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Prenatal Infections with *Theileria sergenti* in Calves

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Theileria sergenti is a tick-borne protozoan parasite of cattle. *Haemaphysalis longicornis* (Ishii and Ishihara 1951), *H. cornigera*(Ishihara 1971) and *H. mageshimaensis* (Fujisaki et al. 1988) are known as biological vectors for *T. sergenti*. *Tabanus trigeminus* is acting as a vector for the mechanical transmission of *T. sergenti* under limited conditions (Takahashi 1983). Recently, a relatively high rate of *T. sergenti* infection was observed in animal houses with paddock, especially during winter season when vectors are inactive. Mechanical transmission of *T. sergenti* by the long-nosed cattle lice, *Linognathus vituli* especially in winter season has been reported (Fujisaki et al. 1993). In the epidemiological study on *T. sergenti* infection carried out during winter season in 1993, we observed prenatal infections with *T. sergenti* as well as in-house infections after birth.

One hundred calves including 22 Hereford, 41 Angus, 30 Japanese Black, 4 Holstein and 3 calves of mixed breed, which had been reared in Shintoku Animal Husbandry Experiment Station, Hokkaido Prefecture were investigated. Blood samples were collected from the calves 1 or 2 days after birth and several days before grazing in pasture. Thereafter, blood samples were examined monthly from March to July and September, 1993. Fifty five pregnant cows of which calves were included in this investigation, were also tested before calving in January 1993. Giemsa-stained blood smears were microscopically examined for *T. sergenti* infection. For polymerase chain reaction (PCR) to detect *T. sergenti* infection, DNA was extracted by the method described before (Tanaka et al. 1993) in the same day for blood

smear preparations. PCR-amplification of parasite-specific DNA was carried out according to the method described by Tanaka et al. (1993). PCR-amplified products were examined by agarose gel electrophoresis to detect specifically amplified band of 875 bp.

Five out of 100 blood samples of 1 or 2 days old calves were microscopically positive for *Theileria*-like organisms. Veil and bar structures which are characteristic morphologies of *T. sergenti*, were observed in erythrocytes (Fig. 1A and B). Parasites with four masses completely separated from each other, as mentioned by Kawamoto et al (1990) were also observed (Fig. 1C). Parasitemia levels in these 5 calves were between 0.01 and 0.06%. DNA samples of the 5 Giemza-positive calves and 3 Giemza-negative ones were prepared and tested for PCR. The results of PCR completely agreed with those obtained by Giemza-staining (Fig. 2).

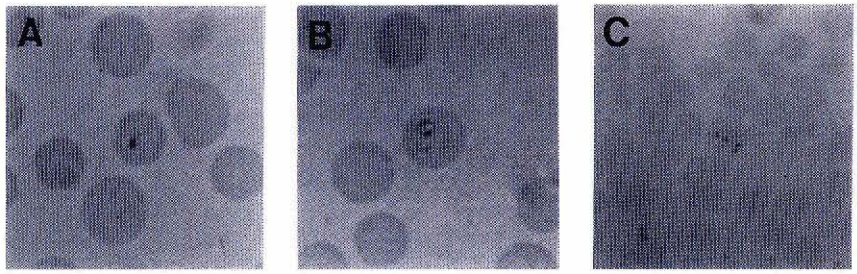

Figure 1 Theilerial parasite detected in Giemza-stained blood smear of a calf one day after birth. A:Comma-shaped form, B:Piroplasm associated with veil and var structures, C:Four basophilic masses.

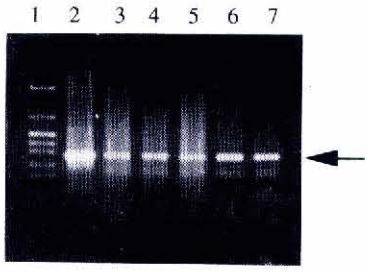

Figure 2. PCR amplification of *Theileria sergenti* DNA fragment from blood samples collected from 1 or 2 days old calves. The PCR products were electrophoresed in a 1% agarose gel. Lane 1: molecular weight marker (4,870, 2016, 1107, 926, 658, 489, and 267 bp). Lane 2:DNA from *T. sergenti* Shintoku stock. Lanes 3-7:Samples from calf No. 102C, 106C, 107C, 214C and 506C. Specific bands of 875 bp as indicated by an arrow were detected by ethidium bromide staining.

