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THEILERIOSIS

IN EASTERN, CENTRAL AND SOUTHERN AFRICA



PROCEEDINGS OF A WORKSHOP ON
EAST COAST FEVER IMMUNIZATION
HELD IN LILONGWE, MALAWI
20-22 SEPTEMBER 1988

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T.T. Dolan

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The International Laboratory for Research on Animal Diseases (ILRAD) was established in 1973 with a mandate to develop effective control measures for livestock diseases that seriously limit world food production. ILRAD's research program focuses on African animal trypanosomiasis and East Coast fever, a form of theileriosis.

ILRAD is one of 13 centres in a worldwide agricultural research network sponsored by the Consultative Group on International Agricultural Research. In 1988 funding for ILRAD's essential research and training activities was provided by the African Development Bank, the Rockefeller Foundation, the United Nations Development Programme, the World Bank (the International Bank for Reconstruction and Development) and the governments of Australia, Belgium, Canada, Denmark, France, India, Italy, Japan, the Netherlands, Norway, Sweden, Switzerland, the United Kingdom, the United States of America and West Germany. Additional research activities were supported by special funding arrangements from the European Economic Community and the World Health Organization and capital funds were provided by the Netherlands Government for construction of a new training and outreach building.

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Foreword

These proceedings are of a workshop that was the third in a series organized jointly by the International Laboratory for Research on Animal Diseases (ILRAD) and the Food and Agriculture Organization of the United Nations (FAO). The 1988 workshop was also supported by the Organization of African Unity/Inter-African Bureau of Animal Resources (OAU/IBAR). These workshops are designed to bring together research workers and those responsible for tick-borne disease control in countries of eastern, central and southern Africa. At the first workshop in this series, held in Nairobi in October 1984, participants provided updates on theileriosis in the countries of the region, reports on immunization trials, and papers expanding on elements of immunization (see Irvin, A.D., *Immunization against Theileriosis in Africa: Proceedings of a Workshop Sponsored by the International Laboratory for Research on Animal Diseases and the Food and Agriculture Organization of the United Nations, Held in Nairobi, Kenya, 1-5 October 1984*, Nairobi: ILRAD, 1985). The establishment of a network for the exchange of information and data on theileriosis immunization and control was also discussed. The second workshop, held in Nairobi in September 1985, emphasized data collection and handling as well as analyses of productivity in relation to immunization (see Irvin, A.D., *Immunization against East Coast Fever: Report of a Workshop on Collection, Handling and Analysis of Performance and Productivity Data, Held in Nairobi, Kenya, 23-25 September 1985*, Nairobi: ILRAD, 1986). This workshop introduced the participants to practical data handling and computer use. The third workshop was originally planned for November 1987 but was postponed due to uncertainty about the future and funding of some of the FAO tick and tick-borne disease control projects. The workshop was eventually held in Lilongwe, Malawi, in September 1988, with considerable support from the Malawi Government.

The topics to be discussed were selected by the workshop's Organizing Committee. The topics included reports on theileriosis and immunization trials carried out in the countries of the region since the workshop in 1985. Papers were also invited on such topics as the cost of immunization, parasite characterization, the role of wildlife in theileriosis epidemiology, the role of regional laboratories in controlling ticks and tick-borne diseases, and the attitude of host governments to regional laboratories. Participants reporting on immunization trials were asked to emphasize problems they had encountered in planning, organizing or executing trials so that these experiences could be passed on and these problems anticipated and avoided, if possible, in future trials. In keeping

FOREWORD

with the themes of the earlier workshops in this series, papers were also invited on productivity and data analysis and these topics were expanded to include epidemiology and geographic information systems.

The Organizing Committee also decided to convene committees to address and make recommendations on safe procedures for handling ticks and preparing stabilates, co-ordination of research and funding, and devising a logical and consistent system of nomenclature of *Theileria parva*. The safety issue has been discussed informally for a long time, particularly in relation to the risk of transmission of other animal pathogens by the infection-and-treatment method of immunization. The risk of disease transmission from ticks to man was highlighted a few years ago by the deaths of two people in South Africa from Crimean Congo haemorrhagic fever. Funding for disease control projects is often limited or, if from donors, of short or uncertain periods. Sustained support for tick-borne disease control projects is critical for their success. The common use of sub-species names for *T. parva* and location names in the identification of isolates and stocks, the isolation of cloned parasites and the use of a range of new techniques for the characterization of these clones all demand that a logical and consistent system of nomenclature be adopted. Thus, committees were convened under the chairmanships of Drs. S.P. Morzaria (Safety), S. Magembe (Co-ordination) and A.S. Young (Nomenclature), and met during the course of the workshop. The committees' recommendations were put before the full meeting and debated under the firm chairmanship of Dr. W.N. Masiga. The final, approved, recommendations appear at the end of these proceedings.

The Organizing Committee was composed of Drs. T.T. Dolan and J.K. Lenahan (ILRAD), R.C. Makandawire (Malawi), W.N. Masiga (OAU/IBAR) and F.L. Muisi (FAO, Malawi). This committee never met but communicated effectively nonetheless by telephone, telex and letter. The lines of communication were maintained through the office of the FAO Representative in Malawi, Mr. G.K. Mburathi. The success of the meeting was guaranteed by the continuous support of the veterinary staff in the Malawi Ministry of Agriculture, particularly of the Chief Veterinary Officer, Dr. Thyangathyanga, and the support and encouragement of Dr. P.J. McCosker, of the Food and Agriculture Organization of the United Nations, in Rome.

Participation at the meeting was sponsored by Coopers Animal Health (UK); FAO; German Agency for Technical Cooperation; the governments of Belgium, Ireland and Malawi; ILRAD; OAU/IBAR; Overseas Development Administration (UK); and Oxfam. The Malawi Government was extremely generous in the welcome and support it gave to participants in the form of staff, infrastructure, transport and hospitality. The FAO office in Lilongwe acted as a conduit for exchanges among the committee members in the months preceding

the meeting. The FAO/Netherlands-funded East Coast Fever Vaccine Production and Quality Control Project (GLP/RAF/247/NET) provided office equipment and support staff throughout the meeting. Malawi Pharmaceuticals hosted a very enjoyable cocktail party.

Many people assisted in the preparation of these proceedings. I am particularly grateful to Drs. Norval, Perry and Young for reviewing the manuscripts. Dr. Peter Gardiner provided very constructive criticism on sections of the text. The artwork was done by Mr. John Ayienga and photographs were provided by the Malawi National Information Centre, Mr. David Elsworth, Mr. Francis Shikhubari, Drs. Jane Walker and M.D. Corwin (covers), and Dr. Geu Grootenhuis. Dr. Jim Lenahan and his assistant, Ms. Catherine Munyua, were extremely patient and helpful both in making arrangements for the meeting and in preparing these proceedings.

*Thomas T. Dolan
The International Laboratory for
Research on Animal Diseases
Nairobi, June 1989*



Left to right are Dr. S. Magembe, from the Southern African Development Coordination Conference; Mr. B.M. Ndisale, Secretary for Agriculture, Malawi, delivering the opening address at the meeting; and Dr. G.A. Thyangathyanga, Chief Veterinary Officer, Malawi.

Opening address

B.M. Ndisale

Secretary for Agriculture
Ministry of Agriculture
Lilongwe, Malawi

Mr. Chairman; Director of the Inter-African Bureau for Animal Resources of the Organization of African Unity, Dr. Masiga; Your Worship the Mayor of the City of Lilongwe, Councillor Msosa; distinguished guests; ladies and gentlemen:

I am honoured and most privileged to have the opportunity to welcome you to Malawi on behalf of the government and the people of Malawi and to officiate at this inaugural sitting of the international meeting on East Coast fever immunization. In this vein, I extend to each and every one of you a warm welcome. We feel greatly honoured that you chose Malawi as the venue of this meeting. It is an honour for us to meet some of the world's distinguished scientists in the field of livestock production. You are most welcome here and you are free to go anywhere you like and to talk to anyone you like. Should time permit, feel free to extend your stay.

Distinguished delegates, ladies and gentlemen, allow me a little self-indulgence and to talk about Malawi. I note with gratitude that the agenda for the meeting has included a field visit to the laboratory and animal facilities. There you will be able to see what we are doing in Malawi. As you probably know, Malawi is basically an agricultural country and therefore places great emphasis on all efforts aimed at improving agricultural production. This improvement in agricultural production is aimed not only at crop production but also at livestock production. It is the aim of the government to improve and expand the contribution from the livestock sector to the overall national agricultural output. Initially the increased output from livestock should help the country meet the ever-growing demand for protein due to the fast-growing human population, but eventually the government wishes to export any surplus of the primary products, such as milk, beef and beef by-products.

Livestock, and in particular cattle production, plays a vital role in the agriculture sector. As you all know, we keep cattle primarily for beef and milk, but I think you will agree with me that these animals play a vital part in cultivation and haulage within the rural areas, not forgetting the manure and the social function. The population of cattle in Malawi is currently estimated at just over 1 million and the majority of these are Malawi Zebu owned by the traditional smallholder farmers. In an effort to increase local milk production, the government's policy is to introduce cross-bred dairy cattle to the smallholder farming

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sector through distribution of heifers from government breeding farms and through artificial insemination services. This dairy group, together with some considerable number of beef cattle in Malawi, especially in the central and northern regions, are seriously threatened by East Coast fever (ECF). Mortality in the indigenous young stock was once estimated at 15%, but a 70% loss has been recorded for the exotic and cross-bred cattle. Therefore ECF is a major constraint in the development and expansion of the industry in Malawi. This is further aggravated by the occurrence of other tick-borne diseases: anaplasmosis, babesiosis and heartwater.

The present policy for controlling ECF and other tick-borne diseases is weekly dipping. Pursuing this policy currently costs the government about 3 million kwacha annually for importing acaricides. To this cost we have to add staff costs for supervising dipping and the management of acaricides in general. In addition, we have difficulties in the following:

- a) ensuring timely and adequate supplies of acaricides
- b) ensuring regular attendance at dipping by farmers
- c) ensuring proper dip strength for a variety of reasons
- d) buying curative drugs for ECF

Moreover, even if we managed to have all these problems solved, our infrastructural support is inadequate. With the introduction of cross-bred cattle, expected to be followed by improved milk and beef output, the government hopes to be able to convince farmers having large but less productive herds to reduce the size of the herds and concentrate on fewer but more productive cattle. This should in turn reduce the chances of overgrazing and subsequent soil erosion.

I understand that the experiences and problems I have just outlined are not confined to Malawi alone but all our neighbours and even those beyond are facing these problems in regard to ECF and other major tick-borne diseases, hence the reason why many countries are being represented at this meeting. At this opportune time you scientists from the various national livestock institutions are afforded an opportunity to come together and learn of the problems each one of you is facing in your respective countries.

Realizing the threat posed by ECF and other tick-borne diseases to the cattle production improvement programmes and the ever-increasing costs of acaricides, as well as noting the strides made already in East Africa in search of alternative means to control the disease, Malawi decided to establish a foundation for future regional control of ECF and to investigate the potential of the infection-and-treatment method of immunization in the Malawian context. Consequently, a project on ECF immunization supported with funds from the Danish Agency for International Development (DANIDA) and executed by the Food and Agriculture Organization of the United Nations (FAO) was established. Laboratory facilities and animal accommodation were built and equipped

at the Central Veterinary Laboratory, in Lilongwe. Through a series of experiments, it was demonstrated that our cattle could be protected against ECF using the infection-and-treatment method. The Malawi Department of Veterinary Services with the support of FAO through the Technical Cooperation Programme have, when requested, in a modest way started immunizing cattle on a regular basis on government and some private estates since the termination of the DANIDA support in 1985. To sustain the immunization programme mentioned, Malawi together with other Southern Africa Development Coordination Conference (SADCC) member states affected by ECF have sought external assistance to establish a facility for the production of vaccine against ECF and other major tick-borne diseases. I am happy to announce that our efforts have been rewarded; the government of the Netherlands agreed to support the ECF Vaccine Production and Quality Control Project for two years. This should be followed by support to the SADCC countries from the United Nations Development Programme for a period of five years. Funding for production of vaccines against the other major tick-borne diseases (anaplasmosis, babesiosis and heartwater) is being provided by DANIDA and is being executed by FAO. We hope that when the current negotiations are completed this complementary project will receive funding assistance for a total of five years. The support referred to will go a long way in ensuring that the Organization of African Unity/Scientific, Technical and Research Commission resolution passed in September 1976, recommending the establishment of regional centres in Africa for controlling ticks and tick-borne diseases, is realized. In fact, the OAU in collaboration with FAO identified the Central Veterinary Laboratory, in Lilongwe, as the regional centre for East and Central Africa.

Therefore, on behalf of the government and on behalf of the people of Malawi, I would also like to extend our most sincere gratitude and appreciation to the funding agencies and governments, the OAU, ILRAD and FAO for the support rendered to Malawi on this very important subject. We in Malawi look forward to the continued co-operation with outside partners, not only on the matter of control of ticks and tick-borne diseases, but in all our development efforts.

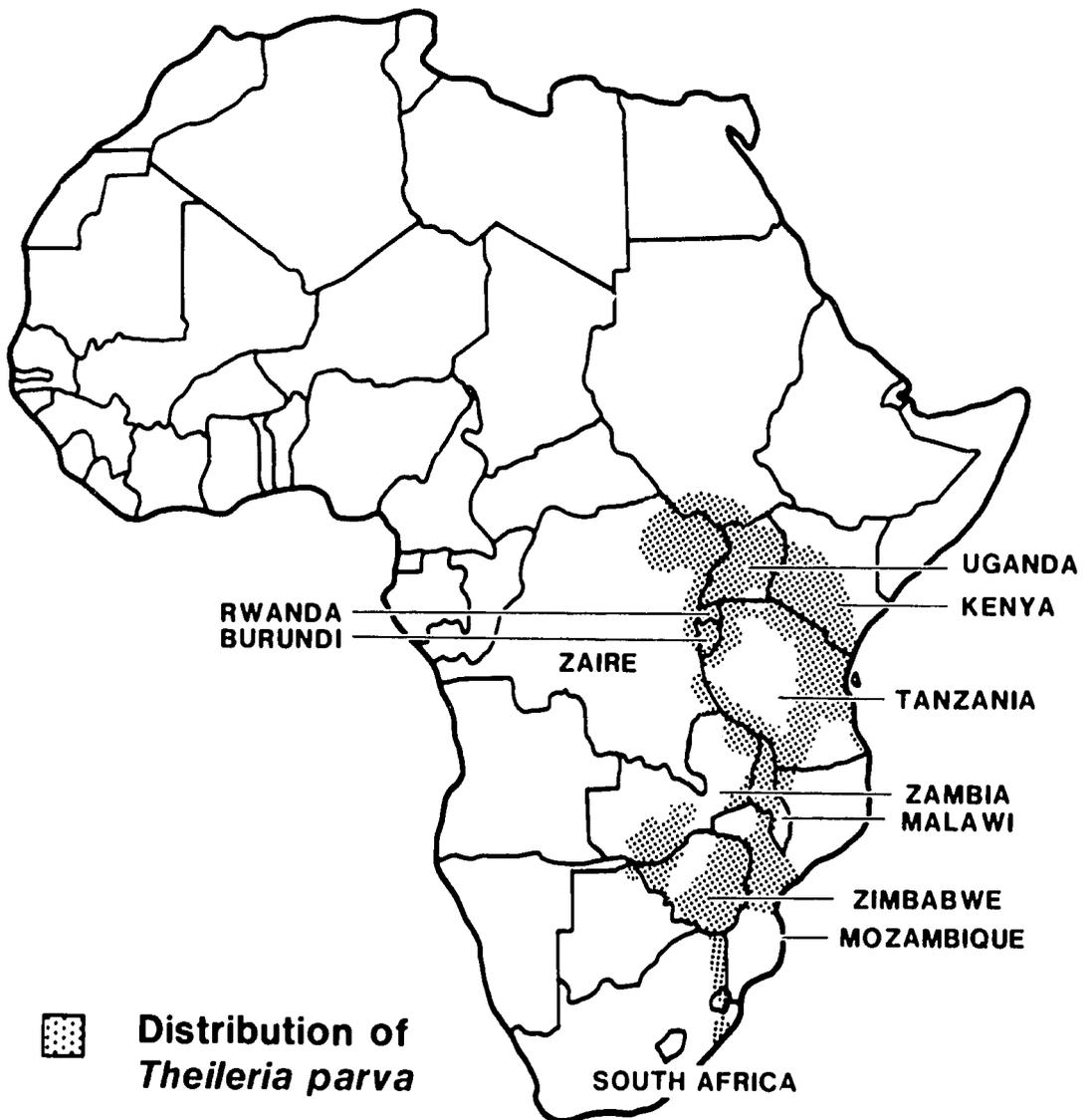
I need not remind you distinguished participants that the task you have before you is not an easy one. The topics listed on the agenda for your deliberations seem appropriately and strategically selected. They are issues of major concern to our livestock and rural development efforts. It is hoped that at this meeting delegates will report on the progress made thus far in their countries in trying to solve the prevailing livestock production problems. Furthermore, I call upon you to come up with realistic strategies and recommendations in the fight against East Coast fever. I am confident that you, the eminent scientists gathered here

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today from participating countries and international organizations, will use the time at your disposal effectively through your interaction to develop strategies aimed at improvements in the livestock field. We must attain high and sustainable livestock production levels for the benefit of the ordinary man and woman whom we are all committed to serve.

Distinguished delegates, I hope you will have an enjoyable stay in our country and that you will be able to combine business with recreation in Malawi, which we boast of as being the Warm Heart of Africa.

Mr. Chairman, ladies and gentlemen, I have the pleasure to declare this meeting officially open.



Distribution of *Theileria parva*. Modified from Lessard, P., L'Eplattenier, R.A., Norval, R.A.I., Perry, B.D., Dolan, T.T., Kundert, K., Croze, H., Walker, J.E., and Irvin, A.D. (in press), Geographic Information Systems in East Coast fever (*Theileria parva*). *Veterinary Record*.

PART 1

COUNTRY REPORTS

Theileriosis in Kenya

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Kenya has a human population of over 21 million (estimated at the end of 1980) and an area of 582670 sq. km. Of the total area, 20% is of high or medium potential and is suitable for crops, intensive forestry and stock raising. The high- and medium-potential zones support 80% of the total human population. The cattle population was estimated in 1986 to be 9 million, 20% of which were exotic and grade cattle. The high-producing dairy and beef cattle are found in the high- and medium-potential zones; indigenous cattle are found mainly in the range areas, which form approximately 70% of the country.

Theileriosis caused by *Theileria parva* is one of the major constraints to the development of the livestock industry in Kenya. The importance of this disease is due to the high rate of mortality it causes in livestock, the productivity losses it causes in animals that recover from the disease and the exclusion of exotic and grade cattle from much of the country. The infection occurs in cattle and buffalo and is transmitted by the ixodid tick, *Rhipicephalus appendiculatus*. The distribution of *R. appendiculatus* correlates closely with areas with the highest concentrations of exotic or grade cattle (Figures 1 and 2, respectively).

CURRENT STATUS OF THEILERIOSIS

Five species of *Theileria* are recognized in Kenya (Table 1). Theileriosis manifests itself in the form of East Coast fever (ECF) (*T. parva parva*) and Corridor disease (*T. p. lawrencei*). *Theileria mutans*, *T. velifera* and *T. taurotragi* infections usually cause at most mild transient fever and anaemia and are not reported as theileriosis in the field. Corridor disease occurs in areas where buffalo are found.

In an extensive survey carried out from 1971 to 1974 in 36 districts, antibodies to *T. parva* were detected in all districts where *R. appendiculatus* was found (Food and Agriculture Organization of the United Nations, 1975). The percentage of cattle showing positive reactions is shown by district in Figure 3. Reported and suspected cases of ECF have been increasing since 1974, probably

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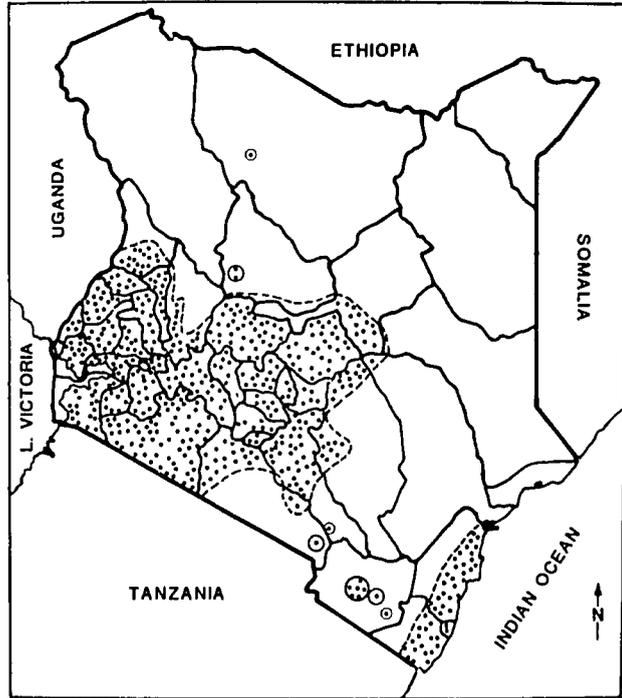


Figure 1. The distribution of *Rhipicephalus appendiculatus*, the vector of *Theileria parva*, in Kenya.

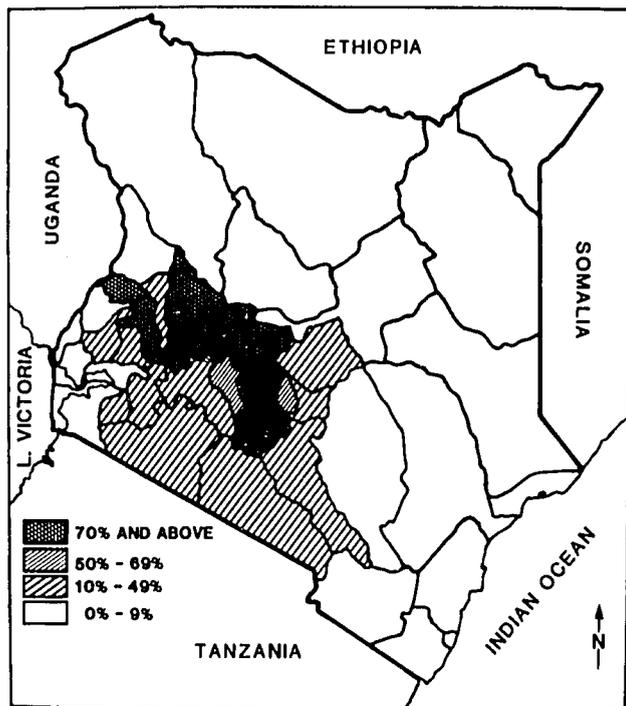


Figure 2. The distribution of exotic beef and dairy cattle breeds in Kenya, showing the percentages by district.

THEILERIOSIS IN KENYA

as a result of improved reporting and diagnosis by the field veterinary staff, the availability of chemotherapeutic drugs and greater awareness of the disease by farmers. Since 1977 there has been an increase in the number of field staff, including veterinarians. Even so, it is suspected that the reported cases represent only 10% of the cases occurring in the field.

Table 1. Species of *Theileria* recognized in Kenyan cattle

<i>Theileria</i>	Disease	Serotype	Vector
<i>T. p. parva</i>	East Coast fever	<i>T. parva</i>	<i>R. appendiculatus</i>
<i>T. p. lawrencei</i>	Corridor disease	<i>T. parva</i>	<i>R. appendiculatus</i>
<i>T. taurotragi</i>	Usually benign	<i>T. taurotragi</i>	<i>R. appendiculatus</i> and <i>R. pulchellus</i>
<i>T. mutans</i>	Usually benign	<i>T. mutans</i>	<i>Amblyomma</i> spp.
<i>T. velifera</i>	Benign	<i>T. velifera</i>	<i>Amblyomma</i> spp.

TICK CONTROL

The strategy of tick and tick-borne diseases control in Kenya has focussed mainly on controlling *R. appendiculatus* to prevent theileriosis transmission and to introduce improved exotic breeds of cattle and upgraded indigenous populations into ECF-affected areas. In these areas tick control measures are enforced by the Cattle Cleansing Ordinance Act, under which the Minister for Livestock Development is empowered to proclaim infected areas and enforce weekly cattle cleansing with government-approved acaricides (Table 2).

Table 2. Approved and registered acaricides in Kenya

Chemical name	Product name	Manufacturer	Recommended concentration
Dioxathion	Delnav DFF	Coopers/Wellcome	0.050% v/v
Quintiphos	Bacdip	Bayer	0.020% v/v
Coumaphos	Asuntol	Bayer	0.100% w/v
Chlorofenvinphos	Supona	Shell	0.050% w/v
Chlorofenvinphos	Steladone	Ciba-Geigy	0.050% v/v
Chlorofenvinphos	Superdip	Coopers/Wellcome	0.050% v/v
Carbaryl	Sevin	May and Baker	0.200% w/v
Diamide	Triatics	Coopers/Wellcome	0.025% w/v

COUNTRY REPORTS

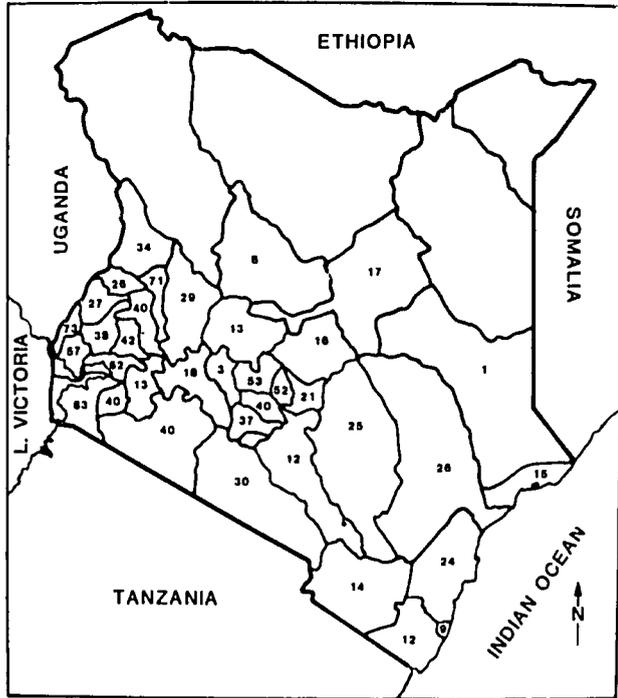


Figure 3. The percentages of cattle with antibodies to *Theileria parva* in Kenya by district.

