Observation on Kinetes of *Babesia ovata* in the Hemolymph of the Vector Tick *Haemaphysalis longicornis*

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There are many reports on the kinetes of *Babesia* species in the vector ticks [1, 5–6]. However, no detailed observations on developmental stages of *Babesia ovata* [4] in *Haemaphysalis longicornis* have been made yet. The present paper describes the kinetes of *B. ovata* in the hemolymph of *H. longicornis* detached from naturally infected cattle in Japan.

The nymphs engorged of *H. longicornis*, parthenogenetic strain, were collected from naturally infected cattle in Aomori Prefecture, Japan, as shown in Table 1. After a total of 39 nymphs had molted in an incubator at 25°C and 80% relative humidity, the resultant adults were used. IgG antibody titers against *B. ovata* and *Theileria sergenti* in serum of the cattle were estimated by the indirect fluorescent antibody technique [3]. FITC-labelled anti-bovine IgG rabbit serum (1:16 dilution), H and L chain specific, used was the products of Miles, U.S.A.. Hematocrit value (Ht) and infection rate were determined by the conventional

methods. As shown in Table 2, a total of 39 ticks were examined from 18 to 34 days after engorgement. The anterior legs of ticks were cut off and the emerging hemolymph was smeared on slides. The smears were fixed in methanol and stained with Giemsa's stain.

In a Giemsa's stained stamp smear on the 30th day after engorgement, culb forms ($15.70 \pm 1.21 \times 2.64 \pm 0.55 \mu\text{m}$) of the kinete-like stage were observed in the hemolymph of *H. longicornis* (Fig. 1). Most of them were either rod or club shaped with one rounded and the other tapering ends. They had a red-staining cap on the broad anterior end, and their nuclei were irregular in shape and located eccentrically or at the center. Vacuole was almost invariably present at the anterior extremity. They were morphologically similar to the kinetes of *B. bovis* [7], *B. bigemina* [6], *B. occultans* [8] and *B. major* [5]. They were identified morphologically as *B. ovata* kinetes. Table 3 shows the comparative sizes of bovine *Babesia* in tick hemolymph as reported by various authors. Kinetes of *B. ovata* in the hemolymph of *H. longicornis* were larger than those of *B. bigemina* and *B. occultans* and similar to

Table 1. Response of Hereford cattle fed *H. longicornis* nymphs

Case No.	Sex	Age (month)	Ht (%)	<i>B. ovata</i>		<i>T. sergenti</i>	
				IgG antibody titer	Infection rate	IgG antibody titer	Infection rate
9	Female	6	34	<1:20 ^{a)}	0 ^{b)}	<1:30 ^{c)}	0 ^{d)}
35	Male	6	37	1:40	0	1:810	0
45	Female	6	27	1:320	15	1:270	30
62	Male	7	38	1:20	0	1:270	70
110	Male	7	28	1:640	4	<1:30	10
115	Female	6	29	1:160	0	1:90	5
118	Male	6	34	1:80	0	1:90	0
122	Male	6	33	1:160	31	1:90	0
126	Male	7	26	1:320	0	1:90	0
132	Male	6	28	1:160	0	1:810	0
136	Male	7	30	1:160	0	1:2430	15
137	Male	7	26	1:80	0	1:129	6
145	Female	7	35	<1:20	0	1:90	0

a) To *B. ovata* by indirect immunofluorescence.

b) Number of erythrocytes infected with *B. ovata* per 1,000 erythrocytes examined.

c) To *T. sergenti* by indirect immunofluorescence.

d) Number of erythrocytes infected with *T. sergenti* per 1,000 erythrocytes examined.

Table 2. Relationship between the days after engorgement of ticks and the presence of *B. ovata* and *T. sergenti* kinetes in the hemolymph

	Day after engorgement of ticks									
	18	20	22	24	26	28	30	32	34	
Number of ticks examined	4	5	5	5	4	4	4	4	4	
<i>B. ovata</i>	0 ^{a)}	0	0	0	0	0	1	0	0	
<i>T. sergenti</i>	0	0	0	1	1	0	0	0	0	

a) Number of ticks containing the kinetes.

those of *B. bovis* and *B. major* in length and width. On the other hand, piroplasmosis in this region is considered to be induced by a mixed infection with *B. ovata* and *T. sergenti* from the results of the indirect fluorescent antibody technique as shown in Table 1. Table 3 shows the dimensions of *B. ovata* and *T. sergenti* kinetes [2] in the hemolymph smears of *H. longicornis*. The kinetes of *B. ovata* were larger than those of *T. sergenti*. In the present experiments, the kinetes observed in the hemolymph of *H. longicornis* were identified as the kinete of *B. ovata*.

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Fig. 1a and b. Giemsa's stained smears of Kinetes of *B. ovata* in the hemolymph of the adult of *H. longicornis* on the 30th day after engorgement. $\times 2,000$.

Table 3. Comparative sizes of *B. ovata* and *T. sergenti* kinetes in the hemolymph of *H. longicornis*

Species	Number of cases	Length (μm)	Width (μm)
<i>B. ovata</i> (present study)	68	15.70 ± 1.21 (12.00–18.10)	2.64 ± 0.55 (1.70–4.10)
<i>T. sergenti</i> (Higuchi <i>et al.</i> [2])	100	$10.05 \pm 1.75^{\text{b)}$ (6.10–13.5)	1.95 ± 0.62 (1.00–3.30)
<i>B. bigemina</i> (Riek [6])		11.1 (9.0–13.0) ^{a)}	2.6 (2.0–2.9)
<i>B. bovis</i> (Rieh [7])		15.8 (14.3–16.9)	3.0 (2.8–3.5)
<i>B. major</i> (Mozaria and Brocklesby [5])		15.53 (15.20–15.86)	3.00 (2.92–3.13)
<i>B. occultans</i> (Tomas and Marson [8])		12.7 (11.3–14.1)	2.62 (2.4–3.0)

a) Mean (Range)

b) Mean $\pm$ S. D.

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要 約

Babesia ovata のキネートの観察 (短報): 樋口誠一・伊藤直之・川村清市・安田純夫 (北里大学獣医畜産学部) —— 飽血後30日のフタトゲチマダニ・成ダニのヘモリンフ内に見られた *Babesia ovata* のキネートの大きさは $15.70 \pm 1.21 \times 2.64 \pm 0.55 \mu\text{m}$ で、一端は丸く、先端に Cap を有し、他端は次第に細く、核は辺縁もしくはほぼ中央に位置し、空胞が観察された。