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Research Note

Observations on the Development of *Babesia gibsoni* in the Midgut of the Nymphal Stage of the Tick, *Haemaphysalis longicornis*

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There are a number of studies describing the development of *Babesia* species in the midgut of the tick vectors (Higuchi *et al.*, 1989, 1990; Mehlhorn *et al.*, 1980 Mehlhorn and Walldorf, 1988; Shortt, 1973), but few detailed observations on *Babesia gibsoni* since its description by Patton (1910). This paper describes the morphological changes observed during the development of *B. gibsoni* in the midgut of the nymph of *Haemaphysalis longicornis*.

The ticks, collected from a local cow, had been fed on two male New Zealand White rabbits, 7 and 9 months of age. They were maintained in the laboratory, and infected with *B. gibsoni* by feeding on an infected dog. The strain of *B. gibsoni* used was isolated from naturally infected dogs in the Towada area in Aomori Prefecture, Japan (Itoh *et al.*, 1987). Five splenectomized mixed-breed dogs, 7 to 12 months old, were used for infecting tick larvae. Some 10 to 14 days after engorging, the larvae moulted to the nymphal stage in an incubator maintained at 25°C and 80% relative humidity. A total of 420 infected ticks were teased apart in a Ringer's solution formulated for insects, under a dissecting microscope. Each midgut was stained with Giemsa's for microscopic examination.

Merozoites of *B. gibsoni* first appeared 8–14 days after inoculation into the peripheral blood of experimental dogs. Six hr after repletion, merozoites of *B. gibsoni* were observed free of erythrocytes in the midgut contents of the ticks. Within 24 hr, relatively large, round "ring-forms" 2–3 μm in diameter, were seen (Fig. 1). In the ring-forms, the nucleus in the peripheral region of the body and the vacuolated cytoplasm were recognized. Later, the ring-forms developed into spherical-forms which were somewhat elliptic and 4–6 μm in diameter. Relatively large spherical-forms had an eosinophilic nucleus and light basophilic cytoplasm (Fig. 2). Within 2–4 days after-repletion, bizarre-forms 4–5 μm in diameter, were found (Fig. 3). At this stage, the bizarre-forms developed into elongated-forms 6–7 μm in length (Fig. 4). At this time, some binucleated organisms has assumed a form intermediate between the spherical and elongated-forms (Figs. 5, 6). About 5–6 days after repletion, large round- or elliptic-forms (7–9 μm in diameter) were observed in the ticks gut (Fig. 7). The parasite's nucleus was in the peripheral region of the body and its cytoplasm stained light blue with Giemsa. About 7 days after engorgement, the round-shaped protozoans began to decrease gradually in number and finally disappeared from the gut.

Within 24 hr after repletion, concurrent with the appearance of the ring forms, most of the ingested erythrocytes had disappeared. This may be due, in part, to red cell hemolysis caused by enzymes from disrupted eosinophil granules during the process of phagocytosis (Schleger, 1976).

The spherical-forms of the organism, which appeared in the midgut of the tick during the first 24 hr after repletion, previously have been reported in

