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THE INFECTIVITY AND VIRULENCE OF *TRYPANOSOMA EVANSI* IN LOCAL INDIAN DAIRY X KEDAH-KELANTAN CATTLE

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Keywords: Infectivity, Intermittent fever, Local Indian Dairy x Kedah-Kelantan cattle (LID x KK), Parasitaemia, *Trypanosoma evansi,* Virulence.

RINGKASAN

Satu strain *Trypanosoma evansi* (TE) telah diasingkan baru-baru ini dari kerbau sawah di Pusat Ternakan Kerbau MARDI, Bukit Ridan, Pahang, Malaysia Barat. Beberapa kajian telah dilakukan untuk menentukan infektiviti dan kevirulenan parasit ini pada lembu-lembu tempatan. Enam ekor lembu tempatan baka Local Indian Dairy x Kedah-Kelantan (LID x KK) telah disuntik secara subkutin dengan 5.0 x 10^7 TE setiap lembu. Didapati lembu-lembu ini rentan kepada infeksi TE. Parasitemia yang parah berpanjangan selama 34 hari selepas infeksi bermula. Kenaikan suhu tubuh berlaku pada semua haiwan berinfeksi dan kejadian ini dicerap hanya pada peringkat awal infeksi. Walau bagaimanapun didapati kenaikan parasitemia ini tidak berlaku secara serentak dengan kenaikan suhu tubuh haiwan. Di samping itu perubahan dalam nilai-nilai hematologi (RBC, PCV dan Hb) telah juga ditentukan.

Lembu-lembu yang pulih dan menjadi imun dari infeksi awal telah dicabar sekali lagi dengan dos gandaan (iaitu 1.0 x 10^8 TE), 290 hari selepas infeksi awal. Lembu-lembu ini menunjukkan pembentukan parasitemia yang lewat (kelewatan selama tiga hari dari infeksi biasa). Demam yang berulang-ulang telah juga dicerap.

INTRODUCTION

Surra or trypanosomiasis is known to be prevalent amongst horses, camels, cattle and buffaloes in various parts of Africa, India, other Asian countries and many other parts of the world. The disease is invariably fatal in camels and horses. Cattle and buffaloes are usually resistant to the disease, but under stress and in the young, the disease may prove fatal (LINGARD, 1899; MUKHERJI, 1925; LYER and SARWAR, 1935; BRAR and SHARMA, 1962; VERMA, 1973).

For buffaloes, especially in South East Asia, the agent of the disease, the trypanosomes, become one of the most important protozoan parasites although results obtained from experimental infections have been somewhat variable. In Malaysia, the prevalence of trypanosomiasis in cattle and buffaloes is practically unknown and the disease has not received the same attention as in the equines. Surra in horses and cattle has been recorded in this country for the first time at the turn of the century (FRAZER and SAYMONDS, 1909). An outbreak of the disease in horses had occurred in the Equine Unit of the Universiti Pertanian Malaysia, Serdang, Selangor (NG and VANSELOW, 1978). Local cattle and buffaloes act as the reservoir hosts of the parasite (NG and VANSELOW, 1978) and the parasite is considered as non-pathogenic and of no economic importance. These two types of animals have also been reported not to be clinically affected by the parasite (NG and VANSELOW, 1978).

Until recently, buffaloes, calves and weaners at the MARDI Buffalo Breeding Farm, in Bukit Ridan, Pahang were reported to show signs of chronic emaciation, anaemia and even deaths (ABAS MAZNI, ZAINAL-ABIDIN and RAMAKRISHNAN, 1984). Those animals with emaciation also

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showed enlarged prescapular lymph nodes and examination of the blood indicated the presence of *Trypanosoma evansi*. The isolate was found to be very pathogenic to mice, rats and Mongolian gerbils (ZAINAL-ABIDIN, SWAIBAL, SABARINAH, KWONG and ABAS MAZNI, 1982).

This paper reports some of the results of the observations on the infectivity and virulence of this parasite in our local cattle.

MATERIALS AND METHODS

Experimental Animals

Eleven healthy Local Indian Dairy x Kedah-Kelantan (LID x KK) calves of 8–12 months old were used in the study. Microscopic examinations of wet blood films, blood smears and subinoculations of fresh blood into ICR strain mice indicated that they were free from trypanosome infection. The calves were kept in groups of two in a pen under an enclosed building. Each calf received 2 kg commercial concentrate and Napier grass *(Pennisetum purpureum) ad libitum* daily.

