

## Serological Survey of Bovine Theileriosis in Malaysia

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### Abstract

The serological cross reactivity between the Malaysian *Theileria* and the other benign *Theileria* species from Japan and Australia, which are tentatively referred to as *T. sergenti* and *T. buffeli* respectively, was examined. A total of 290 serum samples from cattle were collected from 9 states (25-38 samples from each state) in Peninsular Malaysia and the ELISA was carried out to measure the specific optical density against *T. sergenti* and *T. buffeli* antigens. All the samples except two cattle, reacted more intensely to *T. buffeli* antigen as compared to *T. sergenti* antigen. Therefore, it is suggested that the Malaysian *Theileria* species of cattle bear a serological resemblance to *T. buffeli*, as compared to *T. sergenti*. In addition, the serological survey of bovine theileriosis in Peninsular Malaysia was also carried out. A higher reactivity to *T. buffeli* antigen in ELISA was generally observed in the serum samples collected from the various states. There were, however, a few exceptions which showed a lower reactivity to *T. buffeli* antigen in ELISA in samples from the two states in the east coast of Peninsular Malaysia.

**Discipline:** Animal health

**Additional key words:** ELISA, *Theileria buffeli*, *Theileria sergenti*

### Introduction

The taxonomical classification of the causative agents of bovine theileriosis prevalent in Eurasia is still controversial. According to the collaborative study between Malaysia and Japan undertaken since 1988, the Malaysian *Theileria* species of cattle is morphologically different from *T. mutans* unlike the conclusion of Legg<sup>2)</sup>. Rajamanickam has recently proposed that the correct name of the relevant parasite for the Malaysian *Theileria* be either *T. orientalis* or *T. buffeli* instead of *T. mutans*<sup>4)</sup>. This implies that identification of the organism designated as *Theileria* in Malaysia should be reconsidered in more detail. In this connection, the latest finding that all the blood samples collected from cattle in Peninsular Malaysia had *Theileria* parasites should also be

taken into account<sup>1)</sup>.

This paper presents results of the comparative study on the serological cross reactivity between the Malaysian *Theileria* and the other benign *Theileria* species from Japan and Australia, which are tentatively referred to as *T. sergenti* and *T. buffeli*, respectively. It also provides brief data on the serological survey of bovine theileriosis implemented in Peninsular Malaysia during the period September to December in 1989.

### Materials and methods

A total of 290 serum samples from cattle were collected from 9 states in Peninsular Malaysia, comprising 25-38 samples from each state. A total of 43 EDTA blood as well as of serum samples were collected from cattle in two locations, i.e. Sungai

