

# *Theileria parva* (Kasoba): isolation and challenge of cattle recovered from infection with other *Theileria parva* stocks

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## Key words

Zebu - Cattle - East Coast Fever - *Theileria parva* - Tick - Experimental infection - Immunization - Statistical analysis - Malawi.

## Summary

A pathogenic *Theileria* stock was isolated from control cattle during an East Coast Fever (ECF) field immunization trial at Kasoba near Karonga town in northern Malawi. A stabilate of this stock caused severe fevers and prolonged parasitosis in *Theileria parva* naive cattle, resulting in the death of 5 out of 12 cattle despite treatment. In contrast, this parasite stock caused mild to moderate reactions in 17 cattle immunized with the trivalent *T. parva* stabilate except in 3 animals which developed severe reactions, and one of them died. Another time, cattle immunized with buffalo-derived *Theileria parva* (Serengeti transformed) resisted a potentially fatal challenge, with only mild to moderate reactions being recorded. The parasite stock was morphologically and serologically indistinguishable from *Theileria parva* (Muguga); it was virulent and could cause mortality, particularly in *T. parva* naive cattle. The parasite stock was designated *Theileria parva* (Kasoba).

## ■ INTRODUCTION

East Coast Fever (*Theileria parva* infection in cattle) is endemic in central and northern Malawi. In one study, East Coast Fever (ECF) caused approximately 10 % and 1 % mortalities in bovine calves and adults, respectively (13), and was considered a major constraint on the improvement of dairy cattle industry. Recently, Chinombo *et al.*, (5) showed that ECF continues to be a major constraint and concluded that smallholder dairy farmers would benefit from immunization of their cross-bred cattle against ECF. A pool of cattle-derived *Theileria parva* (Muguga), cattle-derived *Theileria parva* (Kiambu 5) and buffalo-derived *Theileria parva* (Serengeti transformed) stabilates (8) protected cattle against various *T. parva* stocks from Uganda, Kenya and Tanzania (12). D.E. Radley (unpublished data) noted similar protection during cross-immunity studies between the above combination and Malawi *T. parva* stocks. Consequently, the Malawi Government authorized immunization against ECF in the field where it is now routinely done in improved cattle. This paper describes the isolation of *Theileria* (Kasoba) from control cattle at Kasoba near Karonga town, northern Malawi, during an ECF immunization field trial and discusses protection of cattle recovered from infection with other *T. parva* stocks against a challenge with the Kasoba stock.

## ■ MATERIAL AND METHODS

### Cattle and ticks

The cattle used were Friesian-Malawian zebu crosses about two years of age from farms practising strict tick control in southern

Malawi where ECF has not been reported. All cattle were serologically negative to *T. parva* antigen in the immunofluorescent antibody test (IFAT) described by Burridge and Kimber (4). A *Rhipicephalus appendiculatus* (Muguga) tick colony was maintained as described by Bailey (1) and used for picking up the parasite from the control cattle at Kasoba and subsequent stabilate production.

### *Theileria parva* stocks, drugs and immunization

Stabilates of *T. parva* (Muguga) (3), *T. parva* (Kiambu) (7), and buffalo-derived *T. parva* (Serengeti transformed) (14) were pooled to form a trivalent *T. parva* stabilate used to immunize cattle for the first and second *Theileria* (Kasoba) challenge experiments. Immunization was done by administering intramuscularly long-acting oxytetracycline (20 mg/kg body weight) followed by a subcutaneous injection of 0.5 ml of either the trivalent *T. parva* stabilate or buffalo-derived *T. parva* (Serengeti transformed) stabilate into each animal. The tetracycline blocked potentially severe reactions while parvaquone and halofuginone lactate treated overt clinical theileriosis cases.

### Isolation and production of *Theileria* (Kasoba), stabilate No. 66

During an ECF field immunization trial at Karonga (11), laboratory-reared non-infected *R. appendiculatus* nymphae were fed on control cattle reacting to a *Theileria* field infection to pick up parasites. Nine hundred and fifteen adult *R. appendiculatus* moulted from the nymphae and 370 ticks were fed on ears of rabbits for four days to allow parasite maturation. Ten male and 10 female ticks were dissected to estimate infection rates using the Feulgen stain (2). Using the method described by Cunningham *et al.* (6), the remaining fed ticks were prepared into a stabilate to a final concentration of 10 ticks per ml of media.

