



***In vivo* comparison of susceptibility between *Bos indicus* and *Bos taurus* cattle types to *Theileria parva* infection**

S.G. NDUNGU¹, C.G.D. BROWN² and T.T. DOLAN³

ABSTRACT

NDUNGU, S.G. BROWN, C.G.D. & DOLAN, T.T. 2005. *In vivo* comparison of susceptibility between *Bos indicus* and *Bos taurus* cattle types to *Theileria parva* infection. *Onderstepoort Journal of Veterinary Research*, 72:13–22

The objective of this study was to determine whether *Bos taurus* cattle differ from *Bos indicus* in their susceptibility to infection with the Muguga stabilate of *Theileria parva* and in their resistance to the resultant disease. Ten Friesians (*B. taurus*), ten improved Borans (*B. indicus*), ten unimproved Borans (*B. indicus*) and ten Zebus (*B. indicus*) born to dams from an East Coast fever (ECF) endemic area were inoculated with an infective dose₅₀ dilution of *T. parva* Muguga stabilate 147. All the animals except one Friesian and one Zebu developed schizont parasitosis. All the improved Borans, nine of the Friesians, eight of the unimproved Borans and six of the Zebus developed a febrile response. Four of the improved Borans, four of the Friesians and three of the unimproved Borans died of theileriosis. No significant difference ($P > 0.05$) in the prepatent period occurred between the groups, but the Zebus had a significantly shorter duration of schizont parasitosis ($P > 0.05$) and took a significantly shorter time to recover ($P > 0.05$) than the other three groups. There was no significant difference in the two parameters between the other three groups. The study showed that three *B. indicus* breeds and a *B. taurus* breed are equally susceptible to *T. parva* infection. However, Zebus born to dams from an ECF endemic area showed a better ability to control the course of disease than cattle from ECF free areas.

Keywords: Boran, *Bos indicus*, *Bos taurus*, cattle, East Coast fever, Friesian, *in vivo* susceptibility, *Theileria parva*, Zebu

INTRODUCTION

Theileria parva is a protozoan parasite of the African buffalo (*Syncerus caffer*) which also infects cattle causing the disease East Coast fever (ECF) (Uilenberg 1976, 1981). It is transmitted transtadially by the three host tick *Rhipicephalus appendiculatus*.

The disease is prevalent in large areas of East and Central Africa where it causes major economic losses through morbidity and mortality. It is characterised by lymphocyte transformation into continuously dividing *T. parva*-infected lymphoblastoid cells (Hulliger, Wilde, Brown & Turner 1964) which infiltrate other lymphoid and non-lymphoid organs (Barnett 1960; Wilde 1967; De Martini & Moulton 1973). The non-lymphoid organs include the lungs whose infiltration is associated with severe pulmonary oedema (Irvin & Morrison 1987) resulting in asphyxiation and death of the affected animal. In susceptible animals morbidity and mortality may be in the region of 90% (Brocklesby, Barnett & Scott 1961).

¹ Kenya Agricultural Research Institute, National Veterinary Research Centre, P.O. Box 32, Kikuyu, Kenya

² Centre For Tropical Veterinary Medicine, University of Edinburgh, Easter Bush, Roslin Midlothian, EH25 9RG, Scotland

³ International Livestock Research Institute, P.O. Box 30709, Nairobi, Kenya

Cattle of the *Bos indicus* type have been reported to be less susceptible to infection with *T. parva* and more resistant to ECF than *Bos taurus* cattle types (Barnett 1957; Guilbride & Opwata 1963; Radley 1978; Dolan, Njuguna & Stagg 1982; Mutugi, Ndungu, Linyonyi, Martim, Mining, Ngumi, Kariuki, Leitch, D'Souza, Maloo & Lohr 1991; Paling, Mpan-gala, Luttikhuizen & Sibomana 1991). Similar observations have also been made by Young (1981). Moll, Lohding & Young (1984) and Moll, Lohding, Young & Leitch (1986) have also reported low mortality rates in calves in ECF endemic areas resulting from *T. parva* infection. In experimental *T. parva* infections, *B. taurus* cattle have been reported to be more susceptible than *B. indicus* cattle types to infections induced with infected ticks (Barnet 1968; Paling *et al.* 1991), sporozoite stabilate (Dolan & McHardy 1978; Radley 1978; Mutugi *et al.* 1991; Paling *et al.* 1991) and *T. parva* infected lymphoblastoid cell lines (Dolan *et al.* 1982). It must be emphasised that in the fore-going studies, the *B. taurus* types used have been of recent introduction from north western Europe (e.g. Friesians) and not indigenous African cattle breeds (e.g. Ndama).

The objective of this study was to compare the relative susceptibility and resistance to disease of cattle of the *B. indicus* and *B. taurus* breeds in order to determine whether the resistance is due to a lowered infectibility or a raised ability to recover from infection, and to relate these findings to the environmental background of the animals. To achieve this cattle of the European type of *B. taurus* and three different types or "breeds" of East African Zebu (*B. indicus*) cattle were infected with a standard dose of *T. parva* sporozoites. The Zebu cattle were (i) from dams which resided in an ECF endemic area; (ii) from an area known to be ECF free; and (iii) of improved Boran type, which had been maintained for generations free from ECF.

