Experimental Transmission of *Babesia ovata oshimensis* n. var. of Cattle in Japan by *Haemaphysalis longicornis*

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**ABSTRACT.** Transovarial transmission of a newly isolated large intraerythrocytic parasite, *Babesia* sp. 1 by *Haemaphysalis longicornis* was experimentally demonstrated. Larvae of *H. longicornis* were transovarially infected with the parasite by feeding as adults on the calf which had been experimentally infected with *B. sp. 1*. Pirolasms of *B. sp. 1* were observed in peripheral blood of the calf which was infested with the parasite-infected larvae. Based on the transmissibility of the parasite with vector ticks, this parasite was suggested to be a variety of *B. ovata*. Thus, we propose a new variety name for *B. sp. 1* as *B. ovata oshimensis* n. var. — key words: *Babesia ovata oshimensis* n. var., *Haemaphysalis longicornis*, transmission.


We recently isolated a large intraerythrocytic parasite from cattle population of Hokkaido in Japan [9]. The isolated parasite, tentatively referred to as *Babesia* sp. 1 was differed in morphometrics of pirolasms from that description of *B. ovata*, a major causative agent of bovine babesiosis in Japan [9]. Besides the morphological differences, *B. sp. 1* showed unique characteristics in biochemical, immunological and genetical comparisons with other *Babesia* species of cattle, such as *B. bovis*, *B. bigemina* and *B. ovata* [7, 8]. Parasites in genus *Babesia* are solely transmitted by ticks, and other vector insect for the parasites has not been reported so far [1]. The geographic distributions of *Babesia* parasites often correlate with that of their vector ticks [4]. In this paper we experimentally demonstrated a transovarial transmission of *B. sp. 1* by *Haemaphysalis longicornis*, which is a most prevailing tick species in Hokkaido Prefecture, Japan.

The Oshima stock of *B. sp. 1* [9] was used in this study. The parasite stock used had been passed 15 times in calves by subcutaneous injection of the infected blood. The parthenogenetic Okayama strain of *H. longicornis* [3] used in this study was maintained at our laboratory. Holstein calves between 4 and 6 months of age were screened for *B. sp. 1*, *B. ovata*, and *Theileria sergenti* infections by Giemsa-stained blood smear examination and enzyme-linked immunosorbent assay (ELISA). ELISA reaction was performed as previously described [9, 10]. Two animals that were negative for these parasites by those screenings were spleenecotomized at approximately 1 month prior to infection, and they were kept in tick-free individual pens.

One of the two calves (No. 1121) was infected with *B. sp. 1* by subcutaneous injection of the infected blood. Following injection, the animal was tested at intervals of 1–2 days to determine the sequential course of parasitemia by examining Giemsa-stained blood smears. Parasitemia was recorded as the percentage of parasitized erythrocytes in 10,000 erythrocytes. When the parasitemia had reached 1.5% (10th day of the infection), the animal was infested with 50 adult *H. longicornis* using the ear bag method [5] (Fig. 1). One week after the tick infestation, engorged and dropped ticks in an ear bag were collected. The ticks collected were allowed to lay eggs under saturated humidity at 25°C in a shaded incubator. The larvae, that had been hatched from the eggs, were kept at 15°C until use. Presence of the parasites in the ticks was preliminary examined by Nested polymerase chain reaction (Nested-PCR) [8] using a colony of larvae (about 1,000 ticks) as the PCR template. After detecting the parasite-specific DNA amplification from the tick-derived template, more than 30,000 larvae from the same colony were applied on ears of the next calf (No. 1124).

