BOVINE BABESIOSIS IN JAPAN: ITS LIFE CYCLE

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INTRODUCTIO

There are many reports on the development of Babesia species in the host and the vector ticks[1,2,9,11,15]. However, no detailed observations on the developmental stages of Babesia ovata[12] in Haemaphysalis longicornis have been made yet. Babesia species are known to undergo morphological change during the growth in the host and the vector ticks. The present was carried out to study the growth of B. ovata in peripheal blood of host cattle, in the midgut, haemolymph and eggs of the tick, H. longicornis.

MATERIAL AND METHODS

Vector ticks: The parthenogenic Okayama strain of Hemaphysalis longicornis was supplied from the National Institute of Animal Health, Tsukuba, Ibaraki 304, Japan. It was maintained by feeding on rabbits and cattle for several generations in the laboratory.
Protozoa: The strain of Babesia ovata used was isolated from a naturally infected cattle at Shimokita in Aomori Prefecture, Japan, maintained for several generations in splenectomized cows and proved to be free from any other blood-inhabiting cattle disease.
Cattle and rabbits: Tow experimental cattle (P-63, P-64) were used. P-63 was a Holstein, 8 months old, infected with B. ovata (parasitemia: approximately 38.1%). P-64 was a Holstein, 6 months old, not infected with B. ovata. Tow male New Zealand white rabbits which were 9 and 11 months of age weighing 2.72 and 2.88 kg, respectively, were used.
Experimental procedure: Samples were collected from the peripheral blood of the cattle at predetermined intervales after the experimental infection. Some smears were stained with Giemsa and others were examiend by the direct fluorescent antibody technique. The ticks of H. longicornis were fed on cattle(P-63) infected with B. ovata. The ticks dropped from the calf body when its intraerythrocytic parasitemia became approximately 38.1%. A total of 840 ticks and 10^6 eggs were collected and incubated in glass vials at 25°C and 80% relative humidity in desicator. The ticks were dissected in an insect Ringer solution under a dissecting microscope. Each tick's organs were smeared and subjected to methacrylate embedding [18] by using a JMD embedding kit (Polysciences, U. S. A.) to prepare sections. Smears and sections were stained with Giemsa's staining.

RESULTS

Merozoites of B. ovata appeared first 9 days after inoculation in the peripheral blood of experimental cattle(Fig. 1). In 12 hr post-replation, merozoites of B. ovata were observed outside of erythrocytes in the contents of the midgut of ticks. Within 24 hr post-repletion, destoryed erythrocytes were found in the contents of gut. Many released from erythrocytes were seen. Within 24-48 hr post-repletion, relatively large round-forms 2-3 μm in diameter, called "ring-form", were seen (Fig. 2). In the ring-forms, the nucleus located in the