MATERIALS AND METHODS

Cattle

Forty animals consisting of ten Friesians, ten improved Borans (herein referred to as Borans), ten unimproved Borans from an ECF-free area (herein referred to as non-endemic Zebus) and ten Zebus born to dams from an ECF-endemic area (herein referred to as endemic Zebus) were used. The animals were used when they were 1–1.5 years of age.

The Friesians were purchased from a farm in the Nakuru District of Kenya. The farm is located in an

ECF-endemic area, but because of strict acaricidal control, no cases of ECF have been reported over many years. The Boran cattle originated from a farm in Laikipia district, also located in an ECF-endemic area but where strict acaricidal control is practised. The unimproved Borans or endemic Zebus used in the experiment were purchased from cattle traders in Kitui district of Kenya. The animals had been brought south from Northern Kenya, where the vector tick for ECF, *R. appendiculatus*, and therefore the disease, does not occur (Walker 1974).

The endemic Zebus used in the experiment were Nandi Zebus (Mason & Maule 1960, Maule 1990). They were born in Muguga to dams purchased while pregnant from Sergoit Division of Uasin Gishu District where ECF is endemic. The animals were obtained from farms where no or minimal tick control is practised.

During the experiment the cattle were maintained at Muguga, which is an ECF-endemic area where strict tick control has to be practised. For this purpose the pour-on formulation flumethrin (Bayticol^R pour-on, Bayer, Germany) (Stendel 1985) was applied along the backline of the animals from the occiput to the base of the tail by means of a calibrated cylinder at the manufacturer's recommended dose of 1 mg/kg body mass every 10 days.

All animals were bled before purchase and the serum screened for *T. parva* schizont antibodies by the method described by Burrige & Kimber (1972). Only animals with antibody titres of less than 1:40 were selected for use in the experiment.

The animals were fed on hay and supplemented with ranch cubes (Unga Limited, Nairobi) and mineral licks (Macklic, Cooper Kenya Limited, Nairobi). They were dewormed once every month with levamisole hydrochloride (Nilzan, ICI, England).

Theileria parva parasite

A sporozoite stabilate of the Muguga stock of *T. parva* (Muguga) (Brocklesby *et al.* 1961) stabilate 147 was used to infect the cattle. This was stored in liquid nitrogen and had been prepared by Dolan, Young, Lossos, McMillan, Minder & Soulsby (1984) from infected *R. appendiculatus* ticks according to the method of Cunningham, Brown, Burrige & Purnell (1973) as modified by Radley (1978). It has been characterised by Dolan *et al.* 1984).

Before use, the vials containing the stabilate were removed from the liquid nitrogen and thawed rapid-

ly by holding them in a waterbath at 37 °C. After thawing it was left to equilibrate at room temperature for 30 min. It was then diluted to 1:20 in Eagle's Minimum Essential Medium with Earle's Salts and Glutamine (Flow Laboratories, Irvin, Scotland) containing 3.5 % w/v bovine plasma albumin fraction v (Serve Feinbiochemica, GMBH Heindenbergh Co. Germany) and 7.5 % glycerol v/v to give an estimated 50 % lethal dose (LD50) of 1 ml for Boran cattle (Dolan *et al.* 1984).

Experimental design and parameters

The experiment was carried out in five replicate phases each consisting of eight animals, viz. two Friesians, two Borans, two non-endemic Zebus and two endemic Zebus. The animals were inoculated subcutaneously with 1 ml of the diluted stabilate immediately below and in front of the left ear.

Clinical monitoring (temperature response)

Each animal's rectal temperature was recorded every morning from the day of inoculation (day 0) until death supervened or day 28 for those animals that recovered. A temperature of 39.5 °C or above that was associated with schizont parasitosis was considered to be a significant clinical response.

Haematology

Blood was taken from the jugular vein of each animal into Vacutainer tubes containing the sodium salt of ethylenediamine tetra-acetic acid (EDTA) as anti-coagulant. Total white blood cell (WBC) counts and red blood cell (RBC) counts were determined using an electronic particle counter (Coulter Electronics Inc., Florida, USA). Haemoglobin values were determined using a haemoglobinometer (Coulter Electronics Inc.) and the packed cell volume (PCV) was determined by the haematocrit method using a haematocrit centrifuge (MSE Scientific Instruments, Sussex, UK). Thin blood smears were employed to determine by light microscopy the WBC differential counts. These smears were fixed in methanol and stained in 10 % Giemsa stain (BDH Ltd, Essex, UK) in Giemsa buffer (pH 7.2) for 30 min. Two hundred cells were counted and the number of cells of different categories expressed as a percentage of the total white blood cells counted.

Serology

Blood for serology was collected from the jugular vein into serum Vacutainer tubes (Becton Dickinson, UK) 1 day before the parasite inoculation and once

a week after inoculation until death supervened or day 35. Antibody titres were determined using the indirect fluorescent test (Burridge & Kimber 1972) using *T. parva* schizont-infected cells grown in cell culture as antigen. A titre of 1:160 or above was considered a positive response.

