Development of *Theileria sergenti* Schizonts in the Lymph Node of Experimentally Infected Cattle

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**ABSTRACT.** Schizogony of Japanese *Theileria sergenti* of cattle was studied by light and electron microscopy. Schizonts were detected in the draining lymph node between 4 and 8 days after sporozoite inoculation. Macroschizonts (the phase of nuclear division having invaginations) were formed 6 days after inoculation. Subsequently, microschizonts (the phase of merozoite formation displaying rosette-like appearance) were observed 8 days after inoculation. Multiple infections of a host cell with sporozoites were suggested to occur since different stages of schizonts were simultaneously detected in the same cell. Host cells of schizonts were considerably enlarged by parasitism. However, morphological characteristics of the developmental stages of *T. sergenti* schizonts resembled those of malignant *Theileria* species (e.g. *T. parva*). Schizogony of *T. sergenti* observed in this study seems to be the primary generation.—**KEY WORDS:** macroschizont, merozoite, microschizont, schizogony, *Theileria sergenti*.


*Theileria sergenti* is the causative agent of the benign bovine theileriosis in Japan. Schizonts of *T. sergenti* were detected in the cytoplasm of large cells formed in the lymph nodes, liver and spleen 8 to 10 days after tick infestation [14]. The schizonts were located inside the cytoplasm of large (50–200 μm in diameter) host cells, which might be stimulated to be enlarged by infection with *T. sergenti*. The growth pattern of *T. sergenti* was completely different from those of malignant *Theileria* species, i.e. *T. parva* [10] and *T. annulata* [19]. The schizonts of the malignant *Theileria* species stimulated host cells to divide into daughter cells but never caused them to be enlarged to such extent [6]. However, developmental stages of schizonts of *T. sergenti* have not yet been clarified. Schizonts of other benign *Theileria* species, *T. buffeli* [17] and *T. orientalis* [20], possess similar structures to those of *T. sergenti* [12]. Recent comparative studies on these three benign *Theileria* species [3, 7, 18] suggested that *T. sergenti* might be a new species and that *T. buffeli* and *T. orientalis* might be the same species [4, 5].

The present study describes the development of *T. sergenti* schizonts in the lymph nodes of experimentally infected calves.

**MATERIALS AND METHODS**

Four male Holstein calves used in the present experiments were confirmed not to have antibodies against the antigens of *T. sergenti* piroplasm by the indirect fluorescent test before use. All calves were infected with *T. sergenti* (Ikeda stock [2]) by subcutaneous injection of a tick-derived sporozoite suspension at the base of the left ear. The calves were necropsied 2, 4, 6 and 8 days after sporozoite inoculation. For light microscopy, the tissues were fixed in 10% phosphate buffered formalin, dehydrated in a series of alcohol, embedded in paraffin, sectioned and stained with Giemsa. For electron microscopy, blocks from parotid lymph nodes, draining the site of inoculation, were prefixed with a solution (2% glutaraldehyde, 2% paraformaldehyde), fixed with 2% osmium tetroxide, stained with 2% uranyl acetate, dehydrated and embedded in TAAB Epon 812 mixture. Ultrathin sections were stained with uranyl acetate and lead citrate prior to examination with a JEOL 1200 EX electron microscope.

**RESULTS**

Table 1 summarizes the morphological characteristics of the schizonts and their host cells observed in the left parotid lymph nodes. Schizonts were detected from PID (post inoculation days) 4. Spherical young schizonts of about 2 μm in diameter were observed in the enlarged host cell on PID 4 (Fig. 1). The young schizonts which were irregular in shape (about 5 μm in length) and of homogeneous low electron density were detected in the enlarged host cell of about 40 μm in diameter (Figs. 2, 3). A unit membrane was detected in the periphery of the parasite (Fig. 4). The nucleus of host cells was usually invaginated and scattered with a large quantity of chromatin of a length of about 30–50 μm (Fig. 2). Cytoplasmic organelles consisted of numerous mitochondria, a moderate amount of rough endoplasmic reticulum and clusters of free ribosomes. Macroschizonts (the phase of nuclear divisions) were observed in the cytoplasm of huge host cells from PID 6. They were irregular in shape (Fig. 5) and the annulate lamellae [13] were observed around the parasite (Fig. 6). Micropores were observed on the limiting membrane of schizont (Fig. 7). In macroschizonts, the chromatin of the nucleus was increased in quantity, and the nuclei were developed in the following phase. Macroschizonts appeared wrinkled with deep invaginations (Fig. 8) and had mitochondria and vacuoles...
in the cytoplasm. Host cells became huge, about 105 μm × 80 μm, and about 200 μm × 175 μm on PID 6 and 8, respectively. Microschizonts (the phase of merozoite formation) were observed to be basophilic regular-shaped granules by light microscopy on PID 8 (Fig. 9). At this phase, merozoites were budding from the schizonts, therefore the microschizonts appeared rosette-like (Fig. 11). Merozoite-anlagen were also observed in a microschizont. A residual body was irregular-shaped and about 1.5–2.0 μm in size. The nucleus was not detected in some
Fig. 2a. Electron micrograph of schizonts (arrows) in a host cell on PID 4. The nucleus (N) of the host cell is invaginated in the periphery and has much chromatin. Note the increased mitochondria (m) inside the cytoplasm of host cell. Bar=5 μm.

