Theileria sergenti*: Transformation of Zygotes into Kinetes in Vector Ticks, *Haemaphysalis (Kaiseriana) longicornis* and *H. (K.) mageshimaensis

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ABSTRACT. Invagination or transformation of a zygote of *Theileria sergenti* into a kinete was examined with Giemsa-stained smears of the midgut of vector ticks, *Haemaphysalis longicornis* and *H. mageshimaensis*. Zygotes invaginated almost simultaneously with the moulting of host ticks. They were round and oval, and measured 5.4 to 6.7 μm in diameter on average. The kinetes developing in the zygotes were situated in the periphery of cytoplasm and were spindle- or club-shaped.—**KEY WORDS:** kinete, *Theileria sergenti*, zygote.

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In Japan, *Haemaphysalis (Kaiseriana) longicornis* [13], *H. (K.) cornigera* [12], and *H. (K.) mageshimaensis* [8] are known as biological vectors of *Theileria sergenti*, a causative agent of bovine piroplasmosis and a member of the *Theileria sergenti/buffeli/orientalis* group [3]. The development of *T. sergenti* in the midgut [6, 10], hemolymph [11], and salivary gland [4, 5, 9, 15] of vector ticks has already been reported. However, *T. sergenti* zygote invaginating or transforming into the kinete in vector ticks has not been studied. We have investigated the morphological development of *T. sergenti* in the midgut of nymphs of *H. longicornis* and *H. mageshimaensis* to clarify the zygote-kinete transformation.

Nymphs of a parthenogenetic Okayama race of *H. longicornis* [7] were fed on two calves in ear bags [14] which were subcutaneously inoculated with tick-derived sporozoite suspension [15] of Ikeda stock of *T. sergenti* [4] 20 or 30 days after splenectomy. Sixty or 50 engorged nymphs each were collected from the calves with parasitaemia of 48 and 34% (24 and 39 days after infection), respectively. Nymphs of a bisexual Geruma race of *H. mageshimaensis* [8] were also fed on a calf infected with Ikeda stock of *T. sergenti* [4] by the same method as in the above 30 days after splenectomy. Thirty engorged nymphal ticks were collected from the calf with parasitaemia of 11% (25 days after infection). Two to 10 and 1 to 2 engorged *H. longicornis* and *H. mageshimaensis* respectively were examined daily during their nymphal stage from 3 to 19 days after engorgement and after then they moulted to develop into adults in an incubator under the controlled conditions (25°C, 100% RH and continuous darkness). The midguts were dissected out in PBS (0.02 M phosphate buffer and 0.15 M sodium chloride, pH 7.2) under a microscope and diverticula 6 and 7 of midguts were smeared on slides [6]. Some smears were examined by Giemsa staining and others by direct fluorescent antibody staining (DFA) [4] or direct immunoperoxidase staining (DIP) [16].

H. longicornis and *H. mageshimaensis* moulted 11 and 12 days on average after engorgement, respectively (Table 1). In Giemsa-stained gut smears of both tick species, round and oval forms, reddish-violet in colour were seen (Fig. 1). The cytoplasm was diffuse in some of these forms and compact in others. The nucleus was often situated in the periphery but did not always stain. These forms were

detected in the gut through the examination period and increased in size from 2.5 to 8.5 μm (in diameter) in the course of moulting of ticks. They were considered to be zygotes.

In *H. longicornis*, the partial invagination of zygote (Fig. 1), which led to the formation of kinete, was observed in the period of ecdysis of ticks from 9 to 19 days after engorgement. In *H. mageshimaensis*, invaginating zygotes were observed in the period of ecdysis of ticks from 8 to 14 days after engorgement. Such invaginated zygotes were round and oval, reddish-violet in colour, and were $(6.5 \pm 1.3) \times (5.5 \pm 1.0) \mu\text{m}$ (mean $\pm$ SD) ($n=13$) and $(6.7 \pm 1.6) \times (5.4 \pm 1.4) \mu\text{m}$ ($n=13$) in *H. longicornis* and in *H. mageshimaensis*, respectively. There was no significant difference in the size of zygotes between the two species of host tick. The spindle- or club-shaped structure situated in invaginated zygotes at the periphery of cytoplasm was considered the developing kinete and measured an average size of $(5.2 \pm 1.0) \times (1.5 \pm 0.5) \mu\text{m}$ ($n=7$). They were distinctly stained with DFA and DIP, however, both stains appeared not so useful for identification of the parasite, because many residual bodies from imbibed bovine erythrocytes and some symbiotic microorganisms in the smears were stained along with the parasites.