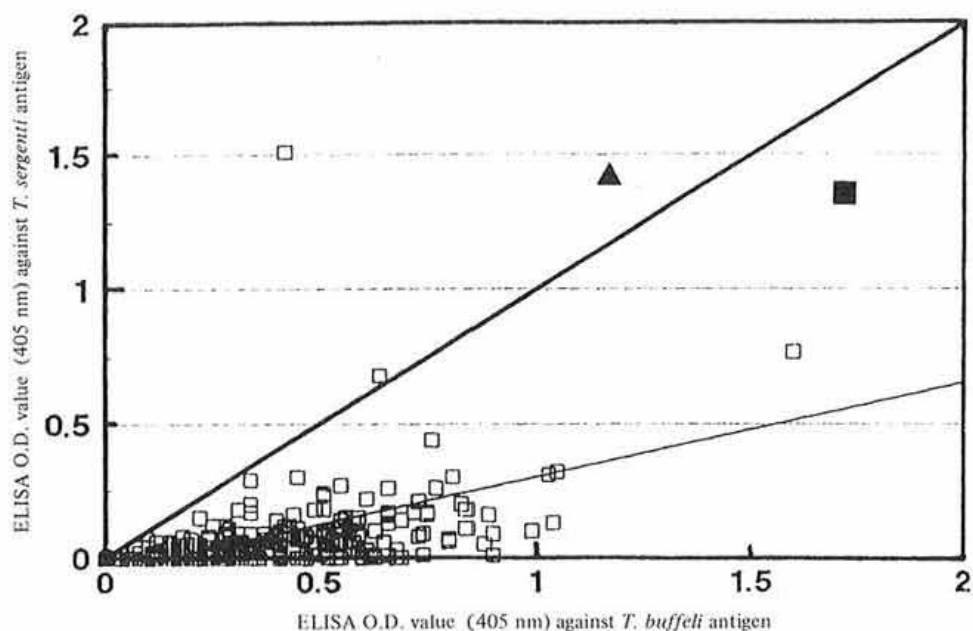


Fig. 1. Relationship between the ELISA O.D. values against *T. buffeli* and *T. sergenti* antigens. The data are taken in 290 field cattle sera collected in Peninsular Malaysia.

- : *T. buffeli* positive reference serum,
- ▲ : *T. sergenti* positive reference serum,
- : Negative reference serum.

Siput and Lenggong both in Perak State. Blood smears were made from EDTA samples and fixed in methanol, stained with Giemsa and examined for intraerythrocytic forms of *Theileria* parasites. The level of parasitaemia was expressed in percentages of parasitized erythrocytes in 1,000 erythrocytes.

The serum samples collected were diluted in 0.01 M phosphate buffered saline (PBS, pH 7.2) containing Tween-20 (0.15%). Enzyme-linked immunosorbent assays (ELISA) were carried out to measure the specific optical density (O.D.) against *T. sergenti* and *T. buffeli* antigens. The ELISA was done according to the method of Shimizu et al.<sup>5)</sup> except for utilizing azinobenzthiazoline (ABTS) as a substrate. Results of the ELISA were expressed in optical densities at wavelength of 405 nm: the samples with an O.D. of 0.2 and above were tentatively classified as serologically "positive" for the respective species of *Theileria* after Shimizu et al.<sup>5)</sup>. The data were subjected to statistical analyses based on the Student's *t*-test.

## Results

The relationship between the O.D.s against *T. sergenti* and *T. buffeli* antigens in 290 field sera tested is demonstrated in a scattergram of Fig. 1. All the samples with two exceptions reacted more intensely to *T. buffeli* antigen as compared to *T. sergenti* antigen. According to this result, those data on the ELISA O.D.s given against *T. buffeli* antigen were used for the analyses in the subsequent experiments.

Fig. 2 shows the distribution of serologically positive cattle in Peninsular Malaysia which are identified on the basis of ELISA O.D.s using *T. buffeli* antigen. There is a distinctive difference between the two states of Kelantan and Trengganu and the other seven states: i.e. fairly low rates (29–32%) in the former, while high rates (63–88%) in the latter.

Table 1 indicates some results of the surveys on blood smears for *Theileria* parasite in 43 field cattle of various ages. Table 2 presents data on the serological testing of the serum samples collected from

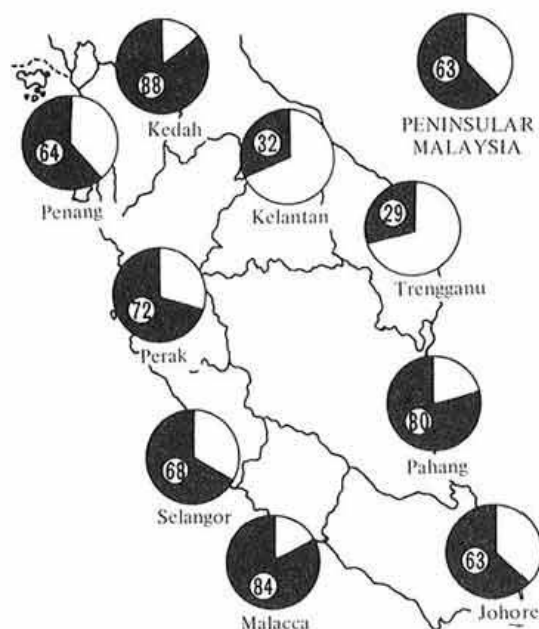


Fig. 2. Distribution of the serologically *Theileria* positive cattle in Peninsular Malaysia