Following tick infestation, blood sample was collected daily from the animal for the blood smear examination to detect pirolasms of the parasite. The pirolasms was detected twice in the animal during 30 days observation. The pirolasms appeared at the first time in the blood smear for three days from 7th day of the tick infestation. The

![Fig. 1](image_url)  

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parasitemia recorded at this time was less than 0.1%. After a week disappearance, the pirolasms reappeared in the smear, and they kept again a low parasitemia of below 0.1% for two weeks. Typical pirolasms observed in this term were shown in Fig. 2. The parasites appeared in the animal was confirmed to be B. sp. 1 by the Nested-PCR using the parasite-specific primers and the infected blood-derived template DNA. Although the parasitemias recorded were very low, the animal showed hyperthermia with the highest temperature of above 40°C, corresponding to the appearances of the parasite in blood. Similar hyperthermias with a lower parasitemia have generally being observed in B. ovata infection in cattle [2]. There was no sign of anemia in visible mucous membrane, however, erythrocyte number, hemoglobin concentration and packed cell volume decreased corresponding to the appearances of the parasite in blood.

Based on morphological, biochemical, serological and genetical identities [7–9], we previously suggested a possibility that B. sp. 1 could be classified into an independent species from B. ovata [6]. However, as transovarial transmission of B. sp. 1 by H. longicornis, which is known to be a vector tick of B. ovata, was shown in this study, the taxonomical status of the parasite became more complex. It appears to be impossible at present to classify the parasite as the distinct species from B. ovata, unless the intra-specific variation within the species B. ovata can be taken into the consideration. To avoid disorders regarding the taxonomy of these Babesia organisms, we propose here that B. sp. 1 should be classified as a variety of B. ovata.

Babesia ovata Minami et Ishihara, 1980

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Diagnosis: Pleomorphic organism in bovine erythrocytes; paired pyriform, pyriform, round, drop-like, budlike, oval and so on. Paired pyriform pirolasms body length 3.40 ± 0.49 (2.07–4.76) μm, width 1.79 ± 0.21 (1.14–2.07) μm, with length to width ratio 1.92 ± 0.36 (1.25–3.09) (n=100). The molecular mass of immunodominant pirolasms protein is 40 kDa.

Type host: Bos taurus

Type strain: Oshima strain isolated in 1993 from Japanese Brown in Oshima area of Hokkaido Prefecture, Japan.

Remarks: The pirolasms of Babesia ovata oshimensis n. var. closely resemble those of Babesia ovata ovata in shape of pirolasms. However, the paired pyriform pirolasms of Babesia ovata oshimensis n. var. is significantly longer than that of Babesia ovata ovata. The paired pyriform pirolasms of the new variety is also wider than that of Babesia ovata ovata and the length to width ratio of the new variety is higher than that of Babesia ovata ovata. Babesia ovata oshimensis n. var. is serologically distinguishable from Babesia ovata ovata in the comparative ELISA. By Western blot analysis, 40 kDa pirolasms protein of the new variety reacted strongly with sera from cattle that had been experimentally infected with Babesia ovata oshimensis n. var., while 28 kDa pirolasms protein of the new variety reacted strongly with sera from cattle that had been experimentally infected with Babesia ovata ovata. The major pirolasms proteins of Babesia ovata oshimensis n. var. revealed by two-dimensional polyacrylamide gel electrophoresis were different from those of Babesia ovata ovata.

Babesia ovata Minami et Ishihara, 1980

ovata Minami et Ishihara, 1980

Diagnosis: Pleomorphic organism in bovine erythrocytes; paired pyriform, pyriform, round, drop-like, budlike, oval and so on. Paired pyriform pirolasms body length 2.69 ± 0.51 (1.86–3.72) μm, width 1.52 ± 0.25 (0.93–2.17) μm, with length to width ratio 1.80 ± 0.39 (1.17–3.10) (n=100). The molecular mass of immunodominant pirolasms protein is 29 kDa.

Type host: Bos taurus

Type strain: Miyake strain isolated in 1967 from grazing cattle in Miyake Island of Tokyo Prefecture, Japan.

Remarks: By Western blot analysis, 34 kDa pirolasms protein of Babesia ovata ovata reacted strongly with sera from cattle that had been experimentally infected with Babesia ovata oshimensis n. var., while 29 kDa pirolasms protein of Babesia ovata ovata reacted strongly with sera from cattle that had been experimentally infected with Babesia ovata ovata.

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