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**Viability of *Theileria* (Kasoba), stabilate No. 66**

Two animals, seronegative to *T. parva* in IFAT, were each inoculated subcutaneously in the front of the right ear with 1.0 ml of the prepared stabilate to assess its infectivity. Development and progress of the infection were monitored daily by recording rectal temperatures, taking lymph node biopsy smears daily when parotid and prescapular lymph nodes became enlarged, and blood smears after detection of *Theileria* schizonts. Lymph node biopsy and blood smears were fixed with methanol on microscope slides, air-dried, Giemsa stained, and examined using normal light microscopy. Schizont parasitosis scoring was as described by Musisi *et al.* (10), namely: Ma(+) - 1 macroschizont per smear; Ma+ - 1 macroschizont per 10 fields; Ma2+ - 1 macroschizont per field; and Ma3+ - more than 1 macroschizont per field at x 1000 magnification. Cattle were bled and sera extracted for IFAT to monitor development of *T. parva* antibody titres on days 0, 14, 21, 28, 35, 42, 49 and 56 post-infection.

**Challenge of trivalent *T. parva* and *T. parva* (Serengeti transformed) immunized cattle**

Each animal from a group of 7 and another of 10 cattle immunized with the trivalent *T. parva* stabilate received subcutaneously a potentially lethal dose of 1 ml *Theileria* (Kasoba); each group had 4 cattle as controls. Parasite and clinical reactions were monitored as before. Another time, 2 control cattle in a group of cattle immunized with buffalo-derived *T. parva* (Serengeti transformed) were challenged with 1 ml of *Theileria* (Kasoba).

**Statistical analysis**

Significance of differences between the mean prepatent periods to detection of schizonts and to fever for the three groups of cattle (table I) was analysed. Similar analysis was done on differences between the mean duration of detection of the schizonts and of fever (table I), and for differences between the degree of parasitosis and of fever (figure 1). Using the t-test, means of 2 samples were compared assuming equal variance. Differences were significant at a value of P<0.05.

**RESULTS**

A 50 % infection rate was recorded in the 10 male and 10 female ticks dissected; the infections were as follows: males 2, 5, 6, 26 acini, and females 3, 7, 21, 32, 53 acini. Thus, the mean number of acini infected was 7.8.

A temperature  $\geq 39.5^{\circ}\text{C}$  denoted fever in cattle. Control group (CG) results (table I) are derived from combining results of the cattle used in infectivity of stabilate No. 66, and of the control

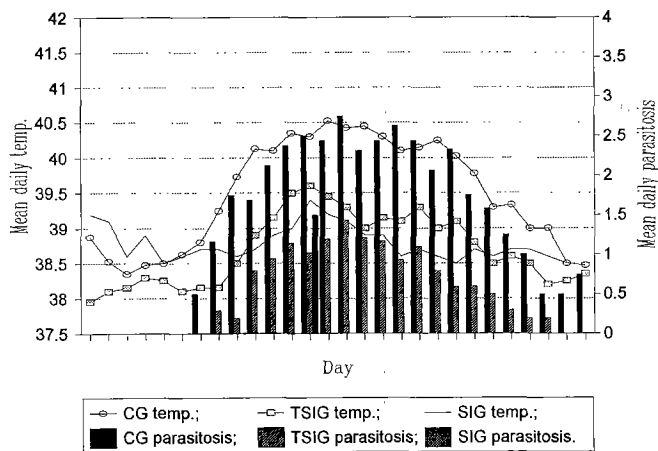


Figure 1: temperatures and parasitosis of *Theileria* (Kasoba) challenged cattle.

groups in the three challenge experiments. *Theileria* schizonts were detected in all 12 animals of the control group. On average, cattle in this group developed schizonts within  $7.9 \pm 1.2$  days for a mean duration of  $15.5 \pm 2.3$  days. The mean period for developing a fever was  $9.0 \pm 1.9$  days spanning a mean period of  $13.5 \pm 3.9$  days. Eleven of the 12 cattle reacted severely and 5 died of ECF; the remaining cattle reacted moderately. All cattle that were alive by day 28 had developed significant serological titres ( $\geq 1:640$ ) to *T. parva* (Muguga) schizont antigen.

