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Occurrence of Microsporidia in Hymenopterans

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ABSTRACT

Microsporidia comprises of an obligate intracellular parasites infecting nearly all animals. Its presence has been reported widely in arthropods of class Insecta and mainly in the order hymenopterans. This paper focuses on the investigation of microsporidian infection in hymenopterans collected from Lucknow, Uttar Pradesh, India. Insects from different families of Formicidae, Vespidae and Apidae were collected and investigated for microsporidian infection. Microscopy and staining technique has been applied for diagnosing the spores of microsporidia and intracellular mode of infection conformity by Scanning Electron Microscope. In the family Formicidae; Black garden ant (*Lasius niger*) showed 81.8% and Red ant (*Solenopsis sp.*) showed 82.6% of microsporidian infection. In the family Apidae; Honey bees (*Apis mellifera*) and Carpenter bees (*Xylocopa* sp.) revealed 94.1% and 90.4% of microsporidian infection. However, Red wasp (*Polistes carolina*) of Vespidae family showed only 1.31% of microsporidian infection. The microsporidian spores observed, had birefringent property, ovo-cylindrical shape with unique Brownian motion. Overall infection percentage in an area that was investigated was 76.9%. In conclusion this study provided with information of the occurrence of microsporidia in Hymenopterans of not only in Apidae family but also its dissemination in Formicidae as well as Vespidae families, eventually affecting the majority of pollinators (bees and wasps) and the regulators of food chain (ants).

KEYWORDS: Obligate, intracellular, arthropods, insect, hymenopterans, pollinators, regulators.

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INTRODUCTION

Microsporidia being an obligate intracellular parasite often causes chronic infections in insects causing significant problems for all types of beneficial hymenopterans from honey bees, ants to biological control agents such as parasitoids. Microsporidia are also frequently found in laboratory-reared beneficial arthropods and often cause chronic disease that reduces host fitness and ultimately affects their biological control value. These are widespread parasite of insects and are commonly found in Lepidoptera and Hymenoptera, causing some well known diseases such as pebrine disease in silkworms (by *Nosema bombycis*) and dysentery in honeybees (*Nosema apis*).The most common method of transmission is through direct oral ingestion of infectious spores found in food or liquids within the insect's immediate environment. Infected insects often exhibit external as well as internal changes as a result of development of the microsporidium. Development of all insect-parasitic microsporidia is restricted to the cytoplasm of the host cell.[1] Microsporidian parasites are emerging parasites and are widely dispersed but its distribution is not well known so it is very important to determine their competitive abilities and population dynamics. Therefore, this paper reports the occurrence of microsporidian parasites in hymenopterans that is collected from Babasaheb Bhimrao Ambedkar University campus, Lucknow (U.P), India.

MATERIALS AND METHODS

a) Sample Collection

Insects sample of order hymenopteras were randomly collected between December 2017-March 2018 in and around Babasaheb Bhimrao Ambedkar University Campus, Lucknow. 239 insect samples belonging to the families Formicidae (Black Garden Ant, *Lasius niger*; Red Ant, *Solenopsis* sp.) Apidae (Honey bees, *Apis mellifera*; Carpenter bees, *Xylocopa* sp.) and Vespidae (Red wasp; *Polistes carolina*) were collected and identified by their standard key.[2]

b) Identification of microsporidian parasite

The insect samples collected were macerated individually in 1ml ddH_2O, filtered by muslin cloth and the homogenate were observed in Light Bright Field microscope under 40X. Each sample was investigated for microsporidian infection that is identified by their high refractive index, fluorescence property and Brownian motion. The Giemsa stain was used to visualize free microsporidian spores in the infected homogenate. For this the smears were air dried, preset with absolute methanol for few minutes and stained with Giemsa stain.[3] Furthermore infected tissues were processed for Scanning Electron Microscopy (Jeol, Japan; JSM 6490 LV). Homogenates were air dried and fixed in 2.5% Glutaraldehyde followed by washing and

post fixation in 1% OsO_4. Again dehydration was performed with different grades of acetone and then mounted on stubs.

c) *Percentage Calculation of Infected Samples:*

Percentage of Microsporidian Infection= Number of Samples found infected ×100
Total number of samples collected

