Clinico-Hematological Observation of Calves Experimentally Infected with *Theileria sergenti*

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Theileria sergenti is a causative agent of Japanese bovine theileriosis. Infected calves show anemia and growth retardation, but usually do not succumb to the infection. The disease is found mainly in grazing cattle, rarely in housing cattle. Stress seems to be a major factor to develop the symptom in *T. sergenti* infection. Splenectomy is necessary to ensure the pathogenesis in healthy cattle. However, clinical course of *T. sergenti*-infected cattle is vague after splenectomy. Here, we report the clinico-hematological observation of splenectomized cattle experimentally infected with *T. sergenti*.

Three splenectomized Holstein calves (No. 1044, No. 1047, No. 1081) were infected with the Ikeda stock of *T. sergenti* [1], by *Haemaphysalis longicornis* (No. 1044, No. 1081) or *H. mageshi-maensis* (No. 1045) [1, 2] by using an ear bag [6]. Before and after the tick infestation serial blood samples were taken from the calves at short intervals to count erythrocytes, leucocytes and parasitized erythrocytes. The counts of erythrocyte and leucocyte, hematocrit value and hemoglobin concentration were determined by the automatic blood cell counter (Coulter Electric Co., USA), and the parasitized erythrocyte and the differential counts of leucocyte were checked by light microscopy with Giemsa stained blood smears. Rectal temperature was checked twice a day throughout the experiment.

As shown in Fig. 1, piroplasms appeared in the peripheral erythrocytes on days 10 (No. 1045) and 12 (No. 1044). On these days the temporary decrease of leucocyte counts occurred coincidentally with the temporary rise of body temperature. After then, leucocyte counts and body temperature recovered. Successively, they continued rising until the parasitized erythrocytes peaked on day 25. When the parasitized erythrocytes lowered they recovered. These changes were not observed in the splenectomized calf with uninfected tick infestation. The temporary

decrease of leucocyte counts and temporary rise of body temperature were not observed in the calves inoculated with parasitized erythrocytes

Fig. 1. Clinical data in splenectomized calves (No. 1044, above; No. 1045, below) inoculated with *T. sergenti*.

Hemoglobin concentration (Hb: □), hematocrit (Ht: ▲), counts of erythrocytes (RBC: ○), leucocytes (WBC: ■), parasitized erythrocytes (PRBC: ⊙) and body temperature (TEMP: —).

intravenously [8, 13]. Minami *et al.* [9] reported that the schizont-like parasites are detected from the lymph nodes at the onset of piroplasm in the peripheral blood. Furthermore, the schizont-like parasites are observed only in this term (Minami's personal communication). These changes might be induced by schizogony. As previously reported, the hematocrit value, hemoglobin concentrations and erythrocyte counts start to decrease after the appearance of piroplasms in the peripheral erythrocyte [8, 13].

After the appearance of piroplasms in the peripheral blood, the unparasitized erythrocytes decreased in number, whereas the parasitized erythrocytes increased. And yet, after the count of parasitized erythrocyte peaked, the erythrocytes decreased in number in spite of the decrease of parasitized erythrocytes (Fig. 1). This

fact could indicate that the erythrocytes were not only destroyed by piroplasms but also by some other causes in *T. sergenti* infection. Indeed, Yagi *et al.* [14] observed that osmotic fragility of erythrocyte increases in *T. sergenti* infected calves. But further investigation is necessary to get the conclusion. As hemoglobinemia or hemoglobinuria was not so observed as in *Babesia* infection in spite of severe anemia in *T. sergenti* infection, it is thought that the destruction rate of erythrocyte might be smaller in *T. sergenti* infection than in babesiosis. After the first peak of parasitized erythrocyte, the counts of parasitized erythrocyte fluctuated with small peaks.

