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Comparative studies on the infectivity of *Theileria parva* in ticks fed *in vitro* and those fed on cattle

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Abstract. Nymphs of the brown ear tick, *Rhipicephalus appendiculatus*, were fed on heparinised bovine blood infected with *Theileria parva* parasites in an *in vitro* feeding system consisting of rabbit skin membranes. The main feeding and development parameters such as the mean attachment rate, feeding duration and engorgement weights of membrane-fed ticks were not significantly different from nymphs fed on cattle. The moulting rate was also comparable although a slight significant difference was observed. Assessment of infection prevalence and abundance with *T. parva* in adults indicated that the membrane-fed ticks acquired infection to the same level as those fed on cattle. Stabilates prepared from both the membrane- and cattle-fed adult ticks were found to be infective and caused severe reactions in susceptible cattle. When the immunised cattle were challenged with a lethal homologous dose of *T. parva* (Marikebuni), they were found to be immune.

Introduction

East Coast Fever (ECF) caused by the haemoprotozoan, *Theileria parva*, is a lymphoproliferative disease of cattle. It is naturally transmitted by the brown ear tick, *Rhipicephalus appendiculatus*. The disease or its variant forms is considered to be an important constraint to the improvement of the livestock industry in large areas of east, central and southern Africa (Norval et al. 1992). It is responsible for high morbidity and mortality in some production systems.

The control of theileriosis has previously been achieved with varying degrees of success in several countries by vector control as part of a general tick and tick-borne disease control strategy (Perry and Young 1995). In recent years however, tick control has become less reliable because of the high cost of purchasing and application of acaricides, development of resistance by some species and poor enforcement of regulations regarding cattle movement and quarantine (Norval and Young 1983; Winrock International 1992). The current shift from public to private provision of animal health services has made the situation worse. Therefore, sustainable ticks and tick-borne disease control strategies that are affordable by resource-limited farmers are desirable.

A vaccination strategy against ECF, known as 'Infection and Treatment method', has been available for some time. It is based on the use of live *T. parva*

organisms and involves the simultaneous inoculation of cattle with live potentially lethal sporozoites and a long acting formulation of oxytetracycline (Radley et al. 1975). To prepare the stabilate used in this method, *R. appendiculatus* nymphs are fed on infected cattle and the resulting adults pre-fed on rabbits for 4 days (to allow for sporozoites maturation) before being ground to release the sporozoites (Cunningham et al. 1973). However, the reliance on experimental animals makes the method expensive and difficult to standardise. There is also the risk of transmitting diseases through the immunisation process as a result of contamination of the stabilate by host factors. Furthermore, there has been growing concern in recent years about the use of animals for experimental purposes and any shift away from this would be most welcome (Eckert 1997). Some of these problems could be overcome if the stabilates were to be prepared in an artificial feeding system.

In this study, we report a method of feeding *R. appendiculatus* nymphs and transmission of *T. parva* parasites through an artificial feeding system using rabbit skin membranes. The infectivity, virulence and the level of immunity conferred by immunisation with the stabilates prepared from these ticks were investigated. Suggestions on the adoption of this method in commercial production of stabilates are discussed.

Materials and methods

Experimental animals

Friesian steers (8–12 months old) were used in all experiments. They were obtained from a farm free of theileriosis through strict tick control. This was confirmed by the indirect fluorescent antibody test-IFAT (Goddeeris et al. 1982). Nymphs were attached on the ears of the steers using the method of Bailey (1960). Body patches of cotton material (40 cm $\times$ 30 cm) were also used. The cloth patches were glued on to the body using Evo-stik impact adhesive glue (Evode Limited, Stafford, England) after clipping the hair. New Zealand white rabbits (3–4 months old) from the National Veterinary Research Centre (NVRC, Muguga) rabbit colony were used for pre-feeding of ticks to induce *T. parva* sporozoite maturation. Membranes for *in vitro* feeding were obtained from rabbits (3–5 weeks old).

Rhipicepthalus appendiculatus nymphs (2–4 months old) of the Muguga stock were used in all the experiments. This is a laboratory colony that has been maintained on rabbits since 1952. For the developmental stages, ticks were maintained in an incubator maintained at $28 \pm 0.5\,^{\circ}$C and 85–90% relative humidity.

Theileria parva (Marikebuni) stabilate NVRC-316 (Anon 1994) cryopreserved at $-186\,^{\circ}$C in liquid nitrogen was used at 1:5 dilution to infect the steers in all transmission trials. Before use, 1 ml of the stabilate was thawed, diluted in 3.5% bovine phosphate albumin, Minimum Essential Medium (MEM) and 7.5% glycerol in a universal bottle. The material was left to stabilise at $37\,^{\circ}$C for 30 min.

Preparation of membranes

Membranes were obtained by euthanising the rabbits and removing the skin aseptically. The skin was cut into 7 cm diameter pieces. These were sterilised in 70% alcohol and rinsed in two changes of sterile distilled water and then in two changes of sterile phosphate buffered saline (PBS: 0.01 M Na_2HPO_4, 0.01 M KH_2PO_4 and 0.3 M NaCl, pH 8.0). They were sterilised again by immersion in a mixture of antibiotics (Penicillin – 300 IU/ml (Hindustan Antibiotics, India), Streptomycin – 300 IU/ml (Gibco, USA) and Gentamycin – 100 IU/ml (Gibco, Scotland) and Mycostatin – 100 IU/ml (Sigma, USA) for 5 min. The membranes were either used fresh or stored at $-20\,^{\circ}C$.