Parasite and the Preparation of Inoculum

The strain of *Trypanosoma evansi* (TE) used in this study was obtained from an infected swamp buffalo from MARDI Buffalo Breeding Farm in Bukit Ridan, Pahang. It was maintained in mice by weekly passage of infected blood.

For the preparation of inoculum, blood from several infected mice having the same degree of parasitaemia was used. The blood was normally drawn at the peak of parasitaemia which occurred in the third or fourth day post infection. The blood was drawn by cardiac puncture and diluted serially using cold normal saline (0.85%) or citrate solution. The final dilution was adjusted to contain 5.0×10^7 TE per millilitre.

Experimental Design

In the first experiment, 11 calves were randomly divided into two groups. Group I consisted of five animals as the control and received 1.0 ml sterile normal saline given subcutaneously. Group II consisted of six animals infected with 5.0×10^7 TE per animal subcutaneously. A second experiment was carried out 290 days after the first infection whereby three calves in Group I and all calves in Group II were given a challenge dose of 1.0×10^8 TE per animal subcutaneously.

Examination of Wet Blood Films and Smears

Wet blood films and thin blood smears were prepared daily using blood obtained from the ear vein of the infected animals. They were prepared early in the morning and used for observation and quantification of parasitaemias. The wet blood films were used for the estimation of degree of parasitaemia using the following adaptations (CABRERA and LUI, 1956):

+	: 1 – 2 trypanosomes per field
++	: 3 – 4 trypanosomes per field
+++	: many trypanosomes per field
++++	: teeming numbers per field

The films were observed under microscope using X40 magnification.

The thin blood smears were fixed in absolute alcohol and stained with the Giemsa's stain and observed under microscope (X100 magnification) to confirm the presence of the parasites.

Observations on Symptoms, Rectal Temperature and Body Weight

Infected animals were examined daily to observe their general body conditions, appetite and behaviour. Daily rectal temperatures were recorded at 8 am and 12 noon, 4 pm and 8 pm. Weekly body weights were also recorded.

Haematological Examinations

Weekly haematological examinations were done on blood samples collected from

the jugular vein into vacuumtainers containing EDTA and analysed for red blood cell count (RBC) and haemoglobin (Hb) concentration using the Coulter Counter ZF 6 system. Packed cell volume (PCV) was determined by the haematocrit method and the plasma protein (PP) was read on the refractometer (Bellingham and Stanley Ltd. England).

Biological Tests

Monthly biological tests were carried out to determine the sterility of the blood in the infected and control animals. For this, blood was collected from the animals and then subinoculated intraperitoneally into mice. Wet blood films were prepared from these mice every other day for a period of up to 20 days and observed for the presence of the parasites.

RESULTS

Clinical Signs of the Infection

All infected animals developed clinical infections and exhibited symptoms associated with a mild chronic form of surra. Preliminary transient rise in the rectal temperature, enlargement of prescapular lymph nodes and occasionally prefemoral lymph nodes and a moderate degree of anaemia were some of the obvious signs *(Plate 1)*. The animals recovered completely after 30 weeks post infection.

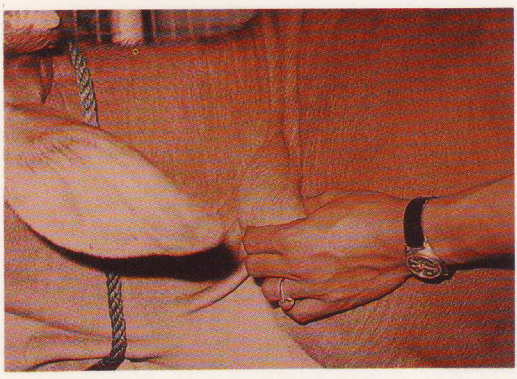

Plate 1. The enlargement of prescapular lymph node in the infected animal.

The rise in the rectal temperature at the early stage of the infection was observed in all infected and challenged animals *(Figure 1)*. This initial rise in the rectal temperature lasted for about four days after which the animals became afebrile. However, there was also individual rise in the rectal temperature at different stages of infection in some of the animals. Five out of six animals in Group II which received parasite challenge in second experiment showed distinct patterns of intermittent fever lasting for about 35 days post challenge *(Figure 2)*.

