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Babesia and *Theileria* Protozoa Detected from Wild Sika Deer (*Cervus nippon yesoensis*) in Hokkaido

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ABSTRACT

A pooled blood sample collected from wild deer (Cervus nippon yesoensis) in Hokkaido prefecture was inoculated to sika deer. Large and small type piroplasms were detected from the blood smear of inoculated deer. The form of large type piroplasm was oval, ring, amoeba or binary fission, and paired pyriforms. The size of this type was 1.00-3.50 um in length, and 0.80-1.50 um in width. The form of the small type piroplasm was like dot, comma, oval or bayonet forms and quadruple fission with the formation of a cross. The size of this type was 1.20-2.50 um in length, and 0.50-1.00 um in width. Clinical signs of fever, anemia and hemoglobinemia occurred with appearance of the large type-piroplasm in peripheral blood, only fever and anemia occurred by the small type-piroplasm. It was considered from morphological features and clinical signs that the large type resembles Babesia sp. and the small type Theileria sp. The two haemoprotozoa did not infect cattle. Babesia sp. were transovary transmitted by Haemaphysalis longicornis to the deer.

INTRODUCTION

There are two studies on sika deer's hemoprotozoa in Japan. One of them describes Theileria damae (Ono et al. 1923), the other Theileria sp. (Bessho et al. 1987). However, there are no detailed reports on morphological features of these parasites and on clinical signs caused by them. The main subspecies of sika deer in Japan is Cervus nippon yesoensis Heude, Cervus nippon nippon Temminck and

Cervus nippon centralis kishida (Kuroda 1979). C. nippon yesoensis is the biggest subspecies in sika deer and lives only in Hokkaido prefecture.

In this paper, we describe morphological features and clinical signs of Babesia and Theileria sp. in C. nippon yesonensis. Babesia sp. of sika deer are recorded in Japan for the first time.

MATERIALS AND METHODS

Parasites: In March 1981, three wild sika deer (Cervus nippon yesoensis) were caught for epidemiological investigations in Nakanoshima Island of Lake Toya, Hokkaido, Japan. Their blood, infected with piroplasms below 0.1% of erythrocytes, was collected from cervical veins. We used this infected blood for the experiments described here.

Experimental animals: Four sika deer (Nos.1-3 male, one year old; No.4 female, 2 years old.) were used for the inoculation tests of deer's piroplasms. Deer No.5 (male, one year old) was used for inoculation tests of Theileria sergenti. The deer was also used for transmission tests of deer's piroplasms by infected larval Haemaphysalis longicornis. The deer were obtained from Asahiyama Zoo in Hokkaido. A calf, one month old, was used for inoculation test of deer's piroplasms. At the preinoculation time, all experimental deer and the calf were negative for Babesia sp., Theileria sp., Anaplasma sp. and Eperythrozoon sp. by repeated examination of blood smears stained with Giemsa's solution.

Inoculation test : Three ml of pooled blood, below 0.1% of parasitemia, collected from three wild deer was inoculated intravenously to deer No.1. Clinical and hematological findings and morphological features of the protozoa were observed for five months after inoculation. More than 1,000 erythrocytes were examined to determine the rate of infected erythrocytes under Giemsa staining. Deer No.1 was splenectomized twenty days after inoculation. 10^8/kg infected erythrocytes collected from deer No.1 at 32 days after infection, were intravenously inoculated to the other three deer (Nos. 2-4), and the deers were observed with the same method. Deer No.2 was splenectomized on day 31 after infection.

Cross infection test: $5x10^{10}$/kg erythrocytes infected with piroplasms of deer No.3, were subcutaneously injected to a splenectomized calf, and infectivity of the parasites was observed by blood smears for one month. Bovine erythrocytes infected with T.sergenti (10^9/kg) were injected to splenectomized deer No.5, and infectivity was observed by the same method for 70 days.

Transmission test using ticks (Haemaphysalis longicornis): Adult H.longicornis, negative against piroplasms, were attached to experimentally infected deer No.1 at 61 days after inoculation. Fifty hatched larval ticks were attached to splenectomized deer No.5. Blood smears stained with Giemsa's solution were observed to confirm the appearence of piroplasm.