Olfactory stimuli

Concentrated ear wash was obtained from adult cattle, which had not come into contact with acaricides for at least 6 months. It was obtained by soaking cotton wool in diethyl ether, scrubbing both the inside and outside of the ears and dipping it back into the ether and squeezing off the wash. This was repeated several times. A total of 250 ml of ether was used to wash both ears of each cattle. The washings from all the animals were pooled and allowed to stand in a beaker for 2 h for the debris to settle. The clear supernatant fraction was collected and sterilised using 0.22 μm filters (Millipore, SA, France). Tick faeces obtained from tick-incubating tubes together with the exuviae of moulted *R. appendiculatus* nymphs were ground into a fine powder. The powder was stored in 5 ml Bijou bottles and sterilised by keeping the bottles in the gas-phase of a liquid nitrogen container overnight prior to use.

The tick-feeding unit

The tick-feeding unit consisted of two cylindrical plastic containers (Figure 1). The lower container (the tick chamber) measured 6.5 cm in diameter by 3 cm high. The bottom end of this container was covered with a black muslin cloth. A sealable hole (1 cm in diameter) was made at the bottom edge through which ticks were introduced. The upper container (the blood chamber) measuring 5 cm in diameter and 5 cm high with a lid on the top end was covered with the skin membrane at the bottom end (with the hairy side of the skin on the outside) and fixed in place by autoclave tape. This container was inserted into the lower container so that the membrane was just inside the container. The two containers were joined together with autoclave tape. The edges of the skin membrane were regularly swabbed with a mixture of antibiotics and mycostatin to prevent contamination and then dried. Blood was introduced into the blood chamber through a 1 cm diameter hole, which was sealed with fine nylon gauge.

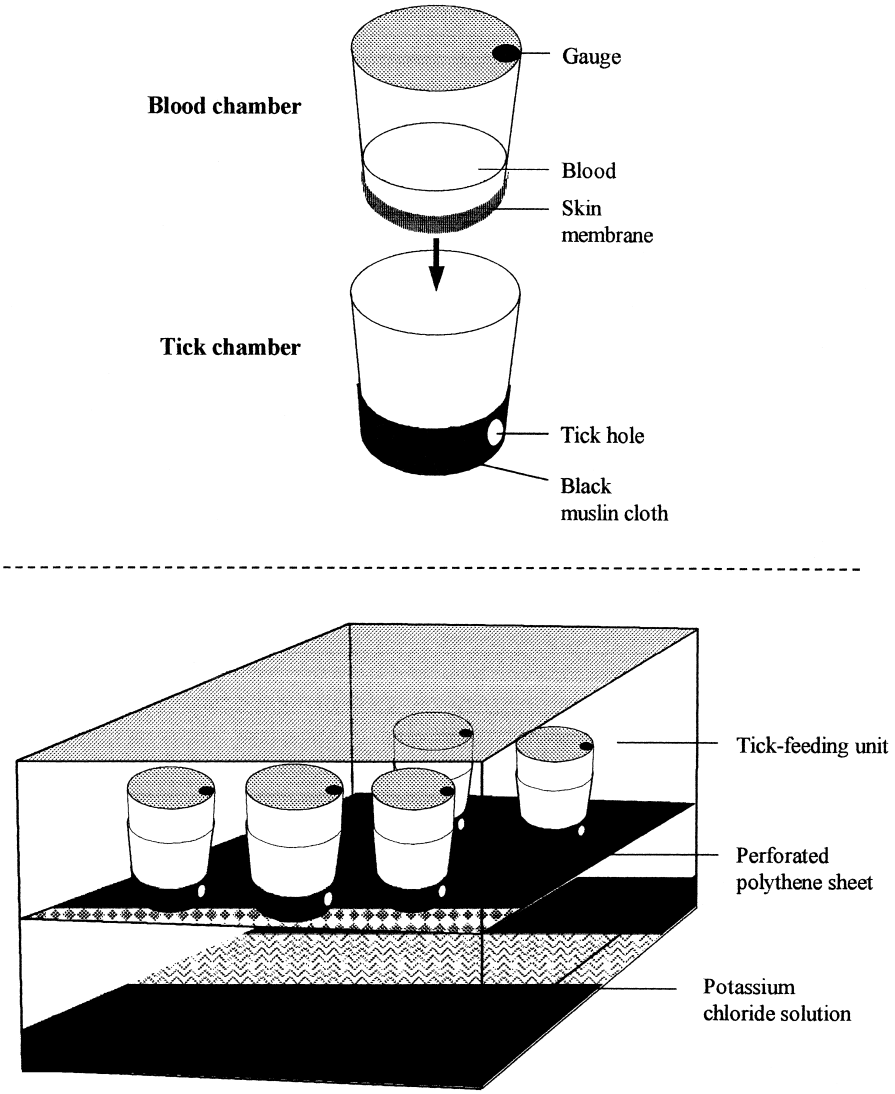

Figure 1. Diagrammatic representation of the *in vitro* feeding system.

The tick-feeding unit was placed on top of a tough polythene paper fixed inside a plastic box measuring $23 \times 25 \times 15$ cm. The polythene paper was perforated in several places to allow the free circulation of air. One litre of saturated potassium chloride (KCl) solution was placed in the bottom of this box to humidify the chamber. Up to 5 tick-feeding units could be placed in one box (Figure 1). The whole apparatus was put inside a dry CO_2 incubator (Flow Laboratories, UK) set at $37 \pm 0.5\,^{\circ}C$ and 3% CO_2.

Jugular blood from either a healthy donor or one infected with *T. parva* was collected once a day. Some of the blood was used immediately after collection and the rest was stored at 4 °C for up to 8 h. Blood was changed twice every day under a sterile hood. After discarding the old blood, the container was rinsed twice with sterile PBS and then with a mixture of antibiotics.

Harvesting of replete ticks

Replete ticks were harvested by disconnecting the two parts of the feeding unit. The ticks were counted, weighed and incubated at 28 °C for further development.