Parasitological observations and scoring

Needle biopsy smears were made daily from the left parotid lymph node, regional to the inoculation site, commencing from day 5 post-inoculation until day 28 from those animals that survived this long. The smears were air-dried and fixed in methanol followed by staining in 10 % Giemsa's stain in Giemsa buffer (pH 7.2) for 30 min. The slides were differentiated in buffer and air dried before examination under a microscope (Leitz, Germany) using the x 50 and x 100 oil immersion objectives for the presence of schizonts. Once schizonts were detected, lymph node biopsy smears were also made daily from the contralateral lymph node, to determine the degree of parasite dissemination by calculating the proportion of infected lymphocytes per field. The degree of parasitosis was recorded on an arbitrary scale as described by Teale (1983):

Ma+ = schizonts detectable in one or only a few microscope fields: parasitosis < 1 %

Ma++ = schizonts detectable in 50 % or more fields examined: parasitosis 1–5 %

Ma+++ = schizonts observed in all or most of the fields examined: parasitosis > 5 %

At the same time blood smears were made daily from blood obtained from the tip of the tail. These were fixed and stained as described for the lymph node biopsy smears. The blood smears were examined for the presence of intraerythrocytic piroplasms and the proportion of infected erythrocytes calculated.

Definition of disease reactions

Definition of disease reaction was, with some modifications, based on the recommendations of a workshop on ECF immunisation (Anon 1989). The day of recovery was considered to be the day after the last schizonts were detected and the febrile reaction had declined to < 39.5 °C. An "inapparent reaction" was where no parasites were seen and there were no apparent clinical signs. A "mild reaction" was considered to be one where low numbers of schizonts (Ma+) were detected with no or transient fever for less than 4 days. A "severe reaction" was

one where schizonts were plentiful (Ma++ or Ma+++), a febrile response was observed for several days and the animal recovered. A “very severe reaction” was one where schizonts were plentiful (Ma++ or Ma+++), fever was persistent and the infection resulted in treatment of the animal or euthanasia *in extremis*.

Post mortem examination

Full necropsy examinations were carried out on all animals that died or were humanely destroyed during the experiment. Impression smears from the spleen, superficial lymph nodes, kidneys, lungs and adrenal glands were made, fixed in methanol and stained in 10% Giemsa stain. They were examined by light microscopy for the presence of schizonts. The presence of schizonts and oedema of the lungs led to the conclusion either that death was caused by theileriosis or that theileriosis necessitated the euthanasia.

Statistical analysis

Analysis of data was performed using the Kruskal-Wallis test (Fowler & Cohen 1990) unless otherwise stated. The Rankit test for normality (Fisher & Yates 1957; De Martin & Moulton 1973) was used to determine the normality of distribution of the data before statistical analysis. Comparison of parameters between any two groups was done using the Mann-Whitney U test (Fowler & Cohen 1990) while comparison of the number of animals that died or recovered was done using the Fisher Exact Test (Fisher 1958).

RESULTS

All the ten Borans, nine of the Friesians, eight of the non-endemic Zebus and six of the endemic Zebus developed a significant clinical febrile response. Four of the Borans, four of the Friesians and three

TABLE 1 Comparisons of clinical parameter responses

Parameters	Groups	Median	Range	K	P	Interpretation
Onset of fever (days)	Friesians	12	9–14	3.6	> 0.05	Similar
	Borans	12	11–15			
	Non-endemic Zebus	12	9–15			
	Endemic Zebus	10.5	9–12			
Peak fever (°C)	Friesians	40.4	39.6–41.3	1.7	> 0.05	Similar
	Borans	40.65	39.7–41.0			
	Non-endemic Zebus	40.15	39.8–41.0			
	Endemic Zebus	40.15	39.8–41			
Days to peak fever	Friesians	15	13–22	10.3	< 0.05	Endemic Zebus different from the other three groups
	Borans	15.5	12–22			
	Non-endemic Zebus	14.5	12–22			
	Endemic Zebu	12	10–14			
Duration of fever (days)	Friesians	9	1–13	4.7	> 0.05	Similar
	Borans	7	4–16			
	Non-endemic Zebus	7	3–14			
	Endemic Zebus	4.5	1–10			
Days to death (animals that died)	Friesians	22.5	17–40	5.5	> 0.05	Similar
	Borans	17.5	16–22			
	Non-endemic Zebus	24	23–26			
Days to death (all groups)	Friesians	0	0–40	4.9	> 0.05	Similar
	Borans	0	0–22			
	Non-endemic Zebus	0	0–26			
	Endemic Zebus	0	0			
Days to recovery	Friesians	22	18–27	4.2	< 0.05	Endemic Zebus different from the other three groups
	Borans	22.5	19–27			
	Non-endemic Zebus	21	18–24			
	Endemic Zebus	17	13–24			

K = Kruskal-Wallis constant; P = Probability

of the non-endemic Zebus developed very severe reactions and died of theileriosis. Four of the Friesians, six of the Borans, four of the non-endemic Zebus and three of the endemic Zebus developed severe reactions and survived. One of the Friesians, three of the non-endemic Zebus and six of the endemic Zebus had mild reactions while one of the Friesians and one of the endemic Zebus had inapparent reactions. Overall, the Borans manifested the most severe clinical disease followed by the Friesians, the non-endemic Zebus and the endemic Zebus (Table 1).