Fig. 2b. Diagrammatic representation of the enlarged host cell shown in Fig. 2a. Arrow head indicates the nucleus of schizont (S). N: Nucleus of the host cell.

Fig. 3. Light micrograph of the enlarged host cell in the left parotid lymph node on PID 4. Giemsa staining. × 100.
Fig. 4. High magnification of Fig. 2a. A schizont (S) in the cytoplasm of host cell on PID 4. A unit membrane (arrows) surrounds the schizont's cytoplasm which is homogeneous and of low electron density. Nuclei (n) are detected in the schizont. N: Nucleus of the host cell. Bar=2 μm.

Fig. 5. Electron micrograph of macroschizonts observed on PID 6. Several nuclei (n) are observed in the cytoplasm of schizonts. N: Nucleus of the host cell. Bar=3 μm.

Fig. 6. Annulate lamellae (arrow) around a schizont (S). Bar=600 nm.

Fig. 7. Longitudinal section through a micropore (arrow) on the limiting membrane of a schizont (S). Bar=200 nm.

Fig. 8. Electron micrograph of a macroschizont in nuclear division before cytoplasmic division. Note the spotted chromatin of nuclei (n) is clearly visible. Micronemes (arrows) are observed in the cytoplasm. N: Nucleus of the host cell. Bar=2 μm.
Fig. 9. Light micrograph of macroschizonts (Ms) and microschizonts (ms) observed in the same host cell. Arrow indicates the residual body. Giemsa staining. × 1,000.

Fig. 10. Electron micrograph of macroschizonts (Ms) and microschizonts (ms) observed in the same host cell on PID 8. Bar=2 μm.

Fig. 11. Electron micrograph of microschizonts during the phase of merozoite (m) formation. Developing merozoites (merozoite-anlagen, arrows) are observed in the periphery of the residual body (R), giving the rosette-like aspect. A merozoite contains mitochondria (arrow heads) and micronemes (mn). Bar=1 μm.
of the residual bodies. Merozoites were round, 0.75 μm in diameter and their round nucleus became clearly visible, 0.45 μm in diameter and relatively homogeneous karyoplasm. Mitochondria and micronemes were observed but no rhoptries were detected in merozoites. Two developmental stages of schizonts, i.e., macroschizonts and microschizonts, could be observed in one host cell (Figs. 9, 10). Most of the organelles of the huge host cells were degenerative. One merozoite was detected in an erythrocyte in the blood vessel on PID 8.

**DISCUSSION**

Figure 12 is the schematic presentation of the developmental course of the schizont of *T. sergenti* proposed by this study. Some schizonts were spherical in shape and others were irregular-shaped, had homogeneous low electron density, and were located in the cytoplasm of enlarged host cells on PID 4. They had the nuclei with scarce chromatin. Macroschizonts (the phase of nuclear division) were detected on PID 6 and 8. They were

![Diagram of schizonts and merozoites](image)