Transmission of T. parva

A naive steer was inoculated sub-cutaneously above the pre-scapular lymph node with 1 ml of a 1:5 dilution of *T. parva* (Marikebuni) stabilate 316. It was monitored by taking daily rectal temperature from day 0, the pre-scapular lymph node biopsy smears from day 5 and blood smears from day 7. The slides were stained in Giemsa solution (Merck, Germany) and used to assess piroplasm and schizont parasitaemia. Piroplasm parasitaemia was calculated by counting the number of infected red blood cells (RBCs) in four fields at $\times 1000$ magnification. The schizont parasitaemia was assessed by estimating the average number of schizonts infected cells in a field at the same magnification. Reactions were classified according to the criteria adopted by the committee on the classification of *Theileria* reactions in cattle (Anon 1989). From day 9 after infection of the steer, ~ 500 nymphs were introduced into each feeding unit and maintained on blood obtained from a clean bovine donor for the first 2 days. From day 3, blood from an infected steer was used until the nymphs engorged and dropped. On day 11 after infection, ~ 2000 nymphs were fed onto the donor steer using ear bags and body patches as described above. Both membrane- and cattle-feeding were synchronised so that ticks fed on blood from roughly the same stage of infection. Replete nymphs dropping each day from either feeding system were collected, counted and incubated separately for further development.

Assessment of infection prevalence and abundance

Four weeks after moulting, 30 males and 30 females from the membrane- and cattle-fed ticks were dissected and the salivary glands of each tick assessed for infection prevalence and abundance (Buscher and Otim 1986). The infection prevalence (the proportion of infected ticks in a batch of ticks assessed expressed as a percentage) and the abundance (number of infected acini/tick) were computed.

Preparation of stabilate

After assessment of the prevalence and abundance of infection, 50 adults from each feeding system were randomly selected and pre-fed on rabbits for 4 days. Based on the prevalence and the abundance of the infection in the male and female ticks fed by the two systems, the number of ticks needed to provide the same number of infected acini in the stabilate was estimated using the formula.

If π_{ij} and λ_{ij} are the prevalence and abundance of infection of the ith sex in the jth feeding system. Further, if $N_j = \Sigma_i n_{ij}$ are the number of ticks in the jth feeding system, where n_{ij} is the number of ticks of ith sex in the jth feeding system.

Then, $N = \Sigma_j \Sigma_i N_j (\pi_{ij} + \lambda_{ij}) = \Sigma_j \Sigma_i n_{ij} (\pi_{ij} + \lambda_{ij})$ is the number of ticks needed to provide the same number of infected acini in the stabilate.

We estimated π_{ij} and λ_{ij} as follows:

$$\pi_{ij} = \frac{x_{ij}}{n_{ij}} \quad \text{and} \quad \lambda_{ij} = \frac{y_{ij}}{n_{ij}}$$

where x_{ij} and y_{ij} are number of infected ticks of the ith sex in the jth feeding system and number of infected acini for the ith sex in the jth feeding system respectively and n_{ij} is as defined earlier.

Using the formula above, 45 (male and female) membrane-fed and 27 (male and female) ticks fed on steers both with a total of 18 infected acini were randomly selected. A stabilate was prepared according to Cunningham et al. (1973).

Four naive steers, 8 months old were each inoculated with 1 ml of a 1:5 dilution of this stabilate. Two of the steers were inoculated with the homogenate from ticks fed on membranes and two with the homogenate from the ticks fed *in vivo*. The steers were monitored as above. In addition blood for haematology was taken three times per week for the duration of the trial. Clinical cases of ECF were treated with Butalex (Pittman-Moore Ltd.).

After recovery each of the four steers and an additional two controls which had been shown to be free of *T. parva* antischizont antibodies were inoculated with a lethal dose of *T. parva*-(Marikebuni) sporozoite stabilate 316 and monitored as previously described.

Data analysis

Data analysis was carried out using Statistical Analysis System (SAS) – release 2000 8.01 TS level 01M0. *T*-test was carried out to compare the feeding and developmental parameters for ticks in the two feeding systems. Multivariate analysis using SAS general linear model (GLM) was used to analyse and compare infection rates, infection abundance and the other parasitological and haematological parameters. Tukey's test was carried out to compare individual means.

Results

Feeding and development parameters of membrane- and cattle-fed R. appendiculatus *nymphs*

Table 1 summarises the feeding and development patterns of the nymphs fed through membranes and those fed on steers. The attachment rate (the proportion of the nymphs applied and those that fed to repletion), the pre-moulting period (the time from harvesting to the start of moulting) and the moulting duration (the time from start to completion of moulting) of membrane- and cattle-fed nymphs were found to be very similar. The engorged weights of nymphs fed on steers were comparable and their means were not significantly different. Further, nymphs feeding through membranes continued to feed for a slightly longer period but these differences were not statistically significant. However, significant difference was observed in the moulting success of the nymphs from the two systems.

Prevalence and abundance of T. parva *infection in membrane- and cattle-fed ticks*

The infection prevalence with *T. parva* in both male and female adult ticks fed as nymphs through membranes or on steers was found to be comparable (Table 2). The infection prevalence in females was 53.3% in the membrane- and 50.0% in cattle-fed ticks, while in males, the values were 30 and 33.3% for membrane- and cattle-fed ticks, respectively. The infection abundance values in females were 1.37 and 1.4 in the membrane- and cattle-fed ticks, respectively. However, the abundance in males was lower in ticks fed through membranes (0.5) than in cattle-fed ticks 2.0. However, the differences in the infection prevalence and infection abundance were not statistically significant.