Higuchi *et al.* [11] reported on kinetes of *T. sergenti* with an average length of 10.0 μm in the hemolymph of *H. longicornis* fed and engorged on infected cattle in the nymphal stage from 25 to 42 days after engorgement, i.e. 7 to 14 days after ecdysis. They classified these kinetes into three types on the basis of their morphological characters of cytoplasm: spindle-shaped, club-like, and granular. The kinetes developing in zygotes detected in this study were similar in shape, but no granules were found in the cytoplasm. The kinetes in zygotes were approximately half the size of those in hemolymph [11]. This difference in size implies immaturity and continuous growth of the kinete within the zygote after it was liberated from the gut epithelium into the hemocoel.

These observations suggested that zygotes of *T. sergenti* might be transformed into kinetes by invagination (Fig. 2). It is well-known that haemosporidian zygotes generally develop into ookinetes by invagination on the cell surface [2, 17], however, the zygote of *Theileria* is transformed into the kinete by invagination of cell content *in situ* [17].

Fig. 1. Zygotes transforming to kinetes in *Haemaphysalis longicornis* 9 and 10 days after engorgement ($\times 2,500$). Big and small arrows show zygotes and kinetes, respectively. Solid triangle shows a nuclear structure. (a) and (b): Zygote invaginating on one side in ticks 9 days after engorgement. (c) and (d): Zygote developing into kinete at the periphery of cytoplasm; ticks 10 days after engorgement.

Fig. 2. Diagrams showing the zygote of *Theileria sergenti* transforming into a kinete by invagination. (a) Zygote with nuclear structure (N) in marginal area. (b) Zygote beginning to invaginate in marginal area. (c) Invaginating zygote. Partially developed kinete (K). (d) Fully developed kinete within a zygote.

The present study shows that *T. sergenti* is identical to other species of *Theileria* in invagination manner. Obviously, the zygotes of *T. sergenti* are transformed into the kinetes during the ecdysis of ticks. In this report *T. sergenti* is identical with *T. taurotragi* (= *Cytauxzoon taurotragi*) [22] where kinete formation occurs almost simultaneously with the moulting of host tick (Table 1).

The number of invaginated zygotes was very small in *T. sergenti* compared with those of other *Theileria* species [17–19, 20–22], irrespective of careful examination, and zygotes were one half to two third of the size of those of the other *Theileria* species (Table 1). In addition, the developing kinetes of *T. sergenti* were situated within zygote and occupied a very small peripheral portion of the

Table 1. A comparison of some characteristics of the transformation of zygotes into kinetes of *Theileria* species in ticks

Species of <i>Theileria</i>	<i>T. sergenti</i> ^{a)}	<i>T. sergenti</i> ^{a)}	<i>T. taurotragi</i> ^{b)}	<i>T. velifera</i> ^{c)}	<i>T. parva</i> ^{d)}	<i>T. annulata</i> ^{e)}	<i>T. mutans</i> ^{f)}
Vector tick species	<i>Haemaphysalis longicornis</i>	<i>Haemaphysalis mageshimaensis</i>	<i>Rhipicephalus appendiculatus</i>	<i>Amblyomma variegatum</i>	<i>Rhipicephalus appendiculatus</i>	<i>Hyalomma anatolicum</i>	<i>Amblyomma variegatum</i>
Stages	Nymph-Adult	Nymph-Adult	Nymph-Adult	Nymph-Adult	Nymph-Adult	Nymph-Adult	Nymph-Adult
Incubation temperature for ticks (°C)	25	25	28	28	28	28	28
Earliest transformation of zygote to kinete (days)	9	8	12	15	20	12	34
Mean size of invaginated zygote (μm)	6.5×5.5	6.7×5.4	12.2×9.0	9-14	9.0×7.0	10	11
Mean time from feeding to moulting of ticks (days)	11	12	14	26	19	21	26

a) Data from present study.

