Trop. Anim. Hlth Prod. (1977) 9, 219–231

EAST COAST FEVER IMMUNISATION TRIALS IN UGANDA: FIELD EXPOSURE OF ZEBU CATTLE IMMUNIZED WITH THREE ISOLATES OF *THEILERIA PARVA*

J. ROBSON,[1] V. PEDERSEN,[2] G. M. ODEKE, E. P. KAMYA and C. G. D. BROWN[1]

Immunological Research on Tick-borne Cattle Diseases and Tick Control Project,[3] *East African Veterinary Research Organization, Muguga, P.O. Box 32, Kikuyu, Kenya*

SUMMARY

Zebu cattle were immunised against East Coast fever (ECF) using three isolates of Theileria parva *inoculated as cryopreserved stabilates of infective particles harvested from* Rhipicephalus appendiculatus. *These isolates included the East African laboratory type strain,* T. parva *(Muguga), and two isolates of* T. parva *from Entebbe, Uganda. Pairs of cattle received an inoculation of stabilate and were allowed to react and recover fortuitously, while groups of five cattle received both stabilate and were protected by chemoprophylactic therapy with oxytetracycline. An IFA serological response was elicited in* 17 *of the* 21 *stabilate recipients.*

These 21 *cattle, along with eight susceptible controls, were exposed to a massive and continuing natural challenge of* T. parva *and* T. mutans, *accompanied by very heavy tick infestation in an ECF enzootic area at Kigungu, Entebbe. All eight controls died of ECF in a mean time of* 25·6 *days. The stabilate recipients were significantly protected, their mean time to death being* 71·3 *days. Only six of these* 21 *cattle died of theileriosis within* 40 *days of exposure and these included three which did not exhibit a serological response following inoculation of stabilate.*

The implications of T. mutans *pathogenesis, unlimited heavy tick challenge and the potential value of complexes of* T. parva *strains are discussed.*

INTRODUCTION

The implementation of chemoprophylactic immunisation against East Coast fever has been described, and the necessity of immunising against a relevant strain or combination of strains of the *Theileria parva/T. lawrenci* complex in order to effect protection emphasised by Radley *et al* (1975a). This need has been illustrated in the field when a trial showed that cattle immunised against *T. parva* (Muguga) were fully susceptible to an ECF syndrome in an enzootic area of Kenya (Snodgrass, Trees, Bowyer, Bergman, Daft and Wall, 1972). These results were in contradistinction to those obtained in a paddock established to provide homologous challenge to *T. parva* (Muguga) immunes under field conditions. In that trial, chemoprophylactically

[1] FAO Staff Members.

[2] Project supported by the United Nations Development Programme with the Food and Agricultural Organization of the United Nations as the Executing Agency, in co-operation with the Government of Uganda.

[3] Project supported by the United Nations Development Programme with the Food and Agriculture Organization of the United Nations as the Executing Agency, in co-operation with the East African Community. The Project is also supported by the Ministry of Overseas Development of the United Kingdom (Research Projects R 2396, R 2494 and R 2845 A and B), the United States Department of Agriculture, the Rockefeller Foundation, the International Atomic Energy Agency and Pfizer International Inc.

immunised cattle were solidly immune to homologous field challenge (Radley *et al*, 1975b).

Similarly, in another field trial, a group of cattle, immunised by exposure to strains of both *T. parva* and *T. lawrenci*, resisted a *T. lawrenci* field challenge which killed four of five cattle only immunised against *T. parva* (Muguga) (Cunningham *et al*, 1974).

The earliest indication that strain characteristics of *Theileria* might be important in immunogenesis and subsequent field protection came from Guilbride and Opwata (1963). In their trial, six cattle, fortuitously immunised against *T. parva* (Muguga) as a result of recovery from an infection induced by 10 infective ticks, died when exposed in an ECF enzootic area at Kigungu, Entebbe. The trial recorded here describes the application of chemoprophylactic immunisation against ECF, using three different isolates of *T. parva*, to groups of zebu cattle and their subsequent exposure in the same Kigungu challenge area used by Guilbride and Opwata.

MATERIALS AND METHODS

Immunisation

Cattle

Boran/Teso (zebu) yearling steers, obtained from the Aswa Beef Production Station, Acholi Ranch, where effective tick control was practised, were used. Giemsa stained smears from the steers were examined for blood parasites and serum was tested for antibodies against *Theileria parva* and *T. mutans* using the indirect fluorescent antibody test described by Burridge (1971), Burridge and Kimber (1972) and Uilenberg, Robson and Pederson (1974). Of the cattle tested, 29 were found to be negative to all the tests and these were used in the experiment. They were held at the Animal Health Research Centre (AHRC), Entebbe, prior to and for 57 days after immunisation and sprayed twice weekly with a long-acting acaricide (0·2 per cent carbaryl[4]). The animals weighed an average of 163 kg, varying between 125 and 215 kg, at the time of immunisation. They were randomised into three groups of seven, inoculated with stabilate, and one group of eight controls.