Infectivity of sporozoites prepared from membrane- and cattle-fed ticks

Table 3 shows the parameters used to evaluate the infectivity of the sporozoites from ticks fed by the two systems. Cattle responses to infection with the stabilates appeared to be similar in all the parameters assessed. The temperature reaction of both pairs of steers was virtually identical (Figure 2). Both stabilates caused severe ECF reactions in the steers requiring treatment on day 9. One of the steers immunised with the stabilate from cattle-fed ticks required a second treatment dose after 3 days due to recurrence of parasitaemia. The trends of the haemoglobin (Hb) levels, the red blood cell RBC counts, white blood cell (WBC) counts and packed cell volume (PCV) for the two pairs of steers are shown in Figures 3–6. All the parameters showed close similarity and the trends were consistent with clinical ECF. This was manifested by a steady decline in all the haematological parameters from day 6–8 post-infection (Figure 5). The WBC count showed a marked rise from

Table 1. Feeding and development of membrane- and cattle-fed *R. appendiculatus* nymphs.

Parameters	Membrane-fed ticks	Cattle-fed ticks
Mean attachment rate (%)	60.40 ± 6.59a	58.5 ± 6.43a
Mean feeding duration (days)	7.4 ± 0.34a	6.2 ± 0.26a
Mean engorgement weight (mg)	6.11 ± 0.05a	6.32 ± 0.13a
Mean pre-moulting time (days)	11.6 ± 0.24a	11.2 ± 0.37a
Mean moulting time (days)	15.8 ± 0.58a	14.8 ± 0.37a
Mean moulting rate (%)	59.73 ± 4.43a	82.58 ± 2.42b

Data represents values mean ± SE (standard error). Means with the same letter in the same row are not significantly different ($P > 0.05$).

Table 2. Infection prevalence and abundance of *T. parva* in adult *R. appendiculatus* fed on membranes and on cattle as nymphs.

	Membrane-fed ticks		Cattle-fed ticks	
	Males	Females	Males	Females
Number assessed	30	30	30	30
Infection prevalence (%)	30.0	53.3	33.3	50.0
Infection abundance	0.5 ± 0.16a	1.37 ± 0.29a	2 ± 0.61a	1.4 ± 0.29a

Data represents values mean ± SE (standard error). Values with the same letter in the same row for each treatment are not significantly different ($P > 0.05$).

Table 3. Infectivity of *T. parva* sporozoites prepared from adult ticks fed on membrane and on cattle as nymphs.

Parameters	Membrane-fed ticks	Cattle-fed ticks
Onset of fever (days)	8	8.5
Maximum temperature attained (°C)	40.8	40.5
Days to peak fever	9	8.5
Duration of fever before treatment (days)	3	2.5
Days to initial appearance of schizonts	9	9.5
Maximum schizont parasitaemia	Ma^{2+}	Ma^{2+}
Days to onset of piroplasms	9	9
Maximum piroplasm parasitaemia (%)	1	1

day 10 post-infection before beginning to fall on day 15. These changes were, however, within the normal range in cattle.

Immunity conferred to steers by T. parva *stabilates prepared from membrane- and cattle-fed ticks*

Table 4 summarises the results of challenge with a lethal dose of *T. parva* on the two pairs of steers previously immunised with stabilates prepared from membrane- and cattle-fed ticks. Neither of the immunised steers showed any febrile reaction

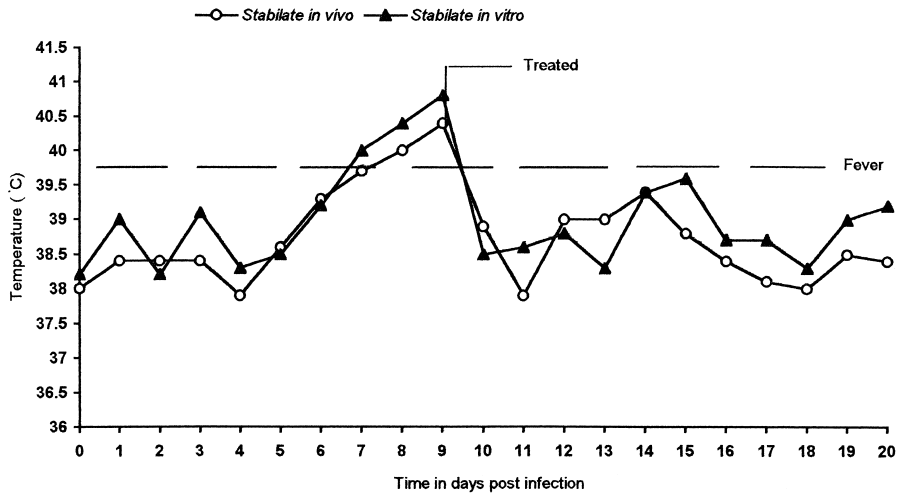

Figure 2. Mean temperature reaction of two pairs of steers infected with *T. parva* (Marikebuni) sporozoites from membrane- and cattle-fed ticks.