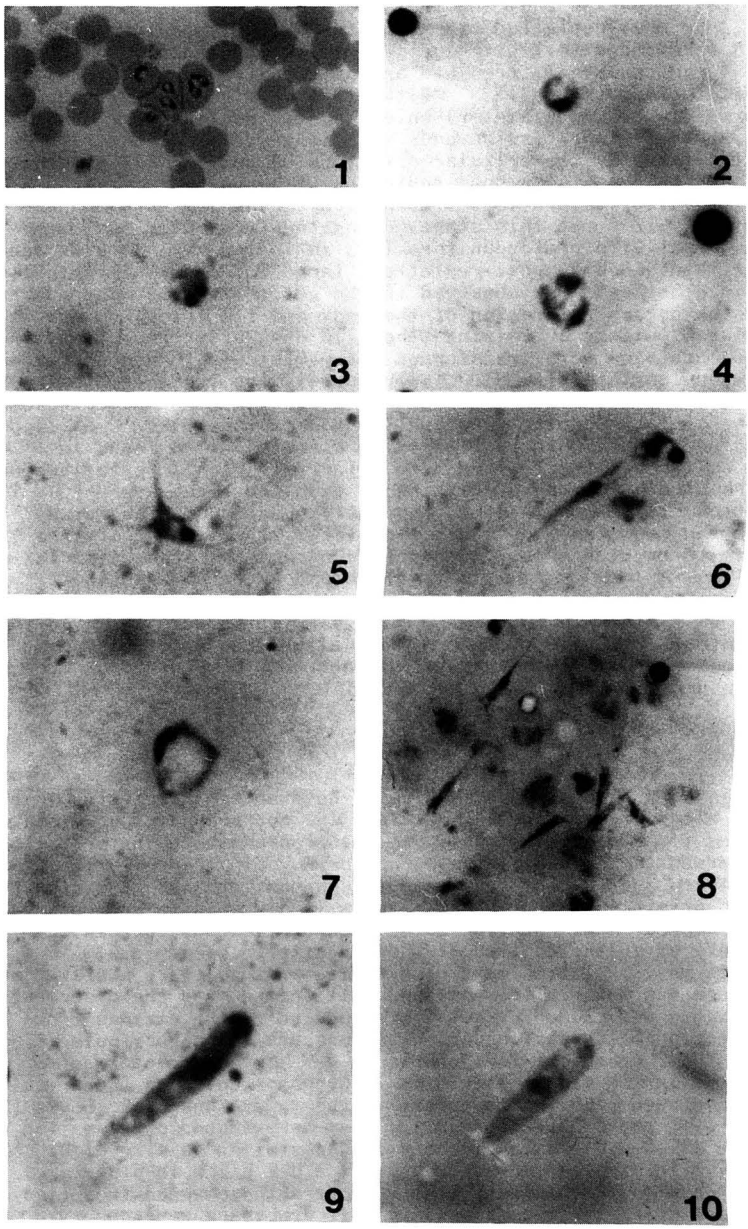

Fig. 1-10. Developmental stages of B. ovata in the host and the vector tick, H. longicornis. See foot-notes on Fig. 11, Table 1.

peripheral region of the body and the basophilic cytoplasm were noted. Within 48-72 hr post-repletion, the ring-forms developed into spherical-forms which were found elliptic and 4-5 μm in in diameter. Relatively large spherical-forms had an eosinophilic nucleus and light basophilic cytoplasm (Fig. 3). Within 3-4 days post-repletion, fission-forms (4-5 μm in diameter) which had two nucleus were observed (Fig. 4). At this time, fission-body (2-3 μm in diameter) emerged from those fission-forms by the cellular divisions on budding-off processes were also seen. Within 4-6 days post-repletion, fission-body developed into bizarre-form (6-7 μm in diameter) which were found bizarrely (Fig. 5). At this stage, elongated-form which projecting threat-like protozoan form (6-8 μm in length) is also seen (Fig. 6). Within 6-8 days post-replation, large round- or elliptic-forms (9-10 μm in diameter) were observed in the gut (Fig. 7). The nucleus was located in the peripheral region of the body and its cytoplasm was stained in light blue by Giemsa's staining. About 10 days after repletion, those round-formed protozoa were transformed into vermicule-formed protozoa (13-15 μm in length)(Fig. 8). Their nuclei were irregular in shape and located eccentrically or at the center, and the cytoplasm contained portions irregularly basophilic and eosinophilic. At 12 days post-replation, round protozoa (1-3 μm in diameter) were found in epithelial cells of the gut. They were relatively rich in cytoplasm and stained dark blue by Giemsa's staining. On the 15th days post-replation, large kinete-like propagative bodies of club forms appeared in the haemolymph of adult ticks(Fig. 9). Most of them were either rod or club shaped with one round and the other tapering end. They had a red or blue-staing cap on the broad anterior, and their nuclei irregular in shape and located eccentrically or at the center. On the 9th day of oviposition, the kinete-like forms were observed in the ooplasm(Fig. 10). Most of them were either rod or club shaped with one rounded and the other tapering ends. These protozoa were not detected in organs of any control ticks.