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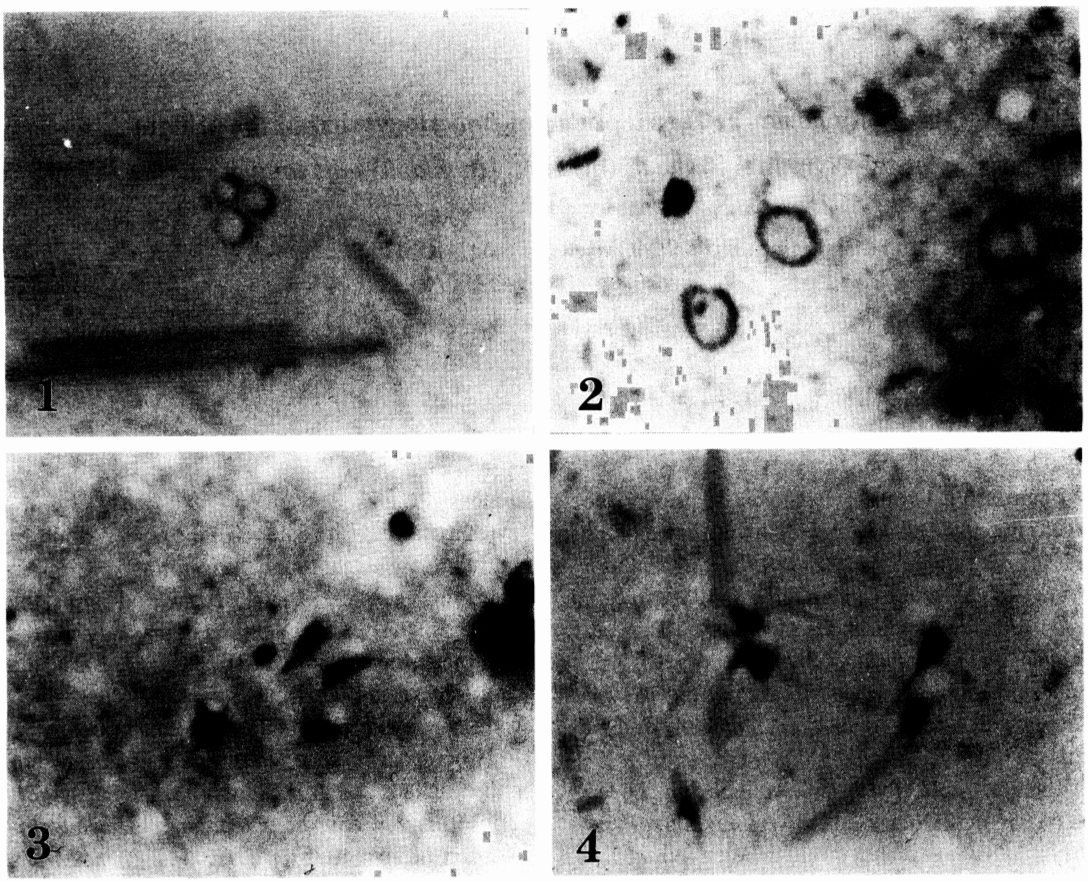
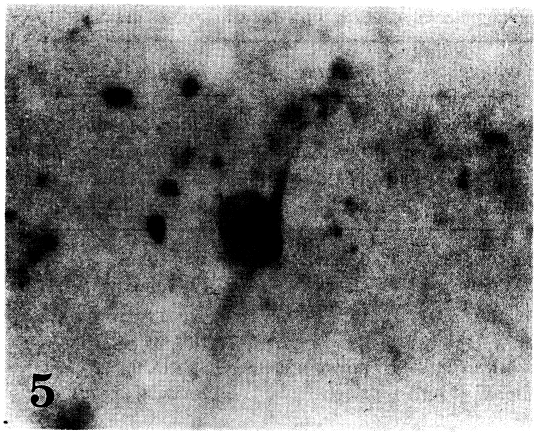
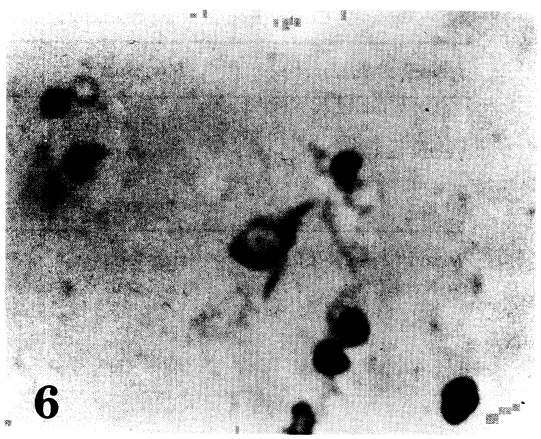


Fig. 1 Relatively large, round shape of the so-called "ring-form" in gut contents; 18 hr post-repletion (p. r.) ×2,000.
Fig. 2 The development of spherical-forms from ring-forms in the gut; 1st day p. r. ×2,000.
Fig. 3 Bizarre-forms, in the gut; 2nd day p. r. ×2,000.
Fig. 4 Elongated-forms in the gut; 3rd day p. r. ×2,000.