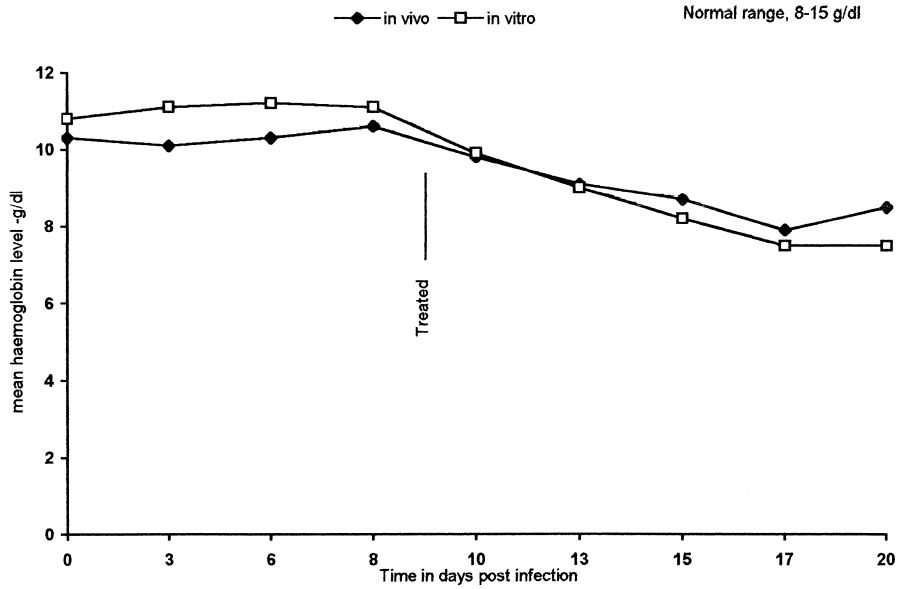

Figure 3. Mean Hb levels for two pairs of steers infected with *T. parva* (Marikebuni) sporozoites from membrane- and cattle-fed ticks.

(Figure 7). The control animals attained a maximum of 40.8 °C on day 13 post-infection. Both groups of immunised steers had only a transient macroshizonts parasitaemia appearing on days 9 and 10 post-infection in the steers immunised

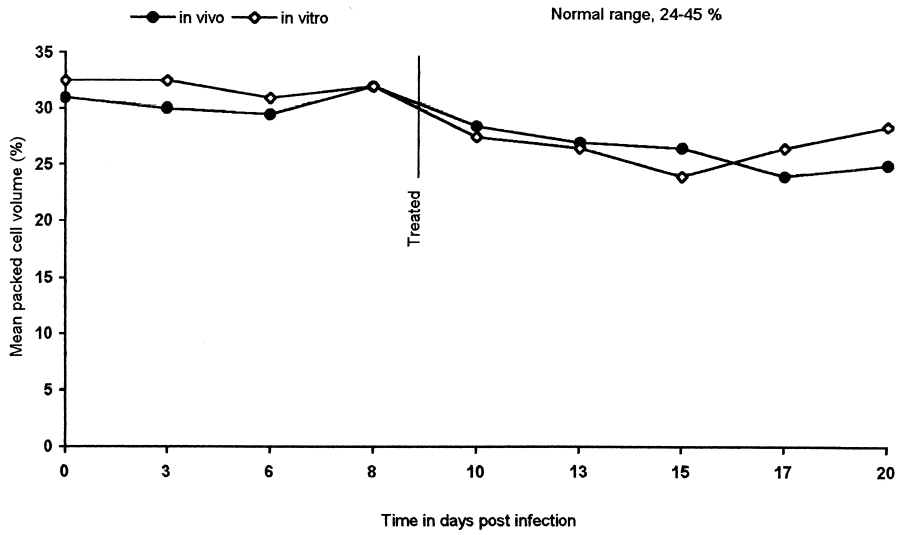

Figure 4. Mean PCV for two pairs of steers infected with *T. parva* (Marikebuni) sporozoites from membrane- and cattle-fed ticks.

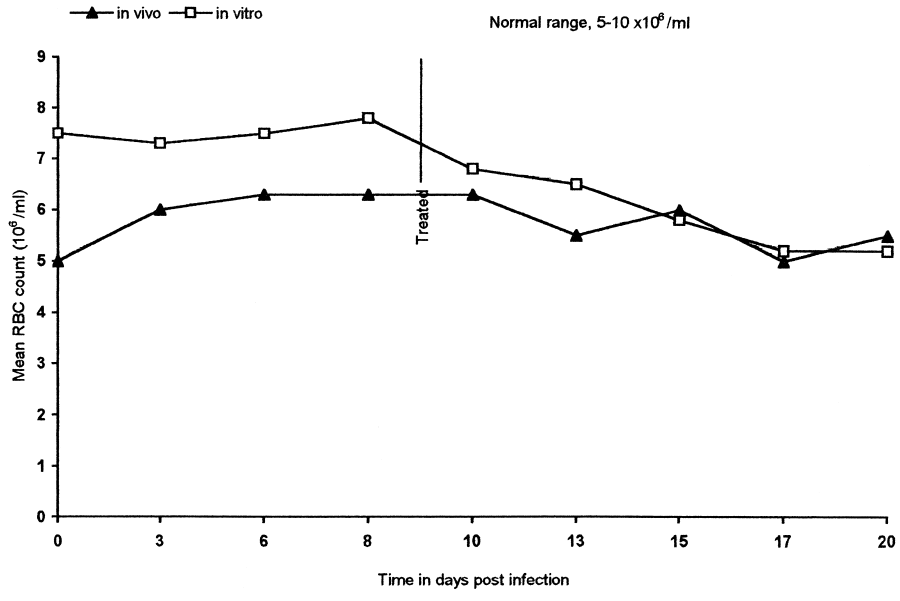

Figure 5. Mean RBC counts for two pairs of steers infected with *T. parva* (Marikebuni) sporozoites from membrane- and cattle-fed ticks.

