Development of *Babesia gibsoni* in the Gut Epithelium of the Tick, *Haemaphysalis longicornis*

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There are many reports on the development of *Babesia* species in the gut of vector ticks [1, 6, 9, 16, 17, 18]. However, no detailed observations on the developmental stages of *Babesia gibsoni* [11] in *Haemaphysalis longicornis* have been made yet. *Babesia* species are known to undergo morphological changes during the growth in the gut of ticks. This study was designed to examine the transformational stages of a zygote to a motile kinete inside the gut epithelial cells of vector ticks.

A parthenogenetic strain of ticks collected from a local cow had been maintained in the laboratory by feeding on two male New Zealand White rabbits which were 6 and 9 months of age, weighing 2.21 and 2.03 kg, respectively, and infected with *B. gibsoni* by feeding on an infected dog. The *B. gibsoni* strain used was isolated from spontaneously infected dogs at the Towada area of Aomori Prefecture, Japan [4]. Five splenectomized mongrel dogs, 9 to 12 months old, were used for transmission of the parasites. The adults of *H. longicornis* were fed on a splenectomized *B. gibsoni*-infected, 9-month-old dog. The ticks were engorged and dropped from the dog, when parasitemia became approximately 23–43%. A total of 240 nymphal ticks were examined daily after they moulted to adults in an incubator under the controlled conditions (temperature 25°C and relative humidity 80%). The ticks were dissected in insect Ringer solution under a dissecting microscope. Guts were each stained with Giemsa’s for microscopic examination.

In the Giemsa-stained smears on day 8 after repletion, the round-formed *B. gibsoni* (7.0–9.0 µm in diameter) (Fig. 1) was found in the gut epithelial cell of tick. The nucleus was located in the peripheral region of organism, and the cytoplasm was stained light blue with Giemsa’s. Such round-forms are also observed in the infection with *B. ovata* [1] and *B. argentina* [13] which has recently been made synonymous with *B. bovis* [3]. The round-forms have been identified as zygotes by other workers [13, 14]. Holbrook *et al.* [2] observed round-forms, that appear to expand and are unfolded to form vermicules, in their study of *B. caballi* in the horse tick *Dermacentor nitens*, and Rick [12] observed similar forms of *B. bigemina* in *Boophilus microplus*. As the roundforms grew inside the gut epithelial cells, the cytoplasm and chromatin were divided into a number of irregularly shaped, vacuolated bodies. During this period the invaginated (Fig. 2), the retort-shaped (Fig. 3), and the club-shaped forms, considered as kinetes, with the broadly rounded anterior tip and the tapering, tail-like posterior end (Fig. 4) were observed. These forms were not observed in the gut epithelium of the control ticks. Such transformation of zygotes (a static stage) into motile kinetes, as noticed in the above and the present *Babesia* species, can be observed also in other species of blood-inhabiting protozoa. Schumacher [15] identified *Plasmodium cathemerium* as ookinete at the same growing stage as of *Theileria* species. Such ookinates as of Plasmodium are observed also in *Leucocytozoon* species [10]. Mehhorn *et al.* [7] pointed out, however, that there is a difference in the transformation of zygotes into kinetes.

![Fig. 1. Round-form, considered as zygote, of *B. gibsoni*, in gut epithelial cells. ×2,000.](image_url)
Fig. 2. Invaginated-form with vacuole, in gut epithelial cells. ×2,000.

Fig. 3. Retort-shaped form, in gut epithelial cells. ×2,000.

Fig. 4. Club-shaped-form, considered as kinete, in gut epithelial cells. ×2,000.
between the species of blood-inhabiting protozoa and those of *Babesia* and *Theileria*. In the developmental course of *Plasmodium* and *Leucozoitozoa* species, a process appears on the surface of a zygote which increases in size and is transformed into a slender ookinete. On the other hand, Mehlhorn *et al.* [5] carried out electron microscopic studies and reported in detail that a kinete of *Theileria parva* is transformed into a process which becomes larger and projects inside a vacuole. In this course, an invagination is found on the peripheral part of kinete and the membrane surrounding the enlarged vacuole becomes the outer membrane of kinete in the later motile stage. In this study, the transformation pattern of *B. gibsoni* in the gut epithelium of adult *H. longicornis* was similar to those of *Babesia* and *Theileria* species reported by Mehlhorn and Schein [8]. Further studies are necessary for determining the fine structure of the zygote at different stages of development, including the course of transfer from the mesenteronic lumen to epithelium.

**REFERENCES**