Journal of Research in Biology

An International Scientific Research Journal

ISSN No: Print: 2231 –6280; Online: 2231- 6299

**Short Communication**

# Histopathology of the gut of the silkworm, *Bombyx mori* Linn. infected with microsporidia

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**ABSTRACT:**

Ultrastructure of gut of the silkworm, *Bombyx mori* infected with microsporidia exhibited cytoplasmic vacuolization in the form of large empty spaces, fewer mitochondria, different spore stages (meronts and spronts) as grayish black spheres and mature spores. The meronts and sporonts measured 0.61 and 0.56 nm and 1.23 and 0.89 nm in length and width respectively. The lightly infected gut, did not show any vacuolization but in the heavily infected gut cell, cytoplasm destruction resulted in the formation of empty spaces.

**Keywords:**

*Bombyx* mori, Microsporidia, Mitochondria, Cytoplasm, Vacuolization.

**Article Citation:**
**Shabir A. Bhat, Ifat Bashir and Kamili AS.**
Histopathology of the gut of the silkworm, *Bombyx mori* Linn. infected with microsporidia
**Journal of Research in Biology (2019) 9(6): 2732-2735**

**Dates:**
**Received:** 18 Feb 2019 **Accepted:** 13 April 2019 **Published:** 21 Aug 2019





Journal of Research in Biology

## INTRODUCTION

A number of microsporidia have been reported as infecting the gut of the silkworm and then passes to other organs via gut wall (Kawarabata, 2003; Bhat, 2006). However, in most cases, reports have been dealt more with the development of the microsporidians with little emphasis on the host parasite interaction. Microsporidia (Msp) isolated from economically important insect silkworm, *Bombyx mori* (Kamilli *et al.,* 2009 and 2011), infects the cytoplasmic cells of the gut causing histopathological alterations. This research describes the ultra structural histopathological changes observed in the microspordian infected tissues of the silkworm.

## MATERIALS AND METHODS

The 5$^{th}$ instar infected silkworms were dissected out dorsally under dissecting binocular microscope (Magnus-MS-24) and the gut was examined under binocular microscope (Olympus-CX31) as fresh mount. For histopathological studies, a portion of gut was cut and placed in fresh 3% (v/v) glutaraldehyde, fixed overnight at 4$^{o}$C. The sample was post fixed in 1% osmium tetraoxide for 2 h, washed, dehydrated in a ascending series of alcohol, passed through propylene oxide and infiltrated with araldite and propylene oxide for 12 h. The samples were centrifuged and the sediment was infiltrated again with fresh araldite and kept at 60$^{o}$C for 48 h. Ultra thin sections were double