RESULTS

Morphological features and clinical signs of deer's piroplasms: Deer No.1: Large type piroplasms were observed from 16 days after inoculation; parasitemia was below 0.1%. After splenectomy, large type piroplasms rapidly increased. 32 days after inoculation, the parasitemia of large type piroplasm reached 1.1% and then decreased rapidly. Packed cell volume (PCV) decreased from 53% to 23% with rising parasitemia, but soon increased with decreasing parasitemia. Small type piroplasms were observed from 42 days after inoculation. Seventy days after inoculation, small type piroplasms reached 6.9% of the maximum parasitemia and PCV decreased to 16.5% (Fig.1)

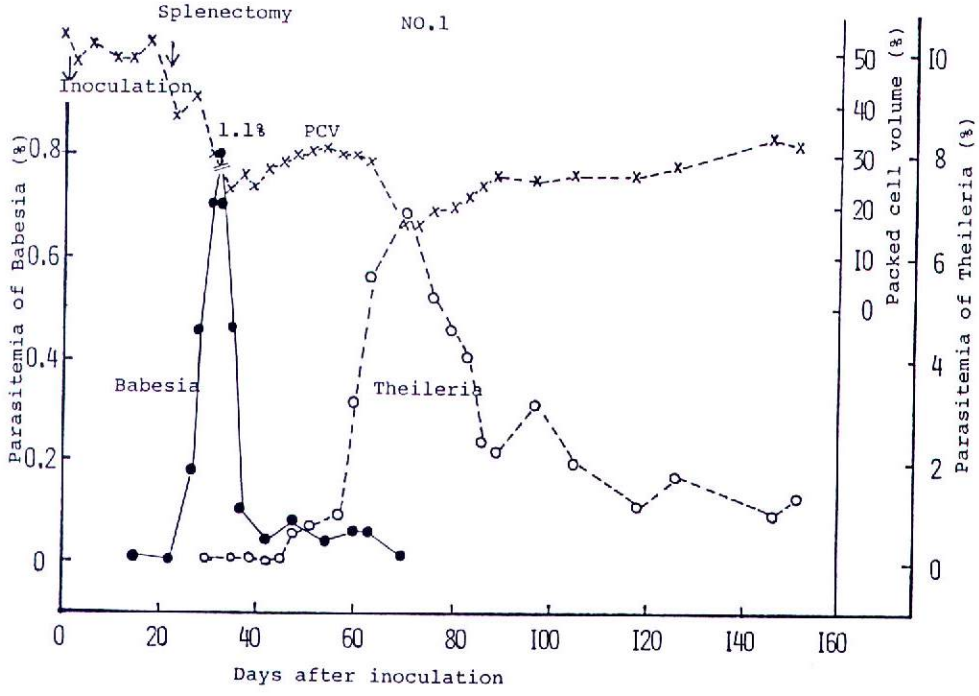

Fig. 1 Changes of parasitemia and packed cell volume(PCV)

Large type piroplasms were observed as oval, ring, amoeba and binary fission forms, and also as paired pyriforms (Fig.2a and 3a). The size of this protozoa was 1.00-3.50 μm in length, and 0.80-1.50 μm in width. Small type piroplasms were like dot, comma, oval or bayonet forms and also quadruple fission with the formation of a cross were observed (Fig.2b and 3b). The size of this protozoa was 1.20-2.50 μm in length, and 0.50-1.00 μm in width. Schizonts were not detected in the smears of peripheral lymphocytes.

Deer Nos.2-4: within three days after inoculation, large type piroplasms increased with fever (approximately 40°C), anemia and hemoglobinemia. After that, anemia decreased with decreasing parasitemia. No.4 died by critical

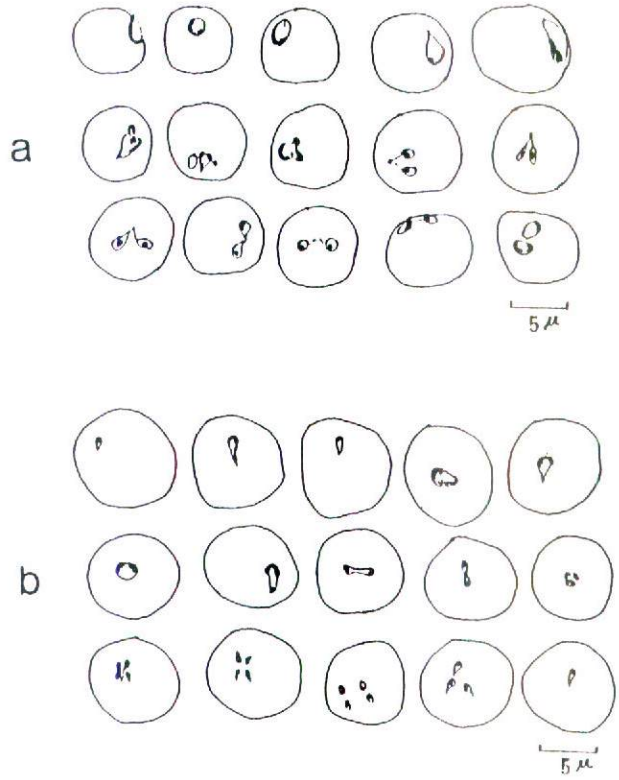

Fig.2. Babesia sp. (a) and Theileria sp. (b) detected in the blood of deer.

