

Research Note

Observations on the Development of *Babesia gibsoni* in the Midgut of  
Adult Female *Rhipicephalus sanguineus* Ticks

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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.*, 1990; 1993; 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 adult female *Rhipicephalus sanguineus* ticks.

The ticks were supplied by the Institute of Animal Health, Naha, Okinawa. The acarids have been maintained for several generations in our laboratory, by feeding on rabbits. In the present study, adults ticks were infected with *B. gibsoni* by permitting them to feed on splenectomized, infected dogs. The strain of *B. gibsoni* used originally was isolated from naturally infected dogs in the Towada area in Aomori Prefecture, and maintained in dogs (Itoh *et al.*, 1987). Five splenectomized mongrel dogs, 7 to 12 months old, were used for infecting ticks. A total of 230 infected female ticks were examined, during nymphal development and after moulting to the adult stage, in an incubator maintained at 25°C and 80% relative humidity. The female ticks were teased apart in a Ringer's solution formulated for insects, under a dissecting microscope. The midgut contents were smeared on glass slides and stained with Giemsa's for microscopic examination.

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Piroplasms of *B. gibsoni* first appeared 7–13 days after the by ticks fed on in the peripheral blood of infected dogs. Six hr after repletion, piroplasms of *B. gibsoni*, free of erythrocytes, were observed in the midgut contents of the ticks. Within 24 hr, relatively large, "ring forms" 2–4 µm in diameter, were seen (Fig. 1). In the ring forms, the peripherally located nucleus and the vacuolated cytoplasm were recognized. Later, the ring forms developed into spherical forms which were somewhat elliptical in shape and 5–6 µm in diameter. The relatively large spherical forms had an eosinophilic nucleus and light basophilic cytoplasm (Fig. 2). Within 2–4 days, bizarre forms 6–7 µm in diameter were found (Fig. 3). At this time, the bizarre forms developed into elongated forms 6–8 µm in length (Fig. 4). About 5–6 days after the blood meal, large round or elliptic forms (8–10 µm in diameter) were observed in the female ticks gut (Fig. 5). The nucleus was in the periphery of the parasite and the cytoplasm stained light blue with Giemsa. About 7 days after engorgement, the round shaped protozoans gradually began to decrease in number and finally disappeared from the gut of the tick.

· 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 female tick during the first 24 hr, previously have been reported in ticks infected with *B. gibsoni* (Higuchi *et al.*, 1990) and *B.*