DISCUSSION

Merozoites of B. ovata appeared first 9 days after inoculation in the peripheral blood of experimental cattle. The morphology of B. ovata was examined to investigate a possible relationship between the varied forms of merozoite and changes in parasitemia. Single pyriform. paired pyriform and budding form were predominant at high parasitemia; incontrast, crisis form at low parasitemia. This results suggests that appearance of some peculiar form of merozoites are correlated with the rate of multhplication of the parasite in blood [3,6].

Within 48h post-replation,many ring-formed protozoa were observed and hemoglobinogenous material disappeared. It seemed that the disappearance of erythrocytes was caused by hemolysis induced by the emzymes from disrupted eosinophil granules during the process of phagocytosis [17] .

In this study, spherical-form protozoa, 4-5 μm in diameter, of B. ovata were observed in the midgut within 48-72 hr post-repletion. Spherical-formed protozoa have been reported to appear in the infections of B. bovis [20], B. caballi [9], and B. bigemina [21]. They were found to be smaller than B. bigemina (5.0-7.0 μm in diameter), and similar to size of B. caballi (4.0-6.0 μm in diameter) and B. bovis (3.0-5.0 μm in diameter). Within 3-4 days post-repletion, fission-body which emerged from fission -forms by cellular divisions on budding-off processes were observed. Those process were identified mutiple budding morphological as B. bigemina [21] and B. bovis [20]. In the present study, it was considered that fission-body divided from fission-form by binary fission on the base of

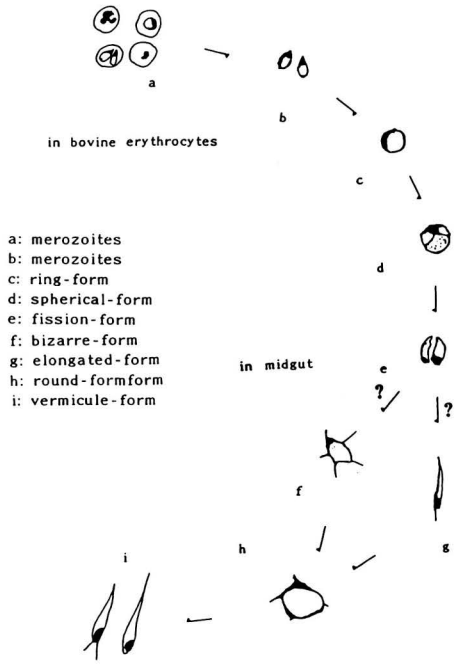

Fig. 11. Schematic diagram of development of B. ovata in the gut of the tick, H. longicornis.

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

Species of *Babesia*	*B. ovata*[a]	*B. bigemina*[b]	*B. argentina*[c]*	*B. bovis*[d]	*B. canis*[e]
Species of vector ticks	*Haemaphysalis longicornis*	*Boophilus microplus*	*Boophilus microplus*	*Boophilus microplus*	*Haemaphysalis leachi*
24~48 hours (h) post repletion (p.r.)	ring-from (2~3μm)	large spherical-form	spherical-form	binary-fission	dividing-form
					ring-form
48~72 h p.r.	spherical-form (4~5 μm)	fission-body (20 μm)	curved cigar-shaped body (2.6~5.6×7.2~13.8 μm)	spherical-form	elongated-form
3~4 days p.r.	fission-form (4~5 μm)	immature-fission body		elongated-form	
			zygote		club-shaped body
4~6 days p.r.	bizarre-form (6~7 μm)	spherical-form	spherical-body	large vermicule	ovid-form
				96 h p.r.	?h p.r.
	elongated-form (6~8 μm)	elongated-organism	24~48 h p.r.		
6~8 days p.r.	round-form (9~10 μm)	24~72 h p.r.			
8~12 days p.r.	vermicule form (13~15 μm)				

a) Data from present study.
b~e) Data from Stewart (1986), Riek (1966), Stewart (1978), and Shortt (1973) respectively.
* *B. argentina* which has recently been made synonymous with *B. bovis* [4].

morphological characteristics. In this study, bizarre form protozoa, 6-7 μm in diameter, of B. ovata were observed in the midgut within 4-6 days post-repletion, and those forms were considered to be macrogametes. Such bizarre form-like protozoa were also proved to appear in the life cycle of the other Babesia species [19-21]. At this time elongated-form protozoa, 6-8 μm in length, are also seen.