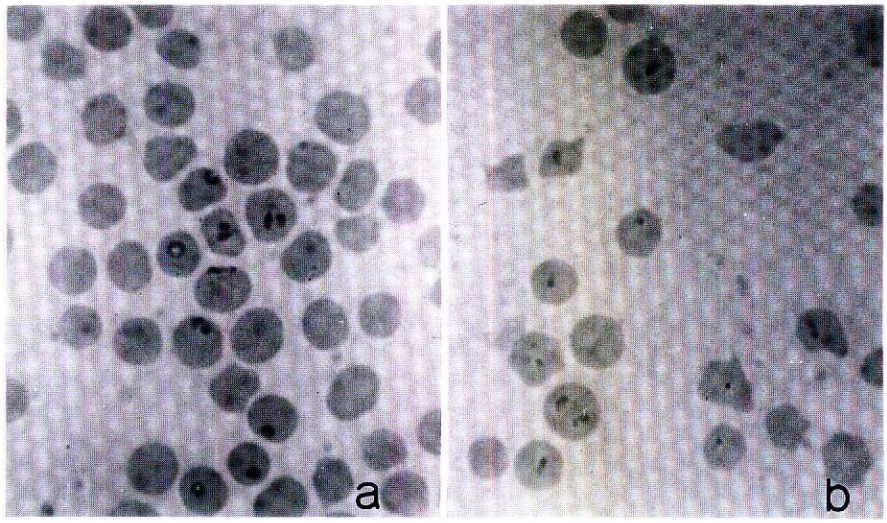

Fig.3. Babesia sp. (a) and Theileria sp. (b) x1280

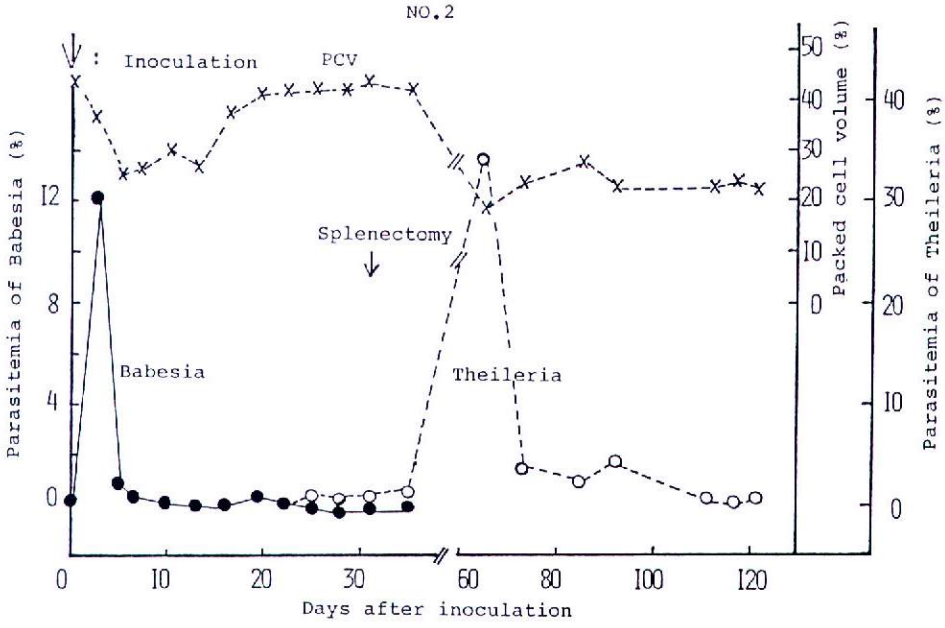

Fig. 4 Changes of parasitemia and packed cell volume (PCV)