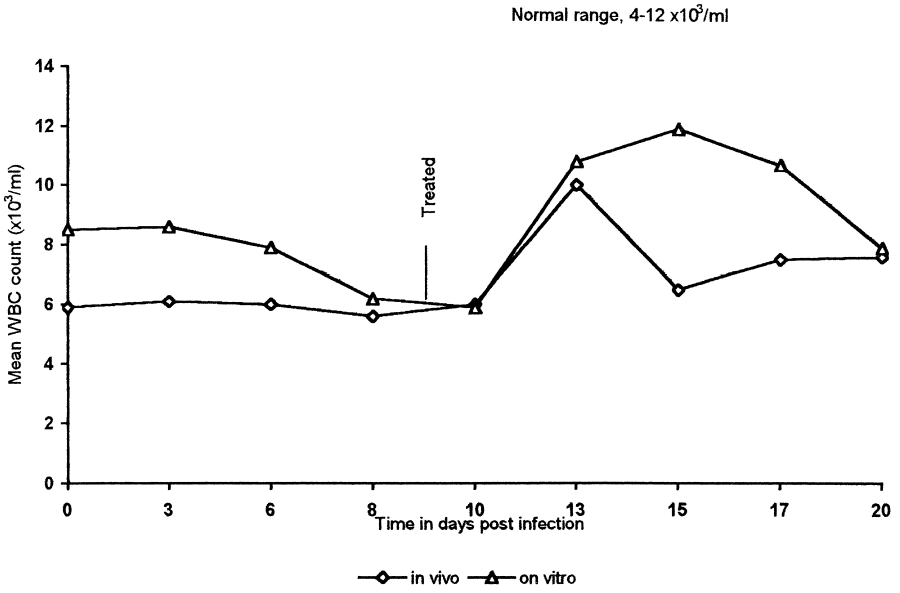

Figure 6. Mean WBC counts for two pairs of steers infected with *T. parva* (Marikebuni) sporozoites from membrane- and cattle-fed ticks.

Table 4. Assessment of the quality of immunity following infection with stabilates prepared from membrane- and cattle-fed ticks after a lethal homologous challenge.

Parameters	Membrane-fed ticks	Cattle-fed ticks	Control
Onset of fever (days)	–	–	10.5
Maximum temperature attained (°C)	38.7	38.5	40.8
Days to peak fever	–	–	13.5
Duration of fever before treatment (days)	–	–	4.5
Days to initial appearance of schizont	9	10	8
Maximum schizonts parasitaemia	Ma	Ma	Ma^{3+}
Days to onset of piroplasms	–	12	13.5
Maximum piroplasm parasitaemia (%)	–	Trace	2

with the membrane- and cattle-fed ticks, respectively. The control animals attained maximum schizont parasitaemia of Ma^{3+} on day 8 and a maximum piroplasm parasitaemia of 2%. One of the steers immunised with the stabilate from cattle-fed ticks showed a trace of piroplasms on day 12.

The RBC count, Hb level and PCV for the four immunised animals (Figures 8–11), remained within their normal ranges but below the mean. However, the values for the control animals followed the same trend as that of immunised animals in the earlier experiment and were consistent with severe ECF reaction. These results indicated that the control animals got a severe ECF

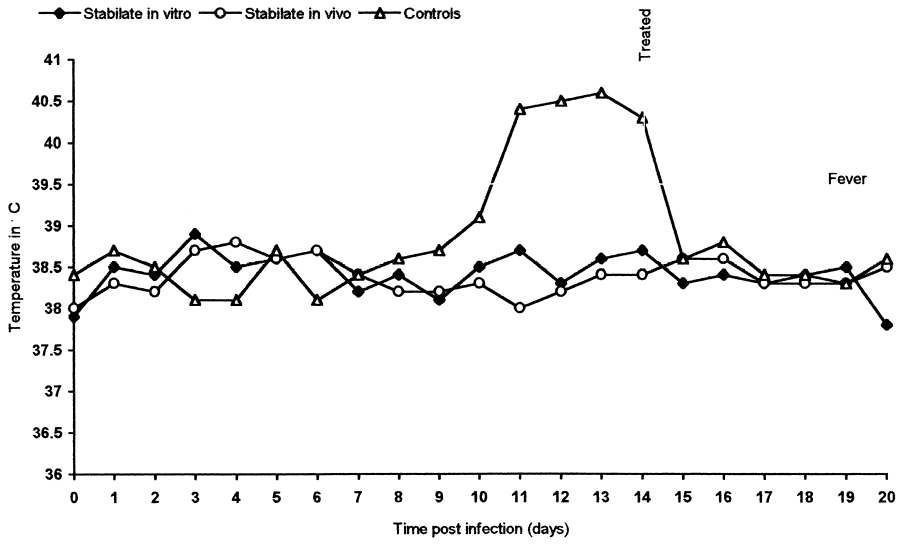

Figure 7. Mean temperature reaction of three pairs of steers challenged with a lethal dose of *T. parva* (Marikebuni) following infection with sporozoites from membrane- and cattle-fed ticks.

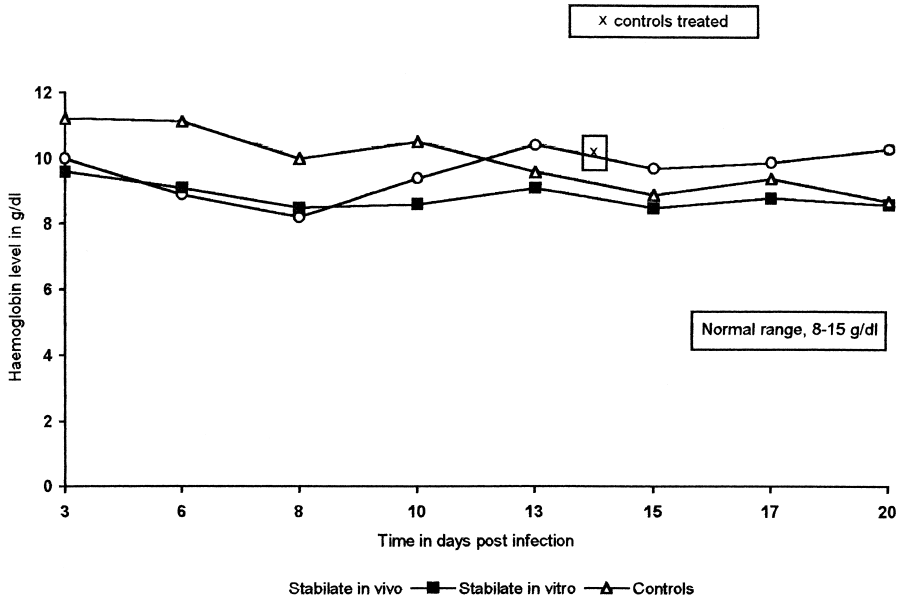

Figure 8. Mean Hb levels for three pairs of steers challenged with a lethal dose of *T. parva* (Marikebuni) following infection with sporozoites from membrane- and cattle-fed ticks.