RESULTS AND DISCUSSION

In our study the occurrence of microsporidia in different families of hymenopterans indicated that it is an emergent pathogen but due to lack of knowledge, these intracellular parasites remained to be unexplored in many regions of India. Our data proposed that microsporidian infection persist in Lucknow region though a very small part has been surveyed. In the family Formicidae, Black Garden ants had 81.8 % of microsporidian infection (45 were found to have microsporidian infection out of total 55 ants collected). Similarly, Red ants showed 82.6% of microsporidian infection (19 out of 23 samples collected were found infected) (Table 1, Graph 1). Earlier Allen and Buren[4] reported the presence of microsporidia in fire ants and found that 1007 colonies of fire ants had microsporidian infection. Following Buren's observation, Allen and Silveira-Guido[5] reported microsporidian infections in the black imported fire ant, *Solenopsis richter.* Also, observation under light bright field microscope revealed that spores had unique Brownian motion having fluorescence property with high refractive index and oval to elongated shapes that are the basic verifying parameters to detect this pathogen (Fig 1). Giemsa staining exposed purple staining of microsporidian spore samples (Fig 2). Similar study was also conducted by Garcia[6] using Giemsa stain for identifying microsporidian spores in the intestinal tract cells. In this manner Giemsa stained preparations became most widely used for microsporidian spore identification. Moreover, to diagnose intestinal parasitic infects many techniques have been evolved but electron microscopy ruined to be a gold standard method to study morphological and structural details.[7] In our study, microsporidian spores were found to adhere to the intestinal wall (Fig 3) thereby representing obligate intracellular relation with its host. Therefore, to disclose the host-parasite interaction scanning microscope application would convey the most descriptive analysis.

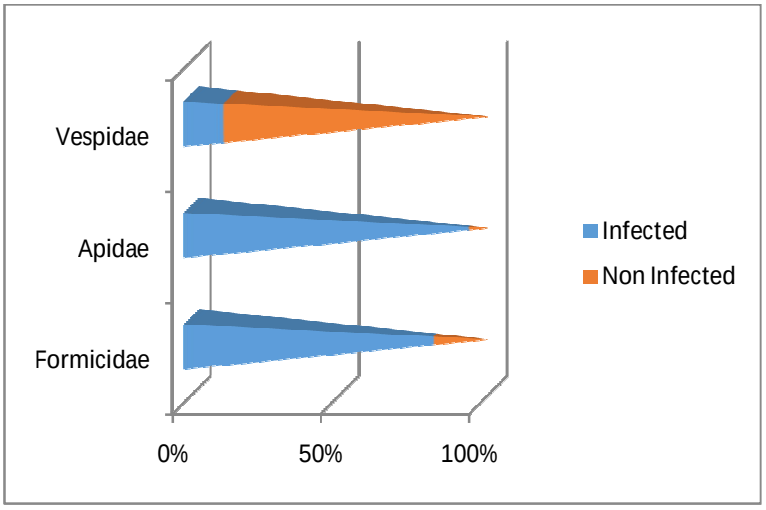
In Apidae family, Honey bees revealed 94.1% of microsporidian infection (96/102 were infected) and Carpenter bees had 90.4% of infection (19/21 were infected) (Table1, Graph 1). In Apidae family, nosemosis disease is widespread and *Nosema apis* and *Nosema ceranae* are parasites infecting the midgut epithelial cells of adult honey bees.[8] Although the microsporidian pathogen of honey bees, *Nosema apis* Zander, has been well-researched, [9] also there have been few studies of microsporidia from bumble bees. Fantham and Porter [10,12] were the first to describe a