ticks infected with *B. gibsoni* (Higuchi *et al.*, 1990), *B. canis* (Mehlhorn *et al.*, 1980), and *B. ovata* (Higuchi *et al.*, 1989). The *B. gibsoni* spherical-forms were smaller than *B. canis* (6.0–7.0 μm in diameter), but similar in size to *B. ovata* (4.0–5.0 μm in diameter) (Table 1). The bizarre-shaped organisms of *B. gibsoni*, which were observed in the midgut within 24 hr after repletion, have been shown to occur during the life cycle of other *Babesia* species as well (Higuchi *et al.*, 1989; 1990; Mehlhorn *et al.*, 1980; Shortt, 1973). Within 2–4 days after repletion, the appearance of elongated organisms emerging from the bizarre-forms, were also seen.

It is difficult to identify the microgametes of *B. gibsoni*. It was considered, however, that the spherical- and elongated-forms of the parasite might be macrogametes and microgametes, respectively, based on morphological characteristics, time of appearance in the gut of the tick, and by comparison with other *Babesia* species (Higuchi *et al.*, 1989, 1990; Stewart, 1978; Stewart *et al.*, 1986). At present, we suggest that the binucleated forms with a projecting thread-like structure may have developed from the fusion of macrogametes (spherical-forms) and microgametes (elongated-forms) resulting in the formation of zygotes. After 5–6 days, round-formed organisms 7.0–9.0 μm in diameter were observed in the *B. gibsoni* infection. This form previously was





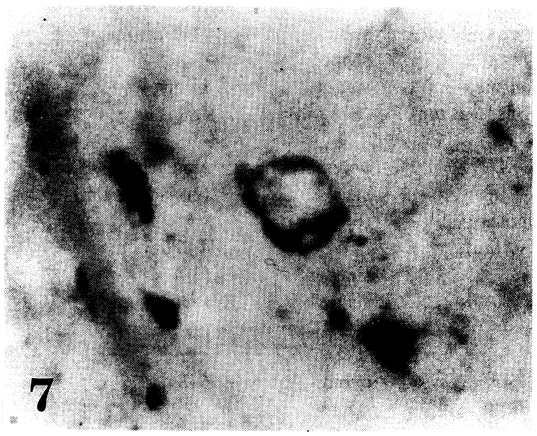


Fig. 5 Fusion-forms in the gut; 4th day p. r. ×2,000.
Fig. 6 Fusion-forms in the gut; 4th day p. r. ×2,000.
Fig. 7 Round-forms, considered as zygotes, in the gut; 5th day p. r. ×2,000.

detected in the adult and nymphal stages of ticks infected with *B. gibsoni* and *B. ovata*, respectively. These round-shaped organisms have been identified as zygotes by other workers (Riek, 1966; Schein *et al.*, 1975). In about 7 days after repletion, these round-forms, which were considered zygotes, gradually disappeared from the midgut of the tick.

A comparison of some developmental characteristics of *B. gibsoni* and other *Babesia* species in the tick are listed in Table 1. The timing of the maturation of the vermicle varies with the *Babesia* species (Higuchi *et al.*, 1989, 1990; Mehlhorn *et al.*, 1980). The morphology of *B. gibsoni* in the midgut of the nymph of *H. longicornis* shows a close similarity to the morphology of *B. gibsoni*, *B. canis* and *B. ovata* in the midgut of adult ticks.

References

1) Higuchi, S., Ezura, K., Hamana, M., Kawamura, S. and Yasuda, Y. (1989): Development of *Babesia ovata* in the midgut of the tick, *Haemaphysalis longicornis*. Jpn. J. Vet. Sci., 51, 1129–1135.
2) Higuchi, S., Simomura, S., Kawamura, S. and Yasuda, Y. (1990): Development of *Babesia gibsoni* in the midgut of the tick, *Haemaphysalis longicornis*. Jpn. J. Parasitol., 39, 492–496.
3) Hoyte, H. M. D. (1976): The tick-fever parasites of cattle. Proc. R. Soc. Qd., 87, 5–13.
4) Itoh, N., Higuchi, S., Ogasawara, T., Ogasawara, A. and Kawamura, S. (1980): An outbreak of canine babesiosis in Aomori Prefecture. J. Jpn. Vet. Med. Assoc., 40, 167–171. (In Japanese)