Stabilates

T. parva (Muguga) Stabilate 68, *T. parva* (Entebbe I) Stabilate 71, and *T. parva* (Entebbe II) Stabilate 66 were prepared by the FAO Project at the East African Veterinary Research Organization (EAVRO) Muguga, and transported to Entebbe at −70°C in solid carbon dioxide. Infectivity control trials with these three stabilates using cattle of *Bos taurus* type, and performed at EAVRO prior to use on this trial gave the following results:

	Stabilate	Volume (ml)	Days to Schizonts	Days to Death
68	*T. parva* Muguga	0·5	Mean 7·57 (7/7) S.E. ±0·48	17·71 (7/7) ±1·27
71	*T. parva* Entebbe I	1·0	Mean 10·44 (9/9) S.E. ±0·53	26·71 (7/9) ±4·33
66	*T. parva* Entebbe II	1·0	Mean 6·78 (9/9) S.E. ±0·32	16·75 (8/9) ±1·16

[4] Sevin (wettable powder), Union Carbide Corporation, USA.

T. parva (Muguga) has been maintained at EAVRO for many years and is the representative East African laboratory strain of *T. parva* (Brocklesby, Barnett and Scott, 1961). *T. parva* (Entebbe I) was an isolate from Kigungu, the area to be used at the exposure site, *T. parva* (Entebbe II) was isolated from a farm a few kilometres from the Kigungu exposure area.

The stabilates were removed from the flask containing solid CO_2, thawed rapidly in a 37°C water bath and held at ambient temperature (approximately 23°C) for 10 min prior to inoculation. Aliquots of 0·5 ml of the selected stabilate were inoculated subcutaneously in front of the right shoulder of each of 21 cattle on day 0 of immunisation. Seven cattle were inoculated with each stabilate and, of these, five in each group were treated chemoprophylactically and two acted as untreated infectivity controls. These 21 cattle were termed "vaccinates".

Treatment

Oxytetracycline hydrochloride,[5] at 5 mg/kg body weight was injected intramuscularly, daily for a total of 6 days (days 0 to 5 inclusive), to 15 cattle, i.e. five recipients of each stabilate. The day 0 treatment was administered approximately 1 min before the inoculation of the stabilate.

Observations

Blood slides and right prescapular lymph node biopsy smears were prepared, air-dried, stained in Giemsa and examined daily after immunisation for 26 days. Thereafter smears were taken 5 days a week to day 57. Rectal temperatures were taken at the same intervals. Twenty millilitres jugular blood were collected weekly from each animal, serum separated and stored at −20°C until used in the IFA test. *T. parva* cell culture schizont antigen was obtained from the FAO Project, EAVRO. *T. parva* and *T. mutans* piroplasm antigens were prepared at the AHRC, Entebbe.

Field exposure

Exposure area

The cattle were exposed to a high natural challenge of both *T. parva* and *T. mutans* in six paddocks of 4 acres each at Kigungu, near the AHRC, Entebbe. The area was that used by Guilbride and Opwata (1963) as their field challenge area and by Matthysse, Colbo and Kamya (1969) for acaricide testing, and described by Robson *et al* (unpublished results). The severity of the ECF challenge in the area was emphasised by the fact that, prior to this experiment, seven susceptible cattle exposed at Kigungu died 24 to 40 days after introduction (mean 28·7 days).

Introduction and handling of cattle

The three groups of seven immunised cattle, together with five susceptible controls, were introduced on day 0 of exposure. A further group of three controls was introduced to the area on day 37 after all cattle in the first group of controls had died. The cattle were herded together within each paddock and moved between paddocks at regular (10-day) intervals so that strains present within the whole area could be evaluated.

Observations

Blood slides, prescapular lymph node smears and rectal temperatures were taken

[5] Terramycin Q Injectable Solution, Pfizer Ltd., Sandwich, Kent, UK.

daily from all cattle for 26 days after exposure and thereafter five times weekly as during immunisation.

Haematrocrit (PCV) levels were determined three times weekly and leucocyte (WBC) counts performed weekly on jugular blood taken in EDTA. Serum was collected weekly and stored at −20°C prior to use in the IFA test.

Diagnostic antigens were prepared from the blood of cattle twice weekly for 2 weeks immediately after piroplasms were first detected. One millilitre jugular blood was collected into 20 ml phosphate buffered saline (PBS) at pH 7·2 and, after washing four times in PBS, smears were made from the packed erythrocytes. These were then used as antigens and the piroplasms examined using the IFA test and titrated dilutions of sera having high antibody titres to *T. parva* or *T. mutans*.