The febrile reactions followed a similar trend with the Borans having the highest mean maximum temperature (40.6 ± 0.4 °C) followed by the Friesians (40.0 ± 0.4 °C), the non-endemic Zebus (39.9 ± 0.8 °C) and the endemic Zebus (39.9 ± 0.6 °C). Comparatively, there was no significant difference ($P > 0.05$) in the time to onset of fever, duration of fever and development of peak fever between the groups. There was, however, a significant difference ($P > 0.05$) in the time it took to reach the peak fever reactions, with the endemic Zebus taking a significantly shorter time to reach these than the other three groups. There was no significant difference in the time it took to reach peak fever between the other three groups. In those that did recover the

endemic Zebus also took significantly shorter time ($P > 0.05$) to recover (i.e. schizonts and fever disappeared) than the other three groups, and there was no significant difference between the other three groups in the time it took to recover.

The clinical manifestations of the disease in the Friesians, the Borans and the non-endemic Zebus were similar. The animals with severe or very severe reactions were listless and inappetent with laboured breathing, and lost condition very rapidly. They had petechial haemorrhages on the visible mucous membranes, were photophobic, and they developed a moist cough and corneal opacities of varying numbers and severity. Of the three Zebus that had severe reactions, only one was listless and inappetent and consequently lost condition. The other two were alert and retained their appetite and condition throughout the duration of the experiment, although they had a moist cough and petechial haemorrhages on the visible mucous membranes. The superficial lymph nodes became enlarged in all the animals that developed clinical disease. Using the Fisher Exact test there was a significant difference in the number of animals that died and conversely, the number of animals that recovered. Significantly more Friesians, Borans and non-endemic Zebus died of theileriosis than endemic Zebus $P > 0.05$.

TABLE 2 Comparisons of parasitological parameter responses

Parameters	Groups	Median	Range	K	P	Interpretation
Day to initial appearance of schizonts	Friesians	10	7–16	1.54	> 0.05	Similar
	Borans	11	6–13			
	Non-endemic Zebus	10	7–13			
	Endemic Zebus	10	8–13			
Duration of schizont parasitosis	Friesians	13	9–17	9.3	< 0.05	Endemic Zebu different from the other three groups
	Borans	10	7–13			
	Non-endemic Zebus	11.5	1–16			
	Endemic Zebus	6	1–19			
Days to peak parasitosis	Friesians	14	10–22	2.4	< 0.05	Endemic Zebu different from the other three groups
	Borans	13.5	11–17			
	Non-endemic Zebus	13	9–18			
	Endemic Zebus	12	9–17			
Days to piroplasm parasitaemia	Friesians	14.5	12–19	3.2		Similar
	Borans	14	10–16			
	Non-endemic Zebus	14	11–16			
	Endemic Zebus	16	12–25			
Duration of parasitaemia	Friesians	10.5	3–16	5.5	> 0.05	Similar
	Borans	9.5	3–19			
	Non-endemic Zebus	12	8–18			
	Endemic Zebus	9	1–12			

K = Kruskal-Wallis constant; P = Probability

TABLE 3 Comparisons of haematological parameter responses

Parameters	Groups	Median	Range	K	P	Interpretation
Total pre-infection WBC x 10 ³ /mℓ	Friesians Borans Non-endemic Zebus Endemic Zebus	12 000 10 250 12 400 10 750	6 700–15 100 4 300–20 200 4 600–18 600 5 500–20 500	0.3	> 0.05	Similar
Days to nadir	Friesians Borans Non-endemic Zebus Endemic Zebus	15.5 16 16 14	12–20 16–19 4–19 12–19	5	> 0.05	Similar
Nadir 10 ³ /ml	Friesians Borans Non-endemic Zebus Endemic Zebu	2 250 1 800 3 600 3 800	900–7 400 1 200–4 700 700–12 600 2 100–7 100	8	< 0.05	Borans different from the other three groups
Lymphoctes: pre-infection 10 ³ /mℓ	Friesians Borans Non-endemic Zebus Endemic Zebus	8 065 8 758 8 822 7 313	4 473–12 835 3 440–15 150 3 818–10 881 4 260–13 223	0.1	> 0.05	Similar
Days of lymphocyte nadir	Friesians Borans Non-endemic Zebus Endemic Zebus	16.5 18 18 14	14–19 14–21 12–19 12–19	8.14	< 0.05	Endemic Zebus different from the other three groups
Lymphocyte nadir	Friesians Borans Non-endemic Zebus Endemic Zebus	1 897 1 431 2 763 3 126	720–5 624 960–3 737 490–7 300 1 750–4 640	7.6	> 0.05	Similar
PCV: pre-infection	Friesians Borans Non-endemic Zebus Endemic Zebus	32.5 40.5 39 37	23–37 29–47 29–45 29–42	9.3	< 0.05	Friesians different from the other three groups
Days to nadir	Friesians Borans Non-endemic Zebus Endemic Zebus	19 19 21 17	10–28 14–28 10–26 12–21	10	< 0.05	Endemic Zebus different from the other three groups
Nadir	Friesians Borans Non-endemic Zebus Endemic Zebus	23 25.5 23 28.5	17–30 16–32 14–33 19–35	4.1	> 0.05	Similar

There was no significant difference in the number of animals that died between the other three groups.