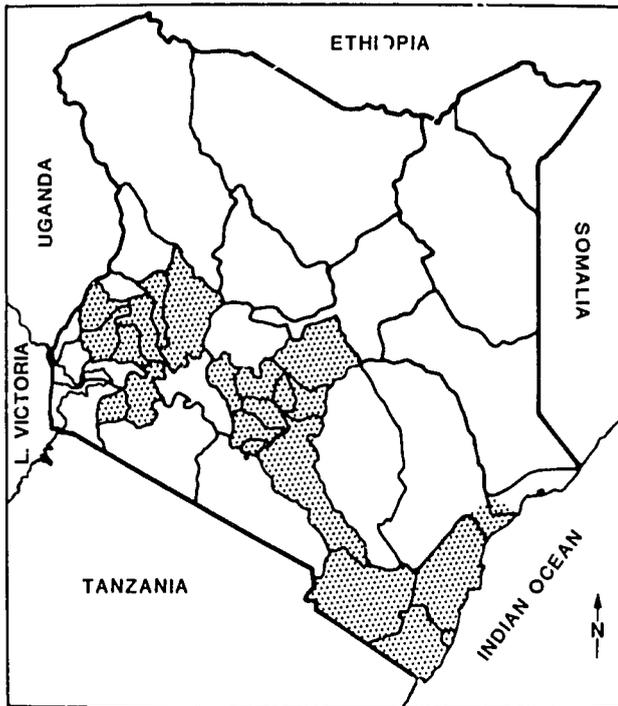


Figure 4. The districts in Kenya included in government tick-control projects.

“Proclaimed areas” are those in which ECF is present and cattle cleansing is compulsory. Before 1977 the Department of Veterinary Services, in the Ministry of Livestock Development, was responsible for supervising the Cattle Cleansing Act but not for managing dip tanks or spray races. Concern over escalating losses due to tick-borne diseases, in particular ECF, and the emergence of acaricide resistance, mainly in the *Boophilus decoloratus* population, accentuated by inefficient acaricide application, caused the Department of Veterinary Services to assume, through a Tick Control Programme, the management of communally operated dip tanks serving small-scale farmers in the proclaimed areas. Under this arrangement, the government provides acaricides for the dip tanks and levies a fee for each animal immersed. Each communal dip tank has a committee, composed of elected representatives from local livestock owners, which is responsible for liaising with the Department of Veterinary Services in running the dip and for providing labour when required. The districts included in the government tick-control projects are shown in Figure 4.

In other areas tick control is voluntary and at the discretion of livestock owners. Under the government tick-control programme there are 3330 dip tanks. The Department of Veterinary Services estimates that a further 1100 tanks are required in the proclaimed areas to ensure complete dipping. In 1985 the total cattle population in the government tick-control programme was just over 6 million, one-third of which were exotic or improved dairy and beef stock.

DISCUSSION

Tick and tick-borne diseases are a major constraint to the development of Kenya's livestock industry. A micro-economic analysis completed recently on eight large and medium-sized farms, with a total of 37 779 head of cattle, in Nakuru District, Central Rift Valley, showed that the cost of acaricides, production losses and losses due to clinical theileriosis and other tick-borne diseases amounted to approximately US\$515 305 or \$13.64 per animal per year (Young et al., in press and this meeting). The current strategy for controlling tick and tick-borne diseases is to attempt to reduce the tick population by applying acaricides, thereby reducing the direct losses due to tick-borne diseases, particularly ECF. Introducing immunization control methods against theileriosis would not only further reduce losses associated with ECF, but also reduce acaricide costs, since acaricides would then be applied strategically rather than universally.

Although the immunization method of infection and treatment, developed at the East African Veterinary Research Organization (EAVRO), Muguga, Kenya, has been approved by the Food and Agriculture Organization of the United Nations and is in use in a number of countries, this method has not been intro-

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duced in Kenya because the Veterinary Department wants some basic assurances and information, which include the following:

- a) information on the *Theileria* strains from various ecological zones
- b) the possibility of identifying a master strain(s) for the whole country
- c) the availability of *Theileria* strain(s) to use in areas with both ECF and Corridor disease
- d) the determination of a safe antigenic dose
- e) the effects of immunization on the productivity of cattle
- f) information on the *T. parva* carrier state

The National Veterinary Research Centre, at Muguga (formerly EAVRO), which is implementing an ECF project conducted jointly by the governments of Kenya and Britain, has been studying infection-and-treatment immunization against theileriosis in the laboratory and in the field, in collaboration with the Department of Veterinary Services, at Kabete, Kenya, and the International Laboratory for Research on Animal Diseases, Nairobi, so as to provide some of the above information and assurances.

Observations on the epidemiology of the disease and on immunization allow us to state the following confidently. Indigenous cattle in theileriosis-endemic areas of Kenya survive in the absence of artificial control measures because endemic stability is established. The carrier state is maintained by cattle that are resistant to the disease but carry a low number of the parasites, thereby providing a constant low source of infection for the tick and parasite challenge for newly born cattle. The introduction of susceptible cattle or tick control programmes in these areas disturbs this stability. Once the stability is disturbed or the tick control measures fail, mortality may be very high.

In areas where exotic and grade cattle are kept, artificial endemic stability of theileriosis can be established by immunization using the infection-and-treatment method. The cattle are immunized by infecting them with a known challenge and then controlling the infection with drugs; any subsequent challenge with a related stock boosts the immunity of the cattle. Livestock that will be moved from one region to another should be vaccinated before the move with a theilerial strain appropriate for the area to which they will be moved. Immunization would not be a problem if there was a master strain that would immunize livestock against all strains in the country. After immunization is implemented, acaricides may be applied strategically, non-conventionally or with reduced frequency.

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Theileriosis in Malawi

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There are just over one million cattle in Malawi, of which the indigenous short-horned Zebu, kept under traditional systems of management, are predominant. Within the national herd, there are about 13000 cross-bred (mainly Fresian crossed with Zebu) dairy cows and heifers kept on government farms, private estates and small holdings in the milk shed areas around Blantyre-Zomba in the south, Mzuzu in the north and Lilongwe-Dowa in the centre of the country.

Tick-borne diseases are endemic throughout Malawi. Of these, East Coast fever (ECF), a *Theileria parva* infection in cattle, is a major cause of cattle mortality. Exotic and cross-bred cattle are more susceptible to ECF than the indigenous cattle. A cattle survey carried out by a Malawian-Danish Project (Knudsen, 1971) showed that 66% of each year's calf crop from indigenous breeds died before two years of age and most of these deaths were attributed to ECF. While the overall national annual mortality among the traditionally managed herds was estimated at 10–15%, mortalities in the ECF-endemic areas of central and northern Malawi in calves and adults were 10% and 1% higher, respectively (Figure 1) (World Bank, 1988).

East Coast fever occurs where the vector tick, *Rhipicephalus appendiculatus*, is established. In the south, the River Shire seems to form a natural boundary for ECF; the only cases reported east of the river have been in cattle recently moved from infected areas. It appears that the disease has not become established in the southern region mainly because of the very low density of the vector in that region. However, if a breakdown occurs in the current efficient tick control practices and the disease is introduced in the area, ECF will likely become established in some parts of the region, particularly in the escarpment areas where the tick is known to occur.

The diagnosis of ECF in Malawi is based on the demonstration of schizonts in lymph node biopsy smears and of piroplasm in blood smears from clinically sick animals or schizonts from spleen smears of dead animals. Where applicable, this diagnosis is complemented by the indirect fluorescent antibody test (IFAT) using schizont antigen. Not all lymph node biopsy smears having schizonts are ECF cases (Malawi Government, 1975).

THEILERIOSIS IN MALAWI

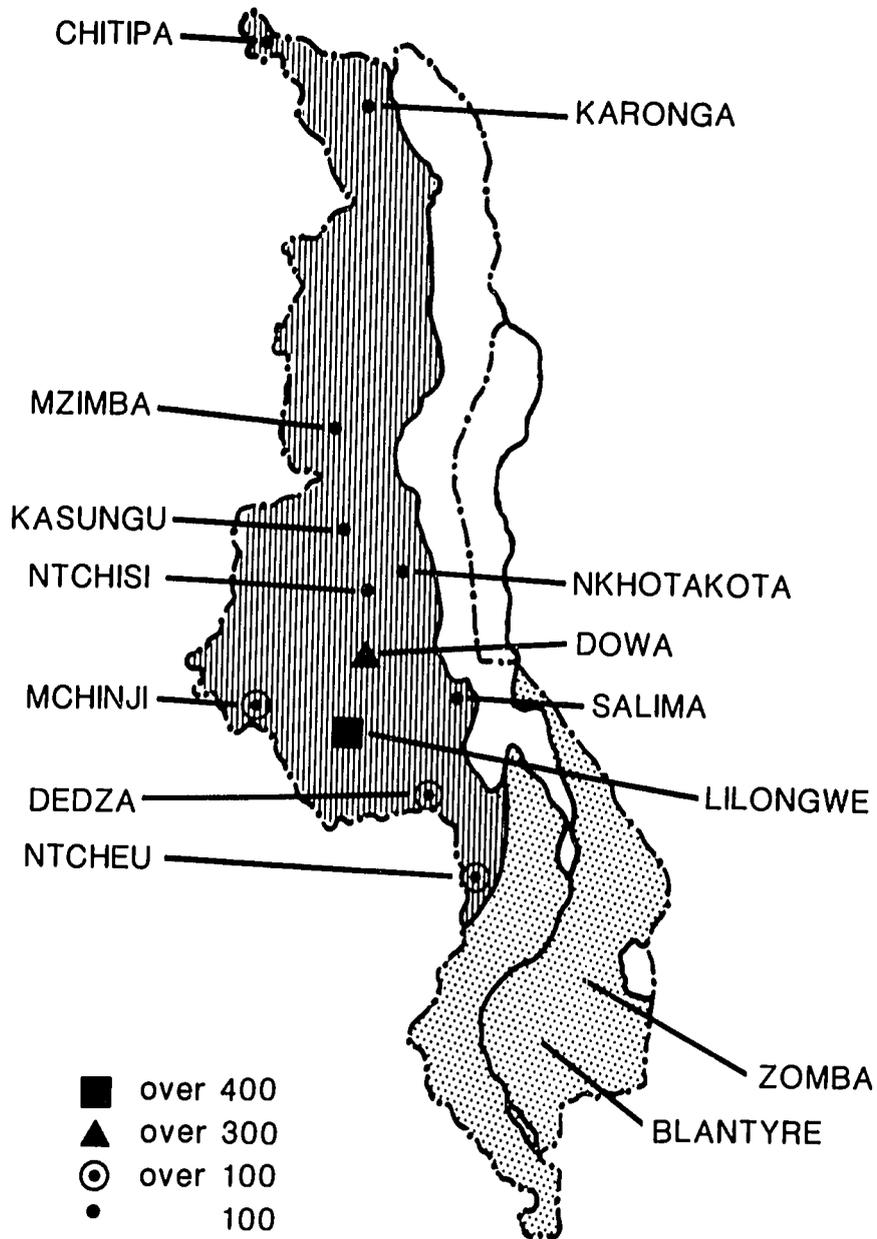


Figure 1. The distribution of *Theileria parva* in Malawi (dark shaded area), with numbers of cases diagnosed at different centres, 1983–87.

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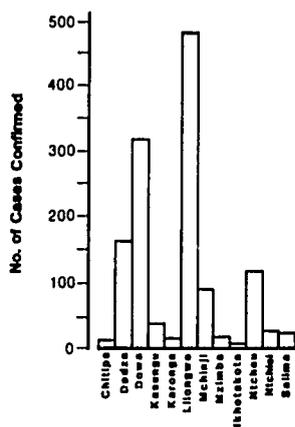


Figure 2. The number of confirmed cases of theileriosis in Malawi by district, 1983-87.

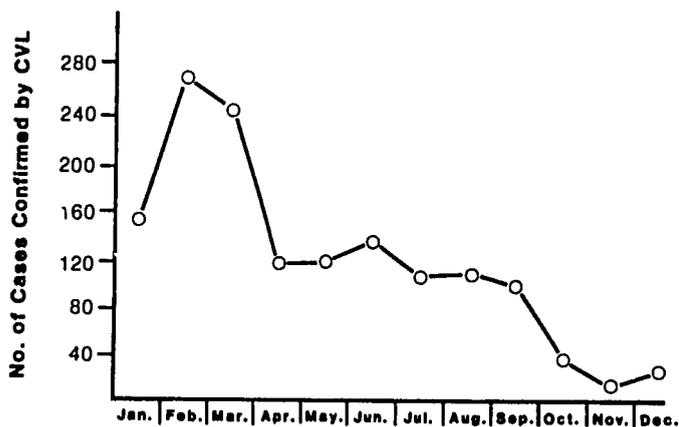


Figure 3. The number of confirmed cases of theileriosis in Malawi by month, 1983-87.

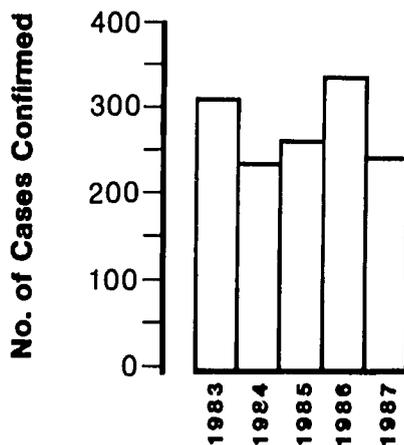


Figure 4. The number of cases of theileriosis confirmed at the Central Veterinary Laboratory, Malawi, 1983-87.

In the past five years, 1412 cases of ECF were confirmed at the Central Veterinary Laboratory (CVL). The cumulative number of cases are shown by districts in Figure 2. It appears that in a given district the number of diagnostic centres as well as the level of awareness in smallholder farmers that they should report dead or sick animals influence the frequency of cases reported. This is evident especially in Lilongwe, Dowa and Dedza districts, which are the districts closest to the CVL. Moreover, in these districts there are more cross-bred cattle, which are usually more susceptible than indigenous cattle to ECF, than in the other districts where indigenous breeds predominate. These confirmed cases represent 6% of the total smears examined at CVL from the districts indicated in Figure 2. Of the total smears examined, 22% were from spleens, of which 23% were positive for theilerial schizonts. There was an overall mortality rate of 5.0%, which was attributed to ECF among the specimens submitted.

The highest number of cases occurs between January and March (Figure 3) and the peak is markedly influenced by rainfall distribution over these months. Transmission at that time of the year is by the adult tick. A small peak occurring between May and July is due to nymphal transmission. This peak depends on the climatic conditions in this period, particularly moisture and temperature. From August to November there is a steady, slow decline in the number of ECF cases detected. In November and December adult tick activity increases with the onset of rains and the number of ECF cases increases as well.

The numbers of confirmed cases of tick-borne diseases at CVL have been steady during the past five years and records show that the distribution of ECF in the country and over the different seasons of the year has not changed. However, there was a slight increase in the number of laboratory confirmed ECF cases in the endemic areas in 1983 and 1986 (Figure 4), while the southern region remained free of the disease.

These observations indicate that ECF continues to be a major constraint to the improvement of the cattle industry in Malawi. Serious losses have been noted even among indigenous Zebu cattle when enzootic stability is disturbed. Controlling ticks and tick-borne diseases with acaricides is of paramount importance in Malawi, but smallholder dairy farmers would benefit more if their cross-bred cattle were immunized against ECF and other tick-borne diseases at the earliest possible opportunity. This and strategic tick control would reduce the costs of purchasing and administering acaricides.

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Theileriosis in Mozambique

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Trypanosomiasis, ticks and tick-borne diseases are major constraints to cattle, goat and sheep rearing in Mozambique. Ticks, particularly those of the genus *Amblyomma*, are indirectly responsible for great losses in calves in the infested areas of the south because of damage to cow teats and udders. Tick-borne diseases such as babesiosis and anaplasmosis are important causes of livestock losses in the commercial sector, and heartwater is of overwhelming importance for goats and exotic breeds of cattle. East Coast fever (ECF) and Corridor disease have been recorded and are of importance in local breeds of cattle in the smallholder sector.

The first recorded outbreak of theileriosis occurred in southern Mozambique in 1901 after the introduction of infected cattle from Tanzania through Beira and Lourenco Marques. The disease spread rapidly and infected cattle in large areas in Mozambique, Zimbabwe and the Republic of South Africa (Koch, 1903, and Gray, 1908, cited by Henning, 1932). In Zimbabwe it is estimated that over half of the cattle population died of ECF in three years after its introduction. In South Africa, where its spread was restricted, some 50 million Rand (US\$137 million at the exchange rate in August 1989) was spent on its control before it was eradicated 50 years later. The disease was present south of the Limpopo River in Mozambique until at least 1917, after which it disappeared due to a combination of strict control measures (Dias, 1977) and, perhaps more importantly, unsuitable ecological conditions for the vector and low cattle density.

East Coast fever remained endemic in Angonia, a northwestern district bordering Malawi, where meteorological and ecological conditions are very suitable for the vector. In this area the brown ear tick, *Rhipicephalus appendiculatus*, and the blue ticks of the genus *Boophilus* are the dominant species, in contrast to

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the main cattle-keeping areas of the south, where the bont tick, *A. hebraeum*, is of greatest importance.

The first outbreak of theileriosis caused by *Theileria parva lawrencei* was recorded in 1960 in Mapai, a southwestern district of Gaza Province. Later outbreaks occurred in Mossurize, Manica and Chimoio in 1970 and 1971 (Dias, 1977).

Few ecological and epidemiological studies on ticks and tick-borne diseases in Mozambique exist. A tick survey has been conducted over a number of years by Dias (1950), but no quantitative data are available. More recently, ecological and epidemiological studies were initiated by Jacobsen (1985) at the Veterinary Research Institute. The potential vector of ECF was shown to have a marked seasonal activity in both Maputo and Gaza provinces, with peak activity in February and March.

The results of a serological survey indicate a low number of ECF-positive animals outside the ECF-endemic area, but these data require further study (Jacobsen, 1985). A high percentage of positive cattle (58%) in the sector in Tete Province indicates a still active focus of ECF.

The lack of quantitative and qualitative data on ECF in Mozambique has been a constraint to the design and implementation of a sound control programme in the endemic area. Both epidemiological and ecological studies are required to define the extent of the endemic area and the risk of disease spread. The distribution of the vector and the seasonal nature of its activity need to be studied to evaluate the viability of an immunization programme combined with strategic tick control.

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Theileriosis in the Equatoria Region of Sudan

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The Equatoria Region is situated in the south of Sudan bordering Kenya, Uganda, Zaire and the Central African Republic. In the last animal census taken in Sudan, in 1976, the region had a cattle population of 1.4 million. Due to the uncontrolled movement of cattle within the region and across the border, including an influx of Ugandan refugees in 1978 and 1979 who brought with them an estimated 15000–18000 head of cattle, this figure is now probably much larger. The region has a widely distributed wildlife population, including buffalo and antelope, whose movements depend mainly on the season and availability of water.

EAST COAST FEVER OUTBREAKS AND INCIDENCES

An East Coast fever (ECF) outbreak was reported in 1950 by Hoogstraal in Kajo Kaji and Yei districts in the Equatoria Region (Hoogstraal, 1956). The disease was not reported again until 1981 (Morzaria et al., 1981), when the presence of *Theileria parva* was confirmed microscopically and serologically in the Chukudum and Awa River areas. Zessein and Baumann (1982) identified *T. parva* antibodies in cattle in the Bahr El Ghazal region. This was surprising in that the vector, *Rhipicephalus appendiculatus*, does not occur in this region, although *R. evertsi* and *R. simus* were identified. Julla (1985) reported a typical outbreak of ECF at Palotaka in the Eastern Bank of the Equatoria Region in 1984, and in 1985 outbreaks of ECF occurred in Nimule and Juba townships. The Numule outbreak affected 4000 cattle and caused a low mortality and a morbidity rate of almost 100%. The disease spread and became established around Juba due to the regular movement of trade cattle in the township area.

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The disease reached the Terekeka area, some 60 miles north of Juba, with the movement of the Mudari people and their cattle away from the ECF problems of Juba. Because of the rapid spread of the disease and the large number of cattle at risk, OXFAM/ACCOMPOLISH in 1987 and 1988 launched an emergency chemotherapeutic control programme using parvaquone and long-acting tetracycline.

DISTRIBUTION OF *RHIPICEPHALUS APPENDICULATUS*

Rhipicephalus appendiculatus is restricted in Sudan to areas of high rainfall and moderate temperature, most of which are located in the green belt area of the Equatoria Region, such as in Kalokaji, Yei, Ngangala, Torit and Katire, where Hoogstraal (1956) found the tick. Morzaria et al. (1981) and Julla (1985) identified *R. appendiculatus* in the areas of Chukudum, Aswa River, Palotaka, Nimule and Juba town. Tick collections between 1985 and 1988 confirmed the presence of *R. appendiculatus* in and around Juba.

CONCLUSIONS

The confirmation of ECF and its vector suggests that the disease has become established in the Equatoria Region. There is an urgent need to conduct epidemiological studies to determine the extent of the disease distribution. Data from these studies could then form the basis of proper control using antitheilerial drugs, tick control measures and immunization by the infection-and-treatment method. The Directorate of Animal Resources has established an East Coast fever survey and control project at Juba University to implement these measures.

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Theileriosis on the Tanzania mainland

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East Coast fever (ECF) has been recorded in Tanzania since the beginning of the century, but its history among traditional herdsman is much older. Local short-horn Zebu cattle have a natural resistance to the disease and have shown a population growth rate of about 1% per year without any specific tick control measures. If improved cattle are introduced, however, tick control methods must be used to prevent serious losses. *Rhipicephalus appendiculatus* is widely distributed in Tanzania (Figure 1) and ECF coincides with its distribution. The government has an established livestock improvement programme that uses livestock multiplying units and artificial insemination to increase the numbers of high-producing dairy and beef cattle and to upgrade the local Zebu stock. In addition, local and cross-bred cattle are being used as draught animals on a wider scale. The cattle population of Tanzania is estimated at 12.5 million, with an improved herd of 200 000 dairy and 100 000 beef cattle.

Annual veterinary reports provide figures for disease diagnosis, treatments, recoveries and deaths. These figures are compiled from data returned mainly by auxiliary veterinary personnel, in most cases from the traditional farming sector. These personnel have very limited equipment and almost no access to laboratory diagnosis. Disease reporting from the commercial sector is more accurate because of better trained personnel and some laboratory support in that sector. Nonetheless, the cases reported still represent only a small proportion of the total. The reported cases of tick-borne diseases and trypanosomiasis in 1986 are presented in Table 1. Tick-borne infections are considered as a group in Table 1 because diagnosis and treatments of these infections are based on similar clinical signs and because mixed infections often occur. Similarly, figures for trypanosomiasis are included because the clinical signs and treatments for trypanosomiasis overlap with those of tick-borne infections, and once again, because mixed infections are common.

CONTROL OF EAST COAST FEVER

The official Tanzanian policy for control of ECF and other tick-borne diseases is tick control. This policy has evolved from one of compulsory free dipping in the

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late sixties and early seventies to the introduction of a dipping fee in the 1980s. This has led to a drop in the number of immersions from 56 million per year in 1972 to 18 million in 1986. Approximately one-third of the 1900 dips in the country are non-functional. Tick-borne disease infections have been increasing, but the rise has not been proportional to the decline in immersions. Thus, due to the high cost of acaricides, their environmental damage and the difficulties in maintaining strict acaricidal control, control methods involving strategic dipping and enzootic stability of cattle to tick-borne infections should now be considered. Such methods would fit in well with an immunization programme for very susceptible herds.

Table 1. Reported cases of tick-borne diseases and trypanosomiasis in cattle in Tanzania, 1986

	Number of cases	Number treated	Number cured	Number dead
East Coast fever	83,961	71,284	64,339	19,562
Anaplasmosis	57,997	51,419	48,509	9,484
Babesiosis	19,355	17,456	16,798	2,557
Heartwater	11,685	11,151	8,979	2,706
Total tick-borne diseases	172,998	151,310	138,625	34,309
Trypanosomiasis	23,592	—	—	9,148

TREATMENT OF EAST COAST FEVER

Several treatment regimes for ECF/tick-borne infections have been used in Tanzania, including the following:

- a) oxytetracycline hydrochloride 10% at a dose rate of 1 ml/10 kg for five days
- b) parvaquone (Clexon, Wellcome) at a dose rate of 10 mg/kg repeated after 48 hours
- c) halofuginone (Terit, Hoechst) at a dose rate of 1.2 mg/kg repeated after 48 hours
- d) a combination of halofuginone and oxytetracycline at the prescribed dose rates

THEILERIOSIS ON TANZANIA MAINLAND

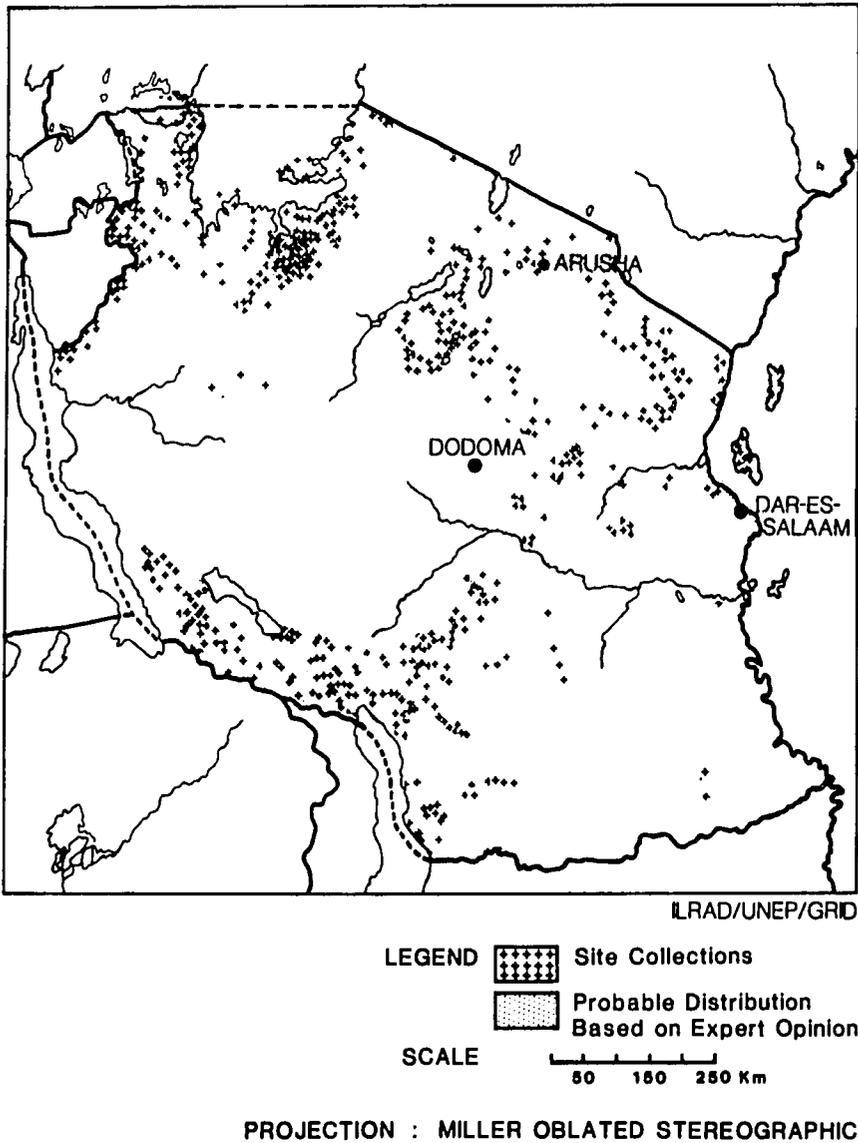


Figure 1. The probable distribution of *Theileria parva* based upon expert opinion and site collections of *Rhipicephalus appendiculatus* in Tanzania. (Map provided by P. Lessard from the International Laboratory for Research on Animal Diseases/the United Nations Environment Programme/Global Resources Information Database Collaboration.)