Adult, nymphal and larval ticks were counted *in situ* on each ear of all cattle 12 times in the first 14 days of exposure and thereafter three times weekly.

Total tick counts, both adults and immatures, were made *in situ* on one animal in each group once a week until the selected animal died. As far as possible estimates of the relative numbers of different tick species encountered were made.

TABLE I

Immunisation of zebu cattle against East Coast fever using three Theileria parva *isolates*

Immunisation group		Animal	Response to immunisation	
Isolate used	Chemoprophylaxis	No.	Schizonts	Serology†
Theileria parva (Muguga)	Untreated controls	92	+	+
		94	+	+
	Treated*	72	−	+
		85	−	+
		86	−	−
		90	−	+
		93	−	+
Theileria parva (Entebbe I)	Untreated controls	83	−	+
		88	−	−
	Treated*	71	−	+
		75	−	−
		89	−	+
		91	−	+
		97	+	−
Theileria parva (Entebbe II)	Untreated controls	60	+	+
		81	−	+
	Treated*	59	+	+
		62	−	+
		68	−	+
		69	−	+
		82	−	+

* Oxytetracycline hydrochloride administered intramuscularly, at 5 mg/kg, daily from day 0 to 5.

† An indirect fluorescent antibody titre of 1:640 or greater against *T. parva* (Muguga) cell culture schizont antigen prior to challenge.

Post-mortem examinations were made of all animals which died and smears prepared from blood, lymph nodes, spleen, liver, kidney, heart, lungs and brain. These were air-dried, stained Giemsa and examined for both schizonts and piroplasms.

RESULTS

Immunisation

Table I summarises the results of observations made on the 21 vaccinates up to day 57 after inoculation of stabilate. All reactions were mild or inapparent, schizonts being detected in only 5 of 21 (3 of 6 controls, 2 of 15 treated cattle). The serological responses of individual cattle evaluated against *T. parva* (Muguga) schizont antigen are also given in Table I. All but 4 of the 21 were positive (an IFA titre of 1:640 or higher). Steer No. 86—*T. parva* (Muguga) chemoprophylaxis and three recipients of *T. parva* (Entebbe I)—Nos. 88, an untreated control and 75 and 97, both treated, did not convert serologically and were considered not to have been immunised.

Group mean serological responses are shown in Table II. Serological responses evaluated against both *T. parva* schizont and piroplasm antigens for all three immunised groups gave means of two or more on the dilution count system (Lutz, 1973), indicating a titre of 1:640 or higher. No significant response was detectable against *T. mutans* piroplasm antigen.

TABLE II

Mean indirect fluorescent antibody responses to immunisation and ECF field exposure of immune and susceptible cattle*

Immunogenic isolate (no. of cattle in group)	Serological responses					
	To immunisation		To field challenge			
	T. parva† schizonts	*T. parva* piroplasms	*T. mutans* piroplasms	*T. parva* schizonts	*T. parva* piroplasms	*T. mutans* piroplasms
T. parva Muguga (7)	2·00‡ ±0·22	3·71 ±0·18	0·29 ±0·29	3·00 ±0·53	3·57 ±0·65	3·96§ ±0·40
T. parva Entebbe I (7)	2·00 ±0·38	3·43 ±0·30	0·86 ±0·40	2·86 ±0·55	3·00 ±0·58	3·86§ ±0·30
T. parva Entebbe II (7)	2·71 ±0·18	4·00 ±0	0·57 ±0·37	3·86§ ±0·14	4·00 ±0	4·00§ ±0
1st group susceptible controls (5)	—‖	—	—	1·00 ±0	1·40 ±0·60	0·80 ±0·49
2nd group susceptible controls (3)	—	—	—	0·33 ±0·33	1·67 ±0·88	3·33§ ±0·33

* Using a dilution count formula on $\log_4$, dilutions with titres of 1:40 or less=0, 1:160=1, etc.
† Antigens used in the IFA test.
‡ Group means and standard errors.
§ Significant ($P<0·05$) increase in titre after field exposure.
‖ All titres 0 (1:40 or less) prior to challenge.

