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Developmental Stages of Protozoan *Piroplasma* Species Endemic in Japan

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ABSTRACT

The development of *Theileria sergenti*, *Babesia ovata*, and *Babesia gibsoni* in their vertebrate hosts, and in the tick vector, *Haemaphysalis longicornis* is investigated. Three forms of *T. sergenti* are noted in host erythrocytes, the bacillus-like, coccus-like and rod-like forms. In the tick vector, *T. sergenti* goes through gametogony (ring, and round forms, microgamete, macrogamete and zygotes) in the gut, and sporogony (primary, secondary and tertiary sporoblasts, and sporozoites) in the salivary glands. Morphologically, merozoites of *B. ovata* are classified into four types: single pyriform, budding form, paired pyriform and crisis form. In tick host infected with *B. ovata,* two growth stages can be observed, gametogony (ring, spherical, fission, bizarre and elongate forms, microgamete and zygote) in the gut, and sporogony (sporont and sporozoites) in the salivary glands. In its tick vector, *B. gibsoni* undergoes gametogony (ring, spherical, fission, bizarre, elongate forms, microgamete, and zygote) in the gut.

INTRODUCTION

Piroplasmosis in Japan except in the Okinawa Prefecture is caused by *Theileria sergenti* (Uilenberge 1981), *Babesia ovata* (Minami and Ishihara 1980), and *Babesia gibsoni* (Patton 1910). All three infectious agents are transmitted by tick species, *Haemaphysalis longicornis* (Ishii and Ishihara 1951). *Theileria* spp. and *Babesia* spp. are known to undergo morphological changes during their growth in their definitive and tick hosts. This paper presents a comparison of the developmental stages of

Piroplasma species endemic in Japan with those of other *Theileria* and *Babesia* species in other countries.

Development of *Theileria sergenti*

A. In cattle, as definitive hosts

In the peripheral blood, merozoites of *T. sergenti* are morphologically classified into ten types and three subtypes (Ishihara and Ishii 1958). Tayama (1975) reported two different intraerythrocytic forms of *T. sergenti.* He found paired forms that were connected at one end. Fine morphologic structures of *T. sergenti* inside erythrocytes are similar to *Theileria parva* (Büttner 1967), *Theileria annulata* (Schein et al. 1978), and *Babesia equi* (Wayne and Holbrook 1974). With scanning electron microscopy, Higuchi et al. (1985) noted bacillus-like, coccus-like and rod-like forms of *T. sergenti.* Also, parasites inside erythrocytes measure 1.0-2.5 μm long, and show distinct nuclei, rough endoplasmic reticulum, vacuoles, mitochondrion-like bodies, cytostome and tubules. It was thought that the parasite seems incapable of taking up nutrients through the cytostome, as well as of excretion via the tubule (Higuchi et al. 1983, 1984a, 1984b).

B. In ticks, as vectors

Koch (1906) and Gonder (1911) noted the sexual phase of *T. parva* in the gut of *Rhipicephalus appendiculatus* nymphs after engorgement on an infected cattle. Attempts to likewise, find a sexual phase of *T. parva* by Nuttall and Hindle (1913), Reichenow(1935, 1937) and Martin et al. (1964) proved unsuccessful. Cowdry and Ham (1932) suggested the presence of a sexual phase without necessarily demonstrating it. The details of the developmental cycle of *T. parva* (Mehlhorn and Schein 1976; Schein et al. 1977) and *T. annulata* (Mehlhorn et al. 1975; Schein and Friedhoff 1978) in ticks have been studied using light and electron microscopy. They observed the formation of microgametes and macrogametes in the gut of nymphal ticks after feeding on *T. parva* and *T. annulata*-infected cattle. The development of *T. sergenti* in the midgut of *H. longicornis* (Higuchi 1987a, 1987b) showed the release of rounded, comma-shaped or spindle-shaped 1.0-2.5 μm merozoites from erythrocytes 10-24 h post-repletion, and then followed by merozoite transformation into ring forms measuring 1-2 μm in dm. Similar ring forms have been reported in *T. annulata* (Schein 1975), *T. parva* (Schein et al. 1977) and *Theileria taurotragi* (Young et al. 1980). Within 48 to 72 h post-repletion, ring forms are transformed into eosinophilic macrogametes (3-4 μm in dm) and spindle shaped microgametes (5 μm long). Round or elliptical zygotes 4-5 μm in dm appear in the gut within three to five d post-repletion. These findings are consistent with those reported in *T. annulata*, *T. mutans* and *T. parva*. About 6 d after repletion, zygotes decrease in number and finally disappear from the gut, and the round forms (4-5 μm in dm) can be found again in the gut epithelial cells.