THEILERIOSIS ON TANZANIA MAINLAND

- e) a combination of parvaquone and oxytetracycline at the prescribed dose rates

Recoveries from combinations of the above treatments are difficult to attribute to a single compound. Before halofuginone and parvaquone became available, recoveries from ECF using tetracycline alone were about 30%. Using combinations, field observations have shown recovery rates of 56% for halofuginone and 55% for parvaquone.

The cost of the new antitheilerial drugs is prohibitive. Moreover, milk from treated animals must be withheld for 14 days after the treatment and treated animals cannot be slaughtered for human consumption for 28 days. Bearing these disadvantages in mind, the case for control by immunization is attractive.

OTHER CONTROL OPTIONS

While tick control and chemotherapy for ECF have their role in the control of tick-borne infections, zero grazing cattle is another effective control method. Few, if any, infected ticks can reach a susceptible animal that does not graze. Of course, not many cattle can be kept in this manner without a heavy capital investment. Immunizing zero-grazed animals against tick-borne infections would probably give these animals near-complete protection against these diseases.

CONCLUSION

Immunization against ECF is desirable. Once cattle are immunized, it is expected that a low ECF challenge will be maintained. This, together with the considerable economic saving, justifies relaxation of the tick control programmes. When tick control is relaxed, however, other theilerioses and tick-borne infections will appear if they have not been included in the immunization programme. Of particular concern is *T. mutans*, which is reported to have caused problems when treatment of ECF was directed only at *T. parva* using halofuginone or parvaquone.

Disease control will be made more effective by making available basic diagnostic facilities, by making more complete records of disease occurrences and by disseminating information about disease situations more thoroughly. Unfortunately, what we have now is a collection of epidemiological information and assumptions from work done long ago, some as far back as 1967. Therefore, along with instituting an immunization programme, there should be a determined attempt to rehabilitate diagnostic services (which is being addressed) and, concerning tick-borne diseases particularly, the information on tick ecology should be updated. Serological surveys should be conducted to determine the

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distribution of theileriosis more accurately. Immunization programmes should be incorporated into the management of all improved commercial dairy and beef herds, as well as draft oxen in areas that can sustain *R. appendiculatus*.

Theileriosis on Unguja Island, Zanzibar

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East Coast fever (ECF) caused by *Theileria parva* continues to be a major killer of cattle in nearly all parts of Unguja Island, Zanzibar, where the disease is enzootic. According to Irvin (1984), ECF has its greatest impact on calves on smallholder farms. On government farms that practise strict tick control, the disease is more commonly seen in adult cattle as a result of occasional breakdowns in acaricidal protection. However, in many field outbreaks both calves and adults seem to be equally susceptible. Pure or exotic breeds and their crosses are more susceptible to the disease, and have a higher mortality rate (6.0% and 18.3%, respectively), than local Zebu cattle.

In a serological survey carried out by Flach (1988) using the indirect fluorescent antibody test, positive animals were detected in all areas sampled, which represented every district of the island (Figure 1).

Many outbreaks of ECF in the rural areas are not reported. Livestock owners may attempt to treat their afflicted animals in traditional ways, such as by burning the swollen lymph nodes of the animals with a hot iron, or they may slaughter the animals. Of reported cases of ECF, the records of the Department of Livestock Development show the yearly average number of cases to be nearly 800.

The numbers of exotic and cross-bred cattle have increased markedly in the past eight years due to a successful artificial insemination programme. Many farmers are becoming disillusioned, however, because of the high mortality in these animals that appears, from descriptions of the disease, to be caused mainly by ECF. In recent years, treatment with parvaquone (Clexon, Wellcome) has been introduced. This drug is effective if treatment is started early, but it is extremely expensive and many farmers cannot afford to have their animals treated. A new approach to ECF control has been explored over the last two years using tick stabilate infection and tetracycline treatment to immunize cattle against *T. parva*. Results of these trials using this infection and treatment were promising. The Department of Livestock Development will continue to explore the potential of this control measure and if satisfied with the protection will start field immunization of cattle.

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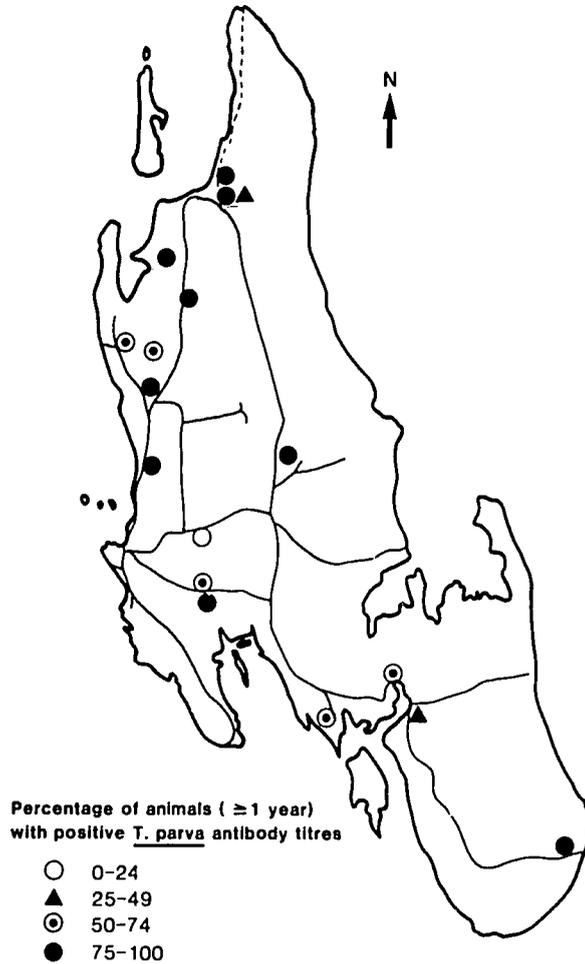


Figure 1. The percentage of animals less than one year old with antibodies to *Theileria parva* in different districts of Unguja Island, Zanzibar.

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Theileriosis in Uganda

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Uganda is a land-locked country of 240000 sq. km lying on the equator. It borders Sudan to the north, Kenya to the east, Tanzania and Rwanda to the south and Zaire to the west. About one-seventh of the country is covered by water. In the south of the country there are two rainy seasons, one in April/May and the other in September/November. In the northern parts of the country there is one long rainy season lasting about five months, usually from May to September. The northeastern part of Karamoja District is arid, having less than 510 mm of rainfall annually. Rainfall in the rest of the country ranges between 1015 and 1525 mm.

About 80% of households depend mainly on agriculture, livestock or fishing for their income. Crop cultivation is by far the most important source of income (55%), followed by mixed farming (20%). Only 5% of the households depend principally on livestock. The cattle population at the end of 1987 was estimated at 3.91 million, the majority of which are indigenous breeds grazed communally.

Tick-borne diseases are the most widespread diseases of cattle and theileriosis is one of the most economically important. The major species of *Theileria* in Uganda are *T. parva parva* and *T. mutans*. The presence of *T. p. lawrencei* is assumed, particularly in areas adjacent to game parks and reserves. *Theileria velifera* is known to occur but it causes no disease. Theileriosis caused by *T. p. parva*, East Coast fever (ECF), is a major killer of cattle and is an important constraint to improving production and productivity of the livestock industry. East Coast fever is enzootic throughout Uganda except in the drier open plains of the Karamoja region. Its distribution coincides with that of the tick vector *Rhipicephalus appendiculatus*. However, ECF epizootics occur from time to time in Karamoja during periods of unusual vector abundance because the majority of cattle lack immunity.

East Coast fever is regarded as one of the most serious causes of economic loss in Uganda, although accurate data on the incidence, case fatality and seasonal occurrence of the disease is lacking. Estimates of losses in indigenous cattle range between 20% and 40% of the calf crop. The mortality in Ugandan breeds of cattle due to ECF recorded during the period 1941–1964 at Serere

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Government Research Station was between 11% and 16%. A study recently concluded among indigenous herds not subjected to tick control at Kigungu, Entebbe, showed that 23% of the calves aged between 2 and 6 months died of ECF.

In a survey of 15226 cattle kept under different management systems and representing all areas of the country, 21% had antibodies to *T. parva*, 65% had antibodies to *T. mutans* and 45% had *Theileria* piroplasms detected in blood smears. Examination of blood and lymph node smears submitted to the Animal Health Research Centre, at Entebbe, showed that 30.8% in 1985, 15.1% in 1986, 27.3% in 1987 and 29.9% in 1988 had *Theileria* parasites. Recorded cases of ECF deaths were 10010 in 1986; these occurred in 18 of the country's 33 districts.

The main method used in Uganda to control tick-borne diseases of cattle, particularly ECF, is intensive tick control with acaricides. Of the 1890 dips, 284 sprays and other types of acaricide application recorded in the country, less than 60% are functional. This problem is compounded by the escalating cost of acaricides and the development of tick resistance to the acaricides. Tick-borne diseases continue to be a major source of cattle losses and a hinderance to the improvement of the livestock industry.

Theileriosis in Zimbabwe

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Tick-borne diseases are a major cause of cattle losses in Zimbabwe. The diseases of importance are babesiosis, anaplasmosis, heartwater and theileriosis. East Coast fever (ECF), caused by *Theileria parva parva*, was introduced into the country at the turn of the century by cattle from East Africa. The disease caused heavy losses initially but was eventually eradicated in 1954 by intensive dipping. Two subspecies, however, remained: *T. p. bovis*, causing January disease, and *T. p. lawrencei*, causing Corridor disease. These two diseases are responsible for about 2000 cattle deaths each year. Outbreaks of *T. p. bovis* occur only in highveld areas infested with the brown ear-tick, *Rhipicephalus appendiculatus*. Outbreaks of *T. p. lawrencei* are associated with the presence of buffalo and occur most commonly in the southern lowveld where the main vector is the lowveld brown ear-tick, *R. zambeziensis*.

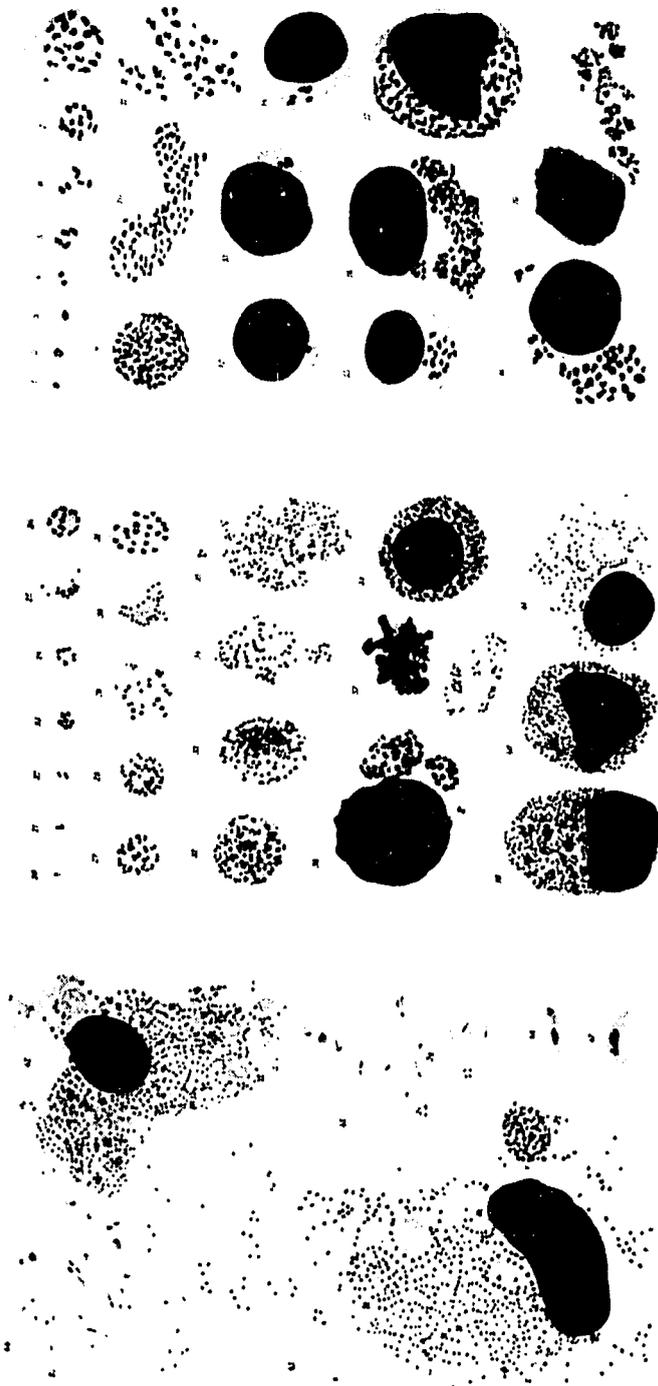
In a serological survey, Norval et al. (1985) found that antibodies to the *T. parva* group of parasites occurred in cattle throughout the country but the percentage of reactors was generally low. Outbreaks of *T. p. bovis* were largely restricted to the commercial farming areas, where *R. appendiculatus* was most abundant. The tick was not common in over-grazed communal farming areas. Clinical cases of *T. p. bovis* were not recorded from areas infested with *R. zambeziensis*.

Outbreaks of *T. p. bovis* in Zimbabwe follow a seasonal pattern and occur most commonly between January and March; this coincides with the activity period of the adult stage of *R. appendiculatus*.

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Illustrations by Dr. Richard Gonder of the life cycle of *Theileria parva*, associating the schizont and piroplasm stages for the first time and identifying gametocytes in erythrocytes. From R. Gonder, 1911. The development of *Theileria parva*, the cause of East Coast fever of cattle in South Africa. *Report of the Government Veterinary Bacteriologist (1909-10)*. Pretoria: The Government Printing and Stationary Office. Plates reproduced under the South African Government Printers copyright authority 9045 of the 30th of October 1989.

PART 2

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East Coast fever immunization in Burundi

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Theileriosis is endemic in Burundi. Local Ankole (*Bos indicus*) cattle are naturally resistant to *Theileria parva*, with a calfhood mortality of less than 10%, although complications with other tick-borne diseases, endoparasites and poor nutrition make accurate diagnosis difficult. Improved cattle are much more susceptible to theileriosis and an estimated 30–40% may die each year of this disease.

Control of theileriosis and other tick-borne diseases in Burundi is achieved mainly by dipping, but this method is becoming less reliable because of the escalating cost of acaricides. Also, because of the expansion of crop agriculture, some livestock owners can no longer walk their cattle to dips. Antitheilerial drugs are used but they are expensive, not generally available and often used without proper diagnosis. Emphasizing crop agriculture, the Burundi Government wishes to increase the numbers of improved cattle (current estimate 5000) while reducing the total cattle population (now about 400000). The use of immunization is an important strategy in this programme of livestock improvement. Infection-and-treatment immunization was introduced in 1981 using a combination of three local isolates and tetracycline treatment. Later it was shown that two of the three stocks were effective against natural challenge. The immunized cattle remained productive, but different treatment regimens were necessary for different cattle types, depending on the proportion of *Bos indicus* in their breeding. The immunization method has been used mainly on young cattle on government farms. These immunized cattle are then distributed in the countryside. Between 1981 and 1987, 560 cattle were immunized and the total to date is 850.

The following conclusions may be drawn about the infection-and-treatment immunization programme in Burundi.

- a) The immunization programme has attracted great attention; demands for its application exceed the ability of the small number of laboratory staff to provide it.
- b) Concern has been expressed about the disease risk that carrier (immunized) cattle may introduce to other improved stock.

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- c) Where dipping intervals have been extended in the western part of the country, sporadic cases of heartwater have occurred.
- d) Occasional cases of blindness have been observed in immunized cattle.

Parasite stocks used for East Coast fever immunization in Kenya

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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.

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- 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 (Burrige 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. Buparvaquone controlled higher concentrations of the stabilate better than the two oxytetracycline formulations. In contrast, both oxytetracyclines controlled

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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
<i>T. p. lawrencei</i> (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	
<i>T. p. lawrencei</i> (Mara III) (Stabilate 202) and <i>T. p. parva</i> (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	
<i>T. p. lawrencei</i> (Mara III) (Stabilate 202)	No drug	—	—	2/3 (1)	
<i>T. p. parva</i> (Kilae) (Stabilate 187)	No drug	—	—	3/3 (3)	
		Undiluted	1:10	1:80	
<i>T. p. parva</i> (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.

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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-immunized cattle, but not in butyrolactone-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

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- 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.

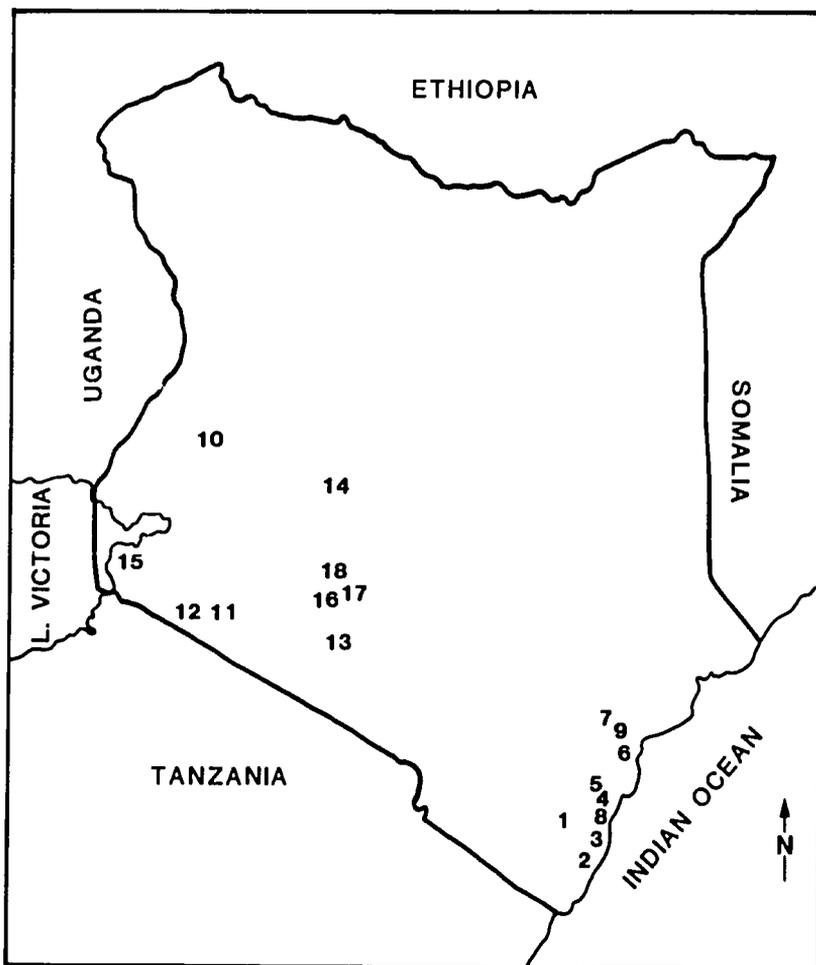


Figure 1. The locations in Kenya where *Theileria parva* stocks were isolated for use in cross-immunity studies. The key to the locations is given in Table 2.

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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.	<i>T. p. parva</i>	(Mariakani)	Stabilate 3029
2.	<i>T. p. parva</i>	(Utange)	Stabilate 223
3.	<i>T. p. parva</i>	(Mtwapa)	Stabilate 2262
4.	<i>T. p. parva</i>	(Kilifi)	Stabilate 1015
5.	<i>T. p. parva</i>	(Kibarani)	Stabilate 2448
6.	<i>T. p. parva</i>	(Kiswani)	Stabilate 2240
7.	<i>T. p. parva</i>	(Magarini)	Stabilate 2365
8.	<i>T. p. parva</i>	(Junju)	Stabilate 1086
9.	<i>T. p. parva</i>	(MARIKEBUNI)	Stabilate 3014
Rift Valley Province			
10.	<i>T. p. parva</i>	(Uasin Gishu 6)	Stabilate 216
11.	<i>T. p. parva</i>	(Kilae)	Stabilate 187
12.	<i>T. p. lawrencei</i>	(Mara III)	Stabilate 202
13.	<i>T. p. lawrencei</i>	(Ngong 1)	Stabilate 2306
14.	<i>T. p. lawrencei</i>	(Ol Pejeta)	Stabilate 199
Nyanza Province			
15.	<i>T. p. parva</i>	(Mbita)	Stabilate 169
Central Province			
16.	<i>T. p. parva</i>	(Ki1)	Stabilate 210
17.	<i>T. p. parva</i>	(Ki3)	Stabilate 213
18.	<i>T. p. parva</i>	(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 sub-clinical 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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A systematic approach to East Coast fever immunization in the Kilifi District of the Kenya Coast

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Since the development of the infection-and-treatment method of immunization against East Coast fever (ECF), *Theileria parva* infection of cattle (Radley, 1981), several field immunization trials have been performed in different countries of eastern, central and southern Africa. Most of these trials, which were carefully planned with appropriate controls and adequate supervision, have demonstrated that over 90% of the immunized cattle could be protected against natural *T. parva* challenge (e.g., Radley et al., 1975; Robson et al., 1977; Uilenberg et al., 1977; Paling and Geysen, 1981).

Immunization strategies and field-trial designs have varied in the different reports. One of the central strategy issues has been the choice of immunizing parasite(s), whether to use local stocks isolated in the area in which the trial is to be conducted or the "Muguga cocktail", a centrally prepared combination of stocks, in the infection-and-treatment schedule (Dolan, 1987). In this paper we report a systematic approach to immunization against ECF, using locally isolated stocks of *T. parva* in a geographically defined area in the Coast Province of Kenya. In this approach, a series of stepwise studies were performed, which involved selection of the trial site, a preliminary survey of the ticks and tick-borne diseases, isolation and laboratory characterization of *T. parva* stocks, selection of appropriate immunizing stocks, field immunization and productivity trials and the final production of bulk stabilate for a large-scale immunization in the chosen area.

THE STUDY AREA

The study area was the Kilifi District, which lies in the Coast Province of Kenya and covers an area of approximately 3000 sq. km. The area represents the northern coastal belt of Kenya and stretches 30 km inland. The annual mean rainfall is 1077 mm and the daily mean temperature varies between 24 and 32°C. Because of the high rainfall, agricultural potential is high. The cattle population

at the time of the survey was approximately 150000, most of which were of the *Bos indicus* type. Ten percent of the cattle population was classified as grade, which consisted mainly of *Bos indicus* crossed with *Bos taurus* and pure milking breeds such as Jerseys, Ayrshires and Guernseys.

The main reasons for selecting the area were the following:

- a) ECF was present in the area
- b) the disease was delineated by geographical and ecological boundaries
- c) the coastal strip has a high potential for supporting intensive dairy and beef production systems
- d) ECF is not complicated by *T. p. lawrencei* because the buffalo, which is a reservoir host of this parasite, is absent in this area
- e) there was an increasing number of ECF-susceptible cattle due to regular dipping in the newly constructed dip tanks in the area
- f) there was a good local infrastructure, with a newly built veterinary investigation laboratory at Kilifi

SURVEY OF TICKS AND *THEILERIA* PARASITES

Ninety-two farms in the regions of Malindi, Marikebuni, Kiswani, Kilifi, Bamba, Kaloleni and Mariakani were visited and 100 herds comprising altogether about 18000 cattle and representing approximately 12% of the Kilifi District's cattle population were sampled. Ticks were collected from 531 cattle for the identification and isolation of parasites and sera were collected for an examination for antibodies to *T. parva* and *T. mutans*.

The tick survey revealed the presence of *Amblyomma gemma*, *A. lepidum*, *A. variegatum*, *Boophilus decoloratus*, *B. microplus*, *Hyalomma marginatum rufipes*, *Rhipicephalus appendiculatus*, *R. evertsi*, *R. parvus* and *R. pulchellus* (A.D. Irvin, R.S. Chumo and P.R. Spooner, unpublished).

The serological investigations (Goddeeris et al., 1982) demonstrated a high prevalence of *T. parva* and *T. mutans* antibodies at all sites sampled. There was a good correlation between the distribution of *R. appendiculatus* and *Amblyomma* ticks and that of the animals serologically positive to *T. parva* and *T. mutans*, respectively.

ISOLATION OF *THEILERIA* STOCKS

Isolation of *Theileria* parasites was attempted either by the application of field-collected ticks to susceptible cattle in the laboratory followed by nymphal pick-up with the laboratory stock of *R. appendiculatus*, or by exposure of susceptible

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naive cattle in the field followed by nymphal pick-up from the infected bait animals.

Seven *Theileria* isolates were made, of which five were identified as *T. parva*, one as *T. mutans* and one as *T. taurotragi*. The *T. parva* stocks were designated Marikebuni, Mariakani, Kilifi, Junju and Mavueni. The *T. mutans* and *T. taurotragi* stocks were referred to as Mitangoni and Malindi, respectively.

CHARACTERIZATION OF THE *T. PARVA* ISOLATES

Stocks of the *T. parva* field isolates were prepared from sporozoite suspensions derived from the laboratory infected ticks and cryopreserved as reference stabilates. They were characterized using *in vitro* and *in vivo* infectivity tests, cross-immunity trials, sensitivity to antitheilerial drugs and monoclonal antibody profiles (Minami et al., 1983). Initial cross-immunity studies showed that the mixture of Marikebuni and Kilifi stocks protected cattle against all the other stocks isolated. Therefore, a combination of these stocks was selected for field trials. Later cross-immunity studies showed that the Marikebuni stock protected cattle against all the stocks from the Kenya coast and several other stocks isolated from different parts of Kenya, and therefore the remaining field trials in Kilifi District were performed using only the Marikebuni stock.

FIELD TRIALS

Three sites were selected for immunization trials. These sites were selected on the basis of the presence of *R. appendiculatus* and ECF, accessibility, presence of adequate infrastructure (such as water, grazing and sampling facilities) and the potential for intensification of the cattle industry. The main objectives of the trials were to test (a) if different breeds (*Bos taurus*, *Bos indicus* and crosses) can be immunized with the selected parasite stocks and withstand unlimited tick challenge, (b) whether different cattle age groups can be successfully immunized and (c) whether 12- to 18-month-old cattle, immunized at 4 to 6 weeks of age and kept in a tick-free environment, could withstand field challenge. The three sites selected were the following:

- a) Coast Agricultural Research Station, Mtwapa
- b) Kibarani Farm, Kilifi
- c) ADC Farm, Kiswani

In the first trial, at Mtwapa, young Jersey calves less than 1 year old were immunized against the Kilifi and Marikebuni stocks and all survived the field

challenge, while the controls died of ECF. In the second trial, at Kibarani, 12- to 18-month-old Boran (*Bos indicus*) cattle from Kapiti were used. These were immunized with the Marikebuni stock when they were between 4 and 6 weeks old and kept tick-free before exposure. All the immunized cattle survived the challenge, while the susceptible controls died of ECF.

