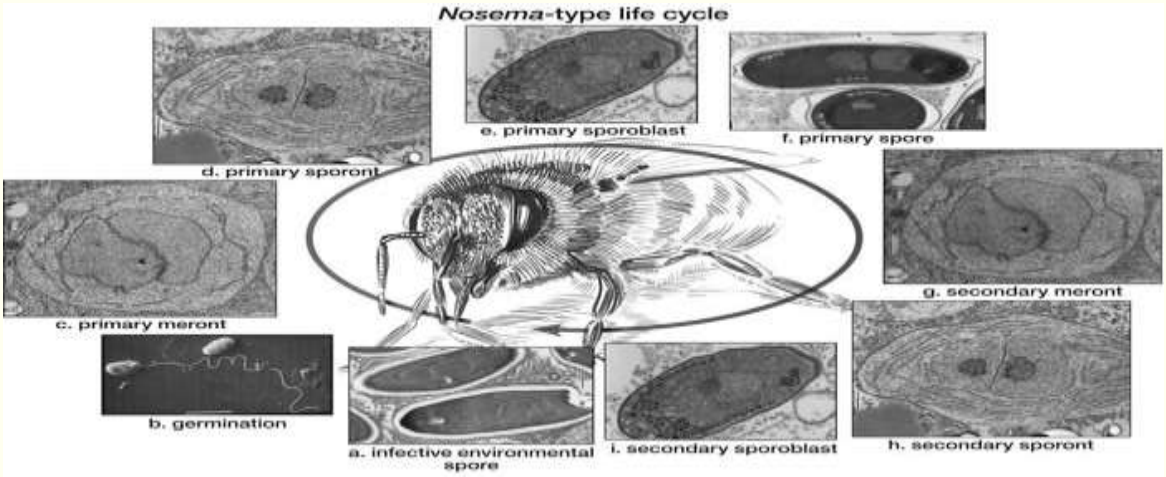

Figure 1.Typical life cycle of a microsporidium in the genus *Nosema*. Infective environmental spores (a) are eaten by a host, and then germinate by extruding the polar filament and injecting the contents of the spore, the sporoplasm, into the host midgut cells (b). The primary meront (c) is the first vegetative stage and divides one or more times before spore formation begins. The sporont (d) is the first stage that is committed to form spores; it divides once to form two sporoblasts (e), which are immature spores. In this first cycle of reproduction, primary spores (f) are formed. These spores have thin walls and short polar filaments, and germinate inside the cells to infect adjacent cells. A second cycle of reproduction occurs when secondary meronts (g) develop from sporoplasms extruded from the primary spores and divide to form secondary sporonts (h). Each sporont produces two secondary sporoblasts (i), before maturing to form environmental spores (j). These spores exit the host, primarily in the feces, to infect other hosts.

[Scanning electron micrograph of germinating spore courtesy the Society for Invertebrate Pathology; transmission electron micrographs of other stages by J. Vavra, courtesy Wiley Publishing Co.; art work by K. Helms.]