Results of the first and second challenge experiments were pooled to form the trivalent stabilate immunized group (TSIG) shown in table I and schizonts were detected in 15 of the 17. The mean prepatent period was  $10.0 \pm 1.2$  days for a mean duration of  $9.0 \pm 4.1$  days. Thirteen of the 17 cattle had a mean period to fever detection of  $12.1 \pm 3.9$  days and on average the fever lasted  $6.5 \pm 4.8$  days. Three of the 17 cattle reacted severely and 1 died while the remainder did not react or reacted mildly to moderately. Although detection of schizonts in some immunized cattle was prolonged (table I and figure 1), parasitosis was generally low compared to that of control cattle. In the third challenge experiment, schizonts were detected in only two of the *T. parva* (Serengeti transformed) immunized group (SIG) shown in table I; the prepatent period was  $12.5 \pm 0.7$  days with a mean duration of schizont detection of  $3.0 \pm 2.8$  days. Six of the 11 cattle developed a fever whose mean prepatent period was  $9.0 \pm 2.0$  days lasting on the average for  $6.0 \pm 2.0$  days.

TABLE I

**Responses of immunized and non-immunized groups of cattle to challenge with *Theileria parva* (Kasoba)**

Group (No. in group)	Mean prepatent period to		Duration of detecting		Severity of reaction
	schizont	fever	schizont	fever	
	(days)	(days)	(days)	(days)	
CG (12)	$7.9 \pm 1.2$	$9.0 \pm 1.9$	$15.5 \pm 2.3$	$13.5 \pm 3.9$	5F, 6S, 1NM
TSIG (17)	$10.0 \pm 1.2$	$12.1 \pm 3.9$	$9.4 \pm 4.1$	$6.5 \pm 4.8$	1F, 2S, 15NM
SIG (11)	$12.5 \pm 0.7$	$9.0 \pm 2.0$	$3.0 \pm 2.8$	$6.0 \pm 2.0$	11NM

CG: control group; TSIG: trivalent stabilate immune group; SIG: Serengeti stabilate immune group; F: fatal; S: severe; NM: no reaction/mild to moderate reaction.

The post-mortem examination of carcasses revealed a typical ECF post-mortem flagrant picture, namely haemorrhages throughout the tissues, oedematous and ulcerated abomasum, enlarged congested liver, enlarged and friable spleen, oedematous frothy and generally haemorrhagic pulmonary tissues, and congested, sometimes haemorrhagic, kidneys with artifact "infarcts" which are white spots under the capsule full of parasitised lymphocytes.

## ■ DISCUSSION

The mean infection rate was low (7.8 acini) for the batch of ticks used to prepare stabilate No. 66 in comparison to rates observed in *T. parva* (Muguga) infected ticks at the Lilongwe Laboratory (F.L. Musisi, unpublished data). However, despite this apparent low infection rate, the stabilate was infective and caused severe reactions as shown in table I and figure 1. The infectivity and severity of reactions observed in the control group in this study are similar to those for the control cattle in a study by Musisi *et al.* (9) on cross-protection between Kasoba stock and Tanzanian stocks: *Theileria parva* (SAO Hill) and *Theileria parva* (West Kilimanjaro).

The mean prepatent period to schizonts was significantly shorter for CG than for either TSIG or SIG ( $P < 0.001$  in each case). The differences between TSIG and SIG were also significant ( $P < 0.017$ ). The mean duration of parasitosis for CG was significantly longer than for either TSIG or SIG ( $P < 0.001$ ), but was not significant between TSIG and SIG ( $P > 0.05$ ). The mean prepatent periods to detection of fever for CG was significantly shorter than for TSIG ( $P < 0.021$ ), but was not significantly different between either CG and SIG ( $P = 1$ ), or TSIG and SIG ( $P > 0.90$ ). The mean duration of fever for CG was significantly longer than for either TSIG or SIG ( $P < 0.001$ ), but the differences between the immunized groups were not significant ( $P > 0.90$ ). The level of fever (figure 1) was significantly higher in CG than in any of the immunized groups ( $P < 0.0001$ ), but the differences between the immunized groups were not significant ( $P > 0.3918$ ). The degree of parasitosis was significantly higher in CG than in TSIG ( $P < 0.0001$ ); no statistical comparisons were done between the CG, TSIG and SIG because there was only one day with a result for the SIG (figure 1). In conclusion, *Theileria* (Kasoba) generally caused shorter prepatent periods to detection of schizonts, prolonged schizont parasitosis and fever, a greater parasitosis and higher fever in the control group than in the immunized groups. In contrast, mild fevers and very low level transient schizont parasitosis occurred in immunized cattle (figure 1).