Comparison of the size and first appearance of macrogametes, the mean size of zygotes, and similarities in the development of *T. sergenti* and other *Theileria* spp. in their tick hosts is presented in Table 1. *Theileria* spp. develop kinetes when they reach the tick haemolymph, and the kinetes on the basis of their morphology are of three types (Higuchi et al. 1987a). Kinetes decrease in number within 32 d period, disappearing almost completely from the haemolymph 44 d post-engorgement.

There are numerous reports on the development of *Theileria* spp. in the salivary glands. Purnell and Joyner (1968) noted three morphologic forms of *T. parva*; while Schein and Friedhoff (1978) described four types of *T. annulata*. Other authors (Purnell et al.

Table 1. Comparison of the developmental stages of *Theileria* spp. in ticks

Species of *Theileria*	*T. sergenti* a)	*T. mutans* b)	*T. parva* c)	*T. annulata* d)	*T. taurotragi* e)
Species of tick vector	*Haemaphysalis longicornis*	*Amblyomma variegatum*	*Rhipicephalus appendiculatus*	*Hyalomma anatolicum*	*Rhipicephalus appendiculatus*
Length of microgamete (μm)	5	7	10	12	8
Period post-repletion to first occurrence of microgametes (d)	2-3	5	2	3	1-2
Size of macrogametes (μm)	3-4	4	4-5	4	4
Mean size of zygote (dm.)	5.0	6.0	9.0	10.0	12.2x9.0

a) Data from present study

b~e) Data from Warnecke *et al.* (1980), Schein *et al.* (1977), Schein *et al.* (1975) and Young *et al.* (1980), respectively.

1975; Young et al. 1980) also classified the developmental stages of *T. mutans* and *T. taurotragi* into four types. In the process of budding into sporozoites in the salivary glands *T. sergenti* shows three forms of developmental stages (primary, secondary and tertiary sporoblasts) within one to four d (Higuchi 1986a). The sporozoites are apparently formed in the acinar cells and are released from the cytoplasm on the 4th d.

Theileria species are spread primarily by stage to stage transmission but rarely via transovarial route. Ishii and Ishihara (1951) noted a form of transmission involving nymphs and adult ticks of *Haemaphysalis* spp. in infection with *T. sergenti*. Attempts to detect *T. sergenti* from the ovarian follicles and eggs of adult *H. longicornis*, however, showed no parasites, and propagative forms were likewise,

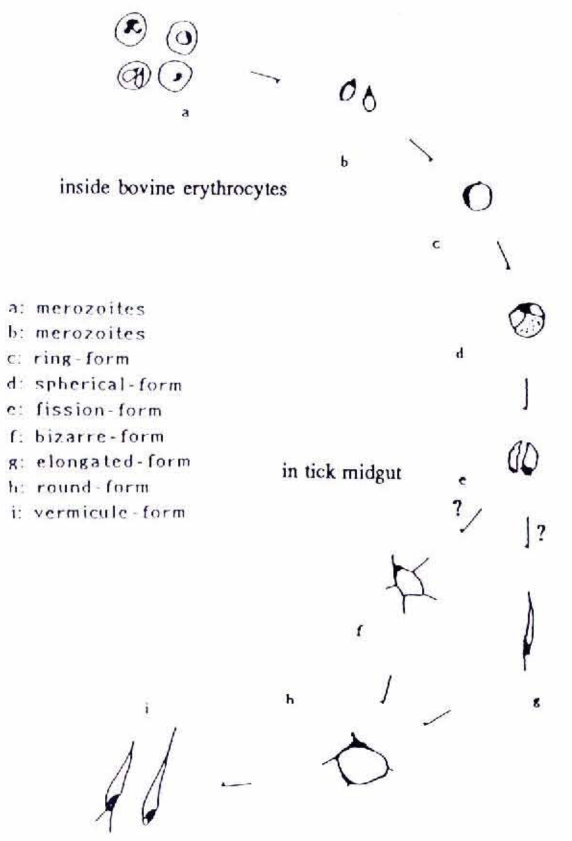

Fig. 1. Schematic diagram of the development of *B. ovata* in the gut of *H. longicornis*

