Development of Babesia gibsoni in the Salivary Glands of the Larval Tick, Rhipicephalus sanguineus

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ABSTRACT. The development of Babesia gibsoni in the salivary glands of larval Rhipicephalus sanguineus ticks was morphologically studied for 4 days of feeding of tick on rabbits. Babesia gibsoni showed two forms (sporont and sporozoite) in the budding process. Sporozoites were formed in the acinar cells of salivary glands and released from the hollow cytoplasmic area of these cells by day 4 of feeding. The morphology of B. gibsoni in the salivary glands of R. sanguineus shows a close similarity to those of B. gibsoni in Haemaphysalis longicornis.—KEY WORDS: Babesia gibsoni, Rhipicephalus sanguineus, salivary gland.


There are many reports on the development of Babesia species in the salivary glands of tick vectors [1, 2, 4, 6, 7, 9, 10]. Authors examined the development of Babesia gibsoni [8] in the salivary glands of Haemaphysalis longicornis tick, and morphologically classified the parasite into sporont and sporozoite forms [2]. However, no detailed observations have been made on B. gibsoni in Rhipicephalus sanguineus ticks. The present paper describes the development of B. gibsoni inhabiting the salivary glands of larval R. sanguineus for 4 days after feeding on a rabbit.

The ticks were supplied from the Institute of Animal Health, Naha, Okinawa 900, Japan. It was maintained by feeding on rabbits for several generations 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 [4]. Five splenectomized mixed-breed dogs, 8 to 11 months old, were used for transmission of parasites.

The adults of R. sanguineus received the infection with B. gibsoni by feeding on a splenectomized, B. gibsoni-infected, 10-month-old dog which parasitism reached 21–35%. After engorgement and dropping from the dog, the infected ticks were put in an incubator under the controlled conditions (temperature 25°C and relative humidity 80%) and allowed to oviposit. The pre-oviposition, oviposition, and egg-incubation periods under the conditions mentioned above were 5–10 days, 2–3 weeks, and 1 month, respectively. A total of 730 larval ticks were fed on two rabbits, which were 6 and 8 months old, weighing 2.25 and 2.57 kg, respectively, and collected at 1, 2, 3, or 4 day after the start of feeding. Then, the ticks were dissected in Ringer’s solution for insects to examine their salivary glands under a dissecting microscope. Each salivary gland was stained with Giemsa’s staining for microscopic examination.

A large Babesia inclusion was observed in one salivary cell on days 1 and 2 after feeding on a rabbit. The inclusions that comprise irregular shaped cytomere-like bodies (4–10 μm) with varying number of chromatin pieces and nuclei were observed in the acinar cells of salivary gland (Fig. 1). Maltmann et al. [7] studied the development of B. equi in the salivary gland of tick, and described the sporonts, by ultrastructural study, that were polymorphous bodies with a multi lobed nucleus and numerous mitochondria. It was demonstrated that cyromere-like bodies might be sporonts, based on their morphological characteristics and by comparison with other Babesia species [2, 3, 5, 7]. This form previously was detected in the larval stage of H. longicornis tick infected with B. gibsoni [2]. The B. gibsoni cyromere-like bodies were larger than B. gibsoni (2.0–5.0 μm) in H. longicornis. Sporont stage of B. occultans was also observed by Blouin and Resburg [1]. Mehlhorn and Waldorf [5] divided sporonts of B. canis into two types of young and enlarged sporonts. The developmental stages of B. gibsoni in R. sanguineus tick were morphologically similar to those of B. equi, B. occultans, B. canis and B. gibsoni in H. longicornis.

By day 2–4 of feeding, a very small fission body (1.0–2.0 μm) appeared and grew to mass of discrete organism in the cytoplasm of acinar cell (Fig. 2). The fission body probably may be formed by budding from sporonts, and differentiated into the final stage. Each particle contained a small purple-stained nuclear mass. Schein et al. [9] studied the development of B. canis and described that the shape of parasite was more similar to that of infectious, pyriform sporozoite after many times of binary division. In the present experiments, the fission body in the salivary glands was determined to be sporozoites. Sporozoites were transformed from sporonts which produced several small nuclei, and then developed into the mononucleate stages. The cytological features of sporozoite formation were reported by Weber and Friedhoff [10], Moltmann et al. [6, 7], Blouin and Resburg [1] and Higuchi et al. [2] for B. bigemina, B. ovis, B. equi, B. occultans and B. gibsoni in H. longicornis tick, respectively. Sporozoites of B. gibsoni were morphologically similar to those of B. bigemina, B. ovis, B. equi, B. occultans and B. gibsoni in H. longicornis tick. On day 4 after feeding, many sporozoites began to be released from the acinar cells. After releasing sporozoites, most of the infected acinar cells changed into the empty sacs (Fig. 3).

From the present observations, it was concluded that B. gibsoni might take two developmental forms (sporont and sporozoite) in the salivary glands of the tick, R. sanguineus. The morphology of B. gibsoni in the salivary glands of R. sanguineus shows a close similarity to those of B. gibsoni in H. longicornis.
REFERENCES


Fig. 1. A smear of the salivary gland of infected ticks on day 1 and 2 after feeding on rabbits. Sporonts (⇒) are observed in the acinar cells. Giemsa’s stain, Bar = 10 µm, × 1,000.
Fig. 2. A smear on day 3 after feeding on rabbits. The acinus containing cell infected with numerous sporozoites (⇒). Giemsa’s stain, Bar = 10 µm, × 1,000.
Fig. 3. A smear on day 4 after feeding on rabbits. A few sporozoites (⇒) and empty sacs (✉) are observed in the acinar cells of salivary gland. Giemsa’s stain, Bar = 10 µm, × 800.