Parasite stocks used for East Coast fever immunization in Kenya

J.J. Mutugi, A.S. Young, A.C. Maritim, S.G. Ndungu, A. Linyonyi, P.N. Ngumi, S.K. Mining, B.L. Leitch, D.A. Stagg, J.G. Grootenhuis,* D.P. Kariuki, S.P. Morzaria† and T.T. Dolan†

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

*Wildlife Diseases Section
National Veterinary Research Centre
Kenya Agricultural Research Institute
P.O. Kabete
Nairobi, Kenya

†International Laboratory for Research on Animal Diseases
P.O. Box 30709
Nairobi, Kenya

Following the development of the infection-and-treatment method of immunization against East Coast fever (ECF) by the United Nations Development Programme/Food and Agriculture Organization of the United Nations group at the East African Veterinary Research Organisation, now the National Veterinary Research Centre (NVRC), in Muguga, Kenya, between 1967 and 1977, the Kenya Government was reluctant to sanction the extensive field use of the method. The following concerns were expressed.

a) Immunized cattle might show a reduction in productivity.

b) Insufficient information was available on the various *Theileria parva* parasites prevalent in the country.

c) *Theileria parva* parasites from cattle would not protect cattle against buffalo-derived parasite stocks.

d) Immunized animals might become carriers and thus introduce alien strains of parasites into previously uninfected regions of the country.

e) The infection-and-treatment immunization method might be impractical and/or unsafe.

Since then, work in various laboratories has been undertaken to address these concerns. Presently, a Kenya/British project based at NVRC, Muguga, has been charged with the safe implementation of large-scale ECF immunization in the country. We report here on some laboratory studies carried out, focussing on some of the concerns listed above.

SAFETY STUDIES ON THE INFECTION-AND-TREATMENT IMMUNIZATION

Studies to determine safe and optimal immunizing sporozoite doses for a number of *T. p. parva* and *T. p. lawrencei* stocks were carried out, as well as investigations on the efficacy of treatment with the available immunizing drugs. The studies involved inoculating groups of susceptible Friesian cattle with various doses of *T. parva* sporozoite stabilate dilutions, either singly or in combination. The infections were then treated with one of three anti-theilerial drugs: Medamycin 100 (TechAmerica Group, Inc.), a short-acting oxytetracycline; Terramycin LA (Pfizer Ltd., U.K.), a long-acting oxytetracycline; or a new chemotherapeutic drug, buparvaquone (Butalex^{R}, Coopers Animal Health). Medamycin was given at 10 mg/kg on days 0 and 4 of the immunization. Terramycin LA was given at 20 mg/kg at the same time as stabilate inoculation, and buparvaquone was given at 2.5 mg/kg also at the same time as stabilate inoculation. All drugs were injected intramuscularly.

Following sporozoite stabilate inoculation and appropriate drug treatment, the immunization reaction was monitored using daily clinical and parasitological observations (theilerial schizont parasitosis). On days 28 and 35 after immunization, surviving cattle were examined for *T. parva* antibodies using the indirect fluorescent antibody test (Burridge and Kimber, 1972). On about day 60 after immunization, the surviving cattle were challenged with a lethal dose of homologous parasite. These cattle were later challenged with heterologous parasites.

The results for five titration experiments involving various *T. parva* stocks from geographically separated areas of Kenya are summarized in Table 1. In the titration involving *T. p. lawrencei* (Ol Pejeta) stabilate 199, the highest concentration of 1.0 ml of undiluted stabilate could not be satisfactorily controlled by either Terramycin LA or Medamycin 100. However, at lower concentration (1:100), it was possible to induce subclinical theileriosis with the development of antibodies to *T. parva*. These cattle were immune to homologous challenge. Bupavaquone controlled higher concentrations of the stabilate better than the two oxytetracycline formulations. In contrast, both oxytetracyclines controlled

undiluted and 1:80 stabilate concentrations of the *T. p. parva* (Marikebuni) stock. However, cattle inoculated with the lower dilutions of this parasite stock, as well as surviving controls, were not immune to homologous challenge. The significance of this is discussed later.

Table 1. Reactions of cattle receiving titrated doses of various *Theileria parva* stabilates and treatment with either Medamycin 100 at 10 mg/kg on days 0 and 4, Terramycin LA at 20 mg/kg on day 0 or buparvaquone at 2.5 mg/kg on day 0

Parasite stock	Drug treatments	Cattle reaction (survived immunization)/D28			
		Undiluted	1:10	1:100	1:1000
T. p. lawrencei (Ol Pejeta) (Stabilate 199)	No drug	0/3	1/3	1/3	3/3 (2)
	Terramycin LA	1/3	2/3	3/3	
	Medamycin 100	0/3	3/3	3/3	
	Buparvaquone	1/3	3/3	8/8	
		1:5	1:10	1:100	
T. p. lawrencei (Mara III) (Stabilate 202) and *T. p. parva* (Kilae) (Stabilate 187)	No drug	0/3	1/3		
	Terramycin LA	2/3	2/3	3/3	
	Medamycin 100	2/3	3/3	3/3	
		Undiluted	1:10	1:100	
T. p. lawrencei (Mara III) (Stabilate 202)	No drug	—	—	2/3 (1)	
T. p. parva (Kilae) (Stabilate 187)	No drug	—	—	3/3 (3)	
		Undiluted	1:10	1:80	
T. p. parva (Marikebuni) (Stabilate 3014)	No drug	0/5	4/4 (2)	5/5 (4)	
	Terramycin LA	5/5	5/5 (1)+	5/5 (2)*	
	Medamycin 100	5/5	5/5	5/5 (1)*	

( ) Not immune to homologous challenge.