Leucocyte counts were not done throughout immunisation but, on day 57 after stabilate inoculation, when the cattle were exposed to field challenge, the mean WBC count of the vaccinates as a whole was significantly lower ($P<0·05$) than that of the control, non-immunised group. Of the individual immunised groups, the WBC counts

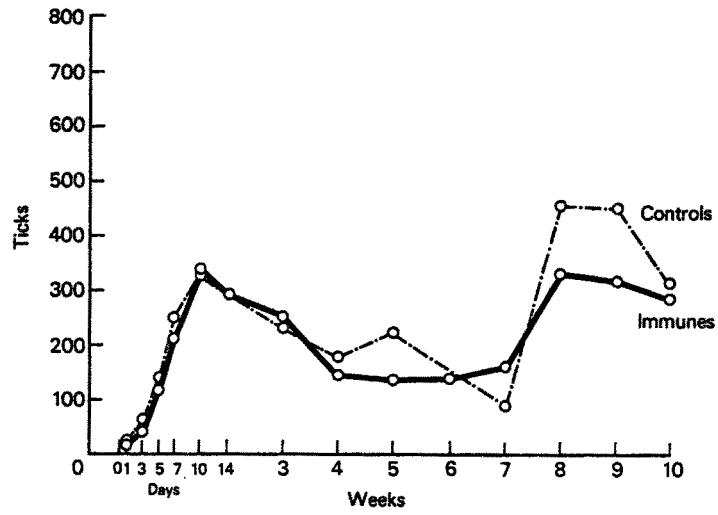


FIG. 1. Mean adult tick counts on the ears of control and immunised cattle during field exposure.

of both the Entebbe I and II recipients were significantly lower than the controls whereas the Muguga group was not different.

Field challenge

Figure 1 shows the mean adult tick counts on both ears of all cattle during the first 10 weeks of exposure. These counts were paralleled by the figures for nymphal ticks on the ears of the cattle. Throughout exposure the ear tick numbers from all vaccinated groups were not significantly different from those on the controls. Whole body counts done on individual animals within each group gave numbers in excess of 1,000 ticks on each animal by day 12 of exposure, the highest figure recorded being over 12,000. The majority of adult ticks on the cattle were *Rhipicephalus appendiculatus* with numerous *Amblyomma variegatum*, fewer *R. evertsi* and rare *Boophilus decoloratus*.

In Table III the individual times to death after exposure are given, while mean figures are recorded in Table V. Schizonts and piroplasms were present on post-mortem examination of all cattle that died before day 40 and these deaths were attributed to theileriosis.

All of eight susceptible controls died of theileriosis with mean time to death of 25·6 days (SE 1·5), while, in 6 of 21 vaccinates, theileriosis could be considered to be the cause of death. Mean times to death of each of the Entebbe vaccinate groups were highly significantly different ($P < 0{\cdot}01$) from that of the controls. The Muguga group differed from the controls at the level $P < 0{\cdot}05$. Examining the vaccinates as a whole, their mean time to death was 71·3 days (SE 8·2), significantly different from the controls at the $P < 0{\cdot}01$ level.

Amongst deaths due to theileriosis, three cattle which were not terminally leucopenic had very low haematocrit levels in the last sample taken prior to death. These were Nos. 86—PCV 7, 72—PCV 10 (both *T. parva* Muguga recipients) and 59—PCV 13 (Entebbe II).

Of the cattle which died after day 40, all were emaciated, anaemic, exhibited very high tick burdens and erythrocytic piroplasms were detectable at varying levels. In these cattle, schizonts could not be detected in post-mortem smears and ECF was

TABLE III

Exposure of zebu cattle immunised with one of three Theileria parva *isolates in an East Coast fever enzootic area at Kigungu*

Immunising isolate	Animal no.	Immunisation response†	Days to death	
			Theileriosis	Other causes
T. parva Muguga	92	+	—	91
	94	+	—	48
	72	+	32	—
	85	+	21	—
	86	−	30	—
	90	+	—	68
	93	+	—	111
T. parva Entebbe I	83	+	—	78
	88	−	29	—
	71	+	—	54
	75	−	—	116
	89	+	—	58
	91	+	—	110
	97	−	23	—
T. parva Entebbe II	60	+	—	98
	81	+	—	65
	59	+	38	—
	62	+	—	143
	68	+	—	84
	69	+	—	139
	82	+	—	62
1st group* susceptible controls	76	−	21	—
	98	−	29	—
	95	−	21	—
	96	−	33	—
	99	−	23	—
2nd group* susceptible controls	84	−	26	—
	87	−	25	—
	100	−	27	—

* Controls introduced—1st group day 0, 2nd group day 37.
† As shown by a significant rise in antibody level against *T. parva* Muguga cell culture (IFA titre of 1:640 or above).

excluded as a cause of death. However, *T. mutans* could not entirely be ruled out as a contributory factor to some of these later deaths.

Tables II and IV confirm not only the exposure of the cattle to a *T. parva* challenge but also to *T. mutans*. The group mean serological responses indicate a significant rise in IFA titre evaluated against a *T. mutans* antigen (Table II). From examination of blood smears of cattle exposed in the field using the diagnostic antigen technique there is evidence that the earliest piroplasms which appeared—at around day 14 to 15 (Table V) were *T. mutans*, since piroplasms in blood smears first reacted with *T. mutans* antiserum some 3 days earlier than against *T. parva* antiserum. In addition, all the immune cattle indicated the presence of *T. mutans* piroplasms, but not necessarily *T. parva*, by day 32 (Table IV).