At the same site, another group of Boran cattle less than one year old were immunized with the Marikebuni stock and 35 days later exposed to natural challenge. All survived the challenge, while 77% of the controls died. Similar trials were carried out on a beef production system at Kiswani, where the cattle used were Sahiwal/Red Poll crosses under 6 months old and the immunizing stock was *T. parva* (Marikebuni). All the immunized cattle survived the challenge, while 71% of the controls died of ECF.

Several animals (not in the trials) became infected with ECF despite being sprayed twice weekly, suggesting that either the spraying was inefficient or the ticks were transmitting the parasite soon after attachment. To investigate this further, a pilot trial was carried out in which the effect of reducing the acaricide spraying frequency on immunized and unimmunized cattle was studied. One immunized group was sprayed twice weekly while the other was sprayed once every three weeks. Susceptible control cattle were included in each group. At the end of the exposure period, all the immunized cattle survived, while 83% and 67% of the susceptible cattle sprayed once every 3 weeks and 2 times weekly, respectively, died of ECF. The detailed results of these trials are presented in Morzaria et al. (1987).

During these trials several *T. parva* stocks isolated from the control cattle were characterized. All these isolates were found to be of *T. p. parva* type and belonged to one of the three monoclonal antibody groups as defined by Minami et al. (1983).

PRODUCTIVITY

The immunization trials showed that the Marikebuni stock provided immunity against a wide spectrum of *T. parva* challenge, suggesting that this stock would be appropriate for use in the Kilifi District. However, before wide-scale immunization could be recommended it was important to study the effect of immunization on productivity. Further, for planning rational tick control programmes it was necessary to assess the effect of different tick-control regimens on ECF immunity and the productivity of ECF-immunized cattle. With these objectives in mind, a productivity trial was carried out using beef cattle at Kiswani in which appropriate parameters for cost/benefit analyses were measured in different treatment groups.

The detailed results have been reported by Morzaria et al. (1988). Briefly, the immunized cattle showed better weight gains than the unimmunized controls.

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Among the immunized groups, cattle under twice-weekly spraying had the highest weight gains, followed by the group with acaricidal ear-tags, the group sprayed once every 3 weeks and the group without any tick control. The unimmunized animals, without tick control or with limited tick control, could not be maintained without significant losses due to ECF. A summary of the cost/benefit analyses of the various treatments are presented by Mukhebi et al. in these proceedings.

DISCUSSION AND CONCLUSIONS

A systematic approach to ECF immunization in the Kilifi District, on the Kenya Coast, was adopted which culminated in the identification of the Marikebuni stock of *T. parva* as providing wide protection against several stocks isolated from the same area. Immunization with this stock protected all cattle exposed to natural challenge at three widely separate sites within the district. The quality of immunity engendered was similar in *Bos taurus*, *Bos indicus* and their crosses. Cattle as young as 6 weeks old were immunized without any side effects. In addition, it was observed that ECF was endemic in the Kilifi District and challenge occurred throughout the year despite seasonal variations in tick challenge. Based on the *in vivo* and *in vitro* characterization of the field isolates, it was concluded that only *T. p. parva* parasites existed in that area and only limited immunogenic types might be present.

During this work several shortcomings in the design of the trials were identified. In all trials, cattle were exposed to unlimited tick challenge without acaricidal intervention. Consequently, other tick-borne diseases, especially babesiosis, anaplasmosis, benign theileriosis and cowdriosis, were diagnosed in the trial animals. In many instances multiple infections (e.g., with *Trypanosoma* spp., *Babesia bigemina*, *Anaplasma marginale* and *T. mutans*), together with heavy tick burdens, caused chronic anaemia and severe losses in productivity. Heartwater was responsible for several deaths. In addition, intercurrent diseases often caused problems interpreting the results of the trials. If cattle are exposed to unlimited tick challenge, it is recommended that very close supervision of the animals be undertaken and other diseases be treated promptly. Alternatively, the trial cattle should be immunized against other tick-borne diseases in the trial area or the tick exposure should be limited.

One problem encountered with the Marikebuni stock was that when some *Bos taurus* cattle or their crosses were immunized using one dose of long-acting oxytetracycline (Terramycin LA, Pfizer), the parasite caused severe ECF and animals had to be treated with parvaquone (Clexon, Wellcome) to prevent losses. The parasite was originally characterized in Boran (*Bos indicus*) cattle, which were safely immunized using one dose of long-acting tetracycline. Because of differences in susceptibility of cattle to *T. parva* infection, it is recommended

that all immunizing stocks for field use be characterized in highly susceptible breeds.

The Marikebuni stock has been prepared as a bulk stabilate and further characterized (see Mutugi et al., this meeting). The systematic approach described has provided sufficient information on its safety and wide immunogenic properties to justify its use on a wide scale in the Kilifi District.

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Three sporozoite stabilate concentrations were used: 1:5 dilution at APRS, Mariakani; 1:10 dilution at CARS, Mtwapa and ADC Home and Top farms; and a 1:50 dilution on milking cows at Home Farm, Kiswani. A long-acting oxytetracycline treatment (Terramycin LA, Pfizer, UK) was given at 20 mg/kg (+10%) at the same time as stabilate inoculation at APRS, Mariakani, and CARS, Mtwapa, farms to control the immunization reaction. At ADC Home and Top farms, a comparison in the immunization treatment was made between two oxytetracycline formulations, Medamycin 100 (TechAmerica Group), a short-acting tetracycline, and Liquamycin LA (Terramycin LA, Pfizer, USA), a long-acting oxytetracycline. Medamycin 100 was given at 10 mg/kg on days 0 and 4 of the immunization, and Liquamycin LA at 20 mg/kg at the same time as stabilate inoculation. All drugs were injected deep into the gluteal muscles.

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Animal Production Research Station, Mariakani. The Animal Production Research Station farm is on the Nairobi–Mombasa Road 30 km from Mombasa. The typical animal is a Sahiwal/Aryshire cross kept on a low nutritional plane on pasture with only occasional feed supplementation given to milking cows. Disease challenge includes *Trypanosoma vivax* and *Trypanosoma congolense* infections, helminthiasis, anaplasmosis and East Coast fever. Tick control was by weekly dipping in quintiophos (Bacdip, Bayer) and trypanosomiasis was controlled by chemoprophylaxis using isometamidium chloride (Samorin, May & Baker).

Two hundred and fifty cattle over 1 month old received 1.0 ml of a 1:5 dilution of the stabilate subcutaneously below the left parotid lymph node and another 20 cattle were inoculated subcutaneously on the shoulder. All were treated simultaneously with Terramycin LA. From day 14 following immunization, clinical parameters were monitored for all cattle. From any animal with a rectal temperature of 39.5 °C or above, the parotid and prescapular lymph nodes were sampled and blood smears were taken, stained with Giemsa's stain and examined for theilerial parasites. A reacting animal also had its packed cell volume estimated and its buffy coat examined for trypanosomes, using the haematocrit centrifugation technique (Woo's Test). An animal with clinical signs of theileriosis, East Coast fever (ECF), shown by a prolonged high schizont parasitosis accompanied by fever lasting longer than 3 days, was designated an ECF reactor and treated with parvaquone (Clexon, Wellcome) and a supportive antibiotic. On day 35 after the immunization, cattle were bled and examined for *T. parva* antibodies using the immunofluorescent antibody test (Burridge & Kimber, 1972).

The results of serological testing are shown in Tables 1 and 2. Of 271 cattle,

Immunization of cattle against East Coast fever in the Coast Province of Kenya: pilot immunization trials on government farms

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Initial epidemiological and cross-immunity studies showed that a theilerial parasite stock, *Theileria parva parva* (MARIKEBUNI), from Kilifi District, Kenya, conferred good protection in immunized cattle against lethal challenge with other theilerial stocks from the district (Irvin et al., 1981, 1983; Minami et al., 1983; Morzaria et al., 1987; Morzaria, this meeting). Following further laboratory characterization of a large stabilate (stabilate 3014) of this stock, involving titration in cattle and extensive cross-immunity studies (Mutugi et al., in press; Mutugi et al., this meeting), *T. p. parva* (MARIKEBUNI) was selected for large-scale immunization in the Coast Province of Kenya. Before proceeding with immunization of smallholder cattle, pilot immunization trials were conducted on government farms in Kilifi District.

The four farms selected were the Animal Production Research Station (APRS), at Mariakani; the Coast Agricultural Research Station (CARS), at Mtwapa; and two farms, Kiswani Home Farm and Kiswani Top Farm, both near Malindi and belonging to the Agricultural Development Corporation (ADC) of Kenya. These trials involved "whole herd" immunization, in which all cattle, including calves 1 month old and older, were vaccinated. Normal farm procedures, such as deworming and vaccinations, were allowed to continue. The effect of ECF immunization on milk production was investigated in a selected group of lactating cows and comparisons were made between the immunization reactions of beef and dairy cattle and between cattle breeds.

The *T. p. parva* (MARIKEBUNI) stabilate was stored in 0.5-ml aliquots in pre-labelled, colour-coded plastic straws in a portable liquid nitrogen container. Before inoculation, the plastic straws were removed from the container, rapidly thawed by rubbing them between the palms of the hands and the contents dispensed into a universal bottle. Appropriate dilutions were made using Eagle's Minimum Essential Medium, with 3.5% bovine plasma albumin and 7.5% glycerol.

260 (96%) developed *T. parva* antibodies following immunization. Two of these (0.7%) had ECF reactions that required treatment with parvaquone. There were seven deaths due to an haemorrhagic *Trypanosoma vivax* outbreak. During this immunization there was also an outbreak of foot-and-mouth disease. Serological results showed that immunized animals that suffered from severe foot and mouth lesions or clinical trypanosomiasis developed antibody titres to *T. parva* antigen that did not differ from antibody titres in immunized animals that did not develop these diseases. There were no obvious differences between the immunized and non-immunized cattle in their milk production.

Table 1. East Coast fever reactors following infection-and-treatment immunization on four farms at the Kenya coast

Farm	Total no. immunized	ECF reactors	% ECF reactor
Mariakani	271	2	0.7
Mtwapa	102	15	14.7
Kiswani Top Farm	617	6	0.9
Kiswani Home Farm	391	16	4.1

Table 2. Serological responses to *Theileria parva* antigen following immunization

	Total no. examined	Positive IFAT*	Positive IFAT (%)
Pre-Immunization			
Mariakani	254	28	11.0
Mtwapa	89	18	20.2
Kiswani Home Farm	139	26	18.2
Kiswani Top Farm	197	46	23.4
After Immunization			
Mariakani	271	260	96.0
Mtwapa	102	100	98.0
Kiswani Home Farm	296	278	93.9
Kiswani Top Farm	197	194	98.5
Kiswani milk herd	89	86	96.6

* Titre greater than 1:40.

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Coast Agricultural Research Station (CARS), Mtwapa. The Coast Agricultural Research Station farm is in the coconut belt 20 km north of Mombasa on the Mombasa–Malindi Road. The cattle are a pedigree Jersey breed comprising at the time of the experiment 39 lactating cows, 13 dry cows, 27 bulling heifers, 27 weaner heifers and 17 calves on milk. Disease challenge included trypanosomiasis, helminthiasis and anaplasmosis among others. A pre-immunization serological sample indicated that 20% of the cattle had *T. parva* antibodies. Tick control was by weekly dipping in quintiophos and isomethamidium chloride was administered every 3 months for trypanosomiasis.

One hundred and two cattle, including calves 1 month old and older, were immunized using 1.0 ml of 1:10 dilution of *T. parva* (Marikebuni) stabilate given subcutaneously below the left parotid lymph node and were simultaneously treated with Terramycin LA. A selected group of milking cows (10 immunized, 10 non-immunized controls) was put on an experiment to investigate the effect of the immunization on milk production. Following immunization, the animals were monitored as described above. Serological examination showed that 100 out of the 102 cattle (98%) had developed antibodies to *T. parva* antigen following the immunization. However, 15 cattle (14.7%) had clinical ECF reactions, 14 of which were treated with parvaquone and recovered uneventfully. Ten of the reactors were weaners. All 17 immunized calves developed antibodies to *T. parva* without undergoing a clinical theilerial reaction. The results are shown on Tables 1 and 2. Immunization appeared to have no significant effects on milk production.

Agricultural Development Corporation Farms, Malindi. The Agricultural Development Corporation (ADC) has two farms in this complex: Kiswani ADC Home Farm, which is just outside Malindi, and Kiswani ADC Top Farm, some 15 km away. The Home Farm is dairy, the Top Farm predominantly beef. The farms together have a total of over 1000 cattle. Because the cattle numbers were large, the immunization operation was conducted in phases starting with the Top Farm, which becomes almost inaccessible after the rains. Both farms practise isomethamidium chloride chemoprophylaxis and regular deworming. Recently, vaccinations against brucellosis, vibriosis and leptospirosis have been instituted. East Coast fever challenge is high on both farms, especially on the Top Farm. A pre-immunization serological survey indicated that 18.7% of cattle on the Home Farm had *T. parva* antibodies. Just before the immunization there was an ECF outbreak on the Top Farm involving 30 cattle; 5 cattle died within a week.

Cattle were inoculated with 1.0 ml of 1:10 dilution of the stabilate subcutaneously on the shoulder (except for some milking animals) on both farms and were treated with either Medamycin 100 or Liquamycin LA. An extra 10% of drug over the calculated dose was given because most weight measurements were by weighband or visual estimation. Animals were then monitored as described above.

ADC Top Farm, Kiswani. Six hundred and fourteen beef cattle, predominantly Boran, ranging from 1-month-old calves to 15-year-old cows were immunized in two phases. In the first phase, 197 cattle consisting of young calves and dry cows were immunized. Clinical monitoring was done from days 14 to 28, and 3 out of the 197 cattle (0.5%) were identified as ECF reactors. Serological examination on day 35 showed that 190 of the 197 cattle (96.5%) had developed significant titres against *T. parva* antigen in the immunofluorescent antibody test. In the second immunization operation, 420 cattle consisting of heifers, dry or in-calf cows, including 16 beef and dairy bulls, were immunized on the Home Farm. Four animals (0.95%) required parvaquone treatment. One of these, a bull, acquired a natural infection, which was diagnosed two days after immunization.

ADC Home Farm, Kiswani. The Home Farm is a dairy operation of some 450 cattle of Sahiwal, Boran, Aryshire and Brown Swiss crosses. At the time of immunization, 120 cattle were lactating. The immunization was conducted in two phases. Initially, 306 cattle consisting of 92 yearlings, 32 weaners, 40 calves over 1 month old, 4 young bulls and 138 dry cows and steers were immunized using a 1:10 dilution of stabilate and treatment with either Liquamycin or Medamycin. Following immunization, 14 (4.5%) became theilerial reactors and were treated with parvaquone. Four animals died during the immunization but only 2 (0.6%) deaths were considered to be due to ECF. Serological examination on day 35 after immunization showed that 278 of 296 (95.6%) had developed *T. parva* antibodies.

In the second phase, 73 lactating cows and 12 young calves were immunized using 1:50 dilution of stabilate with either Medamycin or Liquamycin LA treatment. Following immunization, 2 animals, 1 calf and 1 cow (2.3%), required parvaquone treatment. Preliminary results showed no apparent effect on milk production in immunized animals. The trial continues. Serological examination on day 35 showed that 96% of the cattle had developed significant *T. parva* antibodies.

CONCLUSIONS

After these four trials the following observations were made on the method of immunization.

- a) With the selected doses of *T. p. parva* (Marikebuni) stabilate, over 95% of the cattle developed antibodies to *T. parva* after undergoing inapparent theilerial reactions.
- b) Both formulations of oxytetracycline, a short-acting formulation given in two doses and a long-acting formulation given at the same time as stabilate

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- inoculation, controlled the immunization reaction equally effectively.
- c) Exotic breeds, exemplified by the Jersey cattle, had a higher number of clinical ECF reactions, suggesting an inherently lower resistance to clinical theileriosis compared to the Zebu-European breed crosses. Slightly lower stabilate doses may be recommended for immunization in exotic cattle and a second dose of short- or long-acting formulation of tetracycline may be necessary.
 - d) Slightly higher numbers of ECF reactors were recorded in dairy animals and weaners than in beef cattle, suggesting that the stress associated with the physiological/developmental stage of the animal may have caused a more severe immunization reaction.
 - e) East Coast fever immunization appeared to have no effect on milk production, except for those few animals that developed ECF during the immunization.
 - f) Concurrent extraneous infections with foot-and-mouth disease or trypanosomiasis did not seem to affect the development of *T. parva* antibodies in immunized cattle.
 - g) Calves 1 month old tolerated the immunization well. This augurs well for calthood immunization, with the ideal animal being 1–4 months old.
 - h) In any ECF immunization trial there will be a small percentage of reactors, even with optimal stabilate concentration and drug dosage, due to individual cattle susceptibility to *T. parva* sporozoites and the disease.
 - i) The application of infection and treatment could be speeded up by the use of shoulder inoculation of stabilate and automation of oxytetracycline injection.

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Economics of an East Coast fever immunization trial at the Kenya Coast

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This paper summarizes the results of a financial analysis of an East Coast fever (ECF) immunization trial at the Kenya Coast. The details of the trial methodology, design and bioeconomic data generated are presented by Morzaria et al. (1988) and by Morzaria elsewhere in these proceedings.

Eighty beef cattle were immunized and a similar group of 80 were left unimmunized as a control. The immunized group was divided into 4 sub-groups of 20 animals each, the sub-groups identified as A, B, C and D. The control group was also divided into 4 sub-groups (E, F, G and H) of 20 animals each. Sub-groups A and E were sprayed with an acaricide twice a week, B and F were sprayed once every 3 weeks, C and G had prolonged-release acaricide-impregnated ear tags inserted into each animal ear, and D and H had no tick control treatment.

All groups were maintained and grazed together in an ECF-endemic area on a farm for 9 months, starting in May 1985. The bioeconomic data collected at the end of the trial included starting and ending liveweights; the numbers of surviving animals; the numbers and causes of deaths; and the quantities and costs of materials, drugs, acaricides and veterinary services used for each sub-group. Partial budgeting was used to derive gross benefits, costs that vary among sub-groups, and net financial benefits per kg of starting liveweight for each sub-group. Dominance analysis was used to identify dominant and dominated sub-groups. This was done first by listing the sub-groups in the order of their increasing costs that vary per kg of starting liveweight with corresponding net benefits. Any sub-group that had net benefits that were less than or equal to those of a sub-group with lower costs that vary was identified as dominated. Conversely, any sub-group with net benefits that were equivalent or greater than those of a sub-group with higher costs that vary was identified as dominant. A farmer would not choose a dominated practice over a dominant one, because the former would have higher costs and lower benefits than the latter. It is the dominant sub-groups that are candidates for recommendation to the farmer.

Marginal analysis was used to calculate marginal rates of return of the dominant sub-groups and to identify the sub-group that would maximize financial benefits to the farmer.

The gross benefits for treatments A, B, C, D, E, F, G and H were Kenya shillings (KShs) 12.50, 11.35, 11.71, 11.88, 11.39, 8.71, 9.07 and 8.30, respec-

tively (KShs 16.00 = US\$1, January 1986). The costs that vary were KShs 0.81, 0.55, 0.71, 0.63, 0.62, 0.90, 1.21 and 1.15, respectively. The net benefits per kg of starting liveweight were therefore KShs 11.69, 10.80, 11.00, 11.25, 10.77, 7.81, 7.86 and 7.15, respectively.

Sub-groups C, E, F, G and H were dominated. Sub-groups B, D and A were dominant in that order of increasing net benefits. The marginal rate of return between B and D was 562.5% and between D and A was 244.4%. In financial terms, sub-group A generated the highest benefits to the farmer in this trial.

The analysis shows that the immunized sub-groups yielded greater financial benefits, due to lower ECF mortality and higher weight gains, than the non-immunized sub-groups. Of the immunized sub-groups, the one that was sprayed with acaricide twice a week yielded the highest financial benefits. However, such intensive use of acaricides would not be recommended for widespread adoption, because acaricides are becoming increasingly more expensive and less available for smallholder farmers in many countries affected by ECF.

These results demonstrate that immunization is financially superior to non-immunization, but that the frequency of acaricidal application after immunization for tick and other tick-borne disease control requires further on-farm research under various environmental conditions.

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A pilot immunization scheme against theileriosis with unconventional tick control on a farm in Nakuru District, Kenya

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The Nakuru East Coast Fever Project is a programme jointly conducted by the National Veterinary Research Centre, at Muguga, the Veterinary Laboratories, at Kabete, and the managements of eight large and medium-size farms in Nakuru District, Kenya. The objective in the initial phase of the project was to determine both the costs incurred on these farms for tick control and the effects of tick-borne diseases. The total cost of control and economic losses due to ticks and tick-borne diseases for the farms was estimated to be US\$13.64 per animal (Young et al., in press).

It was agreed that a pilot scheme to introduce immunization against theileriosis should be undertaken on one of the farms, Marula Ranch, and the acaricide control should be altered, removing spraying totally. The known tick-borne disease problems on this farm were Corridor disease, caused by *Theileria parva lawrencei* infection derived from the large resident buffalo populations; East Coast fever, caused by *T. p. parva* infection, probably derived from neighbouring farms; anaplasmosis; babesiosis, caused by *Babesia bigemina* infection; and sweating sickness. Frequent dipping and spraying using coumaphos, (Asuntol, Bayer) or, more recently, diamide (Triatics, Cooper Animal Health) did not prevent 480 clinical cases of theileriosis (8.3% of the total population) and 100 cases of other tick-borne diseases (1.7%) in 1986. The farm's diagnosis of clinical tick-borne diseases was largely confirmed over a 6-month period. The reasons for this lack of control of tick-borne disease are reviewed by Ochanda et al. (1988). Deaths from tick-borne diseases had been reduced dramatically by the use of parvaquone (Clexon, Wellcome) for theileriosis treatment and imidocarb dipropionate (Imizol, Wellcome) for babesiosis and anaplasmosis treatment: in 1986 only 53 animals died from theileriosis and 6 from babesiosis or anaplasmosis.

In the experiment carried out on Marula Ranch, 101 European-Boran cross-bred cattle (50 cows and 51 calves) were selected, bled for serology and

weighed. Two additional groups of 20 cattle each (10 cows and 10 calves) were chosen for sprayed (diamide) weight controls and for infection controls. All cattle were kept on diamide application during the immunization period.

Theileria p. lawrencei and *T. p. parva* stocks from another district were chosen to immunize the cattle because local *T. parva* stocks were not available and because an objective of the experiment was to determine whether non-local stocks gave protection in this area. Mutugi et al. (1988) had shown that *T. p. lawrencei* stocks from the Mara area (Narok District) gave complete cross-immunity with *T. p. lawrencei* stocks from Ol Pejeta (Laikipia District); therefore it was decided to use Mara and Trans-Mara stocks. These were *T. p. parva* (Kilae) stabilate 187 and *T. p. lawrencei* (Mara III), which were diluted at 1:10 and 1:80, respectively, for infection. An inoculation of 1 ml of the combined stabilates was given either subcutaneously in front of the ear or on the shoulder. Injections of a short-acting formulation of oxytetracycline hydrochloride (Medamycin 100, Techamerica Group, USA) were given on days 0 and 4 after inoculation of stabilate at 10 mg/kg bodyweight. The total cost of this immunization (for drug and needles, etc.) was KShs30 (US\$1.66) for a 300-kg animal.

Minimal clinical monitoring was undertaken. Twenty-one of 240 lymph node biopsy smears (8.8%) taken from days 15 to 27 after infection had low schizont parasitoses. The conditions during immunization were poor, with little grazing just before the long rains, and the cattle were in poor condition. Three calves died during the immunization of causes other than ECF. Nine percent of the animals had significant antibody titres to *T. parva* schizont antigen before the experiment, 99% after immunization. After the start of the long rains the condition of the immunized cattle improved rapidly.

The second phase of the experiment was to expose the immunized cattle to tick-borne disease challenge on the ranch. The objectives of the exposure were to determine the following:

- a) the efficacy of the theileriosis immunization
- b) the problems caused by other tick-borne diseases in the area
- c) the efficacy of acaricidal ear tags on tick infestation on the ranch
- d) whether these tags would reduce the tick-borne disease challenge

The acaricidal tags were applied to groups 1–4 on 28 April 1988 and the last acaricidal application was on 3 May 1988. The arrangement of the groups is shown in Table 1. Observations made on the exposed cattle included close clinical monitoring in the morning and evening, with rectal temperatures and lymph node and blood smear samples taken when necessary. A theilerial reactor was defined as an animal clinically sick from theileriosis that, if not treated, would

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probably die. These cases were treated with parvaquone and long-acting tetracycline (Terramycin LA, Pfizer).

Table 1. Experimental design showing numbers, tick control and other manipulations of cattle allocated to different groups

Groups	Conventional acaricide	Immunized cattle (no.)	Control cattle (susceptible)	Remarks
Group 1 Acaricidal ear tag Type 1*	None	10 cows 9 calves	2 cows 2 calves	Weighed at monthly intervals
Group 2 Acaricidal ear tag Type 2*	None	10 cows 9 calves	2 cows 2 calves	Weighed at monthly intervals
Group 3 Acaricidal ear tag Type 3*	None	10 cows 10 calves	2 cows 2 calves	Weighed at monthly intervals
Group 4 Acaricidal ear tag Type 4**	None	10 cows 10 calves	2 cows 2 calves	Weighed at monthly intervals
Group 5 Controls, no ear tags	None	10 cows 9 calves	2 cows 2 calves	Weighed at monthly intervals
Group 6 Weight controls, no ear tags	Diamide sprayed once a week	—	10 cows 10 calves	Weighed at monthly intervals
Group 7 No ear tags	None	—	10 steers	Added to the experiment after 2 ¹ / ₂ months exposure
Group 8 No ear tags, calves born during experiment	None	—	12 calves	

* Acaricidal ear tag Types 1–3: Amitraz, Camco, U.K., monolithic tag 10 or 15% diamide.

** Acaricidal ear tag Type 4: Amitraz, Camco, U.K., liquid reservoir.

In addition, ticks infesting the 4 calves and 4 cows were counted on half the body at weekly intervals in groups 1–5 and group 7. Serum and haematological samples were taken at monthly intervals or as necessary. Records of all interventions, such as treatments, were kept.

One hundred and fifty days after the exposure began only one immunized animal had died of acute babesiosis. The immunization experiment was thus considered a success, especially because the ranch management expected great problems from other tick-borne diseases.

The cattle had to be exposed in a low-challenge area for a month due to flooding in the high-challenge area where there is a resident buffalo population. The tick infestation was very low during the first month. On moving the cattle to the high-risk area, the tick infestation increased but was still considered low. However, the incidence of tick-borne disease became fairly high and complicated.

To summarize the results, 10 of the 20 (50%) control cattle developed clinical disease, probably caused by *T. p. lawrencei*, and required treatment, while only 1 of 97 (1%) of the immunized cattle became a theilerial reactor (perhaps from *T. taurotragi* infection).

A complication was the occurrence of a theilerial syndrome in many of the control and immunized cattle characterized by the presence of few macroschizonts, a transient febrile response and the presence of large piroplasms with veils and bars in erythrocytes. This parasite was tentatively identified as *T. taurotragi*. Anaemia was minimal. There is no cross-immunity between *T. parva* and *T. taurotragi* (Young et al., 1977). This parasite is being isolated for laboratory characterization. The individual groups were analysed for theilerial reactors and the results are shown in Table 2.