The incubation period, prepatent period, peak temperature, length of initial fever and paroxysm of parasitaemia of the infected and challenged animals are shown in *Table 1*. The prepatent periods of Group II animals following their first infection and challenge were 4.67 ± 1.86 and 8.50 ± 1.76 days respectively. The difference in these values was highly significant ($P<0.01$), indicating a distinct delay in the onset of parasitaemia in animals receiving challenge. Following challenge, Group I and Group II animals also showed a significant difference ($P<0.05$) in their incubation periods. However, there was no significant difference in the peak rectal temperature, length of initial fever and duration of infection following challenge between the two groups.

Parasitaemia and the Relationship with Temperature

There were fluctuations in the levels of parasitaemia in all infected animals. Irrespective of the two different infective doses of the parasite, the animals in Group I and Group II had intense parasitaemia at least once, especially at the early stage of the infection. This pattern was observed to occur within 15 days post infection and was followed by low parasitaemia until the 34th day.

The percentage of animals showing parasitaemia post infection in the individual groups is shown in *Figure 3*. In most

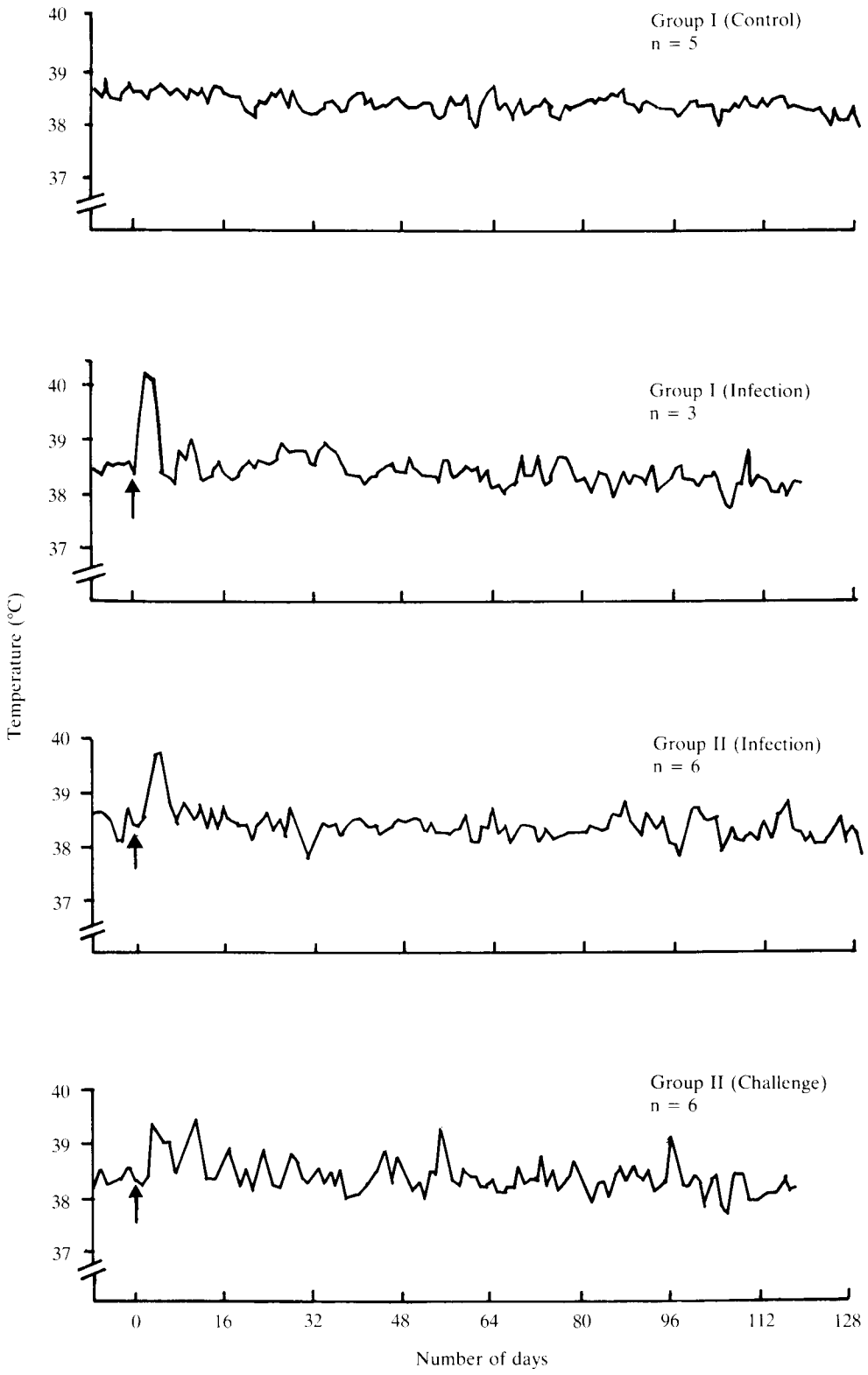

Figure 1. Comparison of mean daily rectal temperature in experimental animals.

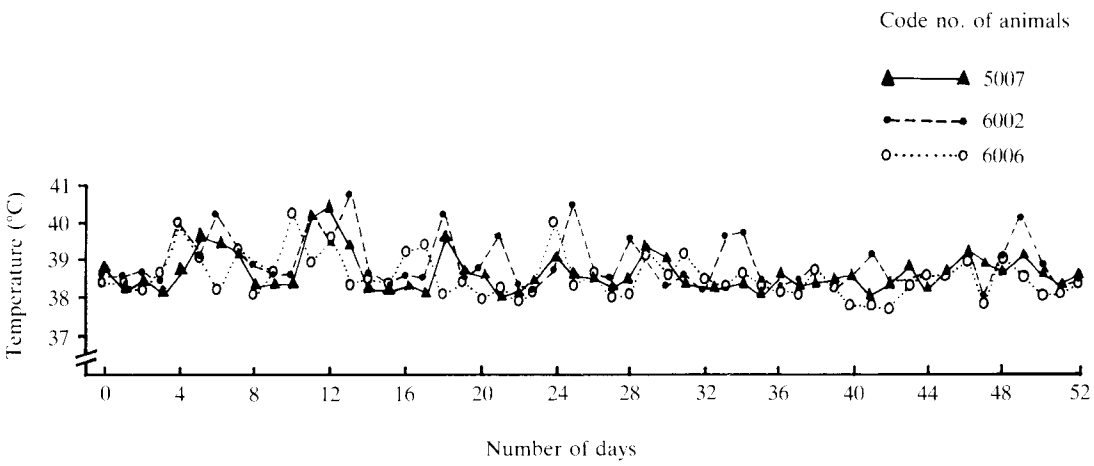

Figure 2. The course of intermittent fever in 3 animals in Group II following challenge.

Table 1. Mean prepatent period, incubation period, peak temperature and length on initial fever of experimental animals