microsporidian from bumble bees, naming it *Nosema bombi*. It also infects *Bombus terrestris* (L.) and other *Bombus* species, *Apis mellifera* (L.) and *Apis florea* (F.). *N. bombi* and *N. apis* are currently considered to be synonymous, but they differ not only in their tissue specificities and spore sizes but also in their merogony and in the number of polar filament coils. In Vespidae family, Red wasp (*Polistes carolina*) showed less percentage of microsporidian infection (1.31%) (5/38 was infected with micropsoridia). Earlier Chapmam et al.[11] investigated *Nosema* sp. (Microsporida: Nosematidae) in cells of several tissues in the abdomen of the parasitic wasp, *Pediobius foveolatus* (Hymenoptera: Eulophidae) by light microscopy. Considering their study, light microscopy has been implicated to study microsporidia in wasp with almost similar findings in their morphology (Fig. 2). Overall, percentatge of microsporidian infection was 76.9% (Graph 2) that was surveyed in small region of Lucknow, U.P. The incidence of occurrence of these parasites warrants further examining and characterisation of microsporidian spores in different insect order from diverse areas of Lucknow regions of U.P.

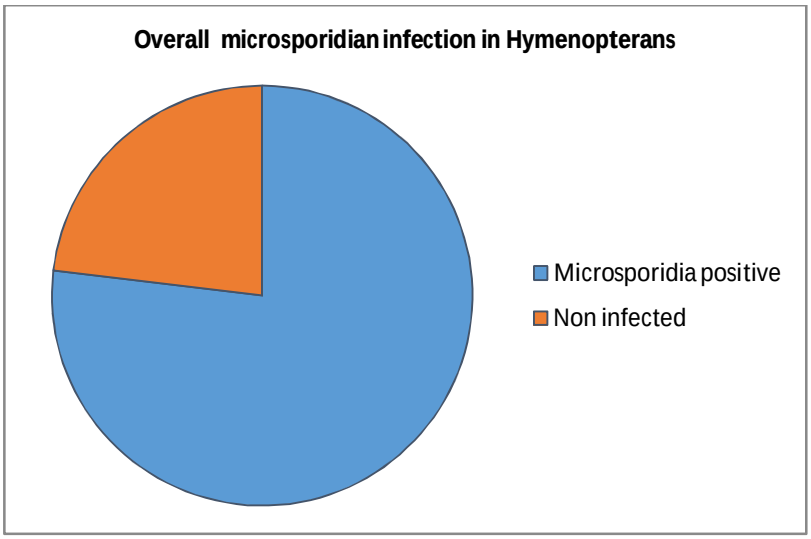
This detection is based on light microscopic and scanning microscope observation. The limiting populations of pollinators and sudden colony decline of wasps and bees somewhere correlates it with the increasing dispersal of microsporidian parasites. The disease has become increasingly more and more complex as more number of microsporidian strains has been identified that resulted from chronic to highly virulent infection. Therefore an attempt has been made to investigate microsporidian infection in Hymenopterans.

Table1. Hymenopterans of families Formicidae, Apidae and Vespidae with Microsporidian infection

Phyllum: Arthropoda					
Class: Insecta					
Order: Hymenopterans					
Family	**Common Name**	**Scientific Name**	**Total no of insect samples collected**	**No. of microsporidia positive samples**	**Percentage of infection (%) observed**
Formicidae	Black Garden Ant	*Lasius niger*	55	45	81.8
	Red Ant	*Solenopsis* sp.	23	19	82.6
Apidae	Honey Bee	*Apis mellifera*	102	96	94.1
	Carpenter Bee	*Xylocopa* sp.	21	19	90.4
Vespidae	Red Wasp	*Polistes carolina*	38	05	1.31



Graph1: Representation of microsporidia infected and non-infected hymenopterans families (Vespidae, Apidae and Formicidae)



Graph2: Showing overall percentage of microsporidian pathogens in Hymenopterans in and around BBAU campus of Lucknow region, U.P