Table 2. The theileriosis reaction of immunized and control cattle under different tick control regimes

Group	Immunized cattle	Controls
1	0/19	2/4
2	0/19	2/4
3	0/20	3/4
4	0/20	0/4
5	1/19	3/4

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On 21 July 1988 an additional group of 10 steers was introduced without tick control and 4 have become theilerial reactors even though the ticks infesting these cattle were removed weekly and applied to a buffalo at Kabete for *T. p. lawrencei* isolation. Twelve calves were born during the exposure and 4 of these have become theilerial reactors. All theilerial reactors recovered following parvaquone treatment, except for one calf in group 8, which died of theileriosis. The acaricidal ear tags reduced the tick infestation considerably compared to untagged controls but did not prevent transmission of theileriosis, with the possible exception of group 4. In this group a new type of linear release tag with a liquid reservoir, containing permethrin, was used.

One of our greatest worries in this experiment was the possible effects of other tick-borne diseases, since the immunized cattle were probably susceptible to them. A number of transient low fever responses were recorded, which appeared to be due to *T. taurotragi*, *Ehrlichia bovis*, *Ehrlichia* (*Cytoecetes*) *ondiri* and *Borrelia theileri* infections, all transmitted by ticks but none, with the exception of Ondiri disease, being fatal to cattle.

Boophilus decoloratus infestations were detected only late in the exposure and no *Anaplasma marginale* infections were detected. One acute *B. bigemina* infection and one *Borrelia theileri* infection did occur, but no other *Babesia* infections were detected. No *Amblyomma* spp., vectors of *Cowdria ruminantium* and *T. mutans*, have been detected infesting cattle to date.

The following points should be stressed about this experiment.

- a) *Theileria* parasites from the Narok District (e.g., *T. p. lawrencei* and *T. p. parva*) gave good protection to cattle immunized with them in Nakuru District, a result already anticipated from laboratory experiments (Mutugi et al., 1988).
- b) The problems with other tick-borne diseases have not been very large.
- c) Acaricidal ear tags gave a good reduction in tick infestation and, in some cases, in tick-borne disease challenge.
- d) The low tick infestation on cattle was undoubtedly due to intensive acaricide control practised on the normal cattle population using the pastures. (The majority of the ticks were derived from wildlife, particularly buffalo and waterbuck, grazing the same pastures.) Reduction of tick control will mean that the tick population maintained by cattle could increase.

The ranch now intends to apply such immunization and tick control on a large scale, which is of great interest since they have a population of 465 dairy (Friesians) and 5291 beef (Boran, Boran/European breed crosses) cattle. Further monitoring will include a cost benefit analysis of the introduction of immunization and reduction of tick control.

ACKNOWLEDGEMENTS

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The fertility of Boran heifers immunized against buffalo-derived *Theileria parva*

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Four hundred Boran (*Bos indicus*) heifers were immunized against *Theileria parva* by infection and treatment on a large ranch in the Kenya highlands. The immunizing stock was prepared as a stabilate (184) from ticks collected from the ground in the riverine areas of the ranch, where resident and migrant buffalo graze. The immunizing infection was controlled in 300 cattle using long-acting oxytetracycline (Terramycin LA, Pfizer) at the time of infection and in 100 cattle using parvaquone (Clexon, Wellcome) on day 8. The cattle were exposed to tick challenge under four different tick control regimes and monitored for disease and productivity parameters for one year (July 1984–July 1985) (Dolan, 1985). The disease challenge was estimated by exposing groups of 10 heifers to unlimited tick challenge. The group of 10 heifers was replaced when they all became clinically ill with theileriosis or developed antibodies to *T. parva*. The disease challenge was potentially 20% lethal for July to September, 60% for October, 100% for November to May and 60% for June. During the year 17 (3.9%) of the immunized heifers developed clinical theileriosis and 67 (15.6%) had anaplasmosis. The theileriosis challenge was due mainly to buffalo-maintained *T. p. lawrencei*.

The rains due in September 1983 and March 1984 had failed and the heifers were healthy but thin and on poor grazing when they were immunized and joined with the bulls in May 1984. The heifers were between 26 and 30 months old. The bulls were removed in August and only 63% of the heifers were in-calf in October. The bulls were re-introduced in January 1985 until mid-March, then introduced again in May. Between November 1984 and March 1985 there were 24 abortions, with the majority occurring in January. In addition, a number of very small calves were born and some of the heifers showed poor mothering ability. There was some rain in July and August 1984 and good and consistent rain fell from September to December. There was almost no rain in January

FERTILITY OF IMMUNIZED BORAN HEIFERS

1985, some in February and March and good rains from April to the end of the trial. The land did not recover rapidly in spite of the plentiful rain, and the cattle were moved to better grazing on another part of the ranch in March 1985. By October 1985, 86% of the heifers had either calved or were in-calf.

During the year and particularly at the peak of abortions in January, samples were taken and examined for infectious causes of abortion or infertility. The heifers had been immunized against *Brucella abortus* and no *Brucella* were isolated from ten freshly aborted fetuses. Paired serum samples were negative for leptospirosis, Rift Valley fever and bovine virus diarrhoea antibodies. A review of records of the other breeding herds on the ranch during the year showed a pregnancy rate of about 70%.

A question that could not be answered at the end of the trial was what effect the immunization had on the fertility of the heifers. They had come on the trial on a falling plane of nutrition and the grazing did not improve for some months, which might have influenced the low in-calf rate, abortions and weak calves. The land did not recover rapidly, probably because of the extended drought from 1983, and mothering ability may also have been adversely affected. An infectious cause for infertility or abortion was not identified and the overall calving rate for the year on the ranch was low (70%). Thus, nutritional influences probably explain the low in-calf rate at 5 months. Certainly the calving/in-calf rate of 86% by October 1985, once the cattle were back onto a good plane of nutrition, indicated that no long-term adverse effects were suffered.

To test the effects of the immunization procedure on similar heifers 26–30 months old on a good plane of nutrition, a group of 166 were selected in January 1986. Fifty were immunized at random using the same stabilate (184) and all 166 were joined with the bulls immediately. In this immunization, tetracycline alone was used because no differences had been detected between tetracycline and parvaquone in the previous trial and a single manipulation of the cattle was desirable. The herd was subjected to once-a-week dipping and detailed records of disease and breeding history were kept throughout the following year. Six animals in the control group died of theileriosis in March/April 1986, two were stolen (one immune), one died while giving birth, one died of haemorrhage and one died of debility. Seven were culled or transferred to other herds and two were sold. A summary of the breeding history of the immunized and control heifers is shown in Table 1.

Thus on a good plane of nutrition and in the absence of the management interventions and disease challenge related to the earlier trial, immunization did not affect the fertility of these Boran heifers. The heifers from the first trial were distributed among other herds on the farm beginning in October 1985 and it was not possible to obtain accurate records of their subsequent fertility. Available records, however, show that these heifers have had normal calving rates and have demonstrated good mothering ability.

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Table 1. Breeding performance of immunized and control cattle (%)

	Immunized	Control
Calving	86.0	80.0
Non-breeders	4.0	5.5
Slow breeders	4.0	5.5
Abortions	2.0	4.0

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An East Coast fever immunization field trial at Kasoba, Malawi

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East Coast fever (ECF), a *Theileria parva* infection in cattle, is a major cause of cattle mortality in Malawi. Moodie (1987) reported that 66% of each year's calf crop was lost before the calves reach two years of age. Most of these losses were attributed to ECF, which is widespread in the central and northern regions of Malawi. In addition, Radley (1981) observed that ECF caused a reduction in productivity and was also costly because of the money and time spent on tick control.

Project RAF/67/077, conducted at the former East African Veterinary Research Organisation, in Muguga, Kenya, demonstrated that a combination of three *Theileria* stocks, *T. p. parva* (Muguga), *T. p. parva* (Kiambu 5) and *T. p. lawrencei* (Serengeti transformed) protected cattle against a variety of *T. parva* parasites isolated from different parts of East Africa, with only a few breakthroughs observed. Cross-immunity experiments were conducted between the East African stocks and isolates from Malawi under project GCP/MLW/018/DEN between 1980 and 1985. On the basis of the findings, the Malawi Government authorized field immunizations against ECF in the central region, using the East African stocks as the base vaccine, but added the proviso that monitoring of the immunized cattle be continued to look for potential breakthrough strains.

In 1986 and 1987 ECF immunization field trials were conducted in Karonga District, in the northern region, to assess the protection afforded by the East African *Theileria* stocks. The first trial was at Kayuni in 1986, but the results were inconclusive because most of the cattle died of other tick-borne diseases. A second trial was conducted in the same district, at Kasoba, a cattle market where many cases of ECF had been reported. This report records the findings during the exposure of ECF-immunized cattle at Kasoba.

MATERIALS AND METHODS

Cattle. The cattle used in the experiment were Friesian-Malawi Zebu cross-breeds approximately 2 years old, which had been raised in the southern region on farms where strict tick control is practised and where active ECF cases have

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not been observed. These cattle were transported to the laboratory and maintained under a strict tick control regime. Prior to immunization, they were found to be serologically negative to *T. parva* schizont antigen using the indirect immunofluorescent antibody test. They were dewormed and vaccinated against blackquarter. During the trial the cattle were monitored for enlargement of lymph nodes, the presence of parasites in lymph node biopsies, the development of fever, serological responses to *T. parva* antigen and survival. The tick numbers on the head and ears were counted.

Stabilates. The following *Theileria* stocks were pooled to provide the immunizing material: *T. p. parva* (Muguga), *T. p. parva* (Kiambu 5) and *T. p. lawrencei* (Serengeti transformed).

Drug. Long-acting oxytetracycline (Terramycin LA, Pfizer, UK) was administered intramuscularly at 20 mg/kg bodyweight shortly before the inoculation of the stabilates, during the immunization process at the Central Veterinary Laboratory, Lilongwe.

Other Vaccines. Twenty ECF-immunized and 15 non-immunized control cattle were immunized against anaplasmosis, babesiosis and heartwater using *Anaplasma centrale*, *Babesia bigemina*, *B. bovis* and *Cowdria ruminantium* vaccines obtained from the Onderstepoort Veterinary Laboratory, South Africa.

Ticks. Clean *Rhipicephalus appendiculatus* nymphs reared at the Central Veterinary Laboratory were applied to reacting control cattle with schizonts in attempts to pick up the field *Theileria* at Kasoba.

RESULTS

The cattle were grazed communally with the local cattle in the area but were kraaled separately in the evening. The cattle were in good condition at the start of the trial but because the grazing was poor and became progressively worse, their diet had to be supplemented with maize bran and mineral blocks.

A generalized lymph node enlargement, which persisted throughout the trial period, was first detected after ten days in the field. The development of fever within the ECF-immune and ECF-naive groups of cattle is summarized in Figure 1. The generalized lymph node enlargement was followed by a fever, which was most prominent in the control group from days 21 to 34 of the exposure; the fever in the immunized group was very mild.

Detection of *Theileria* schizonts also coincided with the generalized lymph node enlargement. The percentages of animals in which schizonts could be de-

tected in the control and immunized groups during the trial are shown in Figure 2. More than 70% of the cattle in the immune group had detectable schizonts between days 12 and 19, compared to 60% in the control group. The percentage of cattle with schizonts in the immune group dropped to 31% between days 20 and 27, while it rose to 80% in the control group in the same period. No schizonts could be detected in the immune group after day 27, while schizonts were detected in the control group up to the end of the trial.

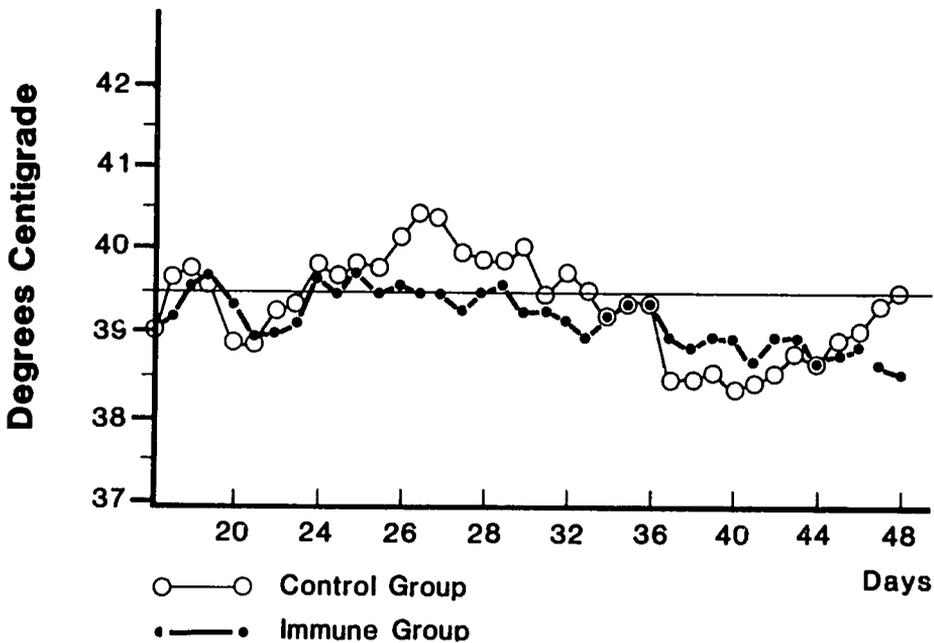


Figure 1. The development of fever in *Theileria parva* immunized and control cattle following exposure to natural disease challenge at Kasoba, Malawi.

Babesia parasites were detected in both the immune and control groups on days 30 and 31, and all cattle were treated with imidocarb dipropionate.

Twelve of the 15 animals in the control group died during the trial, compared to 3 of the 19 in the ECF-immune group. The mean number of days to death for the control group was 28.5, compared to 33 in the immune group. The causes of death in the control group were as follows: 9 to ECF, 1 to a combination of heartwater and ECF, 1 to heartwater and 1 to *B. bovis*. The causes of death in the ECF immunes were as follows: 2 to babesiosis and 1 to a combination of heartwater and babesiosis.

The common findings on gross post-mortem examination in the control group were widespread haemorrhages in the lungs, gastro-intestinal tract, kidneys, myocardium, urinary bladder, gallbladder and spleen. Splenomegaly, hepatomegaly and oedema in the lungs were evident in all cases. The brain was congested and oedematous. In the immune group, jaundice and haemorrhages

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were the most outstanding abnormalities in two of the three carcasses while the third had only minor haemorrhaging along the coronary vessels.

The mean tick counts for the control and immune groups during the trial are shown in Figure 3. The general trend for both groups was similar: there was a rapid build-up in tick numbers in the first two weeks, reaching a peak within the next two weeks, followed by a rapid decline to about half the maximum mean tick counts. The minor differences observed between the groups were not significant.

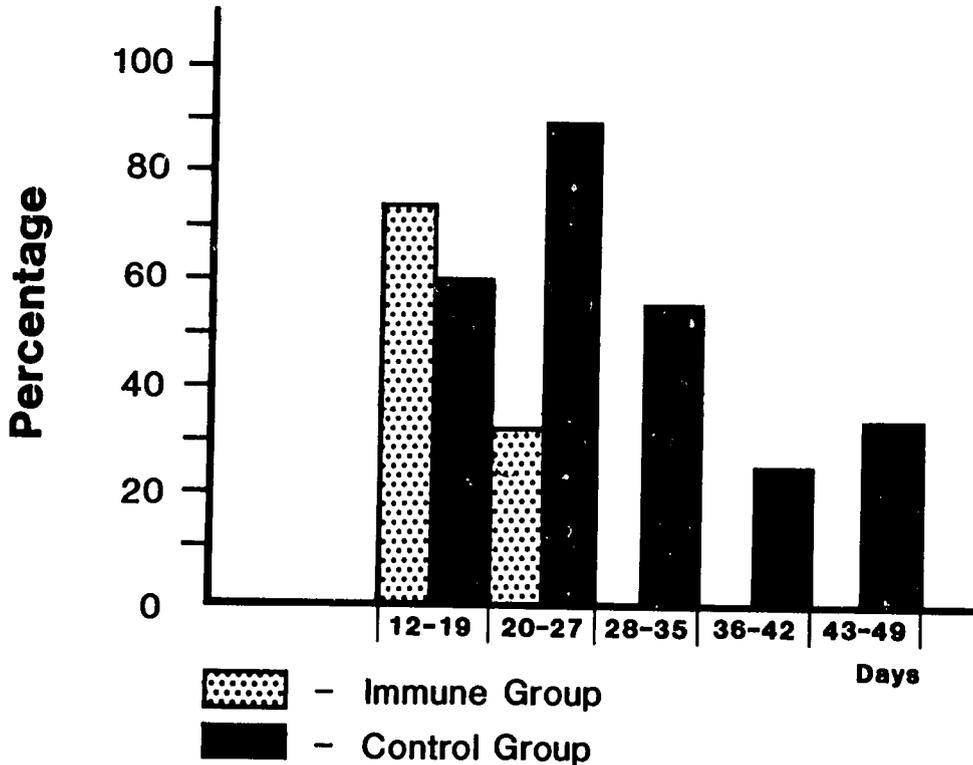


Figure 2. The percentage of *Theileria parva* immunized and control cattle with detectable schizonts following exposure to natural disease challenge at Kasoba, Malawi.

A total of 915 adult *R. appendiculatus*, resulting from clean nymphae applied to the control cattle during the trial, were harvested in the laboratory. These have since been used in laboratory cross-immunity studies.

DISCUSSION

Although *Theileria* schizonts and fever were observed in the immunized group, none of these cattle died of ECF during the trial; 9 out of the 15 control cattle

died of ECF. It was concluded that the immunization was successful and that the *Theileria* stocks used provided protection against the local *Theileria* parasites in the area during the trial period.

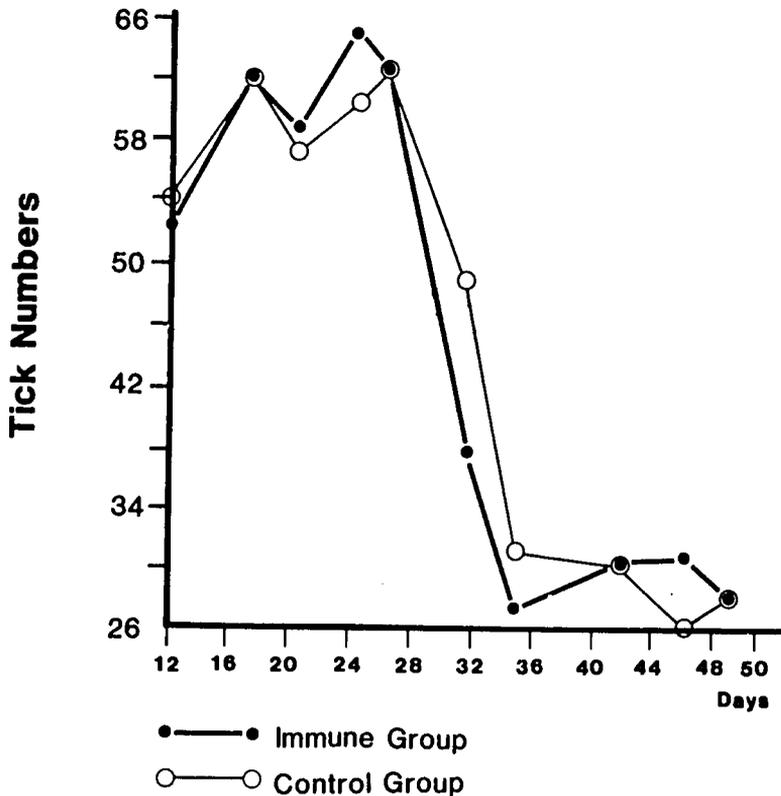


Figure 3. The mean number of ticks counted on *Theileria parva* immunized and control cattle during exposure at Kasoba, Malawi.

The immunization against babesiosis and heartwater was not satisfactory, as evidenced by deaths due to these diseases in both the immune and control groups of cattle. It is not known whether this apparent breakdown in the immunity was due to a failure of the immunization or the existence of entirely different immunogenic strains. It is possible that the poor grazing and generally harsh environmental conditions for these cattle accustomed to stall feeding may have contributed to and exacerbated the situation.

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East Coast fever immunization trials on Unguja Island, Zanzibar

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Immunization against *Theileria parva* on Unguja Island, Zanzibar, has been undertaken jointly by the Department of Livestock Development, Zanzibar, the International Laboratory for Research on Animal Diseases, the Livestock Development Project of the Food and Agriculture Organization of the United Nations and the Overseas Development Administration, U.K. Two stabilates were prepared from ticks collected from sites in the north and south of Unguja Island and characterized at the International Laboratory for Research on Animal Diseases (Morzaria, Irvin and Dolan, 1986). The stabilate, designated Zanzibar South (stabilate 2914), was found to be the more pathogenic of the two stabilates and it protected against challenge with the Zanzibar North stock. The stabilate was effective at a 1:10 dilution when used in infection-and-treatment immunization of cattle with two treatments of long-acting oxytetracycline (Terramycin LA, Pfizer) at 20 mg/kg on days 0 and 4.

In October 1986 an immunization trial (trial 1) was carried out on Unguja using the Zanzibar South stock (stabilate 2914). Twenty-two *Bos taurus* calves were immunized and exposed to natural challenge together with 11 unimmunized controls. The calves were exposed at Kizimbani (Central Unguja), Tunguu (Southern Unguja) and again at Kizimbani over a three-month period. All 33 animals reacted to East Coast fever (ECF) at least once and 5 unimmunized cattle died of uncomplicated ECF. None of the immunized animals died of uncomplicated ECF. Three other controls and 8 immunized animals died of a complicated syndrome involving severe anaemia, which affected most of the trial animals. Low numbers of *T. parva* schizonts could be found in necropsied animals, but in addition, *T. mutans* was detected in all anaemic animals and *Anaplasma marginale*, *Babesia bigemina* and possibly *B. bovis* in some. Other complications were a very high tick challenge at Tunguu (head counts of up to 200–250 ticks), turning sickness (one animal) and heartwater (one animal).

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Sixteen survivors from trial 1 were re-exposed in the second trial (August–December 1987), together with twelve *Bos taurus* calves, six of which were immunized with the other six being controls. They were exposed at Kipange (northern Unguja) for 20 days and encountered a heavy tick challenge of 60–100 adult *R. appendiculatus*. The ECF reactions are summarized in Table 1. The unimmunized control animals had earlier and more severe reactions than the newly immunized cattle. Six survivors from trial 1 reacted and one died of ECF. Two major complications, heartwater and anaemia, were encountered. Heartwater killed six animals, five of which were survivors from trial 1, and anaemia affected the new calves, with marked falls in packed cell volume (to 8%). There was an association between the degree of anaemia and the mean *T. mutans* piroplasm parasitaemia, which suggested that concurrent *T. mutans* and *T. parva* infections were the main causes of anaemia. However, *B. bigemina* and *B. bovis* were detected in some of the animals, despite prophylactic treatment with imidocarb dipropionate (Imizol, Wellcome). One animal had *A. marginale*.

Table 1. Unguja ECF immunization trial 2: exposure reactions

Group	Number of animals	Mean number of days to:				Cause of death
		Schizonts	Fever	Piroplasms	Death	
Immunized	6	21.3 (6)	21.7 (6)	21.3 (6)	40.0 (2)	1 ECF plus anaemia, <i>A. marginale</i> and <i>T. mutans</i> 1 heartwater
Unimmunized controls	6	19.4 (5)	20.3 (6)	20.5 (6)	26.3 (3)	3 ECF
Survivors Trial 1	16	24.5 (6)	23.4 (14)	25.4 (11)	27.9 (7)	1 ECF 5 heartwater 1 heartwater (?)

Numbers in parentheses: number of animals.

In the third trial, 5 survivors from trial 2 and 17 cross-bred (Jersey × local Zebu) calves on loan from smallholder farmers were exposed for 5 weeks at Tunguu (the second exposure site in trial 1). Ten of these cross-bred calves had antibodies to *T. parva* despite farmers' efforts to apply acaricide weekly. Twelve animals, including four which were serologically negative, were immunized. The immunization was well tolerated except for one calf, which had a moderate reaction and was treated with parvaquone (Clexon, Wellcome). The tick chal-

lence at Unguja was low (body counts of 6–10 adult *R. appendiculatus*). *Theileria parva* schizonts were detected in only four animals, two controls and two newly immunized calves. One of the controls died of ECF.

During these trials 40 animals were immunized with the Zanzibar South stabilate; there was only one adverse reaction. Susceptible control cattle had earlier and more severe ECF reactions when exposed to field challenge (Table 2). However, there have been many deaths in both immunized and control groups associated with other tick-borne diseases. During these trials *Cowdria ruminantium* and *B. bovis* were confirmed on the island. The anaemic syndrome, affecting particularly the cattle in the first trial, was in part attributed to *T. mutans* and these cattle appeared to be immune to this syndrome on subsequent exposure. It is clear that ECF can be controlled by immunization but that immunization must be part of an integrated strategy of tick and tick-borne disease control. In pursuing this aim of controlling tick-borne disease through immunization of calves born in the artificial insemination scheme, Ministry of Livestock Development officials wish to adopt ECF immunization together with immunization against heartwater and *T. mutans*, using locally isolated parasites.

Table 2. Summary of Unguja ECF immunization trials

Group	No. of animals	Mean number of days to:				Cause of death		
		Schizonts	Fever	Piroplasms	Death	ECF	other	Other
Immunized	40	27.8 (22)	27.7 (27)	27.3 (31)	40.3 (5)	1	2	15
Controls	22	25.2 (15)	24.8 (17)	25.6 (16)	30.4 (9)	9	2	3

Numbers in parentheses: number of animals.

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An East Coast fever immunization trial on Pemba Island, Zanzibar

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Pemba Island has a population of approximately 50000 short-horned East African Zebu cattle, whose productive performance is extremely low. A major priority of the Ministry of Agriculture and Livestock Development (MALD), Zanzibar, is to improve milk and beef production on the islands of Unguja and Pemba by upgrading indigenous Zebu cattle using artificial insemination from exotic *Bos taurus* and *Bos indicus* semen.

East Coast fever (ECF), caused by *Theileria parva*, has been one of the main causes of the high mortality in first cross *Bos taurus* and *Bos indicus* calves on Pemba. Immunization against ECF using the infection-and-treatment method seems an attractive means of controlling this disease, as recent evidence suggests enzootic stability for other tick-borne diseases, particularly babesiosis, which are known to occur on Pemba. For this reason *Rhipicephalus appendiculatus* ticks were collected from Zebu cattle at two widely separate locations on Pemba and *T. parva* isolates were prepared and characterized. Stabilate 2913, designated *T. parva* Pemba/Mnarani, was chosen for use in an immunization trial to be conducted on Pemba (Morzaria, Irvin and Dolan, 1986).

IMMUNIZATION TRIAL

The characterization of *T. parva* (Pemba/Mnarani) stabilate 2913 involved drug sensitivity tests and homologous and heterologous isolate challenge using Boran (*Bos indicus*) steers. Mild immunization reactions were recorded when a single dose of long-acting oxytetracycline (Terramycin LA, Pfizer) at 20 mg/kg was

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administered along with an immunization dose of 1 ml of stabilate 2913 at a 1:10 dilution.