TABLE IV

Examination of patent parasitaemia by the diagnostic antigen method in cattle reacting to ECF field challenge

Group	Animal no.	Day after exposure first positive*	
		To *T. parva* antiserum	To *T. mutans* antiserum
T.parva	92	24	17
Muguga	94	25	17
immunes	72	19	19
	85	19	19
	86	24	18
	90	25	20
	93	—†	25
Mean		22·67	19·29
SE		±1·17	±1·04
T. parva	83	24	20
Entebbe I	88	20	20
immunes	71	25	20
	75	22	17
	89	32	20
	91	—	20
	97	19	19
Mean		23·67	19·43
SE		±1·91	±0·43
T. parva	60	—	19
Entebbe II	81	24	19
immunes	59	24	18
	62	24	19
	68	—	19
	69	—	19
	82	—	25
Mean		24	19·71
SE		±0	±0·89
Susceptible	76	20	20
controls	98	24	24
	95	20	—
	96	22	25
	99	—	—
	84	23	18
	87	23	18
	100	26	17
Mean		22·57	20·33
SE		±0·81	±1·38

* Piroplasms in blood smears as diagnostic antigens, giving a titre of 1:640 or more to positive antiserum.

† Tested up to day 32 after exposure.

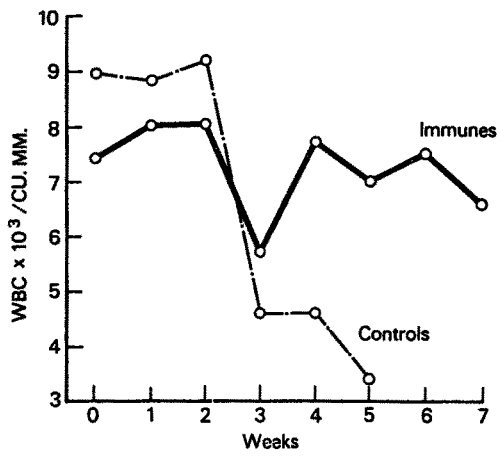
No significant differences were recorded between the groups in the prepatent period, incubation period or time to patent parasitaemia, indicating the uniformity of challenge to which the cattle were exposed (Table V). Moreover, while Table V shows that there was a significant fall in leucocyte counts in all groups ($P<0·05$), Fig. 2 indicates that leucocyte counts in the immunised groups did not differ signifi-

TABLE V

Comparison of group means and standard errors for relevant parameters recorded during ECF field challenge of immunes and susceptible controls

Parameter/ Group	Immunes			Susceptible controls
	T. parva Muguga	*T. parva* Entebbe I	*T. parva* Entebbe II	
Prepatent period (days to first schizonts)	14·43 ±0·43 NS*	16·14 ±1·42 NS	15·86 ±1·03 NS	15·50 ±1·18
Incubation period (days to 39·5°C)	10·71 ±0·36 NS*	7·43 ±1·17 NS	10·57 ±1·21 NS	10·38 ±0·78
Parasitaemia (days to first piroplasms)	15·14 ±0·14 NS*	14·57 ±0·20 NS	14·71 ±0·29 NS	15·13 ±0·35
Days to death	57·29 ±12·84 $P<0·05$	66·86 ±13·80 $P<0·01$	89·86 ±14·98 $P<0·01$	25·63 ±1·45
Percentage fall in WBC count†	39·71 ±3·21 NS*	37·29 ±3·80 NS	29·29 ±6·25 NS	48·20 ±9·41
Percentage fall in PCV‡	57·14 ±6·32 NS*	44·14 ±7·97 NS	60·43 ±3·96 NS	41·80 ±17·22

* Significance of difference from mean of controls.
† Fall in leucocyte count significant in all groups ($P<0·05$).
‡ Fall in haematocrit highly significant in all groups ($P<0·01$).
NS = Not significant.



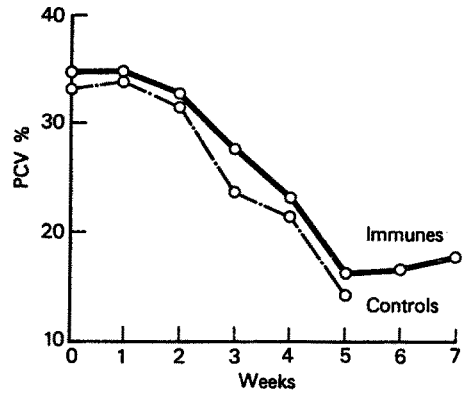


FIG. 2. (left) Mean weekly leucocyte counts of control and immunised cattle during field exposure.
FIG. 3. (right) Mean weekly haematocrit levels of control and immunised cattle during field exposure.

cantly from those of the controls. This was confirmed by evaluation of group means and, except on day 0 of exposure, when the Entebbe groups had significantly lower WBC counts than the controls, the development of leucopenia paralleled that in the controls. After the controls died, however, the WBC counts of the immunes rose again for the period of observation.

