International Journal of Pure and Applied Zoology
Volume 3, Issue 2, pp: 119-121, 2015
http://www.ijpaz.com

ISSN (Print) : 2320-9577
ISSN (Online): 2320-9585
Rishan Publications

Research Article

OBSERVATION OF A MICROSPORIDIAN PARASITE IN THE ACCESSORY SALIVARY GLAND OF *CHRYSOCORIS STOLLII* (WOLFF, 1801) (HETEROPTERA: PENTATOMIDAE)

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Article History: Received 20th January 2015; Accepted 25th March 2015; Published 8th May 2015

ABSTRACT

A species of microsporidian has been established to cause severe tissue damage in the accessory salivary glands of *Chrysocoris stollii* Wolff (Heteroptera: Pentatomidae). While tissue damage by microsporidians in Heteroptera is well known, the present observation in this species of insect is reported here for the first time. The probable route of infection has been postulated through the tissue sections of the gland.

Keyword: Microsporidian, Accessory salivary glands, *Chrysocoris stollii*, Tissue damage.

INTRODUCTION

Microsporidians are unicellular creatures, parasitic with resistant spore and were earlier grouped under Phylum Protozoa. Microsporidians have eukaryotic nucleus and divide mitotically but lack typical mitochondria, peroxisomes and Golgi apparatus. Since 1969, they are classified under the new Phylum Microspora (Sprague, 1969). There are more than 1200 species of known microsporidians (Wittner and Weiss, 1999) and majority of them are found in insects. They cause economic loss of commercially important insects, poultry and cattle farm, even human is not spared. In most cases they cause immune suppression and sometimes death. Infection by microsporidians takes place by ingestion of their spores from environment, particularly through food. In most of the investigations, they are known to infect intestine first and then spread to other organs. On the gut wall, spores are structurally modified to enter into midgut cells (Maddox *et al.*, 1998). Franzen (2008) provided an account of research on microsporidians spanning for about 150 years. Becnel *et al.* (1999) provided an account of microsporidiosis in insects. *Nosema* spp. has specially attracted attention for causing severe damage to the apiculture industry. Lipa (1977a) presented a list of known microsporidians in Heteroptera.

During an examination of histological aspect of the salivary glands of *Chrysocoris stollii*, the authors found that the accessory glands of few specimens were infected by an unknown species of microsporidia. The host insect belongs to the Order Heteroptera, family Pentatomidae. There is no previous report of such infection in this species of insect. Moreover, active cell damage by the pathogen is also established herewith. Lipa (1977a) listed all known microsporidians of Heteroptera; 6 species under genus *Nosema*, 3 under genus *Thelohania* and 2 under genus *Toxoglugea*. Among these, the species *Nosema adiei* infects gut, Malpighian tubules and salivary glands; the rest of the species were not mentioned to infect the salivary gland.

MATERIALS AND METHODS

Live insects were anesthetized by chloroform and fixed on wax tray. Dissections were performed in Ringer solution (0.67%Nacl) under Zeiss binocular. Photography was done by digital camera (Sony Cyber Shot Digital). The accessory salivary glands were dissected out from several specimens of one sample. The tissue was fixed in

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Bouin's fluid. Microtome sections of 5μ thickness were double stained by Haematoxylin-Eosin method and observed under 400 x.

RESULT

The occurrence of a species of microsporidian in *Chrysocoris stollii* has been found for the first time. The protozoan has been found in the accessory salivary gland. The accessory salivary glands are paired tubular and slightly wavy lengthwise. The glands are situated far from the bigger and paired principal salivary glands (Figure 1). The gland is connected with the principal salivary gland by a very thin, long and tortuous duct of accessory gland (Chatterjee, 2014). Therefore it appears that the pathogen may not have travelled by this way to enter the accessory salivary gland. The alternate possibility is that the pathogen entered through food and then to haemocoel crossing the gut layer. The minute spores entered into the accessory salivary glands through their surface. Here they develop at the expense of tissue to become mature schizonts (Figure 2). The schizonts may be discharged on the plant surface during the act of feeding through salivary secretion.