Nineteen pure-bred Jersey (*Bos taurus*) calves and 2 Jersey × New Zealand milking Zebu (NZMZ) cross-bred calves aged 13–15 months were immunized on Pemba as described above. Nine pure-bred Jersey calves and 1 NZMZ calf (Friesian [*Bos taurus*] 75% × Sahiwal [*Bos indicus*] 25%), all of the same age, were used as controls and were given long-acting oxytetracycline at 20 mg/kg only.

Of the 21 immunized cattle, 2 died of clinical ECF following immunization and 1 calf, which was in poor condition at the time of immunization, suffered clinical ECF and was killed by dogs. Nine immunized calves suffered severe clinical reactions but recovered following repeated treatments (in some cases as many as six) with parvaquone (Clexon, Wellcome) at 10 mg/kg. The nine remaining calves suffered mild immunization reactions and recovered without treatment.

Prior to field exposure all calves were treated with a prophylactic dose of imidocarb dipropionate (Imizol, Wellcome) at 2 mg/kg. Field exposure was carried out on rough grazing where indigenous Zebu cattle were known to have grazed recently. Tick challenge was extremely heavy, especially of *R. appendiculatus* and *Boophilus microplus*. Fourteen days after the beginning of field exposure a mean tick count of 107 adult ticks was recorded on the head and ears.

All calves were found to have *T. parva* schizonts in lymph gland biopsy smears from day 15 following exposure. On day 23, 22 of the 28 calves were found to have *B. bovis* and *B. bigemina* piroplasms in blood smears. In addition, piroplasms of *T. mutans* were seen in the blood smears of 25 of the calves and a few had *Anaplasma marginale*. Imidocarb dipropionate treatment was repeated, but in spite of this and parvaquone therapy, three immunized and three control animals died of ECF, complicated by concurrent tick-borne diseases.

DISCUSSION

The 18 calves that survived immunization fell into two distinct categories: 9 calves suffered severe clinical reactions and required parvaquone therapy and 9 calves suffered mild reactions and made spontaneous recoveries. During field exposure 7 of the 9 calves that suffered severe immunization reactions required repeated parvaquone therapy and 2 died of ECF complicated by the other tick-borne diseases. In contrast, of the 9 calves that were immunized and did not require parvaquone treatment, only one suffered a severe clinical reaction during field exposure and this calf died from a combination of babesiosis, anaplasmosis, ECF and *T. mutans* (see Figure 1). It is suggested that most, if not all, of the immunized calves that suffered severe reactions following immunization were harbouring latent, chronic theileriosis and were probably immunosuppressed at

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the time of field exposure. Unfavourable reactions to immunization were almost certainly due to the greater susceptibility of *Bos taurus* cattle to *T. parva*. With hindsight it would have been more appropriate to have characterized the stabi- late using pure *Bos taurus* calves. Babesiosis and anaplasmosis were known to be present on Pemba but it was believed that the imidocarb dipropionate prophylaxis should have controlled these diseases. However, the tick burdens were very heavy and the disease challenge complicated and severe, while the animals were further compromised by the immunization reactions. These complications high- light also the risk of exposure of cattle to unlimited tick challenge and suggest that some level of tick control is justified in trials when certain threshold bur- dens are reached.

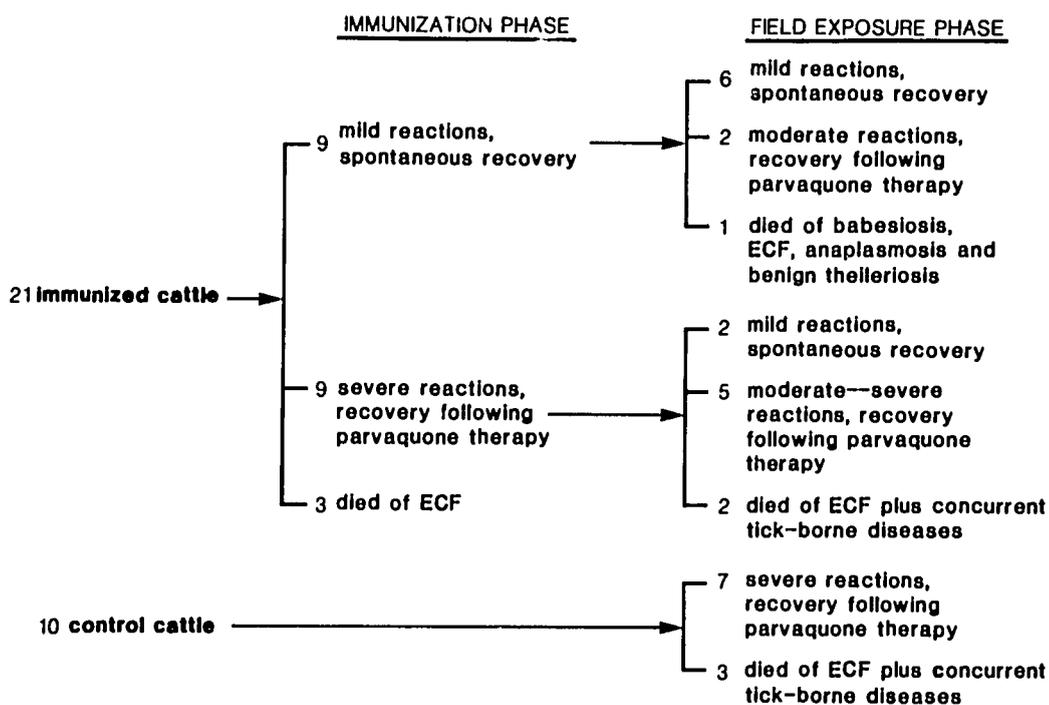


Figure 1. Summary of disease responses of immunized and control cattle on Pemba Island, Zanzibar.

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East Coast fever immunization in the Eastern Province of Zambia

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The Belgian Animal Disease Control Project started its activities in October 1982. Monitoring sentinel herds along an east-west transect and surveying the Eastern Province of Zambia indicated that East Coast fever (ECF) was spreading westward together with its vector (the *Rhipicephalus appendiculatus*/*R. zambeziensis* complex), largely through cattle being moved from epidemic areas to clean areas further west. In 1982/83 a dip-tank barrier was erected in Katete District and compulsory dipping was enforced in the district to stop this spread and to protect Petauke District. Simultaneously a start was made with the isolation of local *Theileria parva* strains in an attempt to control the disease in the area east of the barrier through immunization.

By mid-1987 the disease and its vector had invaded Petauke District despite the control measures, putting another estimated 40000 head of cattle at risk. Currently ECF appears to have reached some form of endemic stability in Chipata, Chadiza and Lundazi districts and parts of Katete District. The disease is mainly confined to calves, and the majority of calves have been in contact with *T. parva* by the age of 30 months, the mean age at first contact being 10 months. ECF kills about 50% of the calves. The area is considered marginal for the vector, resulting in a relatively low tick challenge. An anomalous epidemiological feature is the occurrence of a second generation of adult ticks at the start of the dry season in May/June, increasing the incidence of ECF at this time of the year. The western part of Katete District and the eastern half of Petauke District make up the epidemic area. Large-scale epidemics usually erupt at the start of the dry season, when cattle are allowed to graze on free range in the seasonally waterlogged grasslands called "dambos".

In October 1987 a bulk stabilate was produced and tested in titration trials. A training course for the veterinary assistants to be involved in the immunization campaign was organized. A calf census was undertaken and a representative sample of the calves (about 2000) was ear-tagged to allow an assessment of the immunization campaign. Between November 1987 and January 1988 a total of 4800 calves between 2 months and 1 year old were immunized in Chipata District. The start of the trial was delayed due to the late arrival of essential supplies. The rains had started and a natural ECF challenge was present during most

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of the campaign. The overall turnout of calves was 78% and co-operation from the farmers was generally satisfactory. A certain suspicion about the whole exercise was discernible, and it was obvious that the immunization would be blamed for all fatalities afterwards, even those due to totally unrelated causes. In April and May 1988 a second campaign was undertaken in Chipata District, aimed at the calves over 2 months old. A total of 1100 calves were immunized during this campaign.

The veterinary assistants were asked to make regular follow-up visits to farmers, elicit reports from the farmers on sick and unthrifty animals, submit samples for laboratory diagnosis and provide extra treatment with parvaquone and/or tetracyclines where deemed necessary. Sera from 600 immunized animals were tested for antibodies to *T. parva* one to two months after the immunization to provide further information. Although it was practically impossible to maintain control animals throughout the area, a fairly accurate idea of the natural ECF challenge was obtained. A few farmers who had agreed to have their calves ear-tagged later refused to have them immunized. When they realized the benefits of the immunization, their calves were closely monitored for ECF incidence and treated where required. In other areas, co-operation of the farmers was very poor. Once again, a great deal of information was gathered on the incidence of ECF when they approached project staff for assistance with drugs. The information thus gathered indicated a normal incidence of ECF, with about 70–80% of the calves under 1 year old becoming infected and an estimated 30–40% of fatal cases.

Apart from logistic problems during the actual campaign, which were solved as they cropped up, the main problem was the evaluation of the results achieved. The consensus of the veterinary staff and the farmers was that the immunizations had drastically reduced the calf mortality in the area. It proved impossible, however, to obtain accurate information on the number of fatal cases directly attributable to the immunization and the number of fatal ECF cases in immunized animals. Cases requiring further treatment were most frequently encountered in herds where the general condition of the animals was poor (nutritional status of calves, worm burden, management). It therefore appears dangerous to extrapolate results from well-managed experimental animals directly to a field situation where numerous other factors interfere with the immunization programme.

Too few calves were ear-tagged and the recollections of farmers as to which animals had or had not been immunized were unreliable. Of all the fatalities reported and unequivocally substantiated as being due to ECF, three involved definitely immunized calves. Even in these cases it could not be determined whether the calves harboured an already active ECF infection at the time of immunization or whether their deaths were due to the immunizing infection. A total of about 150 calves received further treatment after the immunization, but

again it was not possible to determine which animals would have succumbed eventually to ECF and which would have survived without treatment. Therefore systematic record-keeping by the veterinary assistants should be established, the records to include herd size and composition and numbers of births, deaths (plus causes of deaths), sales, purchases and slaughters. This should be done for all farmers and villages involved in the programme and the record-keeping should be monitored regularly.

In one particular area (Chikando-Sairi) other tick-borne diseases were sporadically recorded in immunized calves. Of a total 800 immunized calves, 3 confirmed cases of babesiosis were reported and an estimated 45 calves subsequently died during an outbreak of sweating sickness in April 1988. The latter was confirmed clinically and *Hyalomma truncatum* adults were present in abnormally high numbers with an average of 10–15 per sick calf examined, compared to less than 1 per animal in previous years. This might have been the result of the unusually heavy rains during March and April, but was more likely due to the fact that farmers had stopped dipping their animals completely after the introduction of a dipping fee. These outbreaks emphasize the necessity of maintaining some form of strategic tick control, even when the incidence of ECF has been reduced or eliminated.

Finally, the question of financing the immunization programme remains. The total cost of such a programme includes the actual production cost of the stabilate, including the purchase of chemicals, and the cost of the drugs to be used during the campaign. Furthermore, transport and facilities and equipment needed for the immunization have to be budgeted for. Lastly, the veterinary assistants and their associates need vehicles (motorcycles and fuel or bicycles) to enable them to prepare for the campaign, assist researchers during the campaign and monitor the immunized calves afterwards, especially during the first years of an immunization programme, when most problems arise. The importance of this aspect was underlined in the second campaign, when a considerable number of farmers refused to present their calves for immunization because they feared they would be charged for the drugs in the same way that they were charged for dipping their animals.

An attempt was made in July and August 1988 to collect detailed information on the effect of the immunization campaign on the overall calf mortality in Chipata District. The results from the veterinary camps for which sufficient information was gathered are summarized in Table 1. This table indicates that a significant reduction in mortality (about 80%) occurred in the immunized calves, and the results confirm the general impressions of the veterinary assistants and the cattle owners.

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Table 1. Percentage mortality in immunized and non-immunized calves in Chipata District, Zambia

Veterinary camp	Total no. of calves	Percentage immunized	Mortality	
			No. immunized	No. non-immunized
Chiwale Zule	651	75	8	49
Mtenguleni	855	62	3	14
Mnoro	504	94	2	10
Msandile	164	76	0	14
Chiparamba	304	84	5	60*
Kalichero	377	83	3	Unknown
Kanyanja	706	37	18**	50

* Mortality of non-immunized calves: percentage of calf mortality in the sentinel herd between December 1982 and November 1986. (Nine calves were immunized in this herd; all are still alive.)

** The mortality figures have not yet been verified.

Immunization against theileriosis in the Southern Province of Zambia

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Tick-borne diseases, particularly theileriosis, pose a serious threat to the development of the cattle industry in Zambia. The first recorded case of theileriosis was in the Nakonde area of Northern Province in 1922. According to the annual reports of the Veterinary Department of Zambia, no cases of the disease were diagnosed within the country from 1928 to 1945. In 1946 theileriosis was diagnosed in Mbala District, Northern Province, and in 1947 in Chipata District, Eastern Province. Since 1947 theileriosis has spread within the Northern and Eastern provinces, throughout much of which it is now established enzootically. In 1977/78 a malignant form of theileriosis was detected in the Hufwa area of Monze District, in Southern Province. It is fairly certain that prior to this time Southern Province was free of the disease. The disease has since then become endemic in this region, which is an important cattle-raising area containing 900 000 head, which is approximately 45% of the national herd.

In Zambia theileriosis manifests itself in the form of Corridor disease, caused by *Theileria parva lawrencei*, and East Coast fever, caused by *T. p. parva*. Corridor disease appears mostly in Central, Lusaka and Southern provinces. East Coast fever is found in the Northern and Eastern provinces of the country, where high rainfall is common. The current cattle population of Zambia is 2.6 million, out of which 1 259 000 are at risk to theileriosis.

Despite concerted efforts by the government to control theileriosis, it is becoming obvious that control of this killer disease at economically accepted levels will be difficult to achieve. Considering the problems with current control methods—such as the rising costs of acaricides and antitheilerial drugs, the long distances to many dip-tanks, which discourage farmers from regular dipping, and resistance to acaricides by ticks—it became necessary to look for alternative methods of controlling theileriosis.

In view of the above the government of Zambia, with the help of the Food and Agriculture Organization of the United Nations, embarked on a theileriosis immunization programme in 1985 in the Southern Province. This project was designed to assess the following:

- a) the protection provided to groups of cattle immunized using the "Muguga cocktail" against laboratory challenge with three field isolates and exposure to natural field challenge

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- b) the "Muguga cocktail" protection for traditionally managed cattle against natural theileriosis challenge in Northeast Choma in the Southern Province, where the disease is endemic

The results of laboratory challenge and field exposure have been very successful. The (b) phase was equally successful and the data are being analysed. In this immunization programme a total of 1184 cattle 4–15 months old were ear-tagged and 950 of these were immunized by the infection-and-treatment method, 834 with the Muguga cocktail and 116 with *T. parva* (Mandali), a local isolate. Both groups were treated simultaneously with 20 mg/kg of long-acting oxytetracycline. Two hundred and thirty-four cattle served as ear-tagged controls in addition to the untagged non-immunized cattle in the area. The ear-tagged cattle were divided into two groups, group A consisting of 200 immunized and 200 control cattle and group B consisting of 750 immunized and 34 control cattle for convenience of monitoring. Group A cattle were monitored for haematocrit and serology, liveweight changes and tick control and survival; group B was monitored only for survival and serology. Forty-five cattle of the 834 Muguga cocktail immunes died between September 1986 and March 1988. Fifteen of the 45 died of theileriosis; this seems to have been due to either underdosing with long-acting oxytetracycline or overdosing with *Theileria* sporozoites. These cattle died in late February and early March 1987. Only one of the 116 *T. parva* (Mandali) immunized cattle died of theileriosis. From September 1986 to March 1988, 100 of the 234 ear-tagged control cattle died. Between December 1986 and March 1988, a total of 465 cattle died in the trial area. At least 92% of the post-mortem examinations confirmed theileriosis as the cause of death. An additional 286 cattle were treated for theileriosis during the same period from these herds.

The following problems were encountered in this project.

- a) The application of immunization was slowed down considerably by the lack of suitable crushpens. These delays lead to loss in viability of the stabilates, wastage of materials and uncertainty about the success of immunization.
- b) Accurately estimating the weights of animals was a problem in some cases and at times resulted in either underdosing or overdosing with the long-acting oxytetracyclines.
- c) Although farmers were advised to bring cattle for inspection on specific days after the immunization, several failed to do so.
- d) The immunization was organized so that farmers whose cattle had been ear-tagged on a previous occasion would bring the animals on a given day at a pre-arranged time. Unfortunately, on a number of occasions farmers

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did not gather all the animals to be immunized, causing a waste of time and stabilates.

- e) There was a great demand for immunization of adult animals, which caused difficulty: the design for this phase of the project deliberately discouraged immunizing adults because it was difficult to get proper histories of these animals and the project resources did not cater for these cattle.

These trials have demonstrated clearly that in the Southern Province, immunization against Corridor disease by infection and treatment using the Muguga cocktail and a local isolate, *T. parva* (Mandali), can be carried out successfully. Furthermore, immunization against theileriosis would mean dipping animals less frequently. We therefore believe that in tick-borne-disease endemic areas if cattle are immunized against theileriosis when they are young and relatively resistant to other tick-borne diseases, dipping intervals can be safely increased. This would allow limited tick-parasite challenge without the fear of lethal theileriosis breaking through. This is desirable as limited tick/parasite challenge also provides a biologically stable situation where immunity to ticks and tick-borne diseases is continually reinforced.

Immunization of cattle against *Theileria parva bovis* in Zimbabwe

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East Coast fever caused by *Theileria parva parva* was eradicated from Zimbabwe in the 1950s, but a less virulent form of disease, caused by *T. p. bovis* and known as January disease or Zimbabwe malignant theileriosis, has persisted. *Theileria p. bovis* is of considerable economic importance to Zimbabwe, not because of the number of cattle deaths which it causes (1000–2000 each year), but because of the cost of its control by intensive dipping. Immunization offers an alternate and probably more economic means of control. This paper reports an immunization trial that was carried out in Zimbabwe in the 1986/87 and 1987/88 rainy seasons, using local isolates of *T. p. bovis*.

METHODS

The trial was carried out at Willsbridge Farm, approximately 100 km north of Harare. The farm was selected because it had severe outbreaks of theileriosis in the two rainy seasons prior to the trial. In the 1985/86 season four of five susceptible “bait” cattle exposed on the farm died of theileriosis.

Cattle for immunization were obtained from a farm without a history of pathogenic theileriosis. All were Sussex (*Bos taurus*) weaners, approximately 14 months old. Immunization using tick-derived stabilates was carried out at the laboratory and the cattle were moved to the farm and exposed to natural challenge at the start of the first rainy season (November 1986). Fifteen animals were immunized with the Boleni stock (Lawrence and Mackenzie, 1980) and 15 with a mixture of three isolates from the Willsbridge Farm. There were 10 unimmunized controls. The stabilates were prepared at a concentration of 10 ticks per ml. Doses of 0.1 ml, which had been shown in laboratory titration experiments to cause mild but immunizing reactions in cattle, were used to infect the cattle. No chemoprophylactic treatment was given.

In the 1987/88 rainy season eight susceptible weaners were exposed at the

farm to compare their reactions to those of the cattle immunized and exposed in the previous year.

RESULTS

Severe reactions occurred in four of the Boleni- and two of the Willsbridge-infected animals during immunization and these were treated with parvaquone (Clexon, Coopers). One Boleni-infected animal died because treatment was administered too late in the course of the disease.

The maximum challenge with adults of *Rhipicephalus appendiculatus* in the 1986/87 season was approximately 100 per animal and occurred in late January. It was approximately 70 per weaner in the 1987/88 season, also in late January.

Reactions to *Theileria* infection are summarized in Table 1. In the 1986/87 season both the immunized and control groups became infected with a *Theileria* parasite between days 10 and 23 after initial exposure at the farm. The reactions were all mild and temperatures seldom rose above 39.5°C. A second episode of infection, in which only the control group was affected, occurred between days 25 and 51 after initial exposure. Seven out of 10 had severe reactions with high temperatures and two died of theileriosis. There were no theileriosis reactions in the immunized cattle introduced in 1986/87.

Table 1. Results of exposure of immunized and control cattle at Willsbridge Farm, Zimbabwe

Treatment	Number of animals	First infection		Second infection	
		Severe reactions	Deaths	Severe reactions	Deaths
1986/87					
Boleni stabilate	14	0	0	0	0
Willsbridge stabilate	15	0	0	0	0
Control	10	0	0	7	2
1987/88					
Control	8	4	1		

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In the 1987/88 season four of the eight introduced controls had mild reactions and the other four had severe reactions, and one of these latter died of theileriosis.

DISCUSSION

It was concluded that the first infection detected in the 1986/87 season, in which both the immunized and control groups were affected, was caused by a mild *Theileria* species, possibly *T. taurotragi*. The second infection, which resulted in severe and fatal reactions in the controls, was almost certainly caused by *T. p. bovis*.

The trial showed that both the Boleni and the Willsbridge stocks protected cattle against field challenge. In unpublished laboratory experiments it has also been shown that the two stocks are cross-protective and that the Boleni stock protects against three other pathogenic stocks from different parts of Zimbabwe. The prospects of a vaccine against *T. p. bovis* based on a single stock thus appear to be good.

An important problem in this trial was the severity of some of the reactions to immunization, in which chemotherapy was not used. Severe reactions had not occurred when the stabilates had been tested previously at the immunization dilution in *Bos taurus* cattle. Further work is required to establish a method that is safe in all circumstances.

ACKNOWLEDGEMENT

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The cattle-handling facilities of the East Coast Fever Vaccine Production and Quality Control Project at the Central Veterinary Laboratory, Lilongwe, Malawi. (Picture by Dr. J.G. Grootenhuus.)

PART 3

CONTRIBUTED PAPERS

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The attitude of government to regional centres and co-operation

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The Organization of African Unity/Scientific Technical and Research Commission (OAU/STRC) passed a resolution in September 1976 recommending that regional centres be set up for training and applied research on ticks and tick-borne diseases. These centres would cover aspects of tick control (dip management, acaricide chemistry, acaricide resistance testing, etc.) and tick-borne disease control (epizootiology, immunization against East Coast fever [ECF], anaplasmosis, babesiosis and heartwater). The OAU then mounted two missions to the countries of East and Central Africa to advise on the most suitable place for this regional centre. Following consultations between the OAU and the Food and Agricultural Organization of the United Nations (FAO), it was proposed that the Central Veterinary Laboratory (CVL), Lilongwe, be the site for the centre for East and Central Africa.

Since the passing of the above OAU/STRC resolution, the Malawi Government has strengthened the CVL so that the objectives of the resolution can be achieved. Project GCP/MLW/018/DEN established an infrastructure comprising a laboratory, tick-proof housing, a rabbit colony, spacious cattle accommodation and a farm for growing feed for experimental animals. The project then conducted cross-immunity experiments and field trials using the Muguga cocktail, a combination of *Theileria parva parva* (Muguga), *T. p. parva* (Kiambu 5) and *T. p. lawrencei* (Serengeti-transformed) stocks.

After the end of Project GCP/MLW/018/DEN in September 1985, field immunization continued with support from FAO through Projects TCP/MLW/4505 and TCP/MLW/6652. These immunizations were done on government and private farms. The work was also extended to southern Zambia. The immunization experiments have, among other things, established that the Muguga cocktail can confer an effective immunity against ECF in susceptible livestock and that the cocktail is safe to use.

With assistance from the Dutch Government, a two-year ECF Vaccine Production and Quality Control Project has recently been launched. This project will also be preparation for a five-year regional project of the Southern Africa Development Coordination Conference to be funded by the United Nations

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Development Programme. To address the problems caused by other tick-borne diseases, the Malawi Government has further sought and received assistance from the Danish Agency for International Development to produce *Anaplasma*, *Babesia* and heartwater vaccines for East and Central Africa. Work on this project is already under way.

FACTORS CRITICAL TO A REGIONAL SET-UP

With the developments cited above, the Malawi Government is hopeful and positive that CVL will soon be ready to assume operations on a regional basis. There are, however, a number of issues the co-operating countries in the region may wish to have clarified. These are:

- a) the proper handling of biologicals
- b) research clearance for foreign visitors
- c) information dissemination

Biologicals. In the context of the regional project, the term “biologicals” refers to ticks, stabilates, sera and antigens. Most governments have instituted legislation to prevent the introduction of animal diseases into their countries. For the same reason, the Office International des Epizooties (OIE) advises member countries to observe strictly import and export regulations. Accordingly, the Malawi Government prohibits the importation of biologicals without a valid import permit issued by the Chief Veterinary Officer.

Those governments that will deal with the regional centre should bear the following in mind.

- a) Stabilates for immunization will be screened for known animal and human tick-associated viral pathogens.
- b) Biologicals will be kept under lock and key and accurate records about them will be maintained.
- c) There will be very clear restrictions on movements of personnel from one section of the centre to another.
- d) Both the farm for experimental animals and the vaccine-production laboratories will be double-fenced.
- e) Each applicant wishing to export biologicals to the centre will be given specific instructions on how to package and label such parcels.

ATTITUDE OF GOVERNMENT

Some of these requirements are already met by CVL, but in the coming year construction of the new vaccine production laboratories will be completed and additional security measures will be provided. Participants at this meeting are therefore requested to discuss these requirements freely in the spirit of regional cooperation and point out any security measures to which they want the Malawi Government to pay special attention.

Research clearance for official visits. The Malawi Government requires that official international visitors be given clearance before they come to Malawi. To avoid unnecessary paperwork, most visitors who must enter Malawi often are given "block clearance" for a given period. Official visitors to the country must give the following information to receive research clearance: name, date of birth, nationality, passport number and place of issue, education and professional experience.

Information dissemination. The smooth operation of the regional centre will greatly depend on full exchange of information between member countries and interested organizations involved with research in ECF immunization. Furthermore, the Malawi Government requires scientists to send the Chief Veterinary Officer copies of all scientific reports, excluding routine monthly, quarterly or annual reports, to and from the centre. The publication of scientific findings in journals also needs government approval.

CONCLUSIONS

The following conclusions are drawn.

- a) ECF is a major constraint to the improvement of cattle production in East and Central Africa.
- b) An integrated approach to controlling tick-borne diseases and their vectors is needed and this should include immunizing livestock against the major tick-borne diseases.
- c) Since parasites cross geographical borders and are prevalent in many of our countries, the integrated approach can best be effected on a regional basis.
- d) The Malawi Government upholds the OAU/STRC decision to establish regional centres and strongly supports the choice of CVL, Lilongwe, as the tick and tick-borne disease centre for East and Central Africa.
- e) The Malawi Government is committed to strengthening the CVL so that the regional goals regarding tick-borne disease immunizations can be achieved.