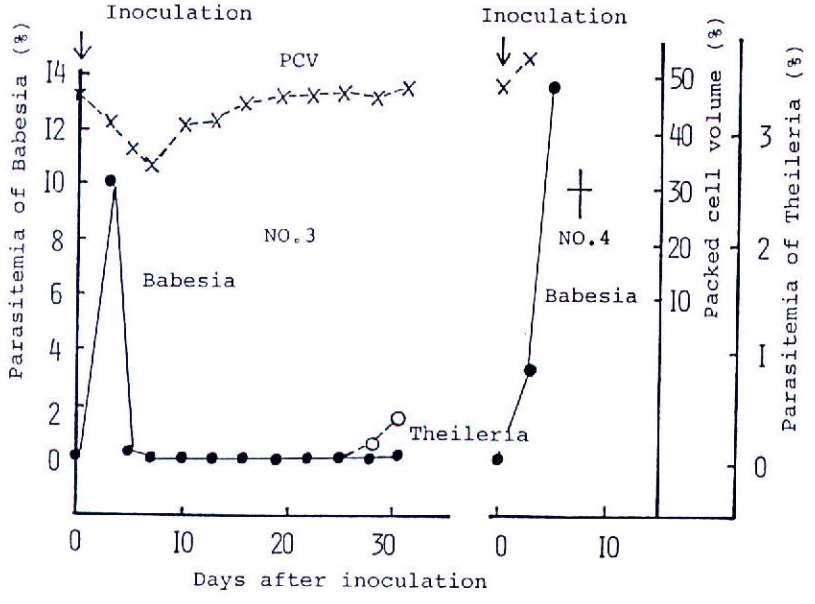

Fig.5 Changes of parasitemia and packed cell volume (PCV)

anemia 4 days after inoculation. Large type piroplasms were hardly observed from six days on after inoculation. In necropsy of deer No.4, jaundice, hemoglobinuria, splenomegaly were observed. In deer No.2 and No.3, twenty six days after inoculation, small type piroplasms were detected but there were no apparent clinical signs of anemia, jaundice and fever. After splenectomy of No.2, small type piroplasms increased with fever over 40°C and anemia. But hemoglobinemia and hemoglobinuria were not observed (Figs. 4 and 5).

Cross inoculation test: Deer's piroplasms did not infect a splenectomized calf, and T.sergenti of cattle did not infect the splenectomized deer No.5, vice versa.

Transmission test by H.longicornis: Large type piroplasms were transmitted to splenectomized deer No.5 by infected larval ticks.

DISCUSSION

From the results of the inoculation tests, large and small type piroplasms were detected in sika deer (Cervus nippon yesoensis) in Nakanoshima Island. It was considered from morphological features and clinical signs that the large type piroplasms were Babesia sp. and the small type piroplasms Theileria sp.

The main clinical signs of this Babesia sp. infection were fever over 40°C, anemia and hemoglobinuria with rising parasitemia, which took a fatal course in deer No.4. These symptoms recovered with decreasing parasitemia, and after recovered anemia only a few parasites were observed in blood smears. It seems that pathogenicity of Babesia sp.is severe in the primary infection, especially in splenectomized deer. This deer will be a carrier after recovery from the symptoms. The detected Babesia sp. is a small type Babesia species and similar to Babesia bovis, B.divergens and Babesia sp. of the deer (Elaphurus davidianus) from morphological features (Davis et al. 1958; Hinaidy. 1987). However, this parasite was not infectious to cattle and thus this species is distinct from B.bovis and B.divergens. Babesia sp. of C. nippon yesoensis and E. davidianus has similar morphological features. It is nesessary for definite identification to compare, by using ticks transmission, cross infection, serological status and DNA probes.

The pathogenicity of Theileria sp. was mild compared with that of Babesia sp. The main clinical signs of Theileria sp. were fever over more than 40°C and anemia with rising parasitemia. From morphological features, the detected Theileria is similar to T.mutans (Rudzinska, 1981). From the result of our inoculation test, this Theileria sp. does not infect cattle, and this species differs from T.mutans and T.sergenti (Ishihara et al. 1978). However, this Theileria sp. should be compared with T.cervi (Robinson, 1967). There is a possibility that the Theileria sp of C. nippon yesonensis is the same species as T. damae detected in C. nippon (Ono et al. 1923), and the detected Theileria sp. the same as the species found in C. nippon

nippon (Bessho et al. 1987).

In Japan, Haemaphysalis longicornis is well known as a common vector of Babesia ovata and T.sergenti in cattle (Ishii, 1951). From our transmission test using infected larval ticks, H.longicornis is a vector of wild deer's Babesia sp. in Japan.

In conclusion, the examination of Giemsa-stained blood smears from deer, and inoculation and transmission tests using ticks suggest that babesiosis and theileriosis are enzootic in Hokkaido prefecture, Japan.

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