Treatment group	No. of animals	Prepatent period (days)	Incubation period (days)	Peak temperature of initial fever (°C)	Length of initial fever (days)
II (Infection)	6	4.67±1.86b**	3.17±0.98ab*	40.56±32.0	4.0±1.26
II (Challenge)	6	8.50±1.76a	3.67±0.52a	40.47±0.44	3.5±0.84^{+}
I (Infection)	3	4.67±3.79ab	2.33±0.58b	40.97±0.47	4.0±1.0

$^{+}$Presence of intermittent fever
*Means in the same column followed by different letter differ at $P<0.05$.
**Means in the same column followed by different letter differ at $P<0.01$.

animals, parasitaemia did not usually coincide with the rise in body temperature. However, intense parasitaemia which occurred at the early stage of infection associated with a marked increase in rectal temperature. At the later stage of the infection, parasites were also seen when there was no fever.

Body Weight and Average Daily Gain

All infected animals showed decrease in body weight during the first two weeks post infection at the time when the fever and parasitaemia were high. However, significant difference ($P<0.05$) in the average daily gain between Group I and Group II animals during the first experiment was only observed in the third month post infection. Following challenge there was also significant difference ($P<0.05$) in the average daily gain during the second and fourth months post infection *(Table 2)* between these animals.

Biological Tests

The biological tests were always positive while the peripheral blood smears were negative on many occasions. Animals in Group II were positive up to fourth month post infection after which only two of them still harboured the parasite up to the fifth month.