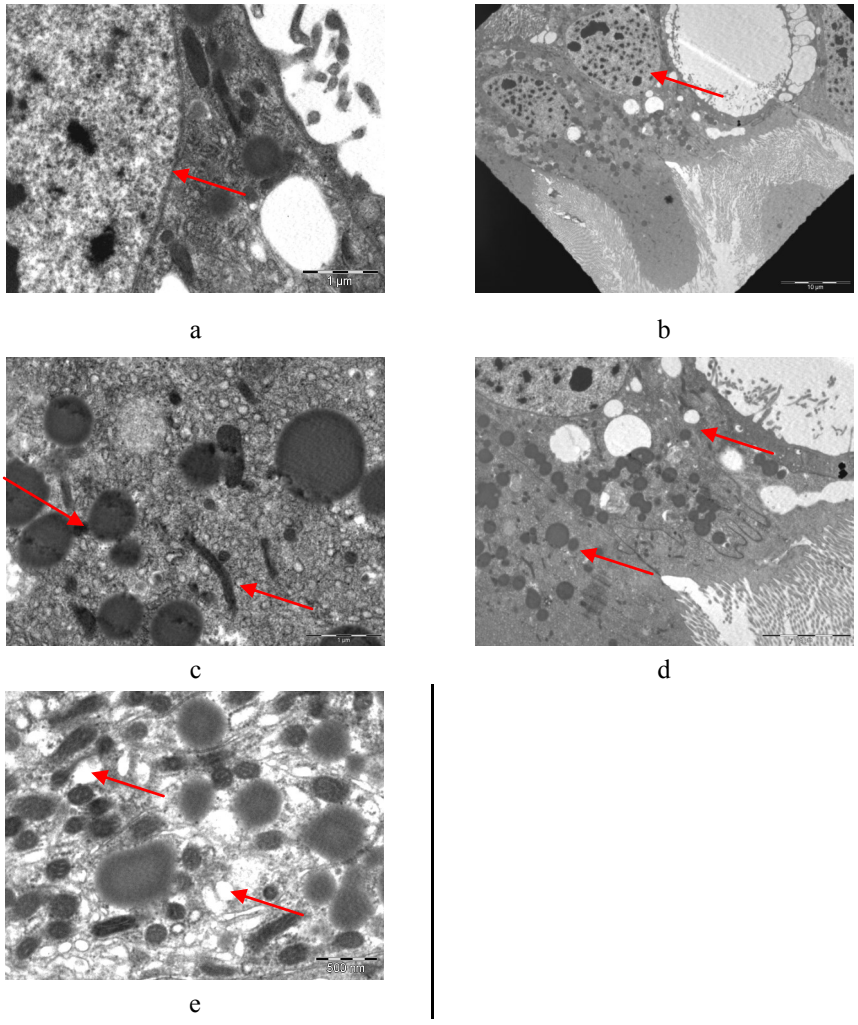


**Figure 1. Histopathology of infected gut of silkworm, a) arrows shows stage of infection b) multiplication of spores in cytoplasm c) arrows shows the rod shaped mitochondria d) meronts and sporots and e) cytoplasm disorganization**

**Table 1. Measurement of developmental stages of microsporidian, in the infected tissues of the mulberry silk worm**

| Developmental stages of the microsporidia | Size (nm ) | | |
|---|---|---|---|
| | Length | Width | Length-width ratio |
| Meront | 0.61 | 0.56 | 1.09:1 |
| Sporont | 1.23 | 0.89 | 1.37:1 |

stained with uranyl acetate and lead citrate and was observed and scanned under 60KVA (JEOL 100CX) electron microscope. The measurement of microsporidia with respect to the different developmental stages was calculated by following the standard formula. The observations were recorded.

## RESULTS AND DISCUSSION

The infected silkworms showed no external sign/ symptom. Hence, they had to be dissected in order to determine their internal changes. So the infection was diagnosed only from fresh smears of the gut. The ultra structural studies showed the presence of the microsporidia spores and its various developmental stages in the infected tissues. Early developmental stages were intact with the gut epithelium and showed degeneration of cell organelles (Figure 1 a and b). Meronts, sporonts and mature spores were observed in infected tissues. The meronts and sporonts measured 0.61, 0.56 nm and 1.23, 0.89 nm as length width respectively. The length-width ration measured 1.09:1, 1.37:1 in the meronts and sporonts respectively (Table 1). It was also observed that microsporidia multiplies in the cytoplasm of the gut cells but not in the nucleus, as the cytoplasm of the infected tissues was full of infection. Rod shaped mitochondria was also observed between the gut epithelium (Figure 1c). Cell degeneration due to large grayish spore developmental stages was obvious (Figure 1d). Sporoblast in the early stages of the differentiation were seen in the infected cytoplasm and an another interesting observation recorded was that the infected cytoplasm showed different developmental stages of the pathogen (Figure 1e). Heavy infection load was observed in the form of different developmental spore stages and mature spores which resulted in marked cell disorganization, empty spaces (without any cell organelle around the microsporidia).

$$\text{Size of structure} = \frac{\text{Magnification of threading}}{\text{Photo magnification}} \times \text{Scale unit selected } (\mu m)$$

The studies conducted on the development of microsporidian infection in silkworm showed chronic effect as the infected silkworm showed normal growth up to spinning and did not exhibit any visible sign of infection externally. Contrary to this study, Baig (1994) made observations on the silkworm infected with *Nosema bombycis* and found that it showed sluggish movement, tiny size, loss of appetite, vomit of gut juice and diarrhea with also pepper like spots on the body of the silkworms. The ultra structural studies of the gut revealed that although the cytoplasmic organelles of lightly infected cells are relatively organized, however, marked disorganization occurred in the heavily infected cell cytoplasm.

The microsporidia usually occur in the cytoplasm but there are few reports of their occasional occurrence in the cell nucleus such as *Nosema apis* in honey bees, *Choristoneura fumiferana* in the spruce and *N. bombycis* in the silkworm (Tanada and Kaya, 1993; Ananthalakshmi *et al*., 1994). It is in conformity with the results of Tanada and Kaya, (1993) that microsporidian (Msp) was also located in the cell cytoplasm. The present study revealed marked cytoplasmic destruction, reduction of cell organelles and occurrence of large empty spaces due to the microsporidia infection in the cell cytoplasm and it is concluded that cell organelle destruction was due to the accumulation of parasite within the tissues and not the consequence of any kind of active function performed by the pathogen. In a similar study Jurad *et al.* (1967)

reported that the microsporidia infection caused marked ultra structural changes in the cells of the salivary gland of the sciarid flies due to deletion, disorganization of cell organelles and vaculation of the cytoplasm. Jyothi *et al.* (2005) reported the large empty spaces around the microsporidia without any cell organelle and the developing stages of the microsporidia spores which are larger in size and occupy enormous spaces and once it reaches its maturity, it shrunk in size and the spaces remain as such or in other words lysis of the host cytoplasm takes place.

**ACKNOWLEDGEMENT**

The authors are thankful to transmission electron microscopy unit of National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, for providing the laboratory facilities to carry out this work.

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