b-f) Data from Young *et al.* (1980), Warnecke *et al.* (1979), Schein *et al.* (1977), Schein (1975), and Warnecke *et al.* (1980), respectively.

cytoplasm, while that of other *Theileria* species occupies almost all the central portion of cytoplasm of zygote [17–19, 20–22]. Thus, the zygote of *T. sergenti* may possibly be different from other *Theileria* species in the maturation manner. Fujisaki [3] suggested that species of *T. sergenti/buffeli/orientalis* group could be discriminated from other *Theileria* species in the kind of host cell and manner of schizogony, and further that all the group might belong to another subgenus and/or genus. There are, therefore, still some obscurities in the taxonomy of the *T. sergenti/buffeli/orientalis* group species, so further studies are needed to clarify whether some disparities in the transformation of the zygote into kinete might be important criteria for the classification of *Theileria* at species and/or genus level.

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REFERENCES

- Coons, L. B., Rosest-Davis, R., and Tarnowski, B. I. 1986. pp. 248–279. In: Morphology, Physiology, and Behavioral Biology of Ticks (Sauer, J. R. and Hair, J. A. eds.), Ellis Horwood, New York.
- Fallis, A. M. and Bennett, G. F. 1961. *Can. J. Zool.* 39: 215–228.
- Fujisaki, K. 1992. *J. Protozool. Res.* 2: 87–96.
- Fujisaki, K., Ito, Y., Kamio, T., and Kitaoka, S. 1985. *Ann. Trop. Med. Parasitol.* 79: 519–524.
- Fujisaki, K. and Kamio, T. 1988. *Jpn. J. Vet. Sci.* 50: 529–536.
- Fujisaki, K., Kamio, T., and Kawazu, S. 1993. *Ann. Trop. Med. Parasitol.* 87: 95–97.
- Fujisaki, K., Kitaoka, S., and Morii, T. 1976. *Natl. Inst. Anim. Health Q. (Jpn.)* 16: 122–128.
- Fujisaki, K., Kamio, T., Kawazu, S., Minami, T., Nakamura, Y., Shimura, K., Shimizu, S., and Henna, M. 1988. *Ann. Trop. Med. Parasitol.* 82: 513–515.
- Higuchi, S. 1986. *Jpn. J. Vet. Sci.* 48: 801–807.
- Higuchi, S. 1987. *Jpn. J. Vet. Sci.* 49: 341–347.
- Higuchi, S., Kawamura, S., and Yasuda, Y. 1987. *Zentralbl. Bakteriologie. Mikrobiol. Hyg., A* 264: 521–526.
- Ishihara, T. 1971. *Bull. Natl. Inst. Anim. Health (Jpn.)* 62: 128–146 (in Japanese).
- Ishii, S. and Ishihara, T. 1951. *J. Jpn. Vet. Med. Assoc.* 4: 289–294 (in Japanese).
- Kamio, T., Fujisaki, K., and Minami, T. 1985. *Proc. Assoc. Acarol.* 14: 1–4.
- Kamio, T., Fujisaki, K., and Minami, T. 1989. *Ann. Trop. Med. Parasitol.* 83: 77–83.
- Kawazu, S., Sato, M., Kamio, T., Yokomizo, Y., Fujisaki, K., Honma, S., and Minami, T. 1991. *J. Vet. Med. Sci.* 53: 743–745.
- Mehlhorn, H. and Schein, E. 1984. *Adv. Parasitol.* 23: 37–103.
- Schein, E. 1975. *Z. Parasitenkd.* 47: 165–167.
- Schein, E., Warnecke, M., and Kirmse, P. 1977. *Parasitology* 75: 309–316.
- Warnecke, M., Schein, E., Voigt, W. P., and Uilenberg, G. 1979. *Tropenmed. Parasitol.* 30: 318–322.
- Warnecke, M., Schein, E., Voigt, W. P., Uilenberg, G., and Young, A. S. 1980. *Z. Parasitenkd.* 62: 119–125.
- Young, A. S., Grootenhuys, J. G., and Leitch, B. L. 1980. *Parasitology* 81: 129–144.