The counts of peripheral lymphocyte increased (Table 1). Extensive lymphocytolysis with profound depletion of lymphocytes in the lymphoid

Table 1. Changes of leukocyte (WBC) counts in peripheral blood and parasitemia in cattle infected with *T. sergenti*

Cattle No.	Days after inoculation	WBC (/μl)	Eosino.* (%)	Neutro. (%)	Lympho. (/μl)	Mono. (%)	Others (%)	Para. (%)
1044	0 ^{a)}	11000	0.5 (60)	17.0 (1870)	76.0 (8360)	3.5 (390)	2.0 (220)	—
	12 ^{b)}	6100	0 (0)	16.0 (980)	77.0 (4700)	6.5 (400)	0.5 (30)	+ **
	24 ^{c)}	13000	0.5 (70)	13.5 (1820)	80.5 (10870)	5.5 (740)	0 (0)	25.9
	31 ^{d)}	11600	0 (0)	5.0 (580)	90.5 (10500)	4.5 (520)	0 (0)	13.5
1045	—1 ^{a)}	14700	1.0 (150)	44.0 (6470)	52.5 (7720)	4.0 (590)	0.5 (70)	—
	10 ^{b)}	6000	0 (0)	58.5 (3510)	32.5 (1950)	7.5 (450)	1.5 (90)	0.1
	24 ^{c)}	14700	1.5 (220)	32.5 (4780)	54.0 (7940)	7.0 (1030)	0.5 (70)	27.1
	30 ^{d)}	12600	0.5 (60)	23.0 (2900)	66.5 (8380)	10.0 (1260)	0 (0)	6.4
1081	0 ^{a)}	12800	3.0 (380)	51.0 (6530)	40.0 (5120)	6.0 (770)	0 (0)	—
	12 ^{b)}	7400	2.5 (190)	19.5 (1440)	70.5 (5220)	7.0 (520)	0.5 (40)	0.4
	23 ^{c)}	12500	0.5 (63)	23.5 (2940)	70.5 (8810)	5.0 (630)	0.5 (60)	25.0
	37 ^{d)}	8900	0.5 (40)	14.0 (1250)	73.0 (6500)	11.0 (980)	1.5 (130)	4.3

*: Percentage with absolute counts of leukocytes in parenthesis; Eosino.: Eosinophile, Neutro.: Neutrophile, Lympho.: Lymphocyte, Mono.: Monocyte, Others: Other cells, Para.: parasitemia.

** : Parasitemia was less than 0.01%.

a) The day of or day before inoculation.

b) The day of piroplasm appearance in peripheral erythrocytes.

c) The day of maximum counts of parasitized erythrocytes.

d) The day of minimum counts of parasitized erythrocytes between 1st and 2nd peaks of parasitized erythrocytes.

tissues and recirculating pool was reported in the last stages of the *T. parva* infection [10]. We previously reported that the antigen of *T. sergenti* piroplasms contains an adjuvant substance [5, 11, 12]. As a result of that bovine lymphoid or myeloid system might be activated by the adjuvant substance of *T. sergenti* piroplasms, the ratio of peripheral lymphocyte, the count of leucocyte and body temperature might change. On the schizont stage of *T. parva* or *T. annulata* infection, clinical signs such as swelling of lymph nodes, pyrexia, anorexia, lachrymation, nasal discharge, diarrhea and dyspnea would occur [3, 4, 7, 10, 15]. But the intralymphocyte schizont of *T. sergenti* has not been reported yet. Although levels of piroplasm parasitized erythrocytes may exceed 50% in cattle infected with *T. parva*, there is only mild anemia [10]. The piroplasm stage of *T. parva* is of minor significance in anemia, but that of *T. sergenti* would be of major significance.

As shown above, it was made clear that the clinical picture of *T. sergenti* infection is very different from that by *T. parva* or *T. annulata*. This is the basal study to solve the mechanism of anemia and immunological aspect in *T. sergenti* infection.

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要 約

小型ピロプラズマ (*Theileria sergenti*) の実験感染摘脾牛における臨床血液学的変化(短報): 清水真也・八木行雄・中村義男・志村亀夫・藤崎幸蔵・小野寺 節・南 哲郎・伊藤進午(農林水産省家畜衛生試験場)——摘脾牛にダニを用い小型ピロプラズマを感染させると、赤内型原虫出現の時期に一致し白血球数の一時的減少と体温の一時的上昇が観察された。また、原虫寄生赤血球数のピークの前後に赤血球数、赤血球容積、ヘモグロビン濃度が減少し、また、白血球数と体温の上昇が認められた。一方、感染経過に伴い末梢血リンパ球数の増加傾向が観察された。以上より、感染が宿主の免疫系や骨髄系に何らかの影響を与えているものと推察された。