**Table 2. Characteristics of schizont of *Theileria sergenti* compared to those of malignant *Theileria* species**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Characteristics</th>
<th>Theileria sergenti</th>
<th>Malignant&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Theileria</th>
</tr>
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<tbody>
<tr>
<td>Host cell</td>
<td>Origin</td>
<td></td>
<td>Lymphocyte (Monocyte)</td>
<td></td>
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<tr>
<td></td>
<td>Transformation</td>
<td>–</td>
<td>+</td>
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<td></td>
<td>Enlargement</td>
<td>+</td>
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<td></td>
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<tr>
<td></td>
<td>Annulate lamellae</td>
<td>+</td>
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<tr>
<td></td>
<td>Size</td>
<td>250 μm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizonts</td>
<td>Shape</td>
<td>Irregular</td>
<td>Irregular</td>
<td></td>
</tr>
<tr>
<td>Macroschizont</td>
<td>Limiting membrane</td>
<td>Unit</td>
<td>Unit</td>
<td></td>
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<tr>
<td></td>
<td>Nucleus</td>
<td>Irregular</td>
<td>Irregular</td>
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<tr>
<td></td>
<td>Mitochondria</td>
<td>+</td>
<td>+</td>
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<td></td>
<td>Rhoptries</td>
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<tr>
<td></td>
<td>Vacuole</td>
<td>+</td>
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<td></td>
<td>Micropore</td>
<td>+</td>
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<td>Size</td>
<td>5 μm</td>
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<td>&lt;5 μm&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Microschizont</td>
<td>Two merozoites</td>
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<td></td>
<td>formation (Rosette-like)</td>
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<td>Several merozoites</td>
<td>formation</td>
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<td></td>
<td>Limiting membrane</td>
<td>Unit</td>
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<td></td>
<td>Mitochondria</td>
<td>+</td>
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<td>Rhoptries</td>
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<tr>
<td></td>
<td>Vacuole</td>
<td>+</td>
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<tr>
<td></td>
<td>Size</td>
<td>0.75 μm</td>
<td>1×0.6 μm</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Data are from Schein *et al.* [15].  
<sup>b</sup> Unknown.  
<sup>c</sup> *T. parva*, *T. annulata* and *T. mutans*.  
<sup>d</sup> *T. lawrencei*.  

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multinuclear and marked invaginations were detected after the nuclear chromatin became clearly visible. Microschizonts (the phase of merozoite formation) were observed on PID 8. Host cells became huge after schizonts were developed, and merozoites were released from host cells and penetrated into the erythrocytes. The primary generation of schizogony of T. sergenti might be achieved in this manner.

Table 2 shows the morphological characteristics of the schizonts of T. sergenti compared to those of other malignant Theileria species such as T. parva, T. lawrencei, T. annulata and T. mutans [15]. Each developmental stage of schizogony, i.e. macroschizont, microschizont and merozoite was detected in T. sergenti as well as the malignant Theileria species [11, 15]. In T. sergenti, host cells were not divided but were enlarged after schizonts were developed. Subsequently the huge host cells were filled with macroschizonts with deep invaginations. Therefore it is difficult to measure the exact size of macroschizonts at this stage of development [15]. Schizonts with deep invaginations are observed in the case of multiple infections of host cells with other malignant Theileria species [15]. In the present study, however, invaginations were often detected in T. sergenti schizonts after nuclear division. Microschizonts of T. sergenti measured up to about 5 μm during merozoite formation, and were the same in size as of T. lawrencei [15]. Schein et al. [15] have reported that two types of merozoite formation were observed in the schizogony of T. parva and T. annulata, i.e. 1) two merozoites formation with rosette-like appearance, 2) more than two merozoites arising from one nucleus of schizont. In this study, only the type 1), merozoite formation displaying rosette-like appearance, was observed. Since concurrent infection with different stages of T. sergenti schizont, the macroschizonts and the microschizonts, was occasionally detected in one host cell, more than two sporozoites might infect one host cell at the same time.

Schizonts of T. sergenti observed by light microscopy resembled those of Cytaxozoon taurorragi reported in an eland [1], however, no multinuclear host cells nor the parasites within the blood vessels of lung were observed in schizogony of T. sergenti, although the cells and parasites were detected in that of C. taurorragi. After Levine [8] described the genus Cytaxozoon as a synonym of Theileria, Stagg et al. [16] cultivated Theileria (Cytaxozoon) taurorragi in macrophages which were stimulated to be enlarged by parasitism. Fujisaki [4] and Fujisaki et al. [5] proposed the necessity of the review of the genus name for the benign Theileria species after the morphological studies of their schizogony.

It is well-known that schizogony of protozoa in Phylum Apicomplexa generally occur pluraly, but there is no finding on this phenomenon in Theileria species. However, parasitaemia by T. sergenti and other Theileria species continues several months, and this might be caused by the successive schizogony, namely, the primary generation of schizogony, the secondary generation of schizogony, and so on. It is, therefore, suspected that the schizogony observed in this study is the primary generation. Further study should be made to clarify if the secondary generation of schizogony would take place in T. sergenti.

REFERENCES