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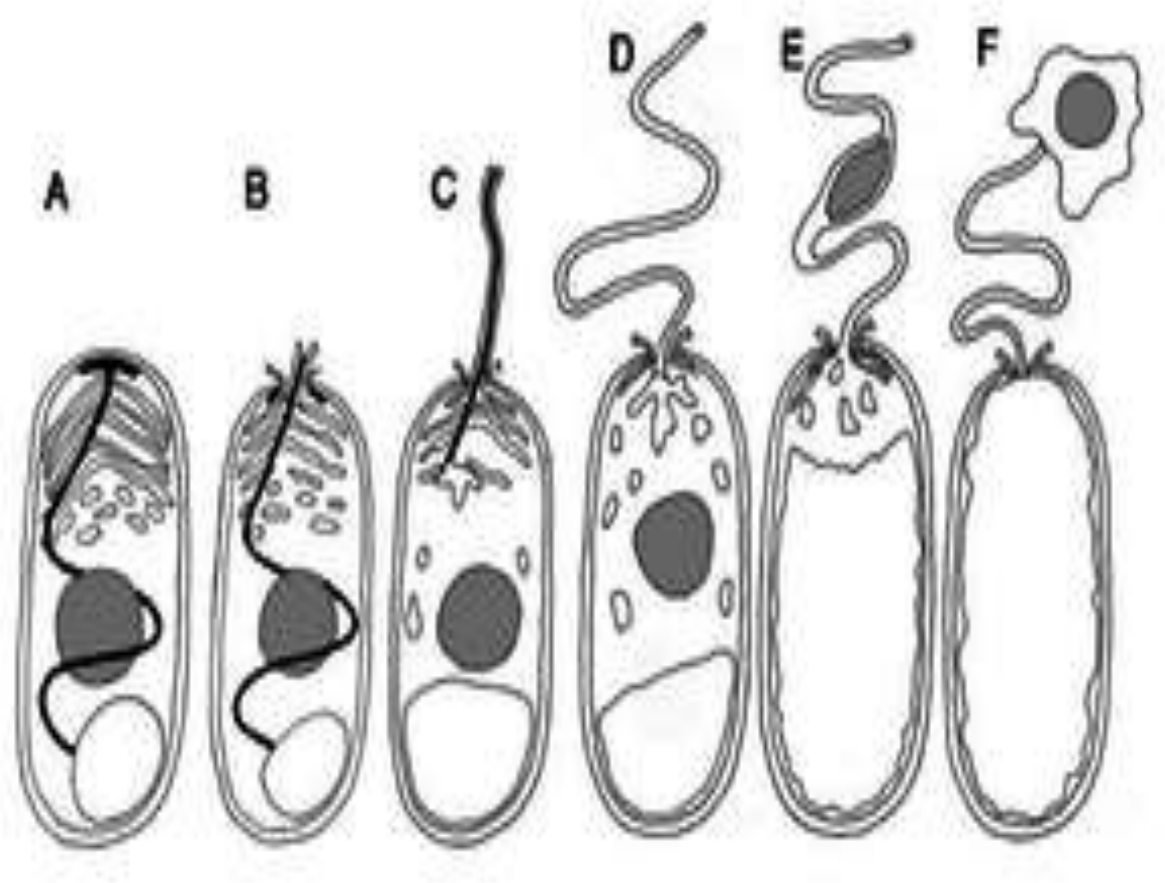

Figure2. Polar tube eversion during spore germination. (A) Dormant spore, showing polar filament (black), nucleus (gray), polaroplast and posterior vacuole. (B) Polaroplast and posterior vacuole swelling, anchoring disk ruptures, and polar filament begins to emerge, everting as it does so. (C) Polar filament continues to evert. (D) Once the polar tube is fully everted, the sporoplasm is forced into and (E) through the polar tube. (F) Sporoplasm emerges from the polar tube bound by new membrane. From Keeling and Fast, 2002.

6. Conclusions:

Parasitology is a growing science and aimed to control and fight parasitic diseases and infections, a quick and reliable diagnosis of the infection agent is necessary. Microsporidia have been studied for over 150 years, but yet many doubts persist regarding their life cycle and biological features, as well as their taxonomy and phylogeny. The detrimental effects of microsporidiosis caused decrease in production in economically significant industries that includes fisheries, honeybee, and silkworm (Wittner, 1999). The way of transmission of microsporidian infection is the determining factor in the evolution of strong host-parasite relationships. It has been verified that parasites that are transmitted horizontally produce higher numbers of transmission stages of the parasite and have higher virulence, often leading to the death of the host (Koella and Agnew, 1997), while parasites that are transmitted vertically are dependent on the host survival and reproduction for their own transmission and survival, thus being less virulent. Furthermore, it is vital to create quick and responsive molecular methods that can identify the parasite pathogen visually and quantitatively for diagnosis, as well as for studying infection characteristics such as epidemiology, pathogenesis and pathogen–host interaction at the molecular level (Klein, 2002).

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