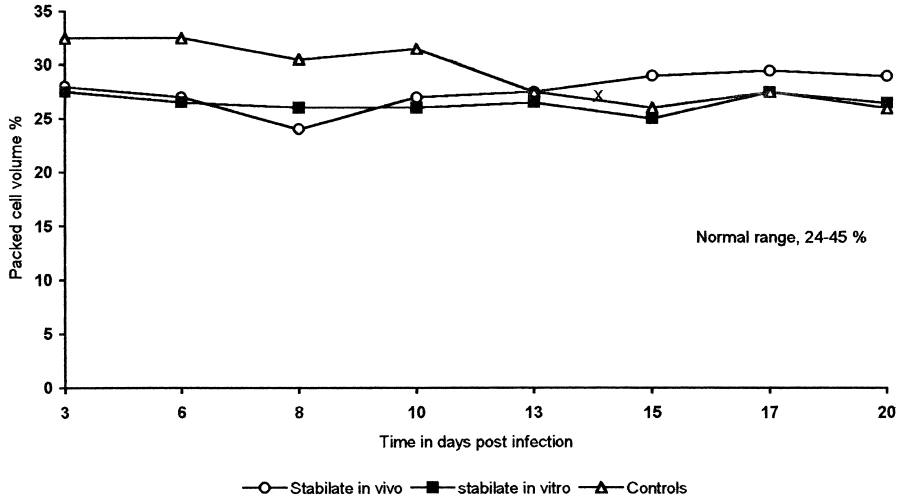

Figure 9. Mean PCV for three pairs of steers challenged with a lethal dose of *T. parva* (Marikebuni) following infection with sporozoites from membrane- and cattle-fed ticks.

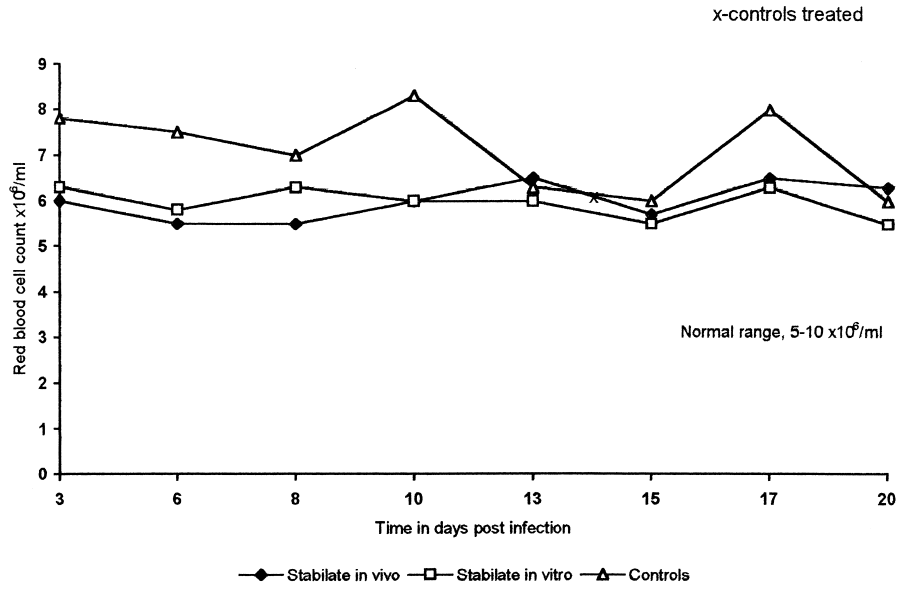

Figure 10. Mean RBC counts for three pairs of steers challenged with a lethal dose of *T. parva* (Marikebuni) following infection with sporozoites from membrane- and cattle-fed ticks.

reaction following a lethal challenge, while the previously immunised steers had no apparent reaction suggesting that they were immune to the homologous challenge.

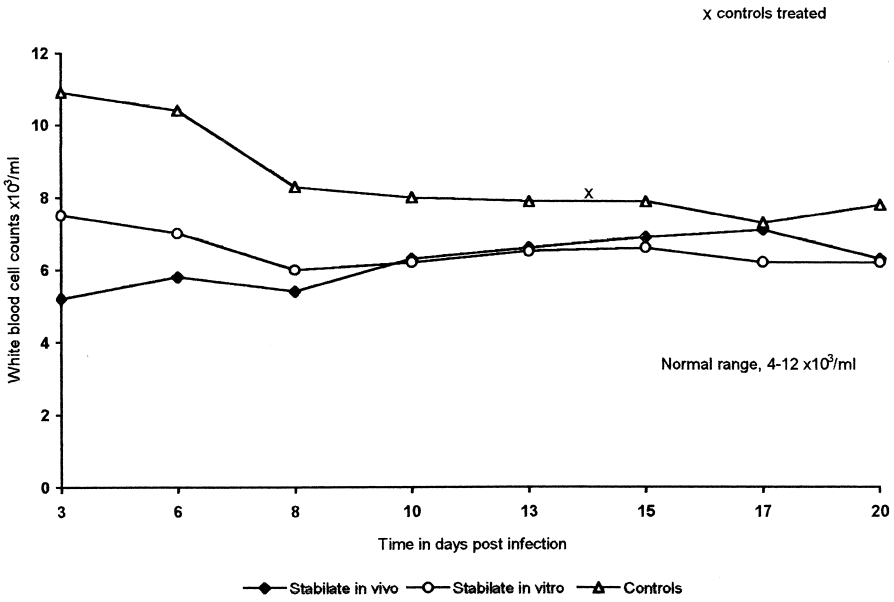

Figure 11. Mean WBC counts for three pairs of steers challenged with a lethal dose of *T. parva* (Marikebuni) following infection with sporozoites from membrane- and cattle-fed ticks.

