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Research Note

Development of *Babesia gibsoni* in the Midgut of the Tick, *Haemaphysalis longicornis*

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Although the development of *Babesia* spp. in the tick midgut has been described by a number of investigators (Higuchi *et al.*, 1989; Mehlhorn *et al.*, 1980; Mehlhorn and Walldorf, 1988; Shortt, 1973; Stewart, 1978; Stewart *et al.*, 1986), detailed observations on the development of *B.gibsoni* (Patton, 1910) in its ixodid host are lacking.

This paper describes the morphological changes observed during the development of *B.gibsoni* in *Haemaphysalis longicornis,* the tick vector. Haemaphysalis ticks, removed from a local cow, were bred and maintained in the laboratory on two male New Zealand White rabbits which were 8 and 11 months old and weighed 2.42 and 2.65 kg, respectively. The adult ticks were infected with *B.gibsoni* by permitting them to feed on splenectomized, infected dogs. The strain of *B.gibsoni* used had been isolated from naturally infected dogs in Towada area Aomori Prefecture, Japan (Itoh *et al.*, 1987). Five splenectomized and infected mongrel dogs, 6 to 12 months old, were used for infecting ticks. A total of 240 infected ticks were examined, during the nymphal stage and after moulting to adults in an incubator maintained at 25°C and 80% relative humidity. The ticks were teased apart in a Ringer's solution formulated for insects, under a dissecting microscope. Each midgut was stained with Giemsa's stain prior to microscope examination.

By 6 hr post-engorgement (PE), merozoites of *B.gibsoni* were observed free of erythrocytes in the midgut contents of the ticks (Fig. 1). Within 24 hr PE, relatively large round-forms 2–3 μm in diameter, the so-called "ring-forms", were detected (Fig. 2). In the ring-forms, the nucleus was located in the ring and the cytoplasm was basophilic. Soon after, the rings developed into spherical-forms which were 3–4 μm in diameter. These spherical-forms had an eosinophilic nucleus and a light basophilic cytoplasm (Fig. 3). Within 2–4 days PE, large bizarre-forms (6–7 μm in diameter) were found (Fig. 4). During this stage some of the bizarre-forms elongated into cylindrical forms 6–8 μm in length (Fig. 5). Within 5–6 days PE, even larger round or ellipitical forms (7–9 μm in diameter) were observed in the gut (Fig. 6). The nucleus was peripheral and cytoplasm stained light blue with Giemsa. About 7 days PE, the large globular organisms gradually began to decrease in number, and finally disappeared from the gut. Within 24 hr PE, concurrent with the appearance of the rings forms, was the disappearance of most of the ingested erythrocytes. This may have been due to red cell hemolysis caused by enzymes from disrupted eosinophil granules during the process of phagocytosis (Schleger, 1976).

The spherical forms, which appeared in the midgut during the first 24 hr of engorgement,

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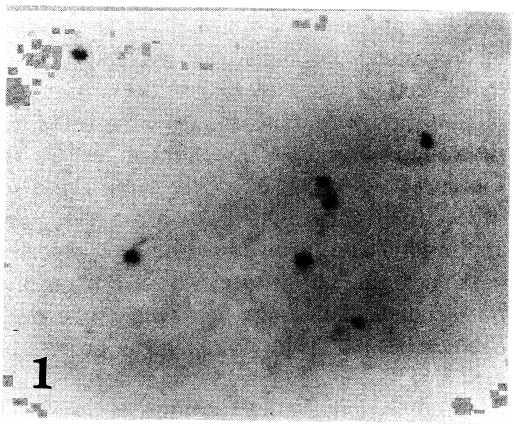

Fig. 1. The merozoite of *B. gibsoni* in the tick midgut ×2,000.

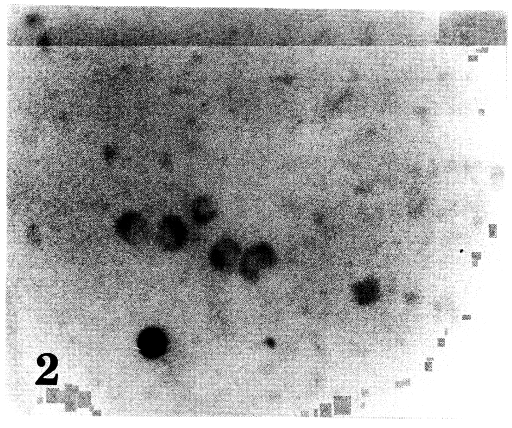

Fig. 2. The round "ring-form" in gut contents ×2,000.