TSIG and SIG showed a degree of resistance to challenge with *Theileria* (Kasoba) as seen from their significantly longer prepatent periods to schizonts when compared to those of CG. The shorter durations of fevers and parasitosis, and the low level fevers and parasitosis in TSIG and SIG confirm this resistance. These results suggest a close antigenic relationship between the *Theileria* (Kasoba) stock and the *T. parva* stocks used to immunize the cattle. The serological identity, in IFAT, of all *T. parva* stocks used in this study further confirms this relationship. The observations from the three challenges show that the buffalo-derived *T. parva* (Serengeti transformed) alone protected at least as well, if not better, than the cocktail of the trivalent stabilates. This leads to the question whether the trivalent combination is really necessary.

The *Theileria* parasite isolated from Kasoba, northern Malawi, produces schizonts and piroplasms that are morphologically indistinguishable from *T. parva* (Muguga). It is serologically indistinguishable from the *T. parva* (Muguga) stock. It causes high

and prolonged fever and parasitosis that tend to result in death of cattle not previously exposed to *T. parva* parasite stocks; the Kasoba stock is therefore virulent. It is a pathogenic *T. parva* stock and is thus designated *Theileria parva* (Kasoba).

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**Résumé**

**Musisi F.L., Quiroga J.C., Kanhai G.K., Kamwendo S.P., Mzoma F.J., Njuguna L.M.** *Theileria parva* (Kasoba) isolée et testée sur du bétail guéri après infection par d'autres stocks de *Theileria parva*

Un stock pathogène de *Theileria* a été isolé à partir de bovins témoins, lors d'un test d'immunisation sur le terrain contre la theilériose bovine à Kasoba, près de la ville de Karonga au Nord du Malawi. Un stabilat issu de ce stock a causé des fièvres graves et une parasitose prolongée chez du bétail n'ayant jamais été infecté par *Theileria parva*, provoquant la mort de 5 animaux sur 12 en dépit du traitement. D'un autre côté, ce stock de parasites a seulement causé des réactions légères à modérées chez 17 bovins préalablement immunisés avec un stabilat trivalent de *T. parva*, excepté chez trois animaux qui ont développé des réactions sévères et l'un d'eux en est mort. Une autre fois, le bétail immunisé avec *Theileria parva* (Serengeti transformé) provenant de buffle a résisté à une inoculation potentiellement fatale en ne montrant que des réactions légères et modérées. Ce stock de parasites s'est avéré morphologiquement et sérologiquement semblable à *Theileria parva* (Muguga) ; il était virulent et pouvait provoquer la mort, en particulier chez du bétail n'ayant jamais été infecté par *T. parva*. Ce stock de parasites a été ainsi appelé *Theileria parva* (Kasoba).

**Mots-clés** : Zébu - Bovin - Theilériose - *Theileria parva* - Tique - Infection expérimentale - Immunisation - Analyse statistique - Malawi.

**Resumen**

**Musisi F.L., Quiroga J.C., Kanhai G.K., Kamwendo S.P., Mzoma F.J., Njuguna L.M.** *Theileria parva* (Kasoba): aislamiento y examen de ganado recuperado de la infección por otros tipos de *Theileria parva*

Se aisló un tipo patogénico de *Theileria* en ganado control, durante un trabajo de campo de inmunización contra la Fiebre de la Costa Este (ECF) en Kasoba, cerca de la ciudad de Taronga en el norte de Malawi. Un inóculo de este patógeno provocó fiebres severas y parasitosis prolongadas en ganado indemne de *Theileria parva*, conduciendo a la muerte de 5 de los 12 animales, a pesar del tratamiento. Por otro lado, este parásito causó reacciones de moderadas a leves en 17 animales inmunizados con el inóculo trivalente de *T. parva*, excepto en 3 animales, que desarrollaron reacciones severas, y 1 sobre 3 murió. Anteriormente, ganado inmunizado con *T. parva* (transformación Serengeti), derivada de búfalo, resistió a una prueba potencialmente fatal, presentando únicamente reacciones moderadas o leves en el ganado inmunizado. Morfológica y serológicamente, este parásito fue imposible de diferenciar de *T. parva* (Muguga). Es virulento y puede causar mortalidad, principalmente en ganado libre de *T. parva*. El parásito fue designado *Theileria parva* (Kasoba).

**Palabras clave** : Cebú - Ganado bovino - Fiebre de la Costa oriental - *Theileria parva* - Garrapata - Infección experimental - Inmunización - Análisis estadístico - Malawi.