Discussion

There is no doubt that an effective *in vitro* feeding system for vectors of veterinary and medical importance would have major benefits. The method would have applications in maintenance of vector colonies, transmission of parasites and research on bioactive substances injected into hosts by the vectors as well as those ingested from hosts (Burtler et al. 1982). Benefits would include reduced costs, precise measurements without the attendant complication of the interactions between vectors, hosts and parasites and the elimination of the discomfort to experimental animals (Voigt et al. 1993).

In vitro feeding systems have been developed for a number of haematophagous parasites and used for a variety of purposes (Waladde and Ochieng 1992; Waladde et al. 1996). Due to the complex and little understood feeding pattern of ixodid ticks, there has been limited success in the development of an *in vitro* feeding system for these species. In recent years, however, some advances have been made in this regard (Waladde et al. 1979, 1991, 1993, 1995; Voigt 1993).

Although it has been demonstrated that tick-borne vectors can be successfully transmitted through artificial feeding membranes (Voigt et al. 1993; Waladde et al. 1995), the results have implied that the parasites transmitted were less virulent than those transmitted naturally. If this were shown to be so then it would put into doubt the value of artificial feeding membranes as a research tool. This study was therefore undertaken to compare the infectivity of *T. parva* sporozoites prepared

from *in vitro*- and cattle-fed ticks. It was also necessary to establish whether the immunity induced by infection with these sporozoites is comparable to that arising from naturally infected ticks. Our study adapted the method of Voigt et al. (1993) on the feeding of *A. variegatum* and that of Waladde et al. (1995) on the feeding of *R. appendiculatus* to optimise the feeding system in order to compare the biological performance of the pathogens transmitted through the system.

The majority of the feeding and development parameters such as attachment, feeding duration and engorgement weight were comparable to those of ticks fed on hosts. This finding confirmed the earlier observations of Waladde et al. (1995). In an earlier study (Voigt et al. 1993), the engorgement weight of nymphs and adults were found to be lower than those of cattle-fed ticks. In our study, successful moulting for membrane-fed ticks was found to be lower than that of cattle-fed ticks. Although the rate was higher in cattle-fed ticks (82.5%), the level was still lower than might be expected. Successful moulting of nymphs can approach 100%. The lower moulting rate may be due to other factors such as the stock of ticks used and climatic factors. For instance, cold weather has been shown to affect the development of ticks (H.K. Kiara, personal communication).

In contrast to *Amblyomma* ticks where membrane feedings did not require the use olfactory stimuli (Voigt et al. 1993), our experience with *R. appendiculatus* in this study was that olfactory stimuli in cattle ear wash facilitated attachment. Semiochemicals found in cattle ear wash are thought to arrest tick movement and induce probing leading to attachment (Waladde et al. 1991). Identification and possibly synthesis of these semiochemicals could greatly improve the *in vitro* feeding of this species. This has been achieved in the case of the aggregation-attachment pheromone of *Amblyomma* ticks (Schoin et al. 1984). Contrary to the view expressed by Waladde et al. (1995) that skin membranes would not be suitable for the feeding of *R. appendiculatus* because of their short mouthparts, our findings indicate that this is not a problem as long as the skins used are derived from rabbits less than 6 weeks old.

The transmission of *T. parva* to nymphs through membrane feeding occurred to the same level as cattle-fed nymphs. Both prevalence and abundance were virtually identical for membrane- and cattle-fed ticks. However, the levels were lower than expected in both cases. Waladde et al. (1995) were able to attain very high prevalence and abundance by their feeding system when they stabilised the humidity of the feeding chamber. Voigt et al. (1993) on the other hand were able to transmit both *T. mutans* and *Cowdria ruminantium* through membrane-fed ticks. But because there was no comparison of the levels of infection between membrane- and cattle-fed ticks in their study, it was not possible to tell whether the ticks became infected to the same extent in the two systems.

A key point of this study was in the assessment of the infectivity and the quality of immunity engendered by the parasites in the membrane-fed ticks with those fed on cattle. In both cases, the parasites appeared to be equally infective and protective since they caused a severe reaction in susceptible cattle which were subsequently protected from a homologous challenge. There was no evidence that the membrane-fed ticks produced parasites of lower virulence than cattle-fed ticks as previously

suggested by Voigt et al. (1993) although a more detailed study involving a large number of animals is required to clarify this point.

The main limitation of membrane feeding so far seems to be the very labour intensive nature of the system. The challenge of adapting it to a large-scale tick production system is enormous. The most tedious part of the system is the need to change blood frequently. Thus, the system could be greatly improved if the blood could be kept for long durations. Stabilate preparation on a commercial scale would require a large number of ticks ($\sim$500,000 nymphs) and a system that allows for continuous blood circulation while minimizing contamination. It should also be possible to induce sporulation of the parasite in infected ticks by pre-feeding them through the *in vitro* system. This would eliminate the use of live animals in the entire system.

In conclusion, this study suggests that there is no major effect on *T. parva* parasites as they go through an artificial feeding system. The results also confirm the findings of Voigt et al. (1993) and Waladde et al. (1995) that *in vitro* feeding system is reproducible and reliable. The system can be adopted as a routine method for parasite maintenance and transmission. The development of artificial membranes suitable for different tick species would be the ultimate goal. In addition, the use of artificial media as a subsitute for blood could greatly improve tick rearing. Although it might not be possible to completely eliminate the use of natural hosts, dependence on them would be greatly reduced.

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