Haematological Changes

Infections have been observed to be associated with the reduction in PCV, Hb and RBC values. In Group II animals, these values deteriorated gradually between the first and 14th weeks post infection. The PCV value of this group dropped by 16.5%, from a preinfection mean of 36.3% to a

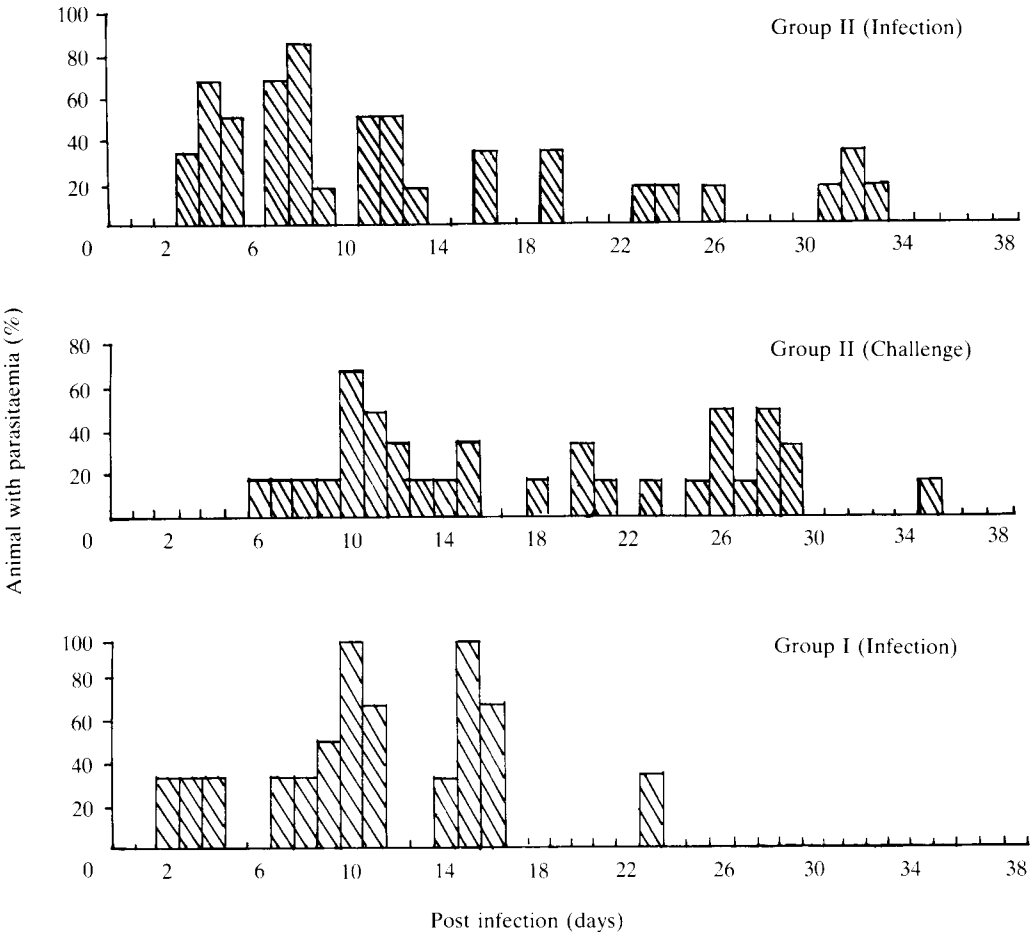

Figure 3. The percentage of animals with parasitaemia for the first 38 days post infection.

Table 2. Average daily gain in all experimental animals

Treatment group	No. of animals	Average daily gain (kg) months post infection					
		1	2	3	4	5	6
I	5	0.04a*	0.18a	0.20a	0.20a	0.21a	0.17a
II	6	−0.26a	−0.01ab	0.07b	0.80a	0.10a	0.10a
II (Challenge)	6	−0.01a	−0.06b	−0.05b	−0.10b	0.02a	0.60a
I (Infection)	3	0.10a	−0.20b	−0.01b	0.03b	0.05a	0.07a

*Means in the same column followed by different letter are significantly different at $P<0.05$.

mean of 30.4% *(Figure 4)*. The PCV value of Group I following infection dropped rapidly by 23.3% from a preinfection mean of 43% to a mean of 35 per cent.