absent in the ooplasm during the period of oogenesis (Higuchi 1985). Higuchi (1986b) also, noted adult ticks unexposed to *T. sergenti* in their larval stage devoid of parasites in the salivary glands, midgut, malpighian tubules, reproductive organs and haemolymph. These findings strongly suggest that transovarian transmission of *T. sergenti* to tick vector is least likely to occur.

Development of *Babesia ovata*

A. In cattle, as definitive hosts

Minami and Ishihara (1980) noted the density of paired pyriform *B. ovata* as indicative of the level of parasitemia. They also, reported statistically significant difference in the number of pyriforms between *Babesia bigemina* (Smith and Kilbourne 1883), *Babesia bovis* (Babes 1888), *Babesia divergens* (M'Fadyean and Stockman 1916) and *Babesia major* (Sergent et al. 1926). There are four morphological types of *B. ovata* merozoites, namely, single pyriform, budding form, paired pyriform and crisis form (Higuchi et al. 1987b, 1991d, 1992a). In a study conducted by Takahashi et al. (1983), they recorded an average detection rate of 23% of the paired pyriform bovine *Babesia* sp. isolated from cattle in Hokkaido. Interestingly, the detection rate did not change with the level of parasitemia. In a related study with *B. ovata*, Higuchi et al. (1987b, 1989c) observed the single and paired pyriform and budding forms of merozoites as predominant forms during high parasitemia, in contrast to the dominance of the crisis form with low parasitemia. These findings demonstrate an apparent correlation between the morphological form of *Piroplasma* in erythrocytes and the rate of parasite multiplication.

B. In Tick vectors

Reports on the development of *Babesia* spp. in tick vectors are numerous such as those of *B. bigemina* in *Boophilus microplus* (Stewart et al. 1986; Higuchi et al. 1992b), *Babesia argentina* in *B. microplus* (Riek, 1966), *B. bovis* in *B. microplus* (Stewart 1978), and *Babesia* canis in *Haemaphysalis leachi* (Shortt 1973). Works of Higuchi et al. (1989a, 1991c, 1991e) on the development of *B. ovata* in *H. longicornis* is summarized in Figure 1. The release of merozoites into the tick midgut occurs within 12 h post-repletion, followed by transformation into ring forms, and within 48-72 h post-repletion ring forms develop into spherical bodies. Fission and bizarre forms appear three to four d, and 4-6 d post-repletion, respectively. During this time they are elongate parasitic forms, and round zygotic forms likewise, appear 6-8 d post-repletion in the gut. Kinetes of *B. ovata* are formed in the haemolymph of adult ticks 15 d post-repletion (Higuchi et al. 1987c, 1989b), and on the 9th d of oviposition, kinetes appear in the ooplasm (Higuchi et al. 1991a). Table 2 shows a listing of some of the characteristics of the developmental stages of *Babesia* spp. in their tick vectors noted in the present study and compared with those reported by other workers.

Table 2. Comparison of the morphology of the developmental stages of *Babesia* spp. 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 h post repletion (p. r.)	ring-form (2-3 μ m)	large spherical-form	spherical-form	binary-fission	dividing-form
48-72 h p. r.	spherical-form(4-5 μ m)	fission-body(20 μ m)	curved cigar-shaped body(2.6-5.6 x7.2-13.8 μ m)	spherical-form	ring form elongate-forms
3-4 days (d) p. r.	fission-form (4-5 μ m)	immature-fission body	zygote	elongate-form	club-shaped body
4-6 d p. r.	bizarre-form	spherical-form	spherical-body	large vermicule	ovoid-form
	(6-7 μ m)				
	elongate-form(6-8 μ m)	elongate-body			
6-8 d p. r.	round-form (9-10 μ m)				
8-12 d p. r.	vermicule form (13-15 μ m)				

a) Data from present study.
[b](Stewart, 1986): morphological stages noted within 24-72 h p.r. ; [c](Riek, 1966) : within 24-48 h p.r. ; [d](Stewart, 1978): within 96 h p.r. ; [e] (Shortt, 1973): not indicated.