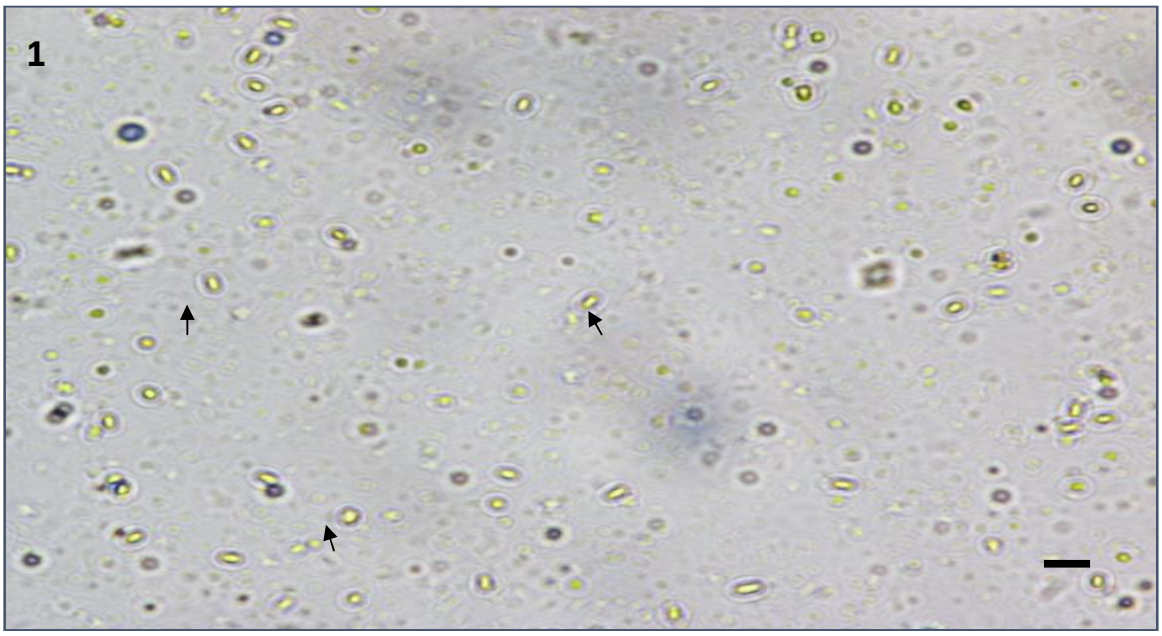


Figure1. Microsporidian parasites in Hymenopterans (black arrow); Scale bar: 10µm

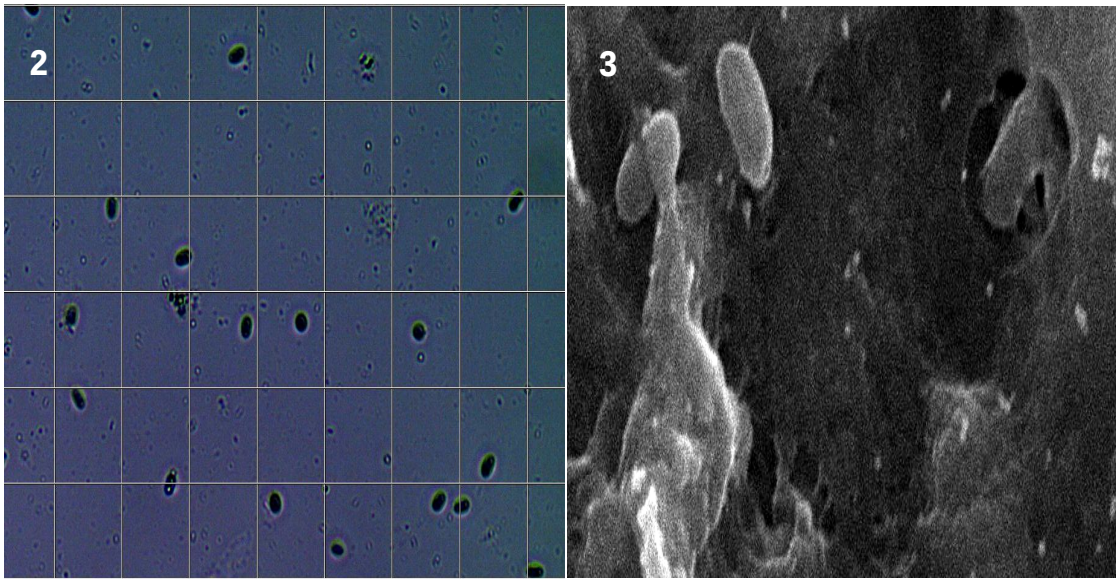


Figure 2 and 3. Giemsa stained microsporidian spores and Scanning micrograph of microsporidian spore in insects gut

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