Figure 3 shows the very marked fall in haematocrit levels in all groups, which was highly significant ($P < 0{\cdot}01$). In the immunes, these did not rise again after the death of the controls but remained significantly depressed.

DISCUSSION

As determined by group mean serological responses, immunisation in the three groups as a whole was effective. However, there were indications that individual animals within the groups were not immunised, as judged by serological conversion evaluated against *T. parva* schizont antigen. Thus, at the time of field exposure it might be considered that six of seven *T. parva* Muguga recipients, four of seven in the Entebbe I group and all seven of the Entebbe II group had been immunised.

Failure to immunise all the cattle might be attributed to either poor stabilate infectivity or low susceptibility of the cattle used. Tests on the stabilates at EAVRO prior to their use indicated them to be highly infective to *Bos taurus* but further control tests were not done on *Bos indicus* nor with aliquots of the material flown to Entebbe. However, work at EAVRO (Radley, pers. comm.) indicates that zebu cattle have a lower susceptibility than *Bos taruus* at the end point of *T. parva* stabilate titration, with apparent loss of stabilate infectivity and immunogenicity. This might, in this instance, be an explanation for the mild reactions exhibited by the untreated controls.

Though the cattle were apparently healthy at the end of the immunisation period, the reduced WBC counts of the animals in the Entebbe groups indicate that they might have experienced longer term effects than were anticipated. It is worth noting that leucopenia was not observed in the Muguga group but only with the two newly isolated and as yet not fully characterised Entebbe strains. Future immunisation trials, using these strains, should perhaps include intensive examination of the long-term effects of this form of immunisation.

The tick counts on the exposed cattle and the consequent clinical parameters recorded would indicate that these animals were exposed to a massive and uniform challenge in the field. Despite this, only 6 of 21 vaccinates died of theileriosis compared with eight of eight controls. Moreover, in three of the vaccinates *T. mutans* could well be incriminated as a significant factor contributing to their death, since they were anaemic and not leucopenic when haematological parameters were last taken. In addition, of the six vaccinates which died, three had not converted serologically post-immunisation. On this basis it was considered that they might not have been effectively immunised. Examining the vaccinates both as individual groups and as a whole, all groups were significantly protected against this field challenge as judged by time to death. Within the groups, four of seven *T. parva* (Muguga), five of seven Entebbe I and six of seven Entebbe II immunes did not die of ECF. Moreover, neither of the two Entebbe I recipients which died had converted serologically during immunisation.

Guilbride and Opwata's *T. parva* (Muguga) recovered cattle all died in 52·3 days at Kigungu and they concluded that *T. parva* (Muguga) had not protected them (1963). Jezierski, Lambelin and Lateur (1959) thought that protection against ECF could best be achieved by immunisation with a local strain or combination of strains. Radley *et al* (1975c), while concluding that *T. parva* (Muguga) afforded significant protection against heterologous stabilate challenge, determined that a multiplicity of strains used to immunise effected better protection against heterologous challenge Radley *et al* (1975a). Thus it was not surprising that this trial indicated that better protection was afforded against field challenge by the local strains than by *T. parva* (Muguga). It was of interest that the Entebbe I strain, isolated from the challenge paddocks themselves, did not appear as effective as the Entebbe II strain. Perhaps this

is more an indication of the poor infectivity of Entebbe I rather than its antigenic characteristics, since it did not appear to infect three of seven subjects.

Comparing the six cattle which reacted and recovered fortuitously, with the 15 chemoprophylactic recipients, it is difficult to discern any differences. The former recovered animals died at a mean time of 68 days compared with the latter group's mean time to death of 73 days. Conversely, in only one of six fortuitously recovered cattle, No. 88, could death be attributed to ECF, whereas 5 of 15 chemoprophylactic recipients died of theileriosis.