Blood samples were collected from the same 100 calves at several days before grazing in pasture and tested. Thirteen samples including 5 neonatally positive ones were both microscopically and PCR-positive, and 10 were only PCR-positive. Their parasitemia rates of these calves were between 0.02% and 2.78% (mean 0.69%). The changes in parasitemia and PCV from the birth to September, 1993 are shown in Table 1. Calves which had been infected before grazing (group A and B in Table 1)

showed similar parasitemia patterns to those of calves which were infected after grazing (Group C). Four pregnant cows which calved infected offspring and 51 which calved uninfected ones were examined for parasitemia and PCV before calving (Table 2). All the cows were infected with *T. sergenti* with parasitemia between 0.02% and 0.70%. There were no significant differences between parasitemia levels of the cows, regardless of the transmission of the parasites to the calves.

Table 1. Changes in parasitemia and PCV of calves infected with *Theileria sergenti*.

Group[1]		Blood examination						
		at birth	March	April	May	June	July	September
A (5)								
	Parasitemia(%)	0.02 ±0.02[2]	0.12 ±0.14	0.99 ±1.01	1.00 ±1.02	1.65 ±1.52	3.61 ±3.20	4.43 ±1.16
	PCV(%)	40.4 ±7.7	33.4 ±7.5	33.2 ±6.4	35.1 ±6.2	33.6 ±5.0	29.4 ±4.1	29.6 ±6.6
B (13)								
	Parasitemia (%)	0	0.08 ±0.21	0.05 ±0.18	0.07 ±0.12	1.05 ±1.42	1.93 ±1.79	1.55 ±1.47
	PCV(%)	38.7 ±5.3	35.6 ±4.6	40.9 ±5.9	35.9 ±7.1	38.0 ±3.8	33.3 ±6.3	34.2 ±5.2
C (82)								
	Parasitemia (%)	0	0	0	0	0.67 ±0.86	4.53 ±3.37	3.12 ±2.22
	PCV(%)	37.8 ±5.3	36.6 ±4.6	38.5 ±4.7	37.3 ±4.1	37.8 ±3.1	30.1 ±6.5	32.4 ±3.7

1) Group A; Prenatally infected, Group B;in-house infected, Group C; infected in pasture.
Number of calves in each group are indicated in the parenthesis. The calves were born in January and February and being grazed in pasture from May to September 1993.
2) Mean ±SD

Table 2. Parasitemia and PCV of pregnant cows.

Cow status[1]	Parasitemia	PCV
Prenatally infected calves (4)	0.34 ±0.25[2]	33.2 ±5.8
Uninfected calves (51)	0.15 ±0.15	34.9 ±3.3

1) Pregnant cows which calved prenatally infected or uninfected animals.
Number of cows in each group are indicated in the parenthesis.
All of the cows tested were *Theileria*-positive.
2) Mean ±SD

The results of this study indicated a possibility of intrauterine transmission of *T. sergenti* from infected cows. It is reported that piroplasms of *T. sergenti* can be detected microscopically at least 7 days after biological or mechanical transmission of the parasite (Ishihara 1971, Fujisaki et al. 1993). By inoculation of infected blood, parasites could be detected by Giemsa-staining 9 days after infection (Tanaka et al. 1992). Even by using more sensitive diagnostic methods including PCR (Tanaka et al. 1993) and dot blot hybridization with DNA probes (Tanaka et al. 1992), parasites could not be demonstrable before 7 days post infection. The detection of piroplasms of *T. sergenti* within a few days of birth in this study strongly suggests prenatal infections with T. sergenti in calves. Prenatal infections of piroplasmosis are known in *Babesia bovis* (Trueman and Mclennan 1987) and *Babesia equi* (de Waal 1992), but our report

is the first record of such an event in *T. sergenti*. Several unpublished observations suggested a possibility of intra-uterine transmission of *T. sergenti*, as mentioned by Ishihara (1971), but no more detailed studies have been done since then.

Fifteen calves which were PCR-negative at the examination of 1-2 days after birth but became positive before grazing were considered to be infected in-house, although we could not specify vectors which transmitted parasite mechanically or biologically. It is proved that low dose of infection induced by syringe passage of infected blood produces resistance against *T. sergenti* infection after grazing (Ishihara 1971), while exact mechanisms of this "vaccination" are still unclear. Regardless of the manners of natural infections, prenatal, in-house or in-pasture, there was no difference in subsequent parasitemia after grazing, which indicate that parasitemia at low levels before grazing may not produce effective immunity against *T. sergenti*. It should be determined whether the calves infected in-house or prenatally allow additional infections of other field strains of parasites in the pasture, or proliferation of the parasites with which they had been infected. It must be noted that the infection rate before grazing is considerably high, showing 13 % in this study. Effective methods to prevent prenatal and in-house infections of the parasite as well as disease transmission in the pasture should be established urgently in order to control bovine piroplasmosis caused by *T. sergenti*.

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