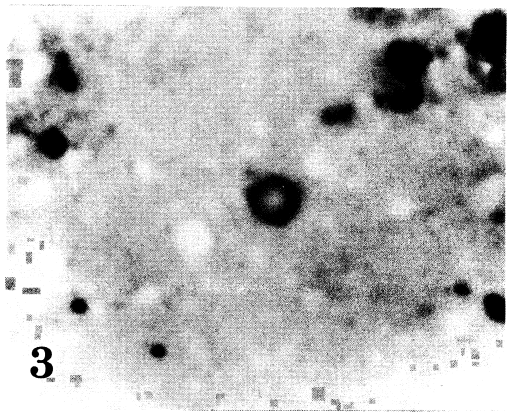

Fig. 3. Rings developing into spherical-forms within the gut ×2,000.

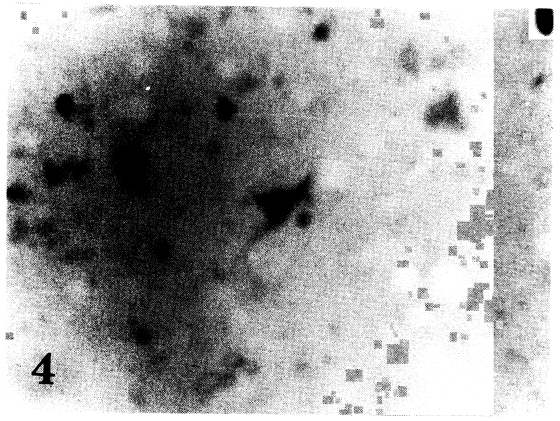

Fig. 4. The so-called "bizarre"-form, in the gut ×2,000.

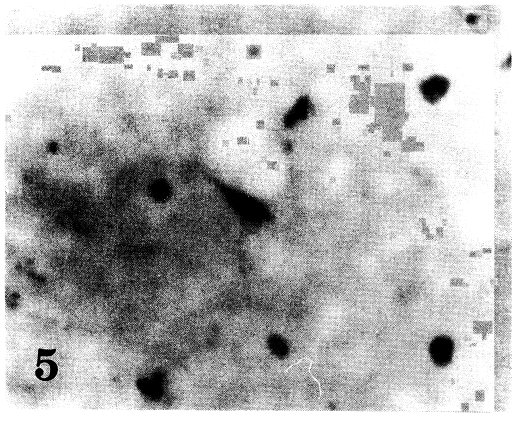

Fig. 5. Elongated organisms in the gut ×2,000.

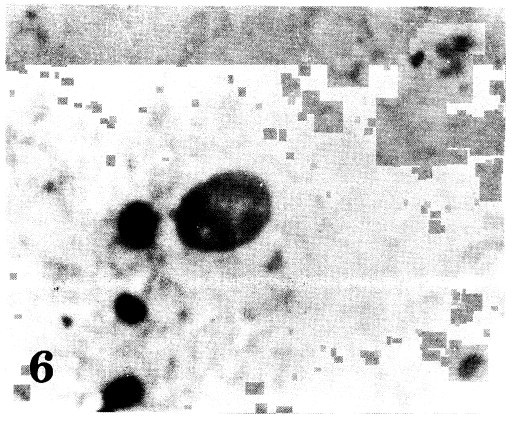

Fig. 6. A Round-form, possibly a zygote, in the gut ×2,000.

likewise have been reported in ticks infected with *B.canis* (Mehlhorn *et al.*, 1980), *B.ovata* (Higuchi *et al.*, 1989), *B.bovis* (Stewart, 1978), *b.caballi* (Holbrook *et al.*, 1968), and *B.bigemina* (Stewart *et al.*, 1986). The *B.gibsoni* spherical forms were smaller than *B.canis* and *B.bigemina,* but similar in size to *B.ovata, B.caballi,* and *B.bovis* (Table 1). The so-called "bizarre" forms of *B.gibsoni,* which we observed in the midgut within 24 hr PE, have been shown to occur during the life cycle of the other *Babesia* species as well (Higuchi *et al.*, 1989; Mehlhorn *et al.*, 1980; Shortt, 1973; Stewart, 1978; Stewart *et al.*, 1986). Within 2–4 days PE, elongated forms of the parasite then appeared. The microgametes of *B.gibsoni* were difficult to identify. It was considered, however, that the spherical and elongated forms might be macrogametes and microgametes, respectively, based on their morphological characteristics, the time of their appearance in the gut lumen of the tick, and by comparison with the other *Babesia* species. Within 5–6 days PE, large globular-shaped organisms, 7.0–9.0 μm in diameter, appeared. These forms have also been reported in the tick phase of the life cycle of *B.ovata* and *B.argentina* (Riek, 1966), now synonymous with *B.bovis* (Hoyte, 1976). Though the process of fusion of the macrogametes was not observed in this study, the round forms of the parasite have been identified as zygotes by other workers (Riek, 1966; Schein *et al.*, 1975). About 7 days engorgement, round, putative zygotes gradually dis-