Perhaps the most important feature of this trial is that it shows that, despite what is evidently highly effective protection against ECF, cattle will still die during field trials if other factors are not also controlled. Notable amongst these in this instance is the *T. mutans* challenge which has previously been incriminated in field trials where *T. parva* immunes are exposed (Snodgrass *et al*, 1972; Irvin *et al*, 1972; Uilenberg *et al*, 1976). Uilenberg *et al* (1974) and Young *et al* (1977, in preparation) have shown that *T. mutans* transmitted by *Amblyomma* spp. may cause severe clinical disease and death associated with anaemia in susceptible cattle. The first of these strains was isolated at Kigungu and, while *T. mutans* might not normally be lethal to zebu cattle it may well have been a factor which contributed to the death of ECF immune cattle in this trial. The highly significant fall in haematocrit in all groups would indicate this. It could prove necessary to immunise cattle against *T. mutans* as well as *T. parva* to help protect them during unlimited exposure to tick-borne pathogens. It is interesting to record, however, that Robson *et al* (unpublished results) exposed five ECF-susceptible cattle recently recovered from tick transmitted *T. mutans*, isolated from Kigungu, at Kigungu, and four died of acute theileriosis at the same time as susceptible controls.

A further factor to be considered is the effect of the ticks themselves. With the massive tick challenge experienced in this trial, the possibilities of some form of tick toxicosis causing death or immunosuppression sufficient to overcome protection against disease must be very great. Riek (1957) showed how heavy infestations with *Boophilus microplus* caused both anaemia and other manifestations of toxicosis. Thomas and Neitz (1958) indicated that tick toxicosis due to *R. appendiculatus* might have an aggravating effect on other syndromes, citing *T. mutans* and Tzaneen disease as one of their examples. These observations were confirmed by van Rensburg (1959). Thus it is evident that an ECF vaccine will not necessarily protect against unlimited challenge with a multiplicity of pathogens. Field trials must therefore be approached, and results interpreted, with caution. Nevertheless, significant protection against ECF has been demonstrated in this trial and it should be followed up. There are arguments for examining the protection afforded by a complex of immunogenic *T. parva* strains, coupled with protection against *T. mutans* and heavy tick infestation.

ACKNOWLEDGEMENTS

We are grateful to our colleagues on the Immunological Research on Tick-borne Cattle Diseases and Tick Control Project, EAVRO, for their advice and assistance in planning and implementing this trial. We would also like to thank Messrs. C. Ongor, O. Lamony, J. Male, H. Luswata, S. Assimwe, J. Obua, I. Alala and S. V. Rubanga for technical assistance.

The Acting Commissioner of Veterinary Services, Mr. H. Kagoda, and the Chief Veterinary Research Officer, Uganda, Dr. A. K. Oteng, are thanked for the facilities provided and for permission to publish. This paper is published by kind permission of the Director, EAVRO.

REFERENCES

BROCKLESBY, D. W., BARNETT, S. F. & SCOTT, G. (1961). *British Veterinary Journal*, **117**, 529–531.

BURRIDGE, W. J. (1971). *Research in Veterinary Science*, **12**, 338–341.

BURRIDGE, M. J. & KIMBER, C. D. (1972). *Research in Veterinary Science*, **13**, 145–455.

CUNNINGHAM, M. P., BROWN, C. G. D., BURRIDGE, M. J., IRVIN, A. D., KIRIMI, I. M., PURNELL, R. E., RADLEY, D. E. & WAGNER, G. G. (1974). *Tropical Animal Health and Production*, **6**, 39–43.

GUILBRIDE, P. D. L. & OPWATA, B. (1963). *Bulletin of Epizootic Diseases of Africa*, **11**, 289–298.

IRVIN, A. D., BROWN, C. G. D., BURRIDGE, M. J., CUNNINGHAM, M. P., MUSOKE, A. J., PEIRCE, M. A., PURNELL, R. E. & RADLEY, D. E. (1972). *Tropical Animal Health and Production*, **4**, 220–229.

JEZIERSKI, A., LAMBELIN, G. & LATEUR, L. (1959). *Bulletin D'Information de L'Ineac*, **VIII**, 1–21.

LUTZ, W. (1973). In **Application of Immunological Methods** (ed. D. M. Weir), Appendix 1, A1.28, Blackwell Scientific Publications, Oxford.

MATTHYSSE, J. G., COLBO, M. H. & KAMYA, E. P. (1969). *Bulletin of Entomological Research*, **58**, 465–485.

RADLEY, D. E., BROWN, C. G. D. CUNNINGHAM M. P., KIMBER, C. D., MUSISI, F. L., PAYNE, R. C., PURNELL, R. E., STAGG, S. M. & YOUNG, A. S. (1975a). *Veterinary Parasitology*, **1**, 51–60.

RADLEY, D. E., BROWN, C. G. D., CUNNINGHAM, M. P., KIMBER, C. D., MUSISI, F. L., PURNELL, R. E., STAGG, S. M. & PUNYUA, D. K. (1975b). *Veterinary Record*, **96**, 525–527.

RADLEY, D. E., BROWN, C. G. D., BURRIDGE, M. J., CUNNINGHAM, M. P., KIRIMI, I. M., PURNELL, R. E. & YOUNG, A. S. (1975c). *Veterinary Parasitology*, **1**, 35–41.