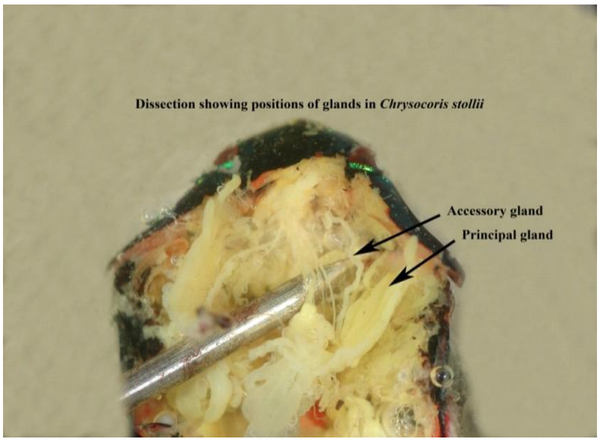

Figure 1. Dissection showing positions of the glands in *Chrysocoris stollii.*

Figure 2. Cross section of accessory salivary gland of *C. stollii* showing the numerous meronts of microsporidian.

DISCUSSION

The accessory glands are connected to the base of the principal glands by a pair of very fine long ducts (Chatterjee, 2014). Based on these observations, the probable routes of entry of the pathogen appear as below:

- Plant sap stylet, common duct of salivary gland, accessory salivary gland.
- Plant sap stylet, common duct of salivary gland, principal gland, duct of accessory salivary gland, accessory salivary gland.
- Plant sap stylet, oesophageal duct, fore gut, hemocoel, accessory salivary gland.

It is not certain if other organs were similarly affected by the pathogen or not. The presence of schizont in accessory salivary gland proves that the pathogen had entered as sporozoite and undergone metamorphic changes in some other organ. In that case, the concept of a direct entry (options I and II above) will be voided.

The cross sections of the accessory salivary gland provide clear evidence that the number of microscopically denser and mature schizonts per section of the gland is variable. The amount of damage varies according to the number of schizonts. The section shows the presence of the new entrants along with proportionally higher tissue damage. At the early stage of infection, the number of immatures are very less and this number increases with the maturity of schizonts. It is unclear if there is any relationship between initial infection and susceptibility in this insect.

One interesting fact is that there are 8-9 smaller pathogens near basal region of the acinus while two are near the luminal region of an acinus. The observations are very similar to that of Lipa (1977b), which indicate their probable entry through hemocoelic circulation. Thus they probably prefer to take up basal position of the acinus and matured schizont migrates to the luminal region of acinus. There was no indication of schizogony in the section. During the investigation none of the section shows the presence of young spores or mature spores or spores with fully extruded polar filament. The amount of tissue damage done by the scizonts indicates the species to be a very serious pathogen and may be treated as a good biological control agent. The morphology of the schizont suggests it may be a species under the genus *Nosema*. Moreover Lipa (1977a) provided detailed evidence on the occurrence of *Nosema* in the different organs of Heteroptera.

Becnel *et al.* (1999) have observed pathology of the epithelium of the gastric caecae, oenocytes, and the fat body after being infected by microsporidians, though they do not support the damage of salivary glands due to this pathogen. Tanada (1953) observed mid-gut epithelium and the Malpighian tubules to be the sites of maximum infestation by the pathogen in case of Cabbage worm, the salivary glands being the least. However, the observations from our experiments provide incidence of definite infection and tissue damage in case of salivary glands by microsporidians. The thick-walled and resistant infective spores during germination deliver its cytoplasm on the surface or into the cell of the host. They can either be phagocytozed by host cell or can escape out by the help of polar tube. Transmission of microsporidians in insects may be vertical ie. transovarial or horizontal ie. through ingestion of spores (Franzen, 2008). Lee *et al.* (2008) support the hypothesis that microsporidians are true fungi, although their exact nature and phylogeny remains obscure.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Zoological Survey of India and Dr. T. K. Mukherjee for laboratory facilities and identification respectively.

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