Table 1 Comparison of developmental stages of *Babesia* species in ticks*

TIME POSTINFECTION*	BABESIA SP AND (TICK HOST)				
	B.gibsoni	*B. canis*	*B. ovata*	*B. bigemina*	*B. bovis*
	(*Haemaphysalis longicornis*)	(*Dermacentor reticulatus*)	(*Haemaphysalis longicornis*)	(*Boophilus microplus*)	(*Boophilus microplus*)
Within 12 hours	ring-forms (2~3 μm)	spherical-stages (6~7 μm)	ring-forms (2~3 μm)	large spherical-forms	binary-fission
12~24 hours	spherical-forms (3~4 μm)	polymorphous stages (5~6 μm)	ring and spherical-forms (4~5 μm)	fission-body (20 μm)	spherical-forms
2~4 days	bizarre-forms (6~7 μm)	spindle-shaped stages (6~8 μm)	fission-forms (4~5 μm)	immature-fission body	elongated-forms
	elongated-forms (6~8 μm)		bizarre-forms	spherical-forms	large vermicules
4~5 days	zygotes (7~9 μm)	polymorphous stages (6~7 μm)	elongated-forms (6~8 μm)	elongated organisms	
5~6 days		slender stages (10~12 μm)	round-forms (9~10 μm)		
6~8 days					
8~12 days			vermicules forms (13~15 μm)		

* Data from: B. canis: Mehlhorn et al. (1980); B. ovata: Higuchi et al. (1989); B. bigemina: Stewart et al. (1986); B. bovis: Stewart (1978).

appeared from the midgut of the tick.

Table 1 compares the developmental characteristics of *B.gibsoni* with other *Babesia* species, in the tick. The morphology of *B.gibsoni* shows a close similarity to *B.canis, B.bigemina* and *B.ovata.* The timing of vermicle maturation varies with the *Babesia* species (Higuchi *et al.*, 1989; Mehlhorm *et al.*, 1980; Stewart, 1978; Stewart *et al.*, 1986). Differences in timing of stages of the several *Babesia* species may be due to differences between the various strains and species of the tick hoste. Based on the present findings, the development of *B.gibsoni in the midgut of the tick H.longicornis,* is diagrammed in Fig. 7.

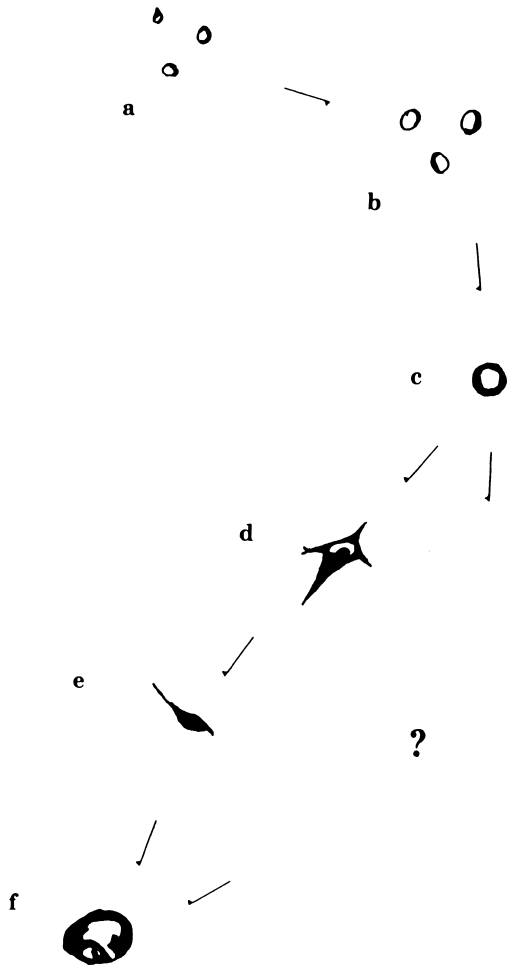

Fig. 7. Schematic diagram of the development of *B. gibsoni* in the midgut of the tick, *H. longicornis.* a: merozoites, b: ring-form, c: spherical-form, d: bizarre-form, e: elongated-form, f: round-form. Refer to the foot-notes of Fig. 1 to 6.

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