The role of regional centres in control of tick-borne diseases and their vectors

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Four regional centres to control tick-borne diseases and their vectors have been proposed for Africa. It is believed that these regional centres will complement the work of the International Laboratory for Research on Animal Diseases (ILRAD), located in Nairobi, Kenya. ILRAD was established in 1973 with a mandate to develop effective control measures for livestock diseases that seriously limit world food production. Its research programme focuses on African animal trypanosomiasis and East Coast fever, a form of theileriosis. ILRAD is expected to liaise with and disseminate its findings to workers in the field who will apply those findings. Most ordinary laboratories are not equipped to conduct the basic research on which ILRAD concentrates.

At the same time, it is believed that expertise should not be concentrated in one laboratory, country or area of the region. The report of the third Food and Agriculture Organization of the United Nations expert consultation on research on tick-borne diseases and their vectors noted that it is neither possible nor desirable to concentrate all expertise at the proposed regional centres in Khartoum, Zaria, Dakar and Lilongwe. It was suggested that the expertise be spread over the countries in each region. These centres were and are still envisaged to embrace the whole spectrum of economically important tick-borne diseases and their vectors.

The proposed regional centres, to be based at already-existing laboratories, will train personnel and conduct applied research in controlling ticks and tick-borne diseases. The centres will cover various aspects of tick control—including dip management, acaricide chemistry and acaricide resistance testing—as well as tick-borne disease control, including epidemiology and use of the immunization-and-treatment method against the economically important diseases.

More specifically, the centres will concentrate on the following:

- a) production and good control of biologicals
- b) provision of biologicals
- c) training in routine techniques and applied research

- d) applied research
- e) liaison with governments in the region and national and international institutes on the use of facilities for training, the exchange of information and biological materials, and the formulation or modification of project proposals
- f) liaison with donors and executing agencies for programmes and projects

To illustrate the above, let us look at the ongoing East Coast Fever Vaccine Production and Quality Control Project in Malawi. This project pays special attention to the following:

- a) the production of good-quality stabilates for immunization
- b) the improvement of the infection-and-treatment method of immunization against East Coast fever, including standardizing the infectivity and dosage of stabilates and drugs, testing the efficacy of new drugs and formulations, determining the optimal age of cattle for immunization and determining the best methods of producing, storing and distributing stabilates and of preventing their contamination by pathogenic micro-organisms
- c) the isolation of breakthrough parasites and characterization of the isolates *in vitro* and *in vivo* in collaboration with other institutions, such as ILRAD
- d) assistance with bulk stabilate preparation and titration of confirmed breakthrough parasites for incorporation into national vaccination programmes
- e) assistance with training staff from countries in the ECF-affected region in field immunization and with monitoring immunized animals
- f) acting as a centre to give advice on immunization procedures, to help monitor ECF-immunized cattle in the region and to co-ordinate immunization against ECF, babesiosis, anaplasmosis and heartwater

It is believed that the regional centres will complement efforts being made by national and international laboratories towards developing effective control measures against tick-borne diseases and their vectors in the following ways: by producing and providing good-quality biologicals, by co-ordinating the distribution of the materials, by training personnel in control methods, by applying research results and by disseminating information.

ACKNOWLEDGEMENT

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Identification of *Theileria* species and characterization of *Theileria parva* stocks

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Many *Theileria* parasites cause diseases in cattle, of which one of the most economically important is East Coast fever (ECF), caused by *T. parva*. The parasite causes high morbidity and mortality in exotic cattle, thus inhibiting the introduction of improved cattle into endemic areas. The only immunization method available against ECF is the infection-and-treatment method (Radley, 1981), in which live parasites are used. For a rational approach to ECF immunization, it is necessary to isolate and characterize *T. parva* stocks from the field before selecting them for immunization. Most field isolates contain a mixture of *T. parva* strains and some contain two or more species of *Theileria*. Furthermore, the immunity engendered following ECF immunization is strain/stock specific. For these reasons, it is necessary to have precise methods for identifying not only different species of *Theileria*, but also different strains of *T. parva*. This paper discusses the various methods and criteria available to differentiate *Theileria* species and *T. parva* stocks. A more comprehensive review of the subject has been published by Irvin (1987).

DIFFERENTIATION OF *THEILERIA* SPECIES

Theileria parva, *T. annulata*, *T. taurotragi*, *T. mutans*, *T. velifera* and *T. orientalis* are the six *Theileria* species known to infect cattle. Various criteria and methods, described below, are used to identify these parasites. In most cases it is necessary to use a combination of these methods to identify a species definitively.

Geographic distribution

The distribution of the two most important cattle species, *T. parva* and *T. annulata*, correlates well with the distribution of their vectors. *Theileria parva* is transmitted predominantly by the brown ear tick, *Rhipicephalus appendiculatus*, which is restricted to eastern, central and southern Africa; *T. annulata* is transmitted by several *Hyalomma* species, which are distributed widely over North

Africa, southern Europe, the Middle East, India, southern Russia and China. There is virtually no overlap of these vectors and therefore the possibility of confusing these two parasites is low.

Vector specificity

In many parts of Africa where more than one *Theileria* species occurs, the vector specificity can be used for differentiation. For example, *T. mutans*, *T. velifera* and *T. orientalis* are widely distributed over Africa and are transmitted by ticks of the genus *Amblyomma*. This group of parasites can be distinguished from *T. parva* and *T. taurotragi*, which are transmitted predominantly by *R. appendiculatus*. *Theileria taurotragi* can also be transmitted by *R. pulchellus*.

Morphology

It is usually difficult to differentiate *Theileria* species solely by examining the morphology of the parasites. Parasites of different species look alike in most piroplasm and schizont stages. Two exceptions to this are *T. velifera*, which has a characteristic veil associated with the piroplasm, and *T. taurotragi*, which has bar-like structures in infected erythrocytes. However, confusion may arise if mixed infections occur. The *T. mutans* schizonts, which occur transiently and in low numbers, have a distinct morphology. They are larger and more diffuse than schizonts of other *Theileria* species and for this reason can be easily differentiated.

Host specificity

It is often difficult to differentiate *T. parva* from *T. taurotragi* because both species are transmitted by the same vector and mixed infections of these parasites occur in cattle. These parasites differ, however, in the range of other mammalian hosts they infect. *Theileria taurotragi* infects eland and sheep (A.S. Young, personal communication), and *T. parva* infects buffalo. *Theileria taurotragi* is also known to infect cells from a variety of ungulate species *in vitro* (Stagg et al., 1983), and therefore *in vitro* infectivity of sporozoites may be useful in differentiating these two species.

Pathogenicity

The virulence of different species of *Theileria* may vary a great deal depending on the strain of parasite, degree of host susceptibility and dose of parasite. *Theileria mutans* is generally known to be benign, although virulent strains have been reported from South Africa (Flanagan and Le Roux, 1957). *Theileria mutans* undergoes limited lymphocytic merogony; its main mode of replication occurs in erythrocytes and in virulent strains this causes a high piroplasm parasitaemia and haemolytic anaemia in the host. In contrast, *T. parva* replicates mainly in lymphocytes and the pathology it causes is associated with destruction of the lymphocytes. Erythrocytic merogony is limited and haemolytic anaemia is

not present. *Theileria annulata* replicates in both lymphocytes and erythrocytes, thus causing disease with severe lymphocytopenia, anaemia and occasionally jaundice. *Theileria taurotragi*, which undergoes lymphocytic and erythrocytic merogony, can be pathogenic in its eland host but is benign in cattle.

Cross-immunity

There is no evidence of cross-species protection among any of the cattle parasites. For example, cattle immunized against *T. annulata* are fully susceptible to *T. parva* and vice versa (Sergent et al., 1945; Neitz, 1957). The cross-immunity test is particularly useful in a situation where *T. taurotragi* and *T. parva* occur together. The lack of cross-protection among *Theileria* species contrasts with the variable degree of protection observed among different immunological stocks of *T. parva* (see "Cross-Immunity" under "Stock Characterization of *T. parva*" below).

Serology

The routine serological test used to diagnose *Theileria* species is the indirect fluorescent antibody test. Using this test, cross-reactions have been observed among *T. parva*, *T. mutans*, *T. annulata* and *T. taurotragi* (BurrIDGE et al., 1974; Grootenhuis et al., 1979). Under experimental conditions and using appropriate controls, the test can be useful in identifying these *Theileria* species. Its usefulness in the field, however, is limited, especially in large areas of eastern, central and southern Africa where *T. parva*, *T. taurotragi* and *T. mutans* occur together.

DNA probes

Conrad et al. (1987a) and Allsopp and Allsopp (1988) have shown that repetitive DNA sequences from *T. parva* stocks, used as radio-labelled probes, hybridize specifically to *T. parva* parasite DNA but not to *T. mutans*, *T. annulata* and *T. taurotragi* DNA. To date, only *T. parva* DNA specific probes are available; probes against other *Theileria* species are urgently needed. This is a powerful and a sensitive technique and may play a significant role in identifying mixed theilerial infections, which occur commonly in cattle exposed to natural tick challenge. The DNA probes can also be used to identify mixed infections in ticks, such as *T. parva* and *T. taurotragi* in *R. appendiculatus*.

Restriction fragment length polymorphisms

Restriction enzymes cleave DNA at specific nucleotide sequences. The DNA fragments thus produced can be resolved on agarose gel by electrophoresis and visualized under ultra-violet illumination following ethidium bromide staining. Using restriction enzymes such as *Sfi* I and *Not* I, which cut *Theileria* DNA infrequently, and separating the digested DNA in pulsed-field gel electrophoresis, unique and characteristic banding patterns of *T. parva* and *T. mutans* have been detected (Morzaria, 1988). This restriction fragment length polymorphism is a

simple way of differentiating *Theileria* species. Comparative DNA profiles of other important parasites, such as *T. annulata* and *T. taurotragi*, have not been studied in this system.

STOCK CHARACTERIZATION OF *THEILERIA PARVA*

With the development of the infection-and-treatment method of immunization and the realization that different immunological strains existed in *T. parva* (Radley, 1981), precise characterization of *T. parva* stocks became necessary. In the last decade several tests have been developed to characterize stocks of *T. parva*; these are described below.

Cross-immunity

Cross-immunity was the first test developed to characterize immunological types of *T. parva*. The test involves immunizing cattle with a stock of *T. parva* using the infection-and-treatment method and challenging the immune animals with a different stock. The breakthrough stocks are classified as immunologically distinct. This test has great value because it enables the identification of a parasite stock or stocks that will provide wide immunity. Both the "Muguga cocktail" and the "Marikebuni" stocks were essentially selected on the basis of the cross-immunity tests (Radley, 1981; Morzaria et al., 1987).

A feature of the cross-immunity between two immunologically distinct stocks is that protection is frequently partial. Usually 30–40% of the immune animals are not protected when challenged with a heterologous stock. This is in sharp contrast to the results of a cross-immunity test using different species of *Theileria*, when no protection is observed. To obtain meaningful results from cross-immunity tests for stock characterization, many animals must be used and thus the test is expensive.

Infectivity

Infectivity is a routine test used in the characterization of *T. parva* stocks. Susceptible cattle are infected with a standard dose of sporozoites and clinical and parasitological parameters—such as the length of prepatent period, the time to fever, the duration of parasitosis and fever, the time to first appearance of piroplasms and the time to death or recovery—are measured. In addition, the schizont parasitosis and piroplasm parasitaemia are estimated. These parameters define the infectivity of a particular stock for a particular breed and age of animal. For example, *T. parva* (Mariakani) and *T. parva* (Marikebuni) stocks, isolated from the Kenya coast, produce unique clinical characteristics in susceptible cattle. The Marikebuni stock usually produces a prepatent period of 8 days and death by day 17. In contrast, the Mariakani stock usually produces a prolonged

clinical reaction with a prepatent period of 9 days and death between days 21 and 25. The fever almost invariably occurs biphasically.

Susceptibility to drugs

Drug sensitivity is also a routine test in which groups of cattle, usually highly susceptible *Bos taurus* breeds, are immunized by the infection-and-treatment method: the cattle are infected with a lethal dose of sporozoite stabilate and treated with one injection of long-acting oxytetracycline. With certain parasite stocks, however, one dose of this drug formulation does not control the infection and either a diluted stabilate dose is used with the same dose of drug or two doses of drug are used with the lethal dose. This test gives additional information on the suitability of a particular stock being used for immunization.

In recent years, buparvaquone (Butalex, Coopers Animal Health) has been found to be effective in immunization against ECF. The drug is administered at 2.5 mg/kg simultaneously with the sporozoite challenge and is useful in immunization where a particular *T. parva* stock cannot be controlled with one dose of a long-acting tetracycline.

Field trials

Depending on the results of the *in vivo* tests, parasites can be selected as putative immunizing stocks and tested further in pilot immunization trials. For example, the *T. parva* (Marikebuni) stock was found to show wide protection in cross-immunity tests and was subsequently used in immunization trials in the Coast Province, Kenya. The results of the field trials substantiated laboratory studies on its ability to provide wide protection (Morzaria et al., 1987; Mutugi et al., in press).

In vitro infectivity

Several *in vitro* tests are routinely used to characterize *T. parva*. After preparing a sporozoite stabilate of a field isolate, *in vivo* and *in vitro* characterizations are performed, usually simultaneously. The test involves infecting a panel of previously characterized susceptible cloned and uncloned cells from cattle and other ungulates with a sporozoite stabilate. The cell lines obtained are a valuable source for amplification of parasite material for further *in vitro* characterization.

Monoclonal antibody profiles

Several *T. parva* antischizont monoclonal antibodies (MAb) have been generated and used in an indirect fluorescent antibody (IFA) test against schizont-infected cells derived from *in vitro* cultures to demonstrate stock-specific diversity. Minami et al. (1983) showed that the presence or absence of binding to MAbs 2 and 3 and to 15 and 16 was a convenient way of dividing *T. p. parva* stocks into three groups. However, extensive studies with more schizont MAbs and several other *T. parva* stocks have revealed that the diversity is much greater than was originally thought. Most *T. p. lawrencei* stocks react with MAb

19, which does not react with *T. p. parva*, and most of the *T. p. bovis* stocks isolated from Zimbabwe do not react with MAb 7.

Minami et al. (1983) showed that the percentage of schizont-infected cells reacting with a particular MAb agrees closely with the percentage of infected cells as identified by Giemsa staining of the antigen slide. However, Conrad et al. (1987b) found that with many *T. p. lawrencei* isolates the percentage of infected cells reacting to certain MAbs was often lower than the percentage of schizont-infected cells. This was subsequently shown to be due to heterogeneity in the stocks studied. Similar findings have also been observed in *T. p. parva* isolates (P.R. Spooner, personal communication).

Irvin et al. (1983) showed in limited cross-immunity tests that if cattle were immunized with a *T. parva* stock and challenged with another parasite stock with an identical MAb profile, good cross-protection was recorded. If the cattle were challenged with a stock of a different MAb profile, the protection was variable. To make a general statement with regard to the relationship between the *in vitro* and *in vivo* tests, more extensive cross-immunity tests need to be performed because it is clear that the MAbs recognize schizont-specific antigens but not necessarily those antigens that may be involved in immunity.

At present monoclonal antibody profiles demonstrate antigenic diversity and are a useful way of characterizing *T. parva* stocks. The characteristic profiles of parasite stocks provide a useful laboratory check where contamination with other parasites is suspected. However, it is important to recognize that the test is performed on parasites that have been cultured *in vitro* and passaged several times. Parasite selection may occur during isolation and *in vitro* maintenance. The MAb profile obtained thus relates to the cultured parasite and may not reflect the true characteristics of the original stock.

Protein analysis

The monoclonal antibody test is essentially a qualitative test. The more precise nature of parasite antigens can be determined by probing western blots of the relevant antigens with the panel of MAbs. Shapiro et al. (1987) showed that MAb 5 identifies a polymorphic schizont antigen in *T. parva* stocks. Certain antischizont MAbs may therefore provide stock-specific markers. This area needs further investigation.

Two-dimensional gel electrophoresis has been used to characterize infection-specific proteins of various *T. parva* stocks (Sugimoto et al., in press). Stock-specific differences have been detected but the technique is difficult to perform and often difficult to interpret. It remains a useful laboratory technique for stock identification.

DNA analysis

Most of the characterization methods described above recognize phenotypic characteristics of *T. parva*. With the advent of recombinant DNA technology,

several workers have attempted to identify polymorphisms in *T. parva* stocks at the DNA level. Conrad et al. (1987a) and Allsopp and Allsopp (1988) have developed DNA probes that differentiate certain *T. parva* stocks. Polymorphism in *T. parva* has also been detected using rare cutter restriction enzymes such as *Sfi* I and *Not* I and by separating DNA fragments on modified pulsed-field gel electrophoresis systems (Morzaria, 1988). Characteristic and unique DNA banding patterns have been detected in several *T. parva* stocks, thus enabling workers to differentiate stocks.

SUMMARY

To conduct epidemiological studies and plan rational immunization programmes against ECF, *Theileria* parasites must be precisely identified and characterized. Several criteria and methods are available to achieve this and with the advent of new biochemical and molecular biological techniques, the task of identifying *Theileria* species is becoming easier. These new techniques are being used increasingly as routine tests to characterize *T. parva* stocks.

A recent development in the field of molecular biology is the polymerase chain reaction technique, which allows amplification of very small (picogram) quantities of DNA to a detectable level. This technique is now being used routinely in diagnostic research, especially to diagnose acquired immune deficiency syndrome (AIDS) in carriers that cannot be easily detected by conventional means. In future the polymerase chain reaction technique will be an important tool in studies of the epidemiology of theileriasis because it can be used to detect *Theileria* carrier animals and it may enable diagnosis at species and strain level.

One of the main reasons for developing *in vitro* characterization tests is to identify markers that will correlate with immunity *in vivo*. To date, none of the modern techniques used fulfil this requirement. However, biochemical and genetic characterization of *T. parva* provides an understanding of the parasite at the molecular level, thus enhancing our knowledge of the basic biology of the parasite. At present, characterization of *T. parva* is being performed on stocks; the aim should be to characterize cloned *T. parva* parasites to define the nature of polymorphisms more accurately. Research should also continue to develop an *in vitro* test to identify the antigenic nature of parasite isolates and stocks. Such a test would simplify the establishment of immunization programmes by doing away with expensive and time-consuming *in vivo* cross-immunity tests.

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IDENTIFICATION OF *THEILERIA* SPECIES

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Tick control in relation to the epidemiology of theileriosis

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Tick control is one of the most important factors influencing the epidemiology of bovine theileriosis in eastern and southern Africa. It was largely through tick control by acaricides that East Coast fever (ECF), caused by *Theileria parva parva*, was eradicated from South Africa, Swaziland, Mozambique and Zimbabwe in the first half of this century. It was the only method of control of the disease in East and Central Africa until the 1970s, when the infection-and-treatment method of immunization was developed. Because of its effectiveness, acaricide control of ticks has been widely adopted for the control of tick-borne diseases and is still the most commonly used control method for the *T. parva* group of diseases. Its effect on the epidemiology of the diseases thus requires examination, particularly in the light of new information on the epidemiology and control of the major vector, *Rhipicephalus appendiculatus*. This paper considers production losses caused by *R. appendiculatus*, the seasonal occurrence of the tick, age-related resistance to *T. parva* group parasites, nymphal transmission of the parasites, resistance to the parasites and their vector in different breeds of cattle, tick control and the role of immunization. There is some bias towards southern Africa because a large amount of research has been carried out in this region in recent years and the situation in East Africa has already been widely reported and discussed.

PRODUCTION LOSSES CAUSED BY *RHIPICEPHALUS APPENDICULATUS*

The rapidly rising costs of tick control make it increasingly important to consider the economics of strategies for the control of ticks and tick-borne diseases. An important economic factor is the effect of ticks *per se* on cattle productivity, particularly where the diseases are controlled by immunization. In these situations the cost of tick control can be weighed against the benefit of increased productivity.

Norval et al. (1988) studied the effects of tick infestation on the growth of Sanga and *Bos taurus* cattle in Zimbabwe. Groups of young cattle were infested with high, moderate and low numbers of larvae, nymphs and adult *R.*

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appendiculatus. The numbers of each stage completing feeding and the liveweight gain (LWG) of the cattle were recorded. Larvae and nymphs had no significant effect on LWG, but each adult female that completed feeding caused a loss of approximately 4 gm. *Bos taurus* cattle had a low resistance to the tick and consequently suffered large losses from adult infestations. The losses in Sanga cattle, which were very resistant to the tick, were insignificant.

The effect of adult *R. appendiculatus* on milk production in Sanga cows was small but statistically significant (Norval et al., in preparation). In a more recent experiment by the same workers, it was found that cows of higher producing breeds were generally less resistant to the tick and the effect on milk production appeared to be greater, but statistical analysis of the data has not been completed.

SEASONAL OCCURRENCE OF *RHIPICEPHALUS APPENDICULATUS*

The pattern of seasonal occurrence of *R. appendiculatus* is determined by climate (Short and Norval, 1981; Rechav, 1982; Floyd et al., 1987a). The seasonal cycle is determined by the adults, which are only active under warm, wet conditions when the photophase (day length) exceeds approximately 11 hours. This means that in locations near the equator, such as Entebbe, Uganda, adults can be active throughout the year if there is no prolonged dry season. As a consequence, larvae and nymphs will also be continuously present and the tick will probably pass through two or more generations each year. If there are two wet seasons, as in the highlands of Kenya, there will be two periods of adult activity and probably two generations each year. Further south, where the seasons are more clearly defined and rain falls only in the summer, there is only one generation each year. Floyd et al. (1987a) have shown that the T3HOST population model, which is climate driven, can be used to predict patterns of seasonal occurrence.

AGE-RELATED RESISTANCE

It is well established that young cattle have an age-related resistance to most tick-borne protozoan and rickettsial diseases. In Zimbabwe, where the ages of cattle that died from *T. p. bovis* infection were recorded from 161 outbreaks in Mashonaland-West Province over an eight-year period, significantly less mortality occurred in calves than in adults or weaners (Koch et al., in preparation). However, in a laboratory experiment the same authors found that age-related resistance was only of short duration (approximately one month), which does not correspond with field observations. The calves used in the experiment were

from non-immune dams and it was concluded that maternal factors are probably of importance in the protection of calves, as indicated by the work of Barnett and Bailey (1955) with *T. p. parva* in Kenya. In a more recent study, on Rusinga Island, in Lake Victoria, antibodies to sporozoites, schizonts and piroplasms have been recorded in the colostrum of immune cows and the serum of their calves (S.P. Morzaria, A.A. Latif and P.B. Capstick, personal communication). In this study, as in that of Barnett and Bailey (1955), it was shown that the majority of calves of indigenous breeds born to immune dams in an enzootic area recover from challenge with East Coast fever and become immune. Those from non-immune dams usually die.

In contrast to the findings with *T. p. bovis*, Barnett and Bailey (1955) and Irvin et al. (unpublished results) found that one-month-old calves from non-immune dams were more susceptible to *T. p. parva* infection than older calves. The reasons for this are not known.

An important factor to consider when interpreting the laboratory experiments on age-related resistance is that the calves were infected by injection of stabilate. This may have resulted in a higher dose of parasites than would normally be experienced in the field, where infection rates in ticks are usually very low (Morzaria and Young, 1987). Young calves also carry far fewer adult *R. appendiculatus* than older cattle (Barnett and Bailey, 1955, and Norval and Colborne, unpublished observations) and must therefore be exposed to lower numbers of *Theileria* parasites. This may be part of the reason for the milder reactions in this age group under field conditions.

In contrast to indigenous breeds, members of all age classes of *Bos taurus* cattle, including calves, usually die on initial exposure to *T. p. parva* infection.

NYMPHAL TRANSMISSION

Nymphs of *R. appendiculatus* transmit *T. p. parva* and play a role in the epidemiology of the disease (Barnett and Bailey, 1955; Neitz, 1956; Purnell et al., 1971). With *T. p. bovis*, however, Matson and Hill (1967) and Lawrence et al. (1983) reported that they had been unable to achieve transmission using nymphs. These findings are supported by the field data of Norval et al. (1985), who reported that of 190 recorded outbreaks of *T. p. bovis* in Zimbabwe in 1981 and 1982, 94.2% occurred from January to March, when adults of *R. appendiculatus* are active and few nymphs are present.

More recently Koch et al. (in preparation) have found that *T. p. bovis* can be transmitted to cattle by large numbers of nymphs of *R. appendiculatus* fed as larvae on reacting animals. The reactions caused were severe or fatal, but the authors were unable to induce mild or unapparent reactions using low numbers of infected nymphs. In field studies on commercial farms in Zimbabwe, however, the authors detected increases in serological titres to *T. p. bovis* schizont antigen late in the dry season, suggesting that nymphs were transmitting subclinical in-

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fection. The implication is that nymphs may play a role in the epidemiology of *T. p. bovis* by transmitting mild but immunizing infections before the onset of the more severe adult-transmitted challenge after the start of the rains.

RESISTANCE TO TICKS AND TICK-BORNE DISEASES

That indigenous Zebu and Sanga cattle are more resistant to ticks (Bonsma, 1944 and 1981; Barnett and Bailey, 1955; Rechav and Zeederberg, 1986; Norval et al., 1988; Spickett, in press), East Coast fever (Barnett and Bailey, 1955; Barnett, 1957; Guilbride and Opwata, 1963; Wilde, 1967; Dolan and McHardy, 1976; Moll et al., 1984, 1986) and other tick-borne diseases, such as babesiosis (anonymous, 1984) and heartwater (Van de Merwe, 1979), has been inadequately exploited in control programmes. In indigenous cattle little or no tick control is required to increase productivity, and mortality due to tick-borne diseases is usually low or insignificant because of enzootic stability. In overgrazed areas, where the suitability of the environment for tick survival is low, *R. appendiculatus* is frequently absent (Yeoman, 1967; Norval, 1977), and the numbers of ticks of other species on indigenous cattle are usually very low (Barnett and Bailey, 1955; Kaiser et al., 1982). In spite of this it is still common in several African countries to subject indigenous cattle to intensive tick control. The practice is uneconomic because increased productivity is minimal, and it is epidemiologically unsound because reduced tick challenge can adversely affect enzootic stability. Pegram and Chizyuka (1987) reported that in Zambia acaricide treatment of Sanga cattle was economically justified only to control occasional heavy infestations of adults of *Amblyomma variegatum*. They recommended the strategic use of acaricides. Floyd et al. (1987b) have shown how computer models can be used to identify the most cost-effective control strategies for different environments and breeds of cattle. Loss of enzootic stability can result in high mortality in adult indigenous cattle if control measures fail (Norval, 1979 and 1981).

As long as *Bos taurus* cattle are kept in areas in which *R. appendiculatus* and the *T. parva* group of diseases occur, the animals will have to be treated regularly with acaricide to control the tick to ensure high productivity. To guarantee their survival, the cattle will have to be immunized against tick-borne diseases.

TICK CONTROL

The aims of any tick control programme should be carefully defined. These are usually to control tick-borne diseases and to increase productivity or to prevent the formation of lesions that can become secondarily infested with screwworm fly (*Chrysomya bezziana*) larvae. Governments and farmers often fail to define

the aims of control programmes and lack an adequate understanding of the epidemiology of the tick-borne diseases that occur in their areas. The result is that many programmes are uneconomic or destabilizing, as explained in the previous section.