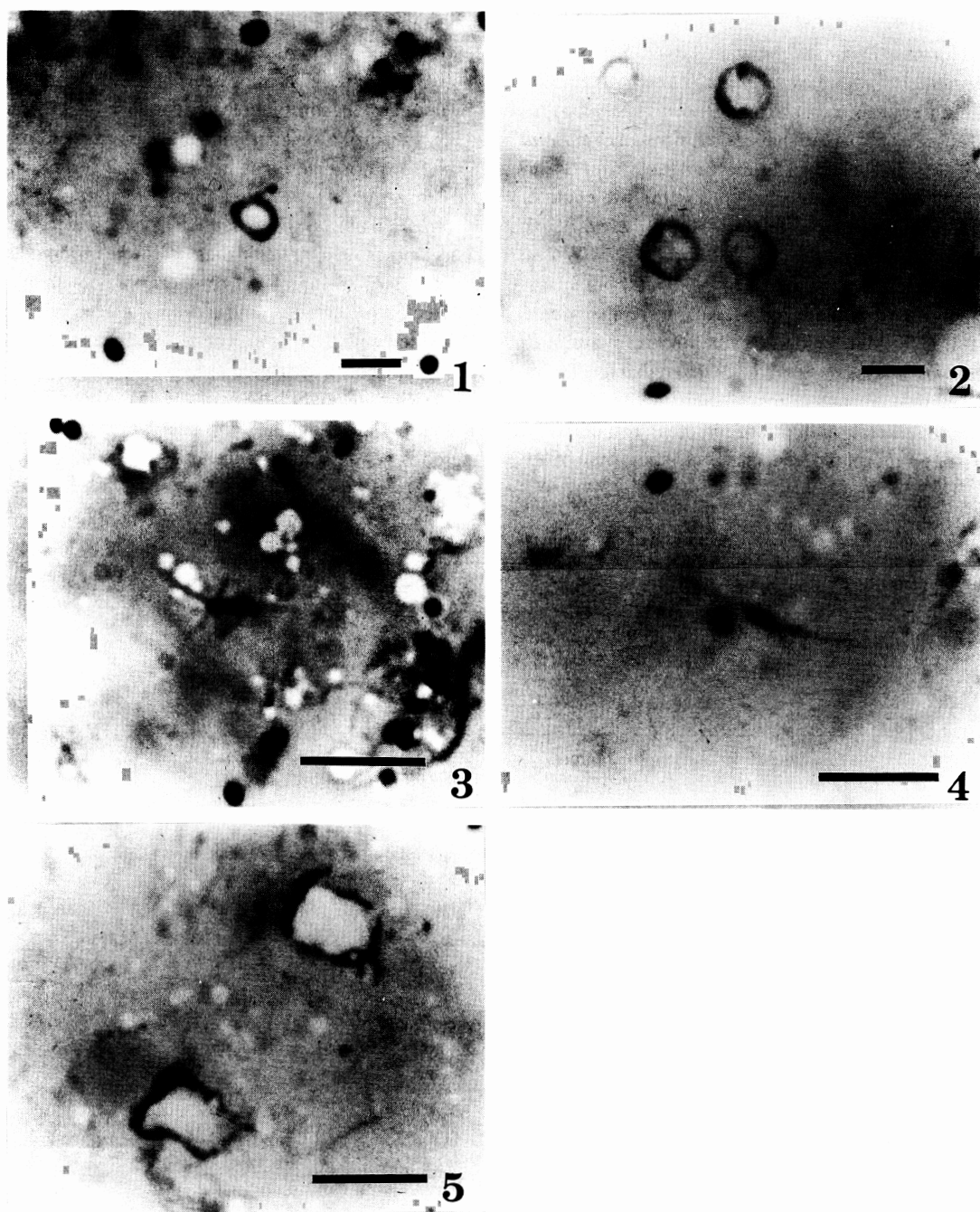


Fig. 1 Relatively large, round shape of the so-called "ring form" of *B. gibsoni* in gut contents; 18 hr post-repletion (p. r.) Bar = 5  $\mu$ m.

Fig. 2 The development of spherical forms from ring forms in the gut; 1st day p. r. Bar = 5  $\mu$ m.

Fig. 3 Bizarre forms, in the gut; 2nd day p. r. Bar = 10  $\mu$ m.

Fig. 4 Elongated forms in the gut; 3rd day p. r. Bar = 10  $\mu$ m.

Fig. 5 Round forms, considered as zygotes, in the gut; 5th day p. r. Bar = 10  $\mu$ m.

*canis* (Mehlhorn *et al.*, 1980). The *B. gibsoni* spherical forms in *R. sanguineus* were larger (5.0–6.0  $\mu\text{m}$ ) than *B. gibsoni* in *Haemaphysalis longicornis* (3.0–4.0  $\mu\text{m}$ ) but similar in size to *B. canis* (6.0–7.0  $\mu\text{m}$ ) (Table 1). The bizarre shaped organisms of *B. gibsoni*, which were observed in the midgut within 24 hr, have been shown to occur during the life cycle of other *Babesia* species as well (Higuchi *et al.*, 1990; 1993; Mehlhorn *et al.*, 1980; Shortt, 1973). Within 2–4 days, 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 ap-

pearance in the gut of the female tick, and comparison with other *Babesia* species (Higuchi *et al.*, 1990; 1993; 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  $\mu\text{m}$  in diameter were observed in the *B. gibsoni* infection. This form previously was detected in the adult and nymphal stages of ticks infected with *B. gibsoni*. These round shaped organisms have been identified as zygotes by other workers (Riek, 1966; Schein *et al.*, 1975). About 7 days after repletion, these round forms, which are considered zygotes, gradually disappeared from the mid-

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

Species of <i>Babesia</i> *	<i>B. gibsoni</i>	<i>B. gibsoni</i>	<i>B. canis</i>
Vector tick	<i>Rhipicephalus sanguineus</i>	<i>Haemaphysalis longicornis</i>	<i>Dermacentor reticulatus</i>
Stage	adult	adult	adult
Time after repletion	ring forms (2~3 $\mu\text{m}$ )	ring forms (2~3 $\mu\text{m}$ )	spherical stages (6~7 $\mu\text{m}$ )
12~24 hr	spherical forms (5~6 $\mu\text{m}$ )	spherical forms (3~4 $\mu\text{m}$ )	polymorphous stages (5~6 $\mu\text{m}$ )
2~4 days	bizarre forms (6~7 $\mu\text{m}$ )	bizarre forms (6~7 $\mu\text{m}$ )	spindle shaped stages (6~8 $\mu\text{m}$ )
	elongated forms (6~8 $\mu\text{m}$ )	elongated forms (6~8 $\mu\text{m}$ )	polymorphous stages (6~7 $\mu\text{m}$ )
5~6 days	zygotes (8~10 $\mu\text{m}$ )	zygotes (7~9 $\mu\text{m}$ )	slender stages (10~12 $\mu\text{m}$ )
			ovoid stages (9x6 $\mu\text{m}$ )

\*Data from present study, *B. gibsoni*: Higuchi *et al* (1990); *B. canis*: Mehlhorn *et al* (1980)

gut of the female tick.

A comparison of some developmental characteristics of *B. gibsoni* and other *Babesia* species in the tick are listed in Table 1. The time regulated for the maturation of the vermicle varies with the *Babesia* species (Higuchi *et al.*, 1990; Mehlhorn *et al.*, 1980). The morphology of *B. gibsoni* in the midgut of the adult female *R. sanguineus* shows a close similarity to that *B. gibsoni* and *B. canis* in the midgut of *H. longicornis* and *Dermacentor reticulatus*, respectively.

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