All animals except one Friesian and one endemic Zebu became infected and developed schizont parasitosis. The Friesian, however, developed a piroplasm parasitaemia, but the endemic Zebu did not. Both animals also developed positive antibody titres indicating they had been infected. All the Friesians and Borans, nine of the non-endemic Zebus and

eight of the endemic Zebus developed piroplasm parasitaemias.

There was no significant difference ($P > 0.05$) in the length of the prepatent period between the groups (Table 2). There was a significant difference ($P > 0.05$) in the duration of schizont parasitosis with schizonts being detectable in the endemic Zebus for a shorter time than in the other three groups. There was no significant difference in the duration

of parasitosis between the Friesians, the Borans and the non-endemic Zebu. There was no significant difference ($P > 0.05$) between the groups in the time to onset of piroplasm parasitaemia, and in the time to peak parasitaemia.

The haematological responses were similar in all the groups. There was a marked fall in the WBC counts in all the four groups. There was no significant difference ($P > 0.05$) in the pre-infection total WBC values between the groups (Table 3), but the Boran WBC values fell to significantly lower levels ($P > 0.05$) than those of the Friesians, the non-endemic Zebus or endemic Zebu. The fall in WBC counts was a panleukopenia involving lymphocytes, neutrophils, eosinophils and monocytes. In all cases there was a recovery of the WBC numbers from the third week of infection in the animals that recovered. For all the animals that died, the WBC counts fell and did not recover. The Friesians had significantly lower ($P > 0.05$) pre-infection PCV values than those of the endemic Zebu although all were within the normal range. The Friesians had significantly lower ($P > 0.05$) pre-infection haemoglobin concentrations than the other three groups. There was no significant difference in PCV values between the other three groups. The PCV values fell slightly during the course of disease, but remained within the normal bovine range in the groups. There was a slight fall in the RBC counts in all the animals groups, but no significant differences were observed in the pre-infection values and those that developed during the course of the disease. In individual animals, six of the Friesians, five of the non-endemic Zebu, two of the Borans and a single endemic Zebu showed variable degrees of anaemia with no signs of regeneration of the RBC. All these animals had severe reactions except the endemic Zebu which had prolonged parasitosis but no fever.

All surviving animals had seroconverted (i.e. had positive antibody titres to *T. parva* schizont antigen) by day 35. Only two of the animals which died, one Friesian and one non-endemic Zebu, had seroconverted. The animals that died showed similar lesions on necropsy. They had various degrees of pulmonary oedema, hydrothorax, hydroperitoneum and hydropericardium. The kidneys revealed the presence of "pseudoinfarcts" typical of ECF and the mucosa of the abomasum had erosions. Ecchymotic haemorrhages were evident on the epicardium and the serosal surface of the rumen. The lymph nodes were enlarged and haemorrhagic. The spleen was of normal size or slightly atrophic. Schizonts were observed in impression smears made from the

spleen, lymph nodes, kidneys, adrenal glands and lungs.

DISCUSSION

In this study no differences were observed between *B. indicus* and *B. taurus* cattle or between any of the Zebu (*B. indicus*) groups in their infectibility with *T. parva* Muguga sporozoite stabilate. All the animals developed a patent schizont parasitosis except one of the Friesians and one of the endemic Zebu. Piroplasms, however, did develop in the blood of this Friesian indicating that the infection had established itself and both these animals seroconverted which confirmed this. The length of the prepatent period in the three *B. indicus* and the *B. taurus* cattle was the same, and as the prepatent period in *T. parva* infection is dose dependent (Jarret, Crichton & Pirie 1969; Cunningham *et al.* 1973; Radley, Brown, Burrige, Cunningham Pierce & Purnell 1974; Dolan *et al.* 1984; Buscher, Morrison & Nelson 1984; Mutugi *et al.* 1991) it is concluded that the animals used in this experiment must have received and effectively been infected by the same dose of the parasite.