Development of *Babesia gibsoni* in tick vectors.

Significant differences in the developmental cycle of different species of *Babesia* in their tick vectors have been reported by various workers. Table 3 shows a comparison of the characteristic developmental stages of *B. gibsoni* in ticks with other *Babesia* spp. *Babesia gibsoni* in the midgut of *H. longicornis* shows some similarities with *B. canis* (Mehlhorn et al. 1980), *B. bigemina* (Stewart et al. 1986) and *B. ovata* (Higuchi et al. 1989a). The timing of the maturation of vermicules varies with *Babesia* spp. (Higuchi et al. 1989a; Mehlhorn et al. 1980; Stewart 1986; Stewart et al. 1978). It is believed that such phenomenon may be controlled by factors associated with differences in tick species and strains.

Table 3. Comparison of the developmental stages of *Babesia* spp in ticks*

Babesia spp.	*B. gibsoni*	*B. canis*	*B. ovata*	*B. bigemina*	*B. bovis*
Tick vector	*Haemaphysalis longicornis*	*Dermacentor reticulatus*	*Haemaphysalis longicornis*	*Boophilus microplus*	*Boophilus microplus*
Within 12 hours (h) post repletion (p. r.)	ring-forms (2~3 μm)	spherical-stages (6~7 μm))	ring-forms (2~3 μm)	large spherical-forms	binary-fission
12~24 h p. r.	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 (d) p. r.	bizarre-forms(6~7 μm)	spindle-shaped (6~8 μm)	fission-forms (4~5 μm)	immature-fission body	elongate-forms
	elongate-forms(6~8 μm)		bizarre-forms	spherical-forms	large vermicules 96 h p.r.
4~5 d p. r.	zygote (7~9 μm)	polymorphous stages (6~7 μm)	elongate-forms(6~8 μm) 4~6 d p.r.	elongate-organisms 24-72 h p.r.	
5~6 d p. r.		slender stages (10~12 μm)			
6~8 d p. r.	round-form (9~10 μm)				
8~12 d p. r.	vermicule form(13~15 μm)				

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

Higuchi et al. (1990a, 1990b, 1991b, 1992 In Press) reported the presence of large rounded forms (=ring form) of *B. gibsoni* in the gut of *H. longicornis* 24 h post-repletion, and then followed with transformation into spherical forms. Within 2-4 d post-repletion, bizarre forms develop into elongate forms, and at 5-6 d post-repletion time large rounded or elliptical forms begin to appear in the gut, which finally reach the gut epithelial cells 8 d post-repletion (Higuchi et al. 1991b). Table 4 presents a summary of the characteristic features of *B. gibsoni* compared with other *Babesia* spp. in ticks. Based on kinete size and time of its appearance in the haemolymph, *Babesia gibsoni* and *B. canis* show the most similarity.

Table 4. Comparison of other developmental characteristics between *Babesia* spp. in ticks

Species of *Babesia*	*B. gibsoni* a)	*B. canis* b)	*B. ovata* c)	*B. bigemina* d)	*B. bovis* e)
Tick vector	*Haemaphysalis longicornis*	*H. leachi* *Rhipicephalus sanguineus*	*H. longicornis*	*Boophilus microplus*	*Boophilus microplus*
Tick developmental Stages	adult	adult nymph	adult, nymph	adult	adult, larva
First detection of kinete in haemolymph	day 10	day 6	day 15	day 3	day 4
Size of kinetes in haemolymph(μ m) (length × width)	14.73×2.20	15.0×2.5	15.68×2.43	11.0×2.5	15.8×3.0

a) Data from the present study.
b~e) Data from Shortt (1973), Higuchi et al (1989), Riek (1964), Stewart (1978), respectively.

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