Table 1 Comparison of some characteristics of developmental stages among *Babesia* species in ticks

Species of *Babesia**	*B. gibsoni*	*B. gibsoni*	*B. canis*	*B. ovata*
Vector tick	*Haemaphysalis longicornis*	*Haemaphysalis longicornis*	*Dermacentor reticulatus*	*Haemaphysalis longicornis*
Stage	nymph	adult	adult	nymph
Within 12 hours (h) post repletion	ring-forms (2~3 μm)	ring-forms (2~3 μm)	spherical-stages (6~7 μm)	ring-forms (2~3 μm)
12~24 h p. r.	spherical-forms (4~5 μm)	spherical-forms (3~4 μm)	polymorphous stages (5~6 μm)	ring-forms; spherical-forms (4~5 μm)
2~4 days p. r.	bizarre-forms (4~5 μm)	bizarre-forms (6~7 μm)	spindle-shaped stages (6~8 μm)	fusion-forms (4~5 μm)
	elongated-forms (6~7 μm)	elongated-forms (6~8 μm)	polymorphous stages (6~7 μm)	bizarre-forms
	fusion-forms		slender stages (10~12 μm)	elongated-forms (6~8 μm)
5~6 days p. r.	zygotes (7~9 μm)	zygotes (7~9 μm)	ovoid stages (9×6 μm)	4~6 days p. r.
			? h p. r.	round-forms (9~10 μm)
				6~8 days p. r.
				vermicule forms (13~15 μm)
				8~12 days p. r.

*Data from *B. gibsoni* (nymphal ticks): present study, *B. gibsoni* (adult ticks): Higuchi *et al.* (1990); *B. canis*: Mehlhorn *et al.* (1980); *B. ovata*: Higuchi *et al.* (1989)

5) Mehlhorn, H., Schein, E. and Voigt, W. P. (1980): Light and electron microscopic study on developmental stages of *Babesia canis* within the gut of the tick *Dermacentor reticulatus*. J. Parasitol., 66, 220–228.
6) Mehlhorn, H. and Walldorf, V. (1988): Life cycles. In Parasitology in Focus, Ed. Mehlhorn, H., Springer-Verlag Berlin Heidelberg New York London Paris Tokyo, 1–48.
7) Patton, W. S. (1910): Preliminary report on a new *Piroplasma* (*Piroplasma gibsoni* sp. nov.) found in the blood of the madras hunt and subsequently discovered in the blood of the jackal *Canis aureus*. Bull. Soc. Pathol. Exot., 3, 274–281.
8) Riek, R. F. (1966): The life cycle of *Babesia argentina* (Lignieres, 1903) (Sporozoa: Piroplasmidea) in the tick vector *Boophilus microplus* (Canestrini). Aust. J. Agric. Res., 17, 247–255.
9) Schein, E., Büscher, G. and Friedhoff, K. T. (1975): Lichtmikroskopische Untersuchungen über die Entwicklung von *Theileria annulata* in *Hyalomma anatolicum exacavatum*. I. Die Entwicklung im Darm vollgesogener Nymphen. Z. Parasitenkd., 48, 123–136.
10) Schleger, A. V. (1976): *Boophilus microplus*: Cellular responses to larval attachment and their relationship to host resistance. Aust. J. Bio. Sci., 29, 499–512.
11) Shortt, H. E. (1973): *Babesia canis*: The life cycle and laboratory maintenance in its arthropod and mammalian host. Int. J. Parasitol., 3, 119–148.
12) Stewart, N. P. (1978): Differences in the life cycles between a vaccine strain and an unmodified strain of *Babesia bovis* (Babes, 1989) in the tick *Boophilus microplus* (Canetrini). J. Protozool., 25, 497–501.

13) Stewart, N. P., Dalgliesh, R. J. and de Vos, A. J. (1986): Effect of different methods of maintenance on the development and morphology of *Babesia bigemina* in the gut of *Boophilus microplus*. Res. Vet. Sci., 40, 94–98.