The severity of disease is also dose dependent and in animals of equal susceptibility, it would be expected that their clinical reactions would be the same. This was not the case in this experiment. The Friesians and the Borans suffered from a markedly more severe disease than the endemic Zebu, while the non-endemic Zebu had a disease reaction mid-way between the Friesians and Borans and the endemic Zebu. Statistically, the Friesians and Borans were similar in their reaction to infection and the non-endemic Zebu and the endemic Zebu were similar to each other but different from the Friesian/Borans. The duration of parasitosis for the non-endemic and endemic Zebus was also similar but different from the Friesians and Borans. The results indicate that *B. indicus* and *B. taurus* are equally infectible by *T. parva* Muguga when simply assessed at one dose level as was done in this experiment. This is in contrast to results obtained using graded doses of the parasite as reported by Jarret *et al.* (1969), Radley (1978) and Dolan & McHardy (1978) where *B. indicus* cattle were observed to be less susceptible to infection than *B. taurus* cattle. Within the *B. indicus* groups, however, with the one dose level as used in our study, the *B. indicus* cattle from ECF-endemic areas were better able to limit the development of clinical disease than the *B. taurus* and both the *B. indicus* cattle

groups form ECF-free areas. This is consistent with the finding of Barnett (1968) who found *B. indicus* cattle from Northern Kenya, where ECF does not occur to be highly susceptible to tick-induced theileriosis. Similar observations have been made by Yeoman (1966) who reported high mortality rates when *B. indicus* cattle from ECF-free areas were introduced into ECF-endemic areas. Stobbs (1966) also reported high mortality of Boran cattle when they were introduced into an ECF-endemic area in spite of chemoprophylaxis for 1–3 months with tetracyclines. It should, however, be noted that in the study by Stobbs (1966) it was not possible to establish the cause of death in some of the animals and several other diseases were prevalent in the region. Calves born through mating of local ECF resistant *B. indicus* (Zebu) heifers to susceptible Boran bulls had an intermediate resistance to ECF that was between that of the parents. Guilbride & Opwata (1963), in a study in which cattle of different genotypes and origin were infected by application of *T. parva* infected ticks, had previously shown that cross-bred calves born of susceptible bulls and resistant dams had a resistance to ECF intermediate between that of the parents. High mortality rates similar to those of *B. taurus* cattle have been reported in *B. indicus* cattle introduced to ECF in an endemic area (Irvin & Morrison 1987). These observations indicate that resistance to ECF in *B. indicus* cattle derived from endemic areas is not innate but acquired through selection of the more resistant members of the cattle population in ECF-endemic areas. This resistance is passed to the progeny since animals from endemic areas which have been kept for several generations under ECF-free conditions retain a high degree of resistance to ECF (Barnett & Bailey 1955b; Barnett 1957; Guilbride & Opwata 1963; Irvin & Cunningham 1981; Paling *et al.* 1991). In our study, the endemic Zebu showed high resistance to the disease despite of having had no prior contact with ECF throughout their lives.

The pathogenic effects of *T. parva* infection are due to its ability to induce uncontrolled proliferation of the infected lymphocytes (Hulliger *et al.* 1964; Malmquist, Nyindo & Brown 1970). The pattern of cellular changes during infection is similar in both *B. indicus* and *B. taurus* cattle (Morrison, Buscher, Murray, Emery, Masake, Cook & Wells 1981) and death of infected animals is brought about by this lymphoproliferation, membrane 'leakage' and infiltration of lymphoid and non-lymphoid organs by parasitized cells which interfere with the normal function of these organs. This is especially so in the lungs, where the infiltration is associated with

severe pulmonary oedema which leads to asphyxiation (Irvin & Morrison 1987). The resistance of the endemic Zebu to ECF seems to be based on the greater efficiency of the immune mechanism to limit the proliferation of parasitized cells as exemplified by a shorter duration of parasitosis. How this is achieved and at what stage in primary infections, is not known. What seems certain is that the effect is not at the sporozoite infection stage since the pre-patent period was the same for both the resistant and susceptible animals and, in effect, all cattle became infected. In field situations Barnett & Bailey (1955a), Young (1981) and Moll, Lodhing & Young (1984) observed that in ECF-endemic areas, calf mortality in indigenous animals due to ECF is very low in spite of morbidity approaching 100%. Colostrum antibodies are not protective (Mining 1992) and neither are serum antibodies (Cowan 1981). These observations lend support to our findings that Zebu from ECF-endemic areas are equally infectible as animals from ECF-free areas, but they have evolved immune mechanisms that efficiently control proliferation of parasitized cells.

In conclusion, no differences in infectibility or susceptibility to infection *per se* were observed between any of the cattle types used in this study. Moreover, *B. taurus* and *B. indicus* cattle from ECF-free areas suffered equally severe reactions when infection was induced with a 1:20 dilution of *T. parva* Muguga stabilate 147, a dose which was calculated to be an LD₅₀ in susceptible, improved Boran cattle. There was, however, a marked difference in resistance to ECF between animals (both *B. indicus* and *B. taurus*) originating from ECF-free areas and *B. indicus* originating from ECF-endemic areas, with the latter being markedly more resistant than the former. Although the mechanism of this resistance is not known, it could be that these animals have greater populations of natural killer cells that inhibit the proliferation of infected cells.

ACKNOWLEDGEMENTS

This study was funded by the United Kingdom Department for International Development (DFID) and the Kenya Government through the KARI-DFID Tick-borne Diseases Project. It is published with the permission of the Director, KARI.

REFERENCES

- ANON. 1989. *Proceedings of a workshop on East Coast fever immunisation, Lilongwe, Malawi, 22 September 1988*, edited by T.T. Dolan, ILRAD, Nairobi, Kenya: 187–188.

