Growth of Microsporidian Parasite in *Aedes albopictus*, Clone C6/36, Cell Cultures

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Abstract: A microsporidian parasite isolated from ovaries of prawns was found to infect *Aedes albopictus*, clone C6/36, cell cultures. Developmental stages of the parasite were observed by electron-microscopy. It is proposed that the mode of infection is by phagocytosis of viable spores, although infection after polar filament eversion may also be considered.

Key words: Microsporidian parasite, *Aedes albopictus* cell cultures

INTRODUCTION

Microsporidian infection in the Philippines has recently been reported and the developmental stages of the parasite have thoroughly been studied (Baticados and Enriquez 1982; Enriquez et al., 1982). Since microsporidians have currently been considered as potential biological control agents against insect pests, it appeared important to know their effects on nontarget organisms as well as their host ranges. It has been shown that several microsporidians can be grown in tissue culture systems (Kurtti and Brooks, 1976). Ishihara (1968) demonstrated the growth of *Nosema bombycis* at 28°C in primary cultures of rat and chicken embryos. Infection of pig kidney cell lines by *Nosema algae* was shown by Undeen (1975).

In this study, the development of microsporidians isolated from *Penaeus monodon* ovaries in *Ae. albopictus*, clone C6/36, cell cultures were investigated by electron microscopy in order to observe *in vitro* development of the microsporidian parasite.

MATERIALS AND METHODS

Microsporidian spores: Specimens isolated from *Penaeus monodon* ovaries were supplied by Dr. Gloria Enriquez, Department of Zoology, University of Philippines. The spores were

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further purified using the triangulation method of Cole as modified by Fowler and Reeves (1974). Spore concentration was adjusted to 10^7-10^8 spores/ml. The spores were primed by incubating them in 0.1 N KOH for 40 min at 37°C.

Ae. albopictus, clone C 6/36 cells: The origin and cultivation of the cells were as described before (Igarashi, 1978).

Infection of cultured cells with microsporidians: Two-tenth volume of the spore suspension was inoculated to *Ae. albopictus*, clone C 6/36, cells suspended in the growth medium of 10% fetal calf serum in Eagle's medium with nonessential amino acids, at the cell concentration of 5×10^5 cells/ml. Then, the cell-spore suspension was distributed in 60 mm Petri dishes (5ml/dish) to make replicate cultures, which were incubated at 28°C in humidified 5% CO_2-atmosphere. Cells were harvested 2, 3, 4, and 5 days after the infection.

Electron microscopic observation: The cells in monolayers were rinsed 3 times with phosphate buffered saline, pH 7.2, and were scraped with a rubber policeman and collected by centrifugation. The cells were fixed by 2% phosphate-buffered glutaraldehyde for 45 min, post-fixed by 1% osmium tetroxide for 1 hour, thoroughly washed and dehydrated by graded series of ethanol. The specimens were then transferred to propyrene oxide, infiltrated and embedded in Araldite resin. Polymerization was done at 60°C for 24 hours. Blocks were sectioned using glass-knives on Ultra-cut E Reichert Ultramicrotome. Specimens were stained with uranyl acetate for 40 min, counter-stained with lead citrate for 10 min, and examined under a JEOL 100CXII electron microscope.

RESULTS

Different stages of the development of the microsporidian spores were observed in the specimens harvested 2-5 days post infection. However, there were not much difference among the specimens according to the days after infection. Therefore, the descriptions on the specimens were limited to 4-5 days after the infection.

Structures of the developmental forms (sporoplasms) were observed as round to oblong or ovoid shape. They had a large nucleus with homogeneous nuclear matrix. The sporoplasm appeared to be surrounded by a single limiting membrane (Fig. 1).

Proliferative forms (Schizonts) were observed as longer and larger forms than sporoplasms and possessed more than a single nucleus. The nuclear matrix appears to be homogeneous, however, more electron-dense than those of sporoplasms and is surrounded by a double membrane. Vesicular structures were observed to be located in a polar position (Fig. 2).

Sporonts in early stages were oblong to elongated form and were seen to be dividing to produce 2 sporoblasts, each containing a single nucleus. As they mature, their walls became thicker and more electron-dense (Fig. 3).

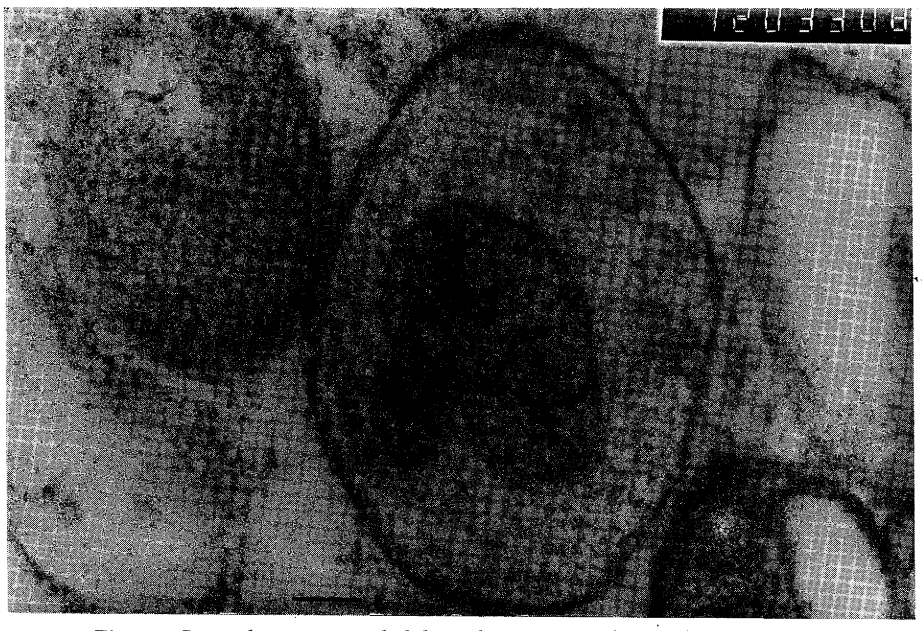

Fig. 1. Sporoplasm surrounded by a limiting membrane, having a large nucleus which appears to be rather homogeneous.
Bar: 100 nm

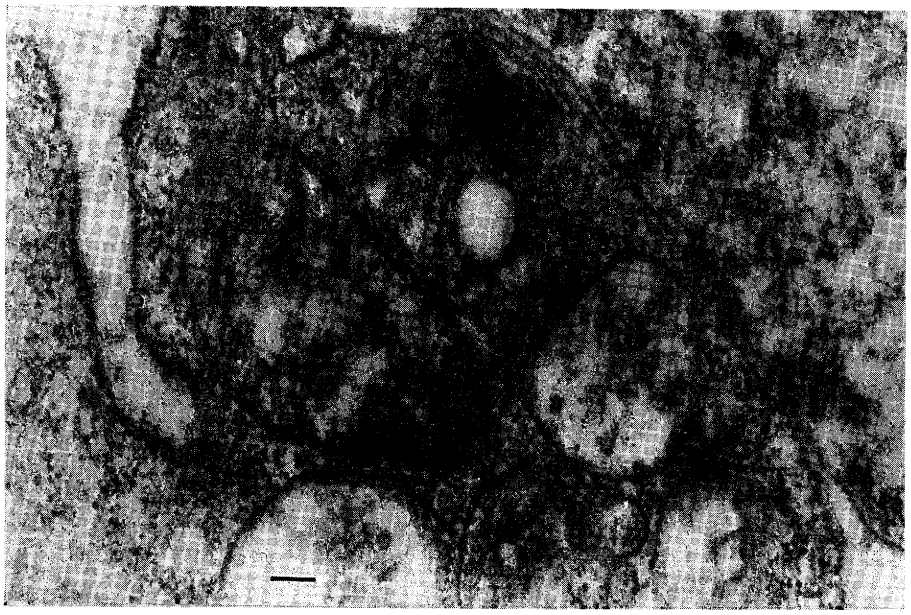

Fig. 2. Proliferative from or schizont showing 2 nucleus having a densely stained nuclear matrix. Vesicles are also observed on the end of the elongated schizont. Bar: 100 nm.

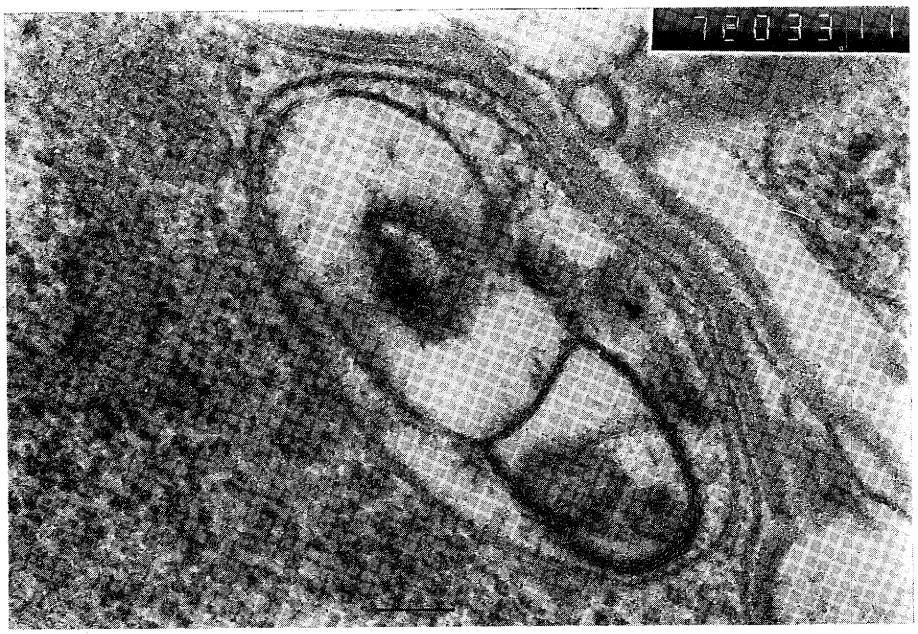

Fig. 3. A sporont which appears to be dividing to from 2 sporoblasts. Bar: 100 nm.

Fig. 4. A young sporoblast showing development of tubular structures probably endoplasmic reticulum. Peripheral membrane appears to be electron-dense. Bar: 100 nm.

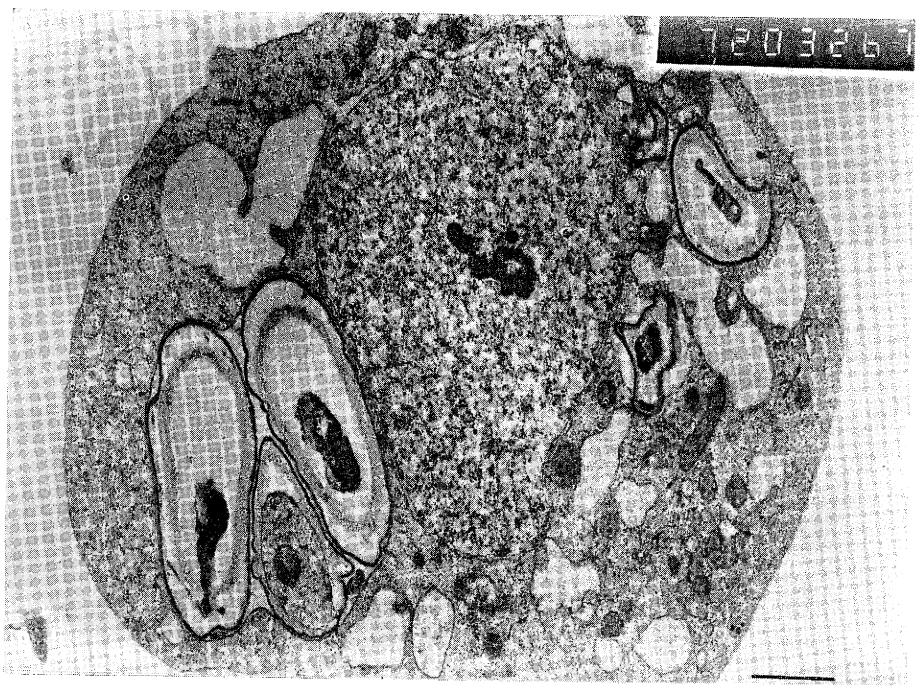

Fig. 5. An *Ae. albopictus*, C6/36, cell showing a more or less centrally located nucleus and mature spores embedded in the cytoplasm. Three spores appear to be enclosed by a very thin membrane, probably pansporoblast membrane. Bar: 1 μm.

Sporoblasts were distinguished by a more electron-dense limiting membrane. As maturation continued, the membrane broadened and resulted in the typical three-layered wall of the mature spore. In this stage of the parasite development, smooth membranous structures suggesting the development of endoplasmic reticulums were observed for the first time (Fig. 4).

Mature spores were observed in the cytoplasm of infected cells surrounded by a limiting membrane. The thick spore wall, composed of 3-layeres, was smooth or undulated and irregular. However, no characteristic spore organelle was observed (Fig. 5).

DISCUSSION

The results indicated that certain microsporidians can be induced to infect and grow in cells derived from organisms other than their natural hosts. Spores contained in phagosomes were observed in the cytoplasm of C6/36 cells, suggesting that the phagocytotic mechanisms for spores to enter into the cells. It is possible that the walls of the intracellular spores are digested by lysosomal enzymes that lead to the release of infectious sporoplasms. The possibility that everted polar filaments penetrated the host cell

membrane and deposit sporoplasms may also be considered. However, this mode of infection was not directly demonstrated in this study.

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ヒトスジシマカ培養細胞クローン C6/36 におけるミクロスポリジアの発育

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エビの卵巣から分離されたミクロスポリジア寄生体がヒトスジシマカ培養細胞クローン C6/36内で発育する事が電子顕微鏡による形態学的観察から認められた．蚊細胞へのミクロスポリジアの感染過程は喰食によると思われるが，ミクロスポリアジアのポラールフィラメントが発芽した后に感染が成立する可能性も除外できない．

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