( )* Negative serology after immunization (D35).

( )+ Positive serology after immunization.

Immunization using mixed *T. parva* stocks, *T. p. parva* (Kilae) stabilate 187 and a *T. p. lawrencei* (Mara III) stabilate 202, worked well. Mixed concentrations of these stabilates at 1:10 and 1:100 dilutions were controlled satisfactorily by both formulations of oxytetracyclines. In a separate titration, stabilate 202 was shown to be more virulent than stabilate 187. *Theileria parva* carrier states were demonstrated in some oxytetracycline-immmunized cattle, but not in buparvaquone-treated animals. Both *T. p. parva* and *T. p. lawrencei* stocks were shown to produce the carrier state.

CROSS-IMMUNITY STUDIES

Since the advent of the infection-and-treatment immunization method, researchers have searched for a *T. parva* stock capable of conferring a wide protection against challenge with other theilerial parasites. An alternative approach was to combine several theilerial parasite stocks to form an "immunization unit" with wide protection. Such a combination of parasites exemplified by the "Muguga cocktail" can be used with a measure of success, as demonstrated in several countries. Recently, Irvin et al. (1983) isolated a theilerial parasite stock, referred to as *T. p. parva* (Marikebuni), from Kilifi District, Kenya, which was shown to provide good protection against severe challenge with other stocks isolated from the district. As the stock is well characterized, it was decided to use this isolate in cross-immunity studies with other *T. parva* isolates from widely separated areas of Kenya.

Table 2 lists the *T. parva* isolates from Kenya used in the studies. Eight of these isolates (all *T. p. parva*) were from Kilifi District, Coast Province, Kenya. Five other stocks, two of *T. p. parva* and three of *T. p. lawrencei,* were isolates from the Rift Valley Province, Kenya. One isolate, *T. p. parva* (Mbita), was from Nyanza Province and three new isolates, Ki1, Ki3 and Ki4, were from Kiambu District, Central Province. The isolation location for each isolate is indicated on the map of Kenya (Figure 1). The isolates were used in cross-immunity studies to challenge cattle immune to the *T. p. parva* (Marikebuni) stock.

The experimental studies required the generation of *T. p. parva* (Marikebuni) immune cattle by immunizing groups of Friesian steers with selected doses of the stabilate and treating the steers with either Medamycin or Terramycin LA. Groups of the Marikebuni immune cattle were challenged in the following ways:

a) challenge of Marikebuni immune cattle by other coastal *T. p. parva* stocks from Kilifi District

b) challenge of Marikebuni immune cattle with *T. p. parva* and *T. p. lawrencei* stocks from elsewhere in Kenya

c) challenge of cattle immune to *T. p. parva* and *T. p. lawrencei* stocks from elsewhere in Kenya with a lethal dose of *T. p. parva* (Marikebuni)

The reactions of the experimental cattle on challenge were described as "inapparent" where no macroschizonts were detected, "mild" where low numbers of schizonts were detected transiently and where a transient fever may or may not have been observed, and "severe" where prolonged schizont parasitosis occurred, usually in high numbers for several days and accompanied by fever. "Very severe" reactions were those where high schizont parasitosis was recorded with a marked development of fever, usually resulting in death. Cattle with inapparent and mild reactions were considered immune. Those with severe and very severe reactions were regarded as not immune, irrespective of whether the animal died.