- BAILEY, K.P. 1960. Notes on *Rhipicephalus appendiculatus* and their infection with *Theileria parva* for experimental transmission. *Bulletin of Epizootiological Diseases of Africa*, 8: 33–43.
- BARNETT S.F. & BAILEY, K.P. 1955a. I. Calf mortality in an enzootic ECF area. *East African Veterinary Research Organisation Annual Report 1954–1955*. Nairobi, Kenya: East African High Commission.
- BARNETT, S.F. & BAILEY, K.P. 1955b II. The susceptibility of Zebu calves to ECF under experimental conditions at Muguga. *East African Veterinary Research Organisation Annual Report 1954–1955*. Nairobi, Kenya: East African High Commission.
- BARNETT, S.F. 1957. Theileriosis control. *Bulletin of Epizootic Diseases of Africa*, 5:343–357.
- BARNETT, S.F. 1960. Connective tissue reactions in acute fatal East Coast fever (*Theileria parva* of cattle). *Journal of Infectious Diseases*, 107:253–282.
- BARNETT, S.F. 1968. Theileriosis, in *Infectious blood diseases of man and animals*, edited by D. Weinman, M. Ristic & T. Volt. New York: Academic Press.
- BROCKLESBY, D.W., BARNETT, S.F. & SCOTT, G.R. 1961. Morbidity and mortality rates in East Coast fever (*Theileria parva* infection) and their application to drug screening procedures. *British Veterinary Journal*, 117:529–531.
- BURRIDGE, M.J. & KIMBER, C.D. 1972. The indirect fluorescent antibody test for East Coast fever (*Theileria parva* infection of cattle). Evaluation of a cell culture schizont antigen. *Research on Veterinary Science*, 13:451–455.
- BUSCHER, G., MORRISON, W.I. & NELSON, R.T. 1984. Titration in cattle of infectivity and immunogenicity of autologous cell lines infected with *Theileria parva*. *Veterinary Parasitology*, 15:29–38.
- COWAN, K.M., 1981. The humoral responses of theileriosis, in *Advances in the control of theileriosis*, edited by A.D. Irvin, M.P. Cunningham & A.S. Young. The Hague: Martinus Nijhoff Publishers.
- CUNNINGHAM, M.P., BROWN, C.G.D., BURRIDGE, M.J. & PURNELL, R.E. 1973. Cryopreservation of infective particles of *Theileria parva*. *International Journal for Parasitology*, 3:583–587.
- DE MARTIN, J.C. & MOULTON, J.E., 1973. Responses of the bovine lymphatic system to infection by *Theileria parva*. 1. Histology and ultra-structure of lymph nodes in naturally infected calves. *Journal of Comparative Pathology*, 83:281–298.
- DOLAN, T.T. & MCHARDY, N. 1978. The chemotherapy of experimental *T. parva* infection, in *Tick-borne diseases and their vectors*, edited by J.K.H. Wilde, University of Edinburgh.
- DOLAN, T.T., NJUGUNA, L. & STAGG, D.A. 1982. The response of *Bos taurus* and *Bos indicus* cattle types to inoculation with lymphoblastoid cell lines infected with *Theileria parva* schizonts. *Tropenmedizin und Parasitologie*, 33:57–62.
- DOLAN, T.T., YOUNG, A.S., LOSOS, G.J., McMILLAN, J., MINDER, C.E. & SOULSBY, K. 1984. Dose-dependent responses of cattle to *Theileria parva* stabilate. *International Journal for Parasitology*, 14:89–95.
- FISHER, R.A. 1958. *Statistical methods for research workers*, 13th ed. Edinburgh: Oliver & Boyd.
- FISHER, R.A. & YATES, F. 1957. *Statistical tables*, 5th ed. Edinburgh: Oliver & Boyd.
- FOWLER, J. & COHEN, L. 1990. *Practical statistics for field biology*, Open University Press.
- GUILBRIDE, P.D.L. & OPWATA, B. 1963. Observations on the resistance of Jersey/Nganda calves to East Coast fever. *Bulletin of Epizootic Diseases of Africa*, 11:289–298.
- HAMEL, H.D. & VAN AMELSFORT, A. 1985. Tick control with flumethrin 1% m/v pour-on under South African field conditions. *Veterinary Medical Review* 1985–1986:132–145.
- HULLINGER, L., WILDE, J.K.H., BROWN, C.G.D. & TURNER, L. 1964. Mode of multiplication of *Theileria* in cultures of bovine lymphocytic cells. *Nature*, 203:728–730.
- IRVIN, A.D. & CUNNINGHAM, M.P. 1981. East Coast fever, in *Diseases of cattle in the Tropics*, edited by M. Ristic and I. McIntyre. The Hague: Martinus Nijhoff Publishers.
- IRVIN, A.D. & MORRISON, W.I. 1987. Immunopathology, immunology and immunoprophylaxis of Theileria infections, in *Immune Responses in Parasite infections: Immunology, Immunopathology and Immunoprophylaxis, Vol. III*, edited by E.J.L. Soulsby. Boca Raton: CRC Press.
- JARRET, W.F.H., CRIGHTON, G.W. & PIRIE, A.M. 1969. *Theileria parva*: Kinetics of infection. *Experimental Parasitology*, 24:9–25.
- JOSHI, N.R., MCLAUGHIN, E.A. & PHILLIPS, R.W. 1957. *Types and breeds of African cattle*. Rome: FAO.
- MALMQUIST, W.A., NYINDO, M.B.A. & BROWN, C.G.D. 1970. East Coast fever: Cultivation of *in vitro* bovine spleen cell lines infected and transformed by *Theileria parva*. *Tropical Animal Health and Production*, 2:139–150.
- MARTIN, L.J. 1973. *Statistical methods for Research workers*, 13th ed. Edinburgh: Oliver & Boyd.
- MASON, I.L. & MAULE, J.P. 1960. *The indigenous livestock of eastern & southern Africa*. Commonwealth Agricultural Bureaux, England.
- MAULE, J.P. 1990. *The cattle of the tropics*. Centre for Tropical Veterinary Medicine, University of Edinburgh.
- MINING, S.K. 1992. *Theileria parva*: The investigation of maternally derived immunity in calves. Ph.D. thesis, University of Liverpool.
- MOLL, G., LOHDING, A. & YOUNG, A.S. 1984. Epidemiology of theileriosis in the Trans-Mara Division, Kenya: Husbandry and disease background and preliminary investigations on theileriosis in calves. *Preventive Veterinary Medicine*, 2: 801–831.
- MOLL, G., LOHDING, A., YOUNG, A.S. & LEITCH, B.L. 1986. Epidemiology of theileriosis in an endemic area in Kenya. *Veterinary Parasitology*, 19:255–273.
- MORRISON, W.I., BUSCHER, G., MURRAY, M., EMERY, D.L., MASAKE, R.A., COOK, R.M., & WELLS, P.H. 1981. *Theileria parva*: Kinetics of infection in the lymphoid system of cattle. *Experimental Parasitology*, 52:243–260.
- MPIRI, D.B. 1990. *Advances in cattle breeding in Tanzania*. Ministry of Agriculture and Livestock Development, Department of Research and Training, Dar-es-Salaam, Tanzania.
- MUTUGI, J.J., NDUNGU, S.G., LINYONYI, A., MARTIM, A.C., MINING, S.K., NGUMI, P.N., KARIUKI, D.P., LEITCH, B.L., D'SOUZA, D., MALOO, S. & LOHR, K.F. 1991. Response to vaccine trial for East Coast fever in two cattle herds at the Kenya Coast. *Preventive Veterinary Medicine*, 10:173–183.
- PALING, R.W., MPANGALA, C., LUTTIKHUIZEN, B. & SIBOMANA, G. 1991. Exposure of Ankole and cross-bred cattle to theileriosis in Rwanda. *Tropical Animal Health and Production*, 23:203–214.
- PAYNE, W.J.A. 1964. The origin of domestic cattle in Africa. *The Empire Journal of Experimental Agriculture*, 32:97–113.