In this study, it was difficult to identify microgametes of B. ovata. It was considered, however, that bizarre- and elongated-form protozoa might be macrogametes and microgametes, respectively, base on the mophological and the time of appearance in the gut lumen of the tick and as compared with the other Babesia species. Within 6-8 days post-repletion, round-formed protozoa 9.0-10.0 μm in diameter were observed in the infection of B. ovata. They were also reported to appear in the infection of B. argentina[15] which has recently been made synonymous with B. bovis [10]. Though the process of fusion of the macrogametes were not observed in this study, these round-formed protozoa have been identified as zygotes by other works[15,16]. About 10 days after repletion, vermicule-forms, which were considered transformed from round-formed protozoa, were observed. They were also reported to appear in the infection of B. argentina and B. bovis. Vermicle and Round-formed protozoa then showed a tendency to disappear gradually from the midgut. They were from the midgut again in basophilic epitherial cells of the gut within 12 day, post- repletion. They were also 1-3 μm in diameter. Similar forms have been detected in the B. bigemina [14]. B. bovis [1] and B. ovis [2]. Those changes were not observed in the gut and epithelial cells of any contorol ticks. Table 1 shows the comparison of some characteristics in the development of B. ovata and other Babesia species in the tick. The morphology of B. ovata in the midgut of nymphal ticks, H. longicornis, shows a close similarity to those of B. bigemina, B. argentina and B. bovis. The timing of the maturation of vermicle varies in the different Babesia species[15,19-21]. Differences in timing of stage of Babesia species may be due to the controlled conditions by the different tick species and strain. Diagram of development of B. ovata in the gut of the tick, H. longicornis, based on the present findings is shown in Fig. 11 [7]. On the 15th days after repletion, large kinete-like propagative bodies of club forms appeared in the haemolymph of adults ticks. They were morphologically similar to the kinetes of B. bovis [20], B. bigemina [2] and B. major [13]. They were identified morphologically with B. ovata kinete [4,5]. On the 9th day afeter oviposition, the kinete-like stages were observed in the ooplasm. Ulrich et al. [22] examined the development of B. ovis in the ovary of the tick, Rhipicephalus bursa, with an electron microscope. They reported that the motile vermicule in the ovary of B. ovis showed development similar to the kinete of Babesia species. They were identified morphological as B. ovata kinete in egg. In conclusion it was clearified morphologically that there was possibility for B. ovata to be transmitted by eggs of the tick, H. longicornis [8].

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SUMMARY

Studies were made on the development of Babesia ovata in their host, cattle and the vector tick, Haemaphysalis longicornis. Morphological characterization indicated the merozoites of B. ovata were generally classified into four types. Within 12 hr post-repletion, merozoites were observed outside of erythrocytes infected with B. ovata in the contents of the midgut of the tick. After that, these merozoites were transformed into ring-forms. Within 48-72 hr post-repletion, ring-form protozoa developed into spherical form. Within 3-4 days post-repletion fission-form which were transformed into fission-bodies. Within 4-6 days post-repletion, fission-bodies developed into bizarre-forms. At this time, elongated form are seen. Within 6-8 days post-repletion, round-formed protozoa which were considered as zygotes were observed in the gut. On 15 days post-repletion, kinete of B. ovata appeared in the haemolymph of adults ticks. On the 9th day of oviposition, the kinete were observed in the ooplasm.