RIEK, R. F. (1957). *Australian Journal of Agricultural Research*, **8**, 209–214.

SNODGRASS, D. R., TREES, A. J., BOWYER, W. A., BERGMAN, J. R., DAFT, J. & WALL, A. E. (1972). *Tropical Animal Health and Production*, **4**, 142–151.

THOMAS, A. D. & NEITZ, W. O. (1958). *Journal of South African Veterinary Medical Association*, **29**, 39–50.

UILENBERG, G., ROBSON, J. & PEDERSEN, VAL. (1974). *Tropenmedizen und Parasitologie*, **25**, 207–216.

UILENBERG, G., SCHREUDER, B. E. C., SILAYO, R. S. & MPANGALA, C. (1976). *Tropenmedizin und Parasitologie*, **27**, 329–336.

VAN RENSBURG, S. J. (1959). *Journal of the South African Veterinary Medical Association*, **30**, 75–95.

EXPERIENCES D'IMMUNISATION CONTRE LA FIEVRE DE LA COTE EST EN OUGANDA: EXPOSITION SUR LE TERRAIN DE ZEBUS IMMUNISES AVEC TROIS SOUCHES DE *THEILERIA PARVA*

Résumé—Des zébus ont été immunisés contre la fièvre de la côte Est (ECF) à l'aide de trois souches de *Theileria parva*, injectées sous forme de stabilats congelés de particules infectieuses récoltées à partir de *Rhipicephalus appendiculatus*. Ces souches comprennent la souche de laboratoire-type d'Afrique de l'Est—*T. parva* (Muguga) et deux souches de *T. parva* d'Entebbe, Ouganda. Des paires de bovins ont reçu une inoculation de ces stabilats et n'ont pas été traités, tandis que des groupes de cinq bovins ont reçu ces mêmes stabilats et ont été protégés par un traitement chimioprophylactique avec de l'oxytétracycline. Une réponse sérologique (réaction d'immuno-fluorescence indirecte) a été détectée chez 17 des 21 animaux inoculés. Ces 21 bovins, avec 8 témoins sensibles, ont été exposés à une infestation naturelle massive et continue par des très grands nombres de tiques infectées de *T. parva* et *T. mutans* dans une région d'enzootie à ECF à Kikungu, Entebbe. Les 8 témoins sont morts de ECF dans un temps moyen de 25.6 jours. Les bovins inoculés avec des stabilats ont été significativement protégés, leur délai de survie étant en moyenne de 743 jours. Seulement 6 de ces 21 animaux sont morts de theilériose en moins de 40 jours d'exposition et parmi eux s'en trouviaent 3 qui n'avaient pas réagi sérologiquement à l'inoculation de stabilat.

Les auteurs discutent des implications de la pathogénie de *T. mutans*, de l'infestation massive et illimitée par les tiques et de la valeur potentielle des combinaisons de souches de *T. parva*.

PRUEBAS DE INMUNIZACIÓN CONTRA LA FIEBRE DE LA COSTA ESTE EN UGANDA: EXPOSICION DE CAMPO DE GANADO CEBÚ INMUNIZADO CON TRES CEPAS DE *THEILERIA PARVA*

Resumen—Un grupo de ganado Cebú fue inmunizado contra theileriosis, utilizando tres cepas de *Theileria parva*, inoculadas con estabilados criopreservados de partículas infectivas extraídas de *Rhipicephalus appendiculatus*. Estos aislamientos incluyeron le cepa patrón del Laboratorio Africano del Este, *T. parva* (Muguga) y dos cepas de *T. parva* de Entebbe, Uganda. Parejas de animales fueron inoculados con estabilados, mientras que grupos de 5 animales recibieron el estabilado y protección quimioterapéutica con oxitetraciclina. Se detectó una respuesta serológica con la prueba indirecta de anticuerpos fluorescentes "IFAT" en 17 de los 21 animales que recibieron estabilados.

Estos 21 bovinos, junto con ocho controles susceptibles, se expusieron a una descarga continua natural de *T. parva* y *T. mutans*, acompañada de una infestación alta de garrapatas en un área enzoótica en Kigungu, Entebbe. Los ocho controles murieron de theileriosis en un tiempo promedio de 256 días. Los animales inmunizados mostraron una protección significativa, muriendo de theileriosis en un tiempo promedio de 71.3 días. Solamente seis de los 21 animales murieron de theileriosis dentro de los 40 días después de la exposición, incluídos tres que no mostraron respuesta serológica después de la inoculación del estabilado.

Se discute también la implicación de la patogénesis de *T. mutans*, la exposición ilimitada a garrapatas y el valor potencial de complejos de *T. parva*.