- RADLEY, D.E., BROWN, C.G.D., BURRIDGE, M.J., CUNNINGHAM, M.P., PIERCE, M.A. & PURNELL, R.E. 1974. East Coast fever: Quantitative studies of *Theileria parva* in cattle. *Experimental Parasitology*, 36:278–287.
- RADLEY, D.E. 1978. *Immunisation against East Coast fever by chemoprophylaxis*. FAO Technical Report 1, AG: DP, RAF67/077, Rome: FAO.
- STENDEL, W. 1985. Experimental studies on the effect of Bayticol[®] pour-on. *Veterinary Medical Review*.
- STOBBS, T.N. 1966. The introduction of Boran cattle into an ECF-endemic area. *East African Agriculture and Forestry Journal*, 31:298–308.
- TEALE, A.J. 1983. The major histocompatibility of cattle with particular reference to some aspects of East Coast fever. Ph.D. thesis, University of Edinburgh.
- UILENBERG, G. 1976. Tick-borne diseases and their vectors and epizootiology of tick-borne diseases. *World Animal Review*, 17:8–15.
- UILENBERG, G. 1981. Theilerial species of domestic livestock, in *Advances in the control of theileriosis*, edited by A.D. Irvin, M.P. Cunningham & A.S. Young. The Hague: Martinus Nijhoff Publishers.
- WALKER, J.B. 1974. *The ixodid ticks of Kenya. A review of present knowledge of their hosts and distribution*. London: Commonwealth Institute of Entomology.
- WILDE, J.K.H. 1967. East Coast fever. *Advances in Veterinary Science*, 11:207–259.
- YEOMAN, G.H. 1966. Field vector studies of epizootic East Coast fever. I. A quantitative relationship between *Rhipicephalus appendiculatus* and the epizooticity of ECF. *Bulletin of Epizootic Diseases of Africa*, 14:5–27.
- YOUNG, A.S. 1981. Epidemiology of theileriosis in East Africa, in *Advances in the control of Theileriosis*, edited by A.D. Irvin, M.P. Cunningham & A.S. Young. The Hague: Martinus Nijhoff Publishers: 38.