



HISTOPATHOLOGY IN THE GUT OF SILKWORM, *Bombyx mori* Linn. INFECTED WITH MICROSPORIDIA

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A number of microsporidia have been reported as infecting the gut of silkworm and then passing to the other organs via gut wall (Kawarabatta, 2003; Bhat, 2006). However, in most cases such reports have been dealt more with the development of microsporidians with little emphasis on host parasite interaction. Microsporidia (Msp) isolated from economically important insect silkworm, *Bombyx mori* infects the cytoplasmic cells of gut causing histopathological alterations (Kamili *et al.*, 2009, 2010). This paper describes the ultrastructural histopathological changes observed in microspordian infected tissues of the silkworm.

The 5th instar infected silkworms were dissected out dorsally under dissecting binocular microscope (Magnus-MS-24) and the gut was examined under a binocular microscope (Olympus-CX31) as fresh mount. For histopathological studies, a portion of gut was cut, placed in fresh 3% glutaraldehyde (v/v) and fixed overnight at 4°C. The samples were post fixed in 1% osmium tetroxide for 2 h, washed, dehydrated in a ascending series with alcohol, passed through propyle oxide and infiltrated with araldite and propyl oxide for 12 h. The samples were centrifuged and sediments infiltrated again with fresh araldite embedded in araldite and kept at 60°C for 48 h. Ultra-thin sections were double stained with uranyl acetate and lead citrate observed and scanned under 60KVA (JEOL 100CX) electron microscope. The measurement of microsporidia with respect to different developmental stages was calculated by following the standard formula (Bhat and Nataraju, 2007). The observations were recorded.

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

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

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. Contrarily to the present study, observations on *Nosema bombycis*

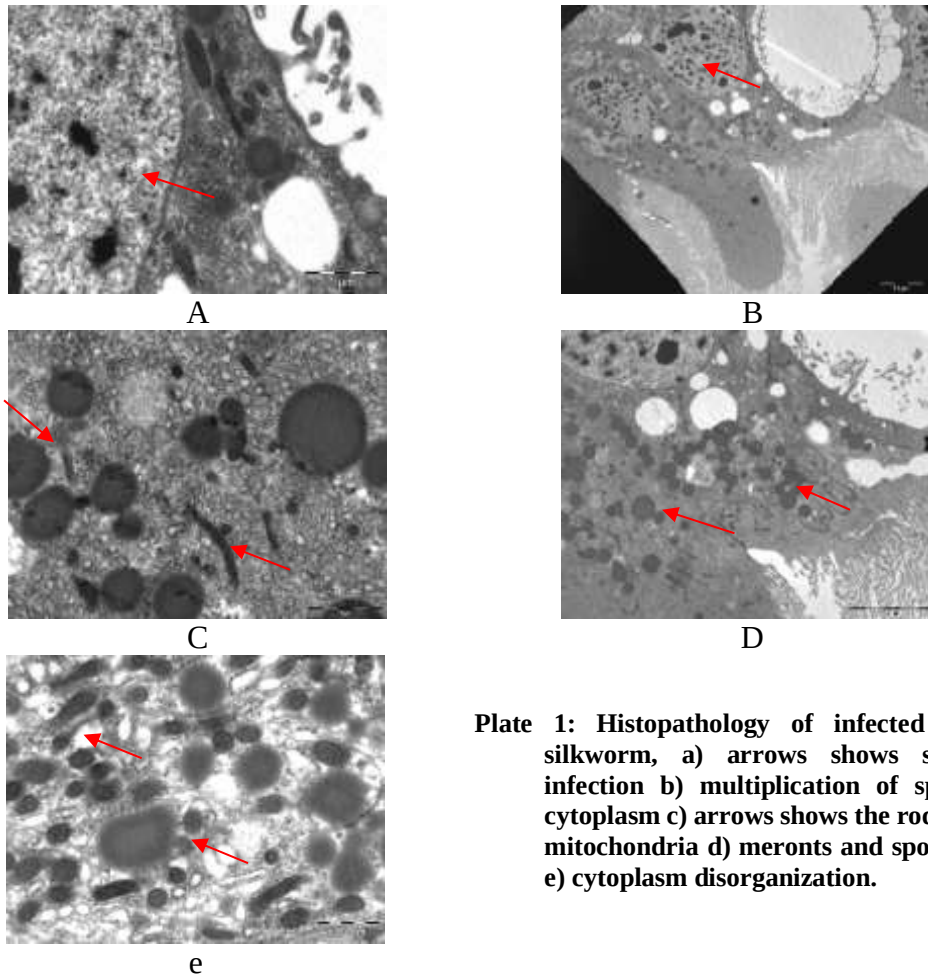


Plate 1: Histopathology of infected gut of silkworm, a) arrows shows stage of infection b) multiplication of spores in cytoplasm c) arrows shows the rod-shaped mitochondria d) meronts and sporonts and e) cytoplasm disorganization.

infected silkworm showed sluggish movement, tiny size, loss of appetite, vomit of gut juice and diarrhea also pepper like spots on the body of silkworms (Baig, 1994). The ultra-structure studies of gut revealed that although the cytoplasmic organelles of lightly infected cells were relatively organized; the marked disorganization occurred in heavily infected cell cytoplasm. The microsporidia usually occur in cytoplasm but there are a few reports of their occasional occurrence in cell nucleus such as *N. apis* in honey bees, *Chorestoneura fumifera* in spruce and *N. bombycis* in silkworm (Tanada and Kaya, 1993). This is in conformity with Tanada and Kaya (1993) that microsporidian (Msp) was also located in cell cytoplasm. The present study revealed marked cytoplasmic destruction, reduction of cell organelles and occurrence of large empty spaces due to the microsporidia infection. The 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 pathogen. Jyothi *et al.* (2005) reported large empty spaces around the microsporidia without any cell organelle, the developing stages of microsporidia spores which were larger in size and occupied enormous spaces and once it reaches maturity it shrunk in size and the spaces remained as such i.e. the lysis of host cytoplasm occurred.

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