Arsenical acaricides were used for at least 50 years in most areas before tick resistance became a problem. Subsequently, organochlorine, organophosphate, carbamate, amidine and synthetic pyrethroid acaricides have been introduced, in roughly that order, to most countries in the region. Tick resistance to organochlorines is now widespread and these compounds have largely been phased out. Organophosphates are currently the most widely used acaricides, but problems with tick resistance are increasing and so their use is likely to decline in the future. The amidines and synthetic pyrethroids are becoming more widely used and have a much longer residual effect than the other acaricide groups but are considerably more expensive. A potential problem with the pyrethroids is cross-resistance between them and the organochlorines; evidence of this has already been reported in *Boophilus decoloratus* in South Africa (Coetzee et al., 1987).

Acaricides are most commonly applied by dipping or spraying, dipping being considered the more effective means of application. In recent years several other methods of acaricide use have been tested, including the slow release of systemic acaricides from implants and boluses; the slow release of conventional acaricides from impregnated ear-tags; "pour-ons", which are applied on the backs of livestock and spread rapidly over the entire body surface; and "spot-ons", which are similar to pour-ons but have less capacity to spread. Neither systemic acaricides nor acaricide impregnated ear-tags have been marketed in Africa. The pour-on and spot-on formulations, which contain synthetic pyrethroids, are now available in some countries and their use is increasing. The advantages of these formulations are ease of application (no physical structures or capital investment are required) and long residual effect.

A recent advance of potentially great importance has been the production, using biotechnology, of an effective vaccine against *B. microplus* (Willadsen and Kemp, 1988). The immunizing agent is a "concealed" tick antigen, an antigen not normally encountered by the host. The immune mechanism it stimulates is different from that stimulated by exposure to feeding ticks. The antigen was derived from a crude extract of partially engorged adult female ticks. It stimulates the production of an antibody that damages tick gut cells and kills the ticks or drastically reduces their reproductive potential. It is likely that similar vaccines will be developed in the future against African tick species of major economic importance (*R. appendiculatus*, *A. hebraeum*, *A. variegatum* and *B. decoloratus*). These vaccines could render the other forms of tick control obsolete and completely alter our approaches to the control of ticks and tick-borne diseases.

EFFECT OF TEMPERATURE ON MATURATION OF SPOROZOITES IN TICK SALIVARY GLANDS

Young et al. (1979, 1984 and 1987) and Ochanda et al. (1988) have shown that exposure of adult *R. appendiculatus* to high temperatures (26 °C and 37 °C) prior to feeding stimulates the maturation of *T. p. parva* parasites in the salivary glands to mature sporozoites. The authors concluded that adult ticks exposed to high temperatures in the field would transmit infection to cattle more rapidly than would otherwise occur. This could reduce the efficiency of acaricide control of East Coast fever and should be considered in the design of control strategies and the selection of acaricides in the hotter areas in which the disease occurs.

DISCUSSION

Immunization offers the best means of protection of *Bos taurus* cattle against theileriosis and other tick-borne diseases. It can be used on indigenous breeds to create stability in enzootically unstable situations created by intensive dipping. Once cattle are immune, tick control can be relaxed and further use of vaccines may be unnecessary because the animals will be immunized against tick-borne diseases by exposure to infected ticks.

A factor contributing to the greater resistance to tick-borne diseases in indigenous breeds is their greater tick resistance. Barnett and Bailey (1955) reported that the recovery rate in calves from immune dams decreased significantly if the numbers of *T. p. parva* infected ticks fed on them were increased. Fivaz et al. (1984) and Leitch (1989) have shown that cattle with a high resistance to *R. appendiculatus* become less severely infected with *T. p. bovis* and *T. p. parva* than tick-naïve cattle, which become heavily infested.

The modelling approach has indicated that the most effective control strategies for *R. appendiculatus* are those directed against the adult stage (Floyd et al., 1987b). These strategies would also reduce the severity of challenge with the *T. parva* group of diseases, because adults are the most important vectors.

Irvin et al. (unpublished observations) have shown that Boran calves can be safely immunized against *T. p. parva* by infection and treatment between 2 and 16 weeks old. There are several advantages to immunizing cattle when young. The animals carry low numbers of ticks and so the risk of acquiring a fatal infection from ticks is low. Calves born to immune dams appear to have some protection from severe reactions due to maternal antibodies and there is evidence of an age-related resistance. Calves are easier to handle than older cattle and the amount of drug required to treat the reaction is low, which minimizes the cost of immunization. Immunizing cattle when they are young also gives greater flexibility in tick control and, in the absence of vaccines for other tick-borne

diseases, exposes calves to infection by ticks while they are still protected by age-related resistance.

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The importance of the carrier state of *Theileria parva* in the epidemiology of theileriosis and its control by immunization

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A full understanding of the carrier state of theilerial parasites in their mammalian hosts is central for a description of the epidemiology of the disease and for assessing the implications of immunization with live parasites. The carrier state of *Theileria* has been defined as the ability of an infected and recovered host to infect ticks which are then able to transmit the parasite to susceptible animals (Levine, 1973; Schwabe et al., 1977; Young et al., 1986). It is possible for an infected host to develop a primary parasitosis and parasitaemia that are eventually cleared, leaving the host with a sterile immunity. However, it is recognized that in some *Theileria*-infected animals, infection persists after recovery and is probably maintained by two phases of the life cycle: first, by the slow proliferation of macroschizont-infected lymphocytes, some of which develop into microschantons and give rise to merozoites infective to erythrocytes, and, second, by the regular division of intra-erythrocyte piroplasms.

Gametocytes of *Theileria*, which develop in the erythrocytes, are believed to be the stage infective for the feeding tick (Gonder, 1911; Mehlhorn and Schein, 1984). Thus the presence or absence of circulating gametocytes determines whether an animal is a carrier at a given time. It is, therefore, possible for an animal with a persistent infection to act either as an initial carrier, a sporadic carrier or a continual carrier, depending on whether the gametocytes infective to ticks are present initially and then lost, or are present intermittently or continuously. Of major importance to the epidemiology of theileriosis is when ticks that became infected by feeding on a carrier have the potential to transmit the parasite to a new host.

There has been no problem in understanding how *T. mutans*, *T. taurotragi*, *T. velifera*, *T. annulata* and *T. orientalis* maintain their carrier state, since both division in erythrocytes and long-term persistence of the piroplasms within the erythrocytes have been demonstrated (Conrad, 1983; Conrad et al., 1985); it is possible for cattle infected with these parasites to maintain their carrier state for several years (Young, 1981). However, the carrier state of *T. parva* in cattle has

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been a subject of much controversy and is complicated by the presence of a number of sub-species of *T. parva*. Immunity to East Coast fever in cattle (*T. parva parva* infection) has until recently generally been considered to be sterile (Du Toit, 1931; Mettam and Carmichael, 1936; Henning, 1956; Neitz, 1957; Barnett, 1968), although both Bevan (1924) and Wilde (1967) considered the evidence to be inconclusive. Although the carrier state of *T. p. lawrencei* has been known to exist in naturally recovered African buffalo and cattle for long periods (Barnett and Brocklesby, 1966), a similar state for *T. p. parva* infection in cattle was only demonstrated relatively recently (Young et al., 1981).

Table 1. Maintenance of persistent infections of theilerial parasites in mammalian hosts

Theilerial species	Long term division		Blood transmission
	Schizont	Piroplasm	
<i>T. mutans</i>	-	+	+
<i>T. taurotragi</i>	+	+	+
<i>T. velifera</i>	-	+	+
<i>T. p. parva</i>	+	-	-
<i>T. p. lawrencei</i>	+	-	-
<i>T. p. bovis</i>	+	-	-
<i>T. sergenti</i>	-	+	+
<i>T. orientalis</i>	-	+	+
<i>T. annulata</i>	+	+	+

The nature and efficiency of the carrier state in cattle of both *T. p. parva* and buffalo-derived *T. p. lawrencei* have important implications for the epidemiology of the diseases these parasites cause. For example, a carrier buffalo infected with *T. p. lawrencei* could be one hundred times more efficient in producing infected ticks than a group of carrier cattle (Young and Grootenhuis, 1985). Acaricide application to such cattle would increase the relative importance of the buffalo in maintaining the disease in an area.

In the South Nyanza District, Kenya, which is a theileriosis endemic area, Young et al. (1986) found that the carrier state of *T. p. parva* approached 100% in adult cattle. Here it was likely that carrier cattle played a greater role in the maintenance of infection than infected ticks, a complete reversal of the epidemiological concepts held for *T. p. parva* infection. This situation has since been shown to be common in the field in Kenya, and a high proportion of *T. p. parva* carriers have been found in other areas of Kenya, such as Uasin Gishu and

Kajiado districts (D.P. Kariuki, personal communication). *Theileria parva* is unusual among theilerial species in that the persistence of infection appears to be in the schizont stage rather than by division of the piroplasm stage or both, as is the case with other species.

An important question is why it took so long for the widespread carrier state of *T. p. parva* to be recognized in the field, while the carrier state of *T. p. lawrencei* and *T. p. bovis* has been recognized for such a long time. In Kenya it appears that too much emphasis has been placed on the results of studies of a laboratory stock, *T. p. parva* (Muguga), one of the few stocks not shown to produce a carrier state (Barnett, 1968). However, it is a fact that in South Africa and Zimbabwe, *T. p. parva* infection has been eradicated by means that certainly would not have eradicated the infection in Kenya. Since the parasite was introduced into South Africa by cattle imported from East Africa, it is possible that by chance the introduced stocks did not produce the carrier state. Alternatively, stocks that did not produce the carrier state may have been selected in a fully susceptible cattle population. It is of considerable interest that in Zimbabwe it has not been possible to eradicate *T. p. bovis*, which has a well-developed carrier state.

An increase in the prevalence of the carrier state of *T. parva* has undoubtedly occurred since the introduction of the curative drugs parvaquone (Clexon, Wellcome) and halofuginone (Terit, Hoechst); Dolan (1986a and 1986b) demonstrated a high prevalence of the carrier state after treatment with these drugs. Mutugi et al. (1988a) have indicated that buparvaquone (Butalex, Coopers Animal Health) treatment used for immunization could reduce the incidence of the carrier state of *T. p. lawrencei* in cattle.

In recent studies, a start has been made to characterize the parasites inducing the carrier state of *T. p. parva* in buffalo and cattle. The carrier state of *T. p. lawrencei* has been characterized by Grootenhuis et al. (1987 and 1987b) and Conrad et al. (1987 and 1989). Conrad et al. (1987) using monoclonal antibodies showed that one cell culture isolate from a carrier buffalo contained at least 5 antigenically different *T. p. lawrencei* schizonts, which demonstrated the antigenic diversity possible in carrier buffalo. It is possible that in the future buffalo-derived *T. p. lawrencei* will be used to immunize cattle in the field. It is important to determine how the introduction of this parasite into an area could influence the epidemiology of theileriosis, given that it can induce a carrier state in immune cattle (Mutugi et al., 1988b) and could result in the production of new antigenic types.

Studies were performed to elucidate the nature of *T. p. lawrencei* in carrier cattle after immunization using the infection-and-treatment method to help predict whether Corridor disease immunization in the field could be hazardous to cattle. These studies focussed on the relationship between the *T. p. lawrencei* isolated from carrier cattle and the original immunizing *T. p. lawrencei* stock using cattle immunized with *T. p. lawrencei* on its own or in combination with

T. p. parva and treated with different formulations of oxytetracycline.

Groups of cattle were immunized with 1:10 dilutions of sporozoite stabilates of either *T. p. lawrencei* (stabilate 199) or *T. p. parva* (stabilate 187) combined with *T. p. lawrencei* (stabilate 202) and treated with oxytetracycline. The animals were examined for persistent infection by cell culture isolation and for carrier state by tick application and examination of tick salivary glands. Of eleven animals examined, six were shown to be infective for nymphal ticks three to four months after immunization by applying the resultant adults to susceptible cattle. Two were carriers, shown by examination of adult tick salivary glands, and two were shown to have a persistent schizont infection by cell culture isolation.

Adult ticks infected with the carrier parasites derived from stabilate 199 or a combination of stabilates 187 and 202 were applied to individual susceptible cattle on nine occasions. All the cattle became infected and showed clinical theilerial reactions with the development of febrile responses. Seven out of nine were treated with parvaquone and recovered. The other two animals died in spite of treatment, showing that the carrier state parasite can be highly pathogenic. The survivors developed significant antibody titres in the indirect fluorescent antibody test. The seven recovered cattle were challenged with a lethal dose (1 ml undiluted) of the homologous immunizing stabilates (199, or 202 and 187, as appropriate), together with control cattle. Six out of seven carrier cattle and all the controls died of theileriosis, while none of the cattle originally immunized with the same stabilates died or showed clinical disease on challenge (Mutugi et al., 1988b). The seventh animal that survived following challenge underwent an unapparent theilerial reaction. The cattle that died on challenge generally showed a longer prepatent period to schizonts and a longer time to febrile response and death than controls, suggesting that some degree of protection may have been provided. When a carrier parasite was isolated and prepared as a sporozoite stabilate (226) and then used to challenge cattle immunized with *T. p. lawrencei* (stabilate 199), the cattle showed unapparent or mild theilerial reactions while the controls died from acute theileriosis (Table 2).

The monoclonal antibody profiles of infected cell lines isolated from the experimental animals were examined as described by Conrad et al. (1987). The stocks used for immunization and the carrier parasites contained mixed antigenic populations. Interestingly, the carrier parasites appeared to be similar to the original immunizing parasites (for example, stabilates 199, 202 and 187) except for the cell line isolated from animal Z639, which was immunized with carrier parasite derived from *T. p. lawrencei* (stabilate 199). This parasite had a markedly different monoclonal antibody profile from other parasites isolated from cattle immunized in the same manner and showed a very restricted pattern of reactivity with the panel of 20 monoclonal antibodies.

The evidence from this study appears to support the hypothesis that in many cases the carrier state is induced by only a part of the immunogenic population

in the stocks, because the carrier parasite immunized cattle were shown to be immune only to homologous challenge while the cattle immunized against the original *T. p. lawrencei* stock were immune to challenge with *T. p. lawrencei* (carrier state) parasite.

Table 2. Results of *in vivo* cross-immunity studies

Previous history	Challenge stock	Stabilate no.	Animal no.	Results			
				Classification of reaction	I*	MR*	SR†
Recovered from <i>T. p. lawrencei</i> carrier parasite	<i>T. p. lawrencei</i> (Ol Pejeta)	199	Z061	—	—	—	+
			Z065	—	—	—	+
			Z639	—	—	—	+
			Z360	+	—	—	—
Controls	<i>T.p. lawrencei</i> (Ol Pejeta)		Z060	—	—	—	+
			Z071	—	—	—	+
			Z649	—	—	—	+
			Z638	—	—	—	+
Recovered from <i>T. p. lawrencei</i> and <i>T. p. parva</i> carrier parasite	<i>T. p. lawrencei</i> (Trans-Mara) and <i>T. p. parva</i> (Kilae)	202/187	Z058	—	—	—	+
			Z059	—	—	—	+
			Z091	—	—	—	+
Controls			Z074	—	—	—	+
			Z075	—	—	—	+
<i>T. p. lawrencei</i> (Ol Pejeta) immune St. 199	<i>T. p. lawrencei</i> (carrier state)	226	Z884	—	+	—	—
			Z885	—	+	—	—
			Z887	—	+	—	—
			Z888	—	+	—	—
			Z889	—	+	—	—
			Z890	+	—	—	—
Controls			Z863	—	—	—	+
			Z894	—	—	—	+
<i>T. p. lawrencei</i> (carrier state) immune St. 226	<i>T. p. lawrencei</i> (Ol Pejeta)	199	Z809	—	+	—	—
			Z817	—	+	—	—

* Immune: I = inapparent reaction, MR = mild reaction.

† Not immune: SR = severe reaction but recovered, VSR = very severe reaction usually resulting in death.

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The next series of experiments is to determine the antigenic nature of carrier parasites of *T. p. parva* and *T. p. lawrencei* in cattle and buffalo infected with cloned parasites to determine whether antigenic variation occurs in carrier hosts and how it is expressed after tick transmission from infected animals.

In Kenya a high prevalence of the *T. parva* carrier state occurs in endemic areas, approaching 100% in adult immune cattle (D.P. Kariuki, personal communication). This prevalence is lower in epidemic areas but even here may reach as high as 10%, as has been shown on farms in some areas, such as Nakuru District (Young et al., this meeting). The effects of immunization against theileriosis using infection and treatment may make all the cattle immune and the majority of the cattle carriers. In such a situation, the carrier state may be beneficial because it maintains immunity both in individual animals and, by tick transmission, in cattle populations.

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The role of wildlife in the epidemiology of cattle theileriosis

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Most African wild Bovidae are carriers of *Theileria* parasites, for many of which the tick vector, host range and pathogenicity are unknown. Buffalo (*Syncerus caffer*) are known reservoirs of *Theileria parva lawrencei* and *T. mutans* and eland (*Taurotragus oryx*) of *T. taurotragi*, all playing an important role in the epidemiology of cattle theileriosis. In spite of the paucity of information on the involvement of wild animals in the epidemiology of cattle theileriosis, the exclusion of wildlife from cattle-grazing environments is advocated as a measure to reduce the risk of disease for cattle. Yet the buffalo, carrying *T. p. lawrencei*, is the only animal that is a proven risk to livestock. The exclusion of wildlife from livestock grazing areas is a drastic measure, which may cause losses in tourism and wildlife as well as ecological damage due, for example, to the loss of genetic resources. This paper discusses the role of wildlife in the epidemiology of theileriosis and immunization of cattle against *T. p. lawrencei*.

THEILERIA FROM BUFFALO

Theileria p. lawrencei, *T. taurotragi*, *T. velifera* and *T. mutans* have been isolated from buffalo and are transmissible to cattle. Of these, *T. p. lawrencei* is the only species highly pathogenic for cattle. The Wildlife Disease Research Project of the Kenya Agricultural Research Institute (KARI) has established breeding herds to breed and rear wild animals in captivity under tick-free conditions to study the role of wild animals in maintaining tick-borne parasites pathogenic to domestic livestock. A question frequently asked is: "Why do researchers work with wild animal hosts if the disease occurs in cattle and the parasites can be studied in domestic animals"? The answer lies in the origin and biology of these parasites.

Carrier state

The buffalo is an indigenous bovine of sub-Saharan Africa and has lived in harmony with *T. parva* and its vector *Rhipicephalus appendiculatus* since long before cattle were introduced into the region. It is assumed that when cattle were

introduced into East Africa many died of theileriosis and that gradually a population of cattle evolved that was resistant or tolerant to this disease. In addition, a sub-population of the *Theileria* parasites transmitted from buffalo to cattle that we now refer to as *T. p. parva* may have become adapted to cattle. However, the major parasite pool is maintained by buffalo and when ticks from buffalo feed on cattle, the cattle are likely to become infected with *T. p. lawrencei* and develop Corridor disease.

In spite of the pandemic of rinderpest in Africa in the 1890s and subsequent rigorous control and culling, buffalo are widespread and commonly share habitats with domestic cattle. Surveys in Kenya, Tanzania and Uganda have shown that almost every buffalo sampled is a carrier of *T. p. lawrencei* (Young et al., 1978a). The carrier state of *T. parva* in cattle has also been well established (Maritim et al., this meeting). However, there are important differences between the carrier state in buffalo and cattle. First, it has been shown recently that not all of the antigenic types of *T. parva* in a stock isolated from a buffalo and used to immunize cattle were maintained in these cattle. Parasites isolated in ticks from the immunized carrier cattle were used to immunize a second group of cattle. Six out of seven of these cattle died on challenge with the original immunizing stabilate (Maritim et al., in press). Second, *Theileria* infection rates in ticks fed on carrier buffalo range from 10% to almost 100%, but infection rates in ticks fed on carrier cattle can be as low as 0.01% to 0.05% (Maritim et al., in press). Third, most cattle challenged with a lethal dose of *T. p. lawrencei* die before the piroplasm stage appears in the blood. Most cattle infected with *T. p. parva* die during a phase of high piroplasm parasitaemia, thus allowing ticks to pick up the parasite and transmit it to other cattle. Therefore, *T. p. parva* can be maintained by a cattle population, but *T. p. lawrencei* might not be. This parasite behaviour necessitates the study of *T. p. lawrencei* in buffalo.

Antigenic diversity

Antigenic diversity of *T. p. lawrencei* from buffalo has been demonstrated in several studies (Young et al., 1977, 1978b; Conrad et al., 1987, 1989; Grootenhuis et al., 1987a). The most striking example is the lack of cross-protection between two *Theileria* stocks isolated in ticks fed on the same buffalo 18 months apart and used to immunize and challenge cattle. Homologous challenge gave complete cross-protection, but some of the cattle immunized with the earlier isolate were susceptible to challenge with ticks infected later (Young et al., 1977, 1978b). Conrad et al. (1987) have shown that cell lines isolated from a single buffalo consist of a mixture of four or five antigenic types identified by monoclonal antibody staining patterns. An experimentally induced carrier state established in a buffalo following a single injection of stabilate resulted in a long-term carrier state from which isolates were made over a two-and-a-half-year period. Monoclonal antibody staining patterns differed among the cell lines isolated (Grootenhuis et al., 1987a). These antigenic differences may explain the

lack of cross-protection among *T. p. lawrencei* types and between stocks of *T. p. lawrencei* and *T. p. parva*.

Nonetheless it has been possible to immunize effectively against natural *T. p. lawrencei* challenge, as shown by trials in the Trans-Mara, Nanyuki and Naivasha areas of Kenya (Young, 1985; Dolan, 1985; Young et al., in preparation). In these trials *T. p. lawrencei* isolates from the local areas were used to avoid introduction of new antigenic types and, more importantly, to immunize against the theilerial antigens of the areas. In the Trans-Mara trials, the *T. p. lawrencei* components were obtained from one buffalo. The success of this immunization suggests that it may be possible to obtain very broadly immunizing stock from one animal. However, the antigenic mixture that is required for this or any other area remains unknown and needs further study.

Transformation

Theileria p. lawrencei has been shown to change its behaviour following serial passage in cattle. After three to six passages it behaves like *T. p. parva*, producing a high schizont parasitosis and piroplasm parasitaemia. The initial passages require large numbers of cattle, since only a small proportion of animals infected with *T. p. lawrencei* from the buffalo will survive and develop a piroplasm parasitaemia. Several studies have described this transformation (Barnett and Brocklesby, 1966; Young and Purnell, 1973; A.C. Maritim, J.J. Mutugi and A.S. Young, personal communication) and the phenomenon was first attributed to a change in parasite behaviour. Recent studies have indicated that this process is not reversible because buffalo infected with the "transformed" parasite develop a transient infection and no carrier state (Grootenhuis et al., 1987a), similar to experimental *T. p. parva* infection in buffalo (Brocklesby, 1964). In addition, there is evidence for antigenic diversity of *T. parva* within individual buffalo as described earlier (Conrad et al., 1987; Grootenhuis et al., 1987b). These studies suggest selection from the diverse antigenic pool of parasites carried by the buffalo as an alternative explanation of *T. p. lawrencei* transformation.

If selection is what takes place, then buffalo contain other antigenic types that are not passaged in cattle to produce the transformed parasite. These other types may account for the high mortality in cattle with classical Corridor disease in which piroplasms are not detected because they die so rapidly. These virulent antigenic types may be essential in an immunizing parasite mixture.

Mechanisms of disease resistance

Buffalo and cattle are related closely phylogenetically, their immunoglobulins cross-react and they share the largest proportion of cell surface markers of all Bovidae (W.C. Davis, personal communication). Their cell mediated immune response to infection with *T. parva* appears to be similar to the response of cattle (Baldwin et al., 1986). Yet buffalo tolerate a *T. parva* challenge that is lethal to

cattle (Brocklesby, 1964). Understanding the mechanism of resistance to theileriosis in the buffalo might provide new avenues for exploitation in the control of cattle theileriosis.

Isolation of parasites from buffalo

The arguments presented above are a strong case for the use of buffalo and their *T. parva* parasites. Three methods have been used to obtain buffalo-derived parasites for use in immunization.

- a) Buffalo calves may be captured from the area where cattle are to be immunized. One- to three-month-old buffalo calves adapt readily to captivity and can be handled as easily as cattle. In our experience, every calf captured has been a carrier of *T. p. lawrencei*. At present we keep nine buffalo from a total of four areas of Kenya.
- b) A carrier state may be induced in *Theileria*-free buffalo born and reared at the laboratory with ticks that have fed as nymphs on wild buffalo in the selected area. Adult unengorged *R. appendiculatus* may be collected from buffalo habitats, or if the tick population density is low, sentinel cattle may be used to collect the ticks. We have infected two parasite-free buffalo with ticks collected from two buffalo habitats in areas where cattle need to be immunized against *T. p. lawrencei* and where buffalo calves could not be captured easily.
- c) Adult unengorged *R. appendiculatus* may be collected from buffalo habitats and fed on rabbits for production of immunizing stabilates.

Methods *a* and *b* have the advantage that a tick feed can always be repeated. Although the antigenic composition of the parasite population from individual buffalo may change over time, experience to date indicates that good immunizing stabilates can be obtained repeatedly from the same buffalo. Tick collections from buffalo habitats are practical only as a "one off" exercise and only if there are high densities of both buffalo and ticks. Another problem encountered was that highly infected salivary glands were uninfective for cattle. These ticks could have been infected with a *Theileria* species from other wild Bovidae inhabiting the area and from a species that could not be transmitted to cattle (T.T. Dolan and D.A. Stagg, personal communication).

THEILERIA FROM ELAND

Theileria parasites initially thought to be mild strains of *T. p. parva* have been isolated in *R. appendiculatus* ticks and in cell culture from cattle in Kenya, Tanzania and Zimbabwe (Burrige et al., 1974; Uilenberg et al., 1977; Koch et al., 1988). These parasites cross-reacted serologically with *T. taurotragi*, a

Theileria species isolated from eland (*Taurotragus oryx*) (Grootenhuis et al., 1981). Although *T. taurotragi* was named for its initial detection in eland (Martin and Brocklesby, 1960; Grootenhuis et al., 1979), it was found later to have a potentially wide host range (Stagg et al., 1983). Experimental infections have been successful in a captured buffalo calf while *in vitro* infection of lymphocytes has been successful in several other species of Bovidae.

Theileria taurotragi from eland causes a mild transient infection in cattle which is not readily detectable. Using an indirect fluorescent antibody (IFA) test, antibodies can be detected in both cattle and eland. Fatal *T. taurotragi* infection has been reported in eland, but infections in cattle are usually subclinical (Grootenhuis et al., 1979). The piroplasm and schizont stages in cattle are usually indistinguishable from those of *T. parva*. One isolate from cattle caused a very mild and transient infection in eland. This group of parasites was called *T. taurotragi* because of these results (Grootenhuis et al., 1981).

Although the reasons for placing these *T. taurotragi*-like isolates in the same group appear to be valid, there are behavioural differences between the cattle and the eland isolates. Eland isolates cause a very mild transient infection in cattle where schizont and piroplasm stages are difficult to detect. Conversely, it is difficult to infect eland with the cