Light and Electron-Microscopical Observations of the Early Developmental Stages of *Theileria sergenti* in the Gut of *Haemaphysalis longicornis*

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ABSTRACT. Morphological characteristics of the early developmental stages of *Theileria sergenti* in the vector tick were observed by light and electron-microscopies and compared with those of other *Theileria* species. Just before the repletion of nymphs, various stages of the parasite were observed in gut contents of tick by light microscopy: the spherical intra-erythrocytic stage, the ring-form stage, the spindle-shaped stage and the round-form stage. Electron microscopy revealed that the spherical intra-erythrocytic stages were 1–2 μm in diameter and were marginally located in host erythrocytes. The organisms liberated from host erythrocytes into the tick gut lumen had a large nucleus, an endoplasmic reticulum, electron dense-organelles and a double-walled, mitochondrion-like organelle. In addition ticks had ray-bodies in the gut lumen. These bodies had short protrusions containing 1–3 microtubules and a labyrinthine structure containing highly electron-dense organelles, so they were considered as the developing microgamont of *T. sergenti*.—**KEY WORDS:** light and electron-microscopy, *Theileria sergenti*, tick.

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The developmental stages of *Theileria* in the gut of vector tick have been investigated by several authors [2, 3, 5, 7, 9, 10]. The developmental stages of *T. sergenti* in particular in the vector tick, *Haemaphysalis longicornis*, have also been described by some workers [1, 8], however the fine-structure of the early developmental stages has not been fully shown. The present study, by light and electron microscopies, illustrated some morphological characteristics of the early developmental stages of *T. sergenti* in the vector tick, and made a comparison with those of other *Theileria* species.

The strain of *H. longicornis* used in the experiment was originally obtained from pastures in Southern Hokkaido, Japan. The vector ticks have since been maintained by feeding on rabbits for several generations in our laboratory. The unfed and the engorged ticks were kept in plastic tubes plugged with cloth at 25°C and over 90% relative humidity. Chitose strain of *T. sergenti* used in this experiment was isolated by the authors from naturally infected cattle in Hokkaido. It has been maintained in our laboratory by passage through splenectomized cattle, in which no protozoans other than *T. sergenti* were detected in the peripheral blood by blood examination. A splenectomized calf (Holstein Friesian) kept in the laboratory under tick-free conditions was inoculated intravenously with parasitized erythrocytes ($5 \times 10^7/\text{kg}$, body weight). Parasitemia of the infected calf reached approximately 20% when nymphs were confined in an ear bag. After about 6 days, just before repletion, the nymphs were removed, and individually dissected in PBS (pH 7.4) on a glass slide under a stereoscopic microscope. The nymphs were cut at the posterior end, and the gut was pulled out of the body by a needle. The specimens for light microscopy were squeezed out of the gut and stained with Giemsa. For electron microscopy, the whole gut was fixed at 4°C for 2 hours in 2.5% glutaraldehyde (v/v), buffered

with 0.1 M phosphate at pH 7.4. The specimens were refixed in 1% (w/v) osmic acid for 1 hour. The fixed specimens were dehydrated in an ascending series of alcohol followed by propylene oxide, and embedded in Quetole 812. The ultrathin sections were stained with uranyl acetate and subsequently lead citrate. The prepared specimens were examined by a JEM-100S transmission electron microscope.

Just before repletion of tick, various stages of parasite were observed in gut contents by light microscopy: the spherical intra-erythrocytic stage, the ring-form stage of 1–2 μm in diameter (Fig. 1-A), the spindle-shaped stage of 4–6 μm in length (Fig. 1-B), and the round-form stage of 2–3 μm in diameter. The spindle-shaped stage was observed in very few numbers in the tick gut lumen. A previous report has described that the developmental stages of *T. sergenti* in the midgut of the vector tick as to be similar to those of *T. parva*, *T. annulata* and *T. taurotragi* [1], and that the spindle-shaped microgamont and the round-formed macrogamont are developed from the ring-form stage within 2–3 days post-repletion. In the present study, the morphological findings by light microscopical observation resembled those in the previous report, however, micro- and macrogamonts were seen within the nymphal gut lumen just before repletion.

Fig. 1. Light micrographs of the developmental stages in the tick gut lumen. A; ring-form stage. B; spindle-shaped stage. $\times 2,000$.

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Electron microscopy revealed that the spherical intra-erythrocytic stages were 1–2 μm in diameter and located in the marginal region of host erythrocytes (Fig. 2), and the nucleus was observed in the peripheral region. A veil-like structure was often observed in the cytoplasm of infected erythrocytes (Fig. 3).

The spherical intra-erythrocytic stages were released into the tick gut lumen following destruction of the host cell membrane (Fig. 3). The liberated organisms were spherical or oval in shape and measured about $1.1 \times 1.4 \mu\text{m}$ (Fig. 4). This stage was surrounded by a unit membrane and had a relatively large nucleus in the peripheral region, and the endoplasmic reticulum, electron dense-organelles and a double-walled structure in the cytoplasm. The possible function of the double-walled structure was discussed in other *Theileria* spp. [4, 5] and it was speculated that this organelle corresponds to a mitochondrion or a mitochondrion-like organelle.

In the lumen also were seen the ray-bodies, approximately 1–2 μm in diameter, and with a short protrusion and a labyrinthine structure containing highly electron-dense organelles (Fig. 5). The protrusion contained one to three microtubules, 50 nm in diameter, which appeared to derive from bundles in the cytoplasm and extended through the entire length of the protrusion (Fig. 5). Mehlhorn and Schein [4] reported that the microgamont of *T. parva* is bounded by a unit membrane and has a stileto-like apex or thorn, a labyrinthine structure found near the base of the stileto-like structure, several flagella-like protrusions and a slender posterior pole with some

microtubules. The labyrinthine structure was also recognized in the microgamont of *T. annulata* in the tick gut stage [5]. In this study, the labyrinthine structure and the

Fig. 3. Electron micrograph of the organism liberated into the tick gut lumen. The spherical organism is released into the lumen following destruction of the host cell membrane. Bar = 0.5 μm . $\times 28,000$.

Fig. 2. Electron micrograph of the spherical intra-erythrocytic stage in the tick gut lumen. Bar = 0.5 μm . $\times 20,000$.

Fig. 4. Electron micrograph of the organism liberated in the tick gut lumen. Bar = 0.5 μm . $\times 54,000$.

Fig. 5. Electron micrograph of the ray-body in the tick gut lumen. The short protrusion contains a microtubule and a labyrinthine structure. Bar = 0.5 μ m. $\times$ 42,000.

ER = Erythrocyte
 er = Endoplasmic reticulum
 UM = Unit membrane
 DO = Dense organelle
 DW = Double walled structure
 LS = Labyrinthine structure
 MT = Microtubule
 N = Nucleus
 P = Protrusion
 VS = Veil-like structure

short protrusion with microtubules were seen only in the ray-body. Therefore, this stage was considered as the developing microgamont of *T. sergenti*.

In the present study, microgamonts were observed by light and electron-microscopies within the nymphal gut lumen just before repletion. Thus, it is suggested that the sexual stages of *T. sergenti* are produced during the feeding process of vector ticks.

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