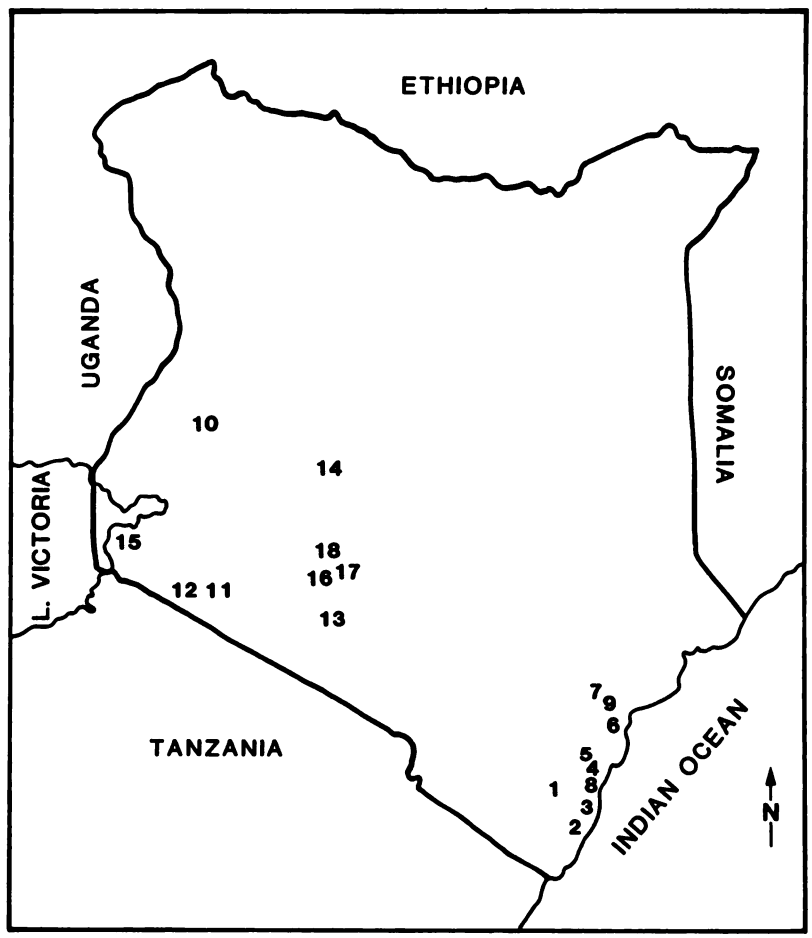

Figure 1. The locations in Kenya where *Theileria parva* stocks were isolated for use in cross-immunity studies. The key to the locations is given in Table 2.

The results of the cross-immunity experiments are shown in Table 2. *Theileria p. parva* (Marikebuni) immune cattle were protected against challenge with seven *T. p. parva* stocks from Kilifi District and four *T. p. parva* stocks from other areas of Kenya and showed partial protection against challenge by *T. p. lawrencei* stocks. Furthermore, cattle immune to various *T. parva* stocks were protected against lethal challenge with *T. p. parva* (Marikebuni).

Table 2. *Theileria parva* stocks used in cross-immunity experiments

Coast Province			
1.	*T. p. parva*	(Mariakani)	Stabilate 3029
2.	*T. p. parva*	(Utange)	Stabilate 223
3.	*T. p. parva*	(Mtwapa)	Stabilate 2262
4.	*T. p. parva*	(Kilifi)	Stabilate 1015
5.	*T. p. parva*	(Kibarani)	Stabilate 2448
6.	*T. p. parva*	(Kiswani)	Stabilate 2240
7.	*T. p. parva*	(Magarini)	Stabilate 2365
8.	*T. p. parva*	(Junju)	Stabilate 1086
9.	*T. p. parva*	(Marikebuni)	Stabilate 3014
Rift Valley Province			
10.	*T. p. parva*	(Uasin Gishu 6)	Stabilate 216
11.	*T. p. parva*	(Kilae)	Stabilate 187
12.	*T. p. lawrencei*	(Mara III)	Stabilate 202
13.	*T. p. lawrencei*	(Ngong 1)	Stabilate 2306
14.	*T. p. lawrencei*	(Ol Pejeta)	Stabilate 199
Nyanza Province			
15.	*T. p. parva*	(Mbita)	Stabilate 169
Central Province			
16.	*T. p. parva*	(Ki1)	Stabilate 210
17.	*T. p. parva*	(Ki3)	Stabilate 213
18.	*T. p. parva*	(Ki4)	Stabilate 214

Note: See Figure 1 showing location of isolation site.

From the titration and cross-immunity studies the following observations were made.

a) There was an optimal range of sporozoite dose for each stabilate that could be controlled satisfactorily by the antitheilerial drugs, which produced subclinical theilerial reactions, and the cattle were immune to homologous challenge. Sporozoite concentrations above the optimal dose produced severe theilerial reactions and those below either did not infect cattle (as shown by lack of *T. parva* antibodies) or infected cattle with only a propor-

tion of the antigenic components of the stabilate. Such cattle were subsequently shown to be susceptible to homologous challenge. This was probably the result of certain parasite components not being present at these very high dilutions.

b) It was possible to immunize cattle with virulent *T. p. lawrencei* stocks (stabilate 199 killed cattle at 1:1000 dilution), provided the right dilution was selected together with the right combination of drug dose and treatment regimen.

c) Mixed *T. p. parva* and *T. p. lawrencei* parasites could be combined and used in various concentrations in immunization.

d) The use of buparvaquone in immunization with the more difficult *T. p. lawrencei* stocks may be justified in special cases, but for routine *T. p. parva* immunization, especially on a large scale, oxytetracyclines are the drugs of choice. Two doses of Medamycin were comparable to one dose of Terramycin LA, but in the *T. parva* (Marikebuni) titration, the short-acting drug gave slightly superior results. The Medamycin treatment regimen was also cheaper, but required that the animals be mustered twice.

e) Cattle immunized with the *T. p. parva* (Marikebuni) stock were protected against parasites from the Rift Valley, Central and Nyanza provinces and did not break through the immunity provided by *T. parva* stocks from geographically separated areas of Kenya. *Theileria p. parva* (Marikebuni) could provide a master immunizing stock for cattle-derived theileriosis in Kenya.

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