The serological responses were examined by ELISA using *T. buffeli* antigen. Shaded area shows the percentage of positive cattle (ELISA O.D. > 0.2) in various states.

the same animals. A significantly higher ( $P < 0.001$ ) parasitaemia is seen in calves aged less than 3 months (3.52% average) as compared to that of adult animals aged 6 months and above (0.61% average). All the blood smears from calves less than 1 month of age, however, had no *Theileria* parasites. Based on the values of packed cell volume, which were less than 20%, three cattle were considered anaemic. Almost all the animals above 1 month of age had relatively high ELISA O.D.s, but there was no significant difference at  $P < 0.005$  between the calves with the average O.D. of 0.66 and the adults with the average O.D. of 0.74. Some of the serum samples collected from the young calves of less than 1 month old showed high O.D.s as seen in Table 2, while no *Theileria* parasites were seen in the blood smears.

## Discussion

It has been argued that the name of *Theileria mutans* is not appropriate for the *Theileria* species in

Table 1. Blood smears for *Theileria* parasites in 43 cattle of various age groups

Age	Range of parasitaemia (%)				Total no. examined
	—	<1.0	<2.0	>2.0	
Adult <sup>a)</sup>	0	21	6	1	28
1-3 month	0	0	2	5	7
Less than 1 month	8	0	0	0	8
Total	8	21	8	6	43

a): The animals aged 6 months and above were referred to as adult.

Table 2. Serum samples from 43 cattle of various age groups in ELISA used *T. buffeli* as antigen

Age	Range of ELISA O.D. (405 nm)				Total no. examined
	<0.2	<0.5	<1.0	>1.0	
Adult <sup>a)</sup>	0	5	19	4	28
1-3 month	1	0	6	0	7
Less than 1 month	4	4	0	0	8
Total	5	9	25	4	43

a): The animals aged 6 months and above were referred to as adult.

Malaysia and the adequate name for this protozoan should be either *T. buffeli* or *T. sergenti/T. orientalis*<sup>1,4</sup>. As presented above, almost all the serum samples tested by the ELISA reacted more intensely to *T. buffeli* antigen as compared with *T. sergenti* antigen. It is therefore suggested that the Malaysian *Theileria* species of cattle bear a serological reactivity similar to *T. buffeli* rather than to *T. sergenti/T. orientalis*. Uilenberg et al. proposed *T. orientalis* as the species name for the benign *Theileria* of cattle in Eurasia on the basis of his serological and morphological comparison<sup>7</sup>. The classification of *T. sergenti*, *T. orientalis* and *T. buffeli* groups is however still controversial. Therefore, the organism designated as *Theileria* in Malaysia should be identified more precisely on the basis of careful analyses.

A higher reactivity to *T. buffeli* antigen in ELISA was generally observed in the serum samples collected from the various states of Peninsular Malaysia. There were, however, a few exceptions which showed a lower reactivity to *T. buffeli* antigen in the samples

from the two states in the east coast of Peninsular Malaysia. This difference of ELISA reactivity might be attributed to the difference of *Theileria* stocks. The stock variation in *T. parva* which causes East Coast fever in East and Central Africa is widely known<sup>6</sup>.

The results of the examination of blood samples collected from the field cattle of various ages revealed higher parasitaemia in calves of 1-3 months old as compared to that in adult animals. The calves less than 1 month old did not show any parasitaemia in the smears. This evidence suggests that the calves in Malaysia be infected with the *Theileria* parasite at an early age (a few weeks after birth), because this parasite requires an incubation period of at least a few weeks before the appearance in the peripheral circulation<sup>3,7</sup>. Some of the serum samples collected from the calves aged less than 1 month showed a high ELISA O.D., although no *Theileria* parasites were seen in the blood. This suggests the presence of maternal antibody. The effect of the infection by *Theileria* on the cattle production in Malaysia is still not clearly identified. It is also not clear whether the specific antibody against the parasite is protective against the *Theileria* parasite from ticks. Therefore, further research will be necessary to clarify the importance of Theileriosis for livestock in this country from the viewpoint of pathogenicity and epidemiology.

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