The PCV , Hb and RBC values of Group I and Group II after their first infection and challenge were different from the preinfection values of Group I between the first and sixth months post infection *(Figure 5)*. The post infection values became quite similar to the preinfection values only after six months.

Plasma proteins (PP) in Group II were higher as compared to Group I *(Figure 6)*. A

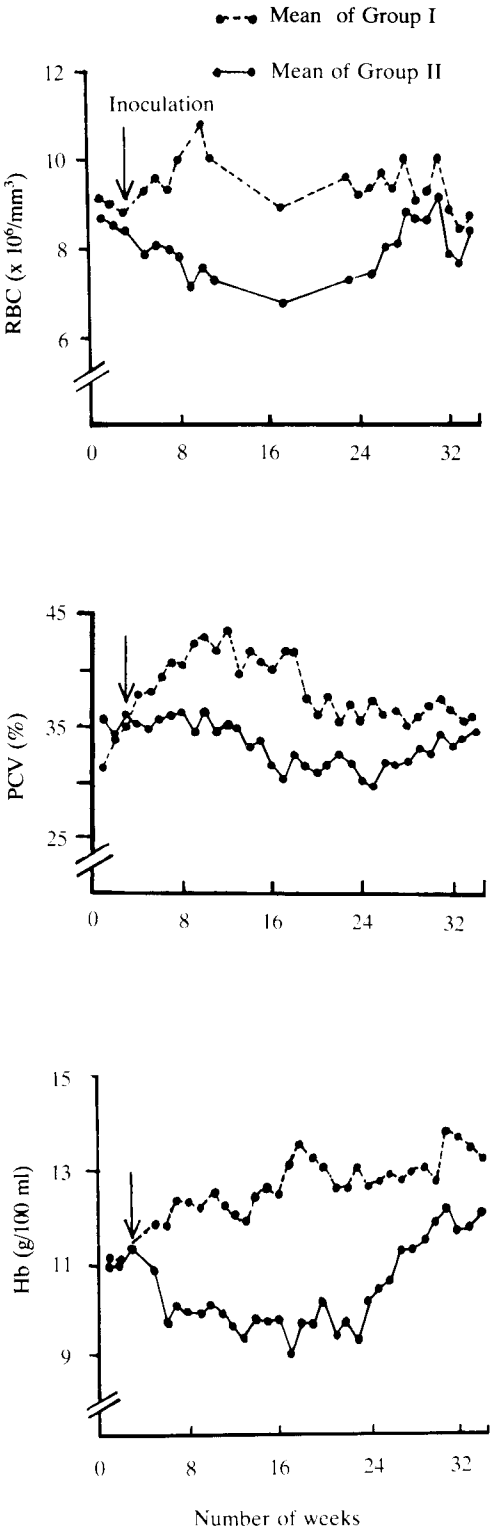

Figure 4. Red blood cell counts (RBC), packed cell volumes (PCV) and haemoglobin (Hb) concentrations of experimental animals.

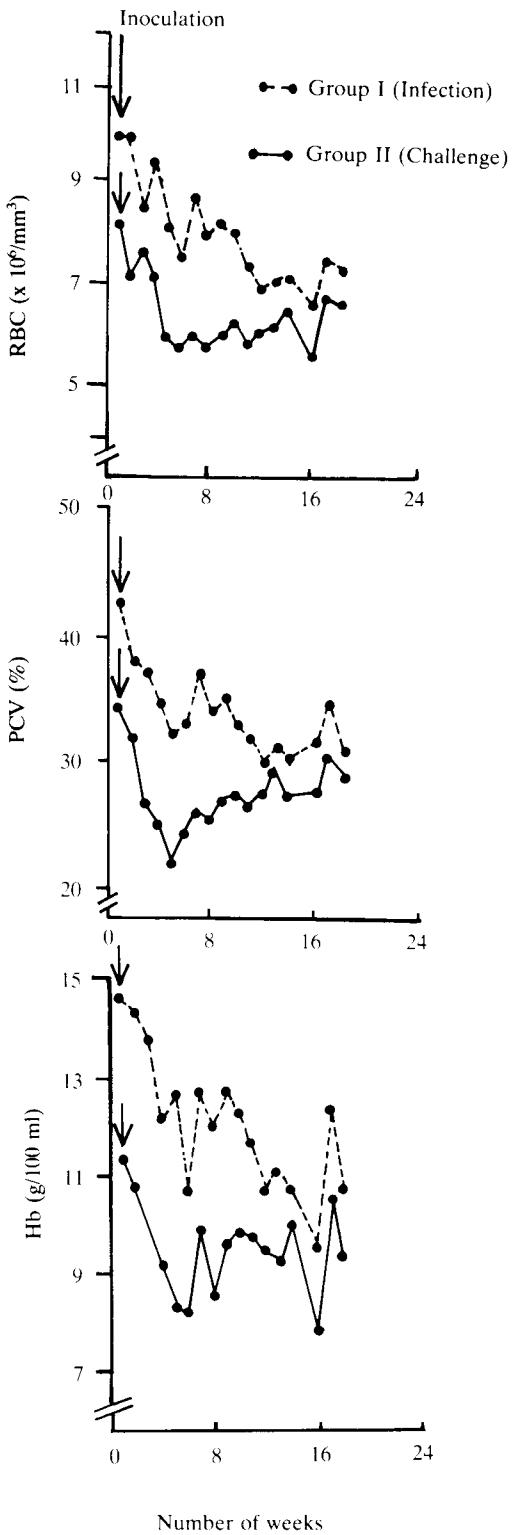

Figure 5. Red blood cell counts (RBC), packed cell volumes (PCV) and haemoglobin (Hb) concentrations of experimental animals.

sudden increase in the PP value of Group II was observed during the period between the first and fourth months post challenge. There was also a significant increase in the PP value ($P<0.05$) of Group I before and after infection during the third and fourth months post infection *(Figure 6)*.

DISCUSSION

The course of the disease due to *T. evansi* infections in LID x KK cattle was of a mild type surra. The main symptoms observed were preliminary rise in rectal temperature, enlargement of prescapular lymph nodes and occasionally prefemoral lymph nodes, decrease in body weight and a moderate degree of anaemia with eventual recovery of the animals. Similar observations have been recorded by other workers and the authors' finding may indicate that cattle used in this study were relatively more resistant to *T. evansi* (LINGARD, 1899; HUTGRA and MALEK, 1912; MUKHERJI, 1925; MAHAJAN, 1934; LYER and SARWAR, 1935). Some of the animals were found positive with the parasite up to the fifth month post infection and this may also indicate the existence of a carrier stage in the infection.

Incidence of parasitaemia in the peripheral blood was intermittent. Fluctuations in the parasitaemia were indicated by periodic peaks and the absence of the trypanosomes from the circulation which was eventually followed by low parasitaemia. In the later stages of the infections, the parasite could only be detected by intraperitoneal injection of the blood into clean mice. Similar findings were also observed by VERMA (1973) and RAZZAQUE, MISHRA and SAHAI (1976). The variation in the parasitaemia noted within the individuals can be related to the capacity of the individual to delay or limit the level of parasitaemia. In the challenged animals (Group II), three of them still showed intense parasitaemia following challenge. Furthermore, it was also found that the high (intense) parasitaemia did coincide with the rise in the body temperature although BRAR and SHARMA (1962), BANSAL (1966), SRIVASTAVA, MALHOTRA and LYER (1969), CHAND and SINGH (1971) and VERMA (1973) had reported that no correlation between parasitaemia and the rise in the body temperature during *T. evansi* infections in buffaloes, dogs, rabbits and donkeys.

In the present study, slight to moderate decrease in the haematological values were also observed in the infected animals during the first to the sixth months

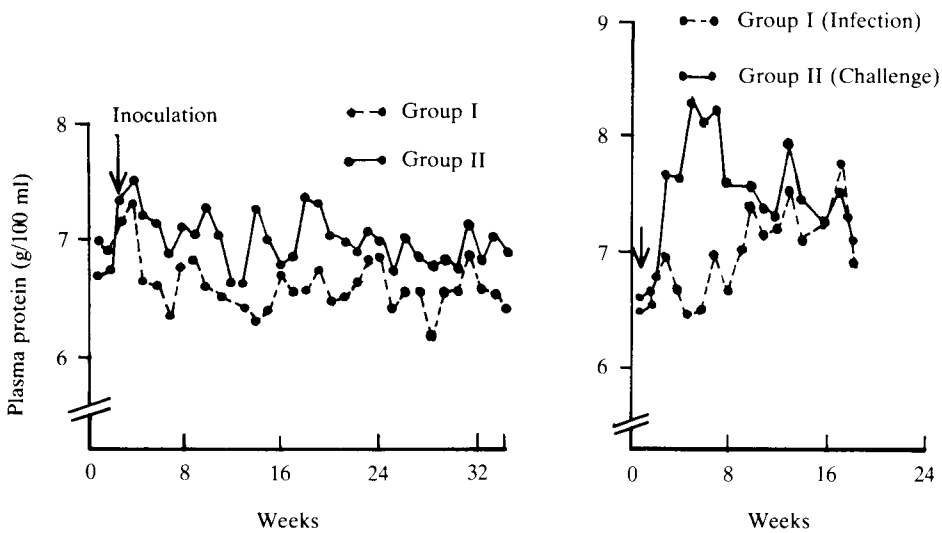

Figure 6. Values of plasma proteins in all experimental animals.

post infection. During this period, these values decreased when compared with those of the control. Then thereafter, the values were found to be within those of the control. Although this parasite did not cause severe anaemia, there was indication that it did decrease the blood values. The anaemia observed was of a progressive type. It fell into two distinct phases : an initial or 'acute' phase, characterized by a rapidly developing anaemia and accompanied by high level of parasitaemia and a 'chronic' phase during which the low PCV levels remained static for the extended period. During this chronic phase there was little or absence of parasites in the peripheral blood. Even when parasites could no longer be detected in the blood, PCV values showed little tendency to recover and it took up to six months for the blood pictures to return to the values of the control. Similar patterns were also recorded in *T. congolense* infection (FIENNES, 1954; 1970).

Anaemia is the most significant factor in the disease process in naturally occurring and experimentally induced bovine trypanosomiasis (HORNBY, 1921; MURRAY, 1974). These mechanisms, acting singly or in concert, have been implicated as the factors underlying the anaemia. These are haemodilution (FIENNES, 1954; NAYLOR, 1971; HOLMES, 1976), increased red cell breakdown (MAMO and HOLMES, 1975; HOLMES, 1976; PRESTON and WELDE, 1976) and reduced cell synthesis (FIENNES, 1954; 1970). There have been reports suggesting that anaemia accompanying *T. evansi* infection is due to the inhibition of erythropoeitic activity in the bone marrow (RICHARDSON and KENDALL, 1962; SRIVASTAVA *et al.*, 1969). There is also a possibility of an immunologically mediated mechanism being responsible for the development of the accompanying anaemia (ASSOKU, 1975).

Animals infected with a massive dose of 1.0×10^8 TE showed a much higher PP value. Similarly there was a marked increase in this value in the challenged animals at the initial stage of the infection. This is probably due to an increase in the globulin level as reported by VERMA (1973) in calves infected with *T. evansi.*

The results from the study indicated that the local LID x KK cattle were susceptible to *T. evansi* with mild severity although it caused emaciation and anaemia with eventual recovery. Earlier studies have indicated that N' Dama breed was trypanotolerant and partially resistant to trypanosomiasis as compared with the Zebu breed, to the same disease (FAO, 1976). From the results of the present study, it can be suggested that LID x KK cattle were also trypanotolerant to *T. evansi.*

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ABSTRACT

A strain of *Trypanosoma evansi* (TE) was recently isolated from swamp buffalloes at Bukit Ridan Station, MARDI, Pahang in West Malaysia. Some studies were carried out on the local cattle to determine the infectivity and virulence of the parasite. Six local cattle, Local Indian Dairy x Kedah-Kelantan (LID x KK), were infected subcutaneously with 5.0×10^7 TE per animal. It was found that the local cattle were susceptible to this parasite. Intense parasitaemia in the blood persisted until 34 days post infection. A rise in the body temperature was observed in all infected animals but only at the initial

stage of the infection. However, it was found that the rise in parasitaemia did not coincide with the rise in the body temperature. Changes in the haematological pictures (RBC, PCV and Hb) were also observed. Recovered animals were challenged with a double dose *i.e.* 1.0 x 10^8 TE, 290 days after the initial infection. It was found that all the animals showed a delayed parasitaemia (*i.e.* three days later) as compared with the parasitaemia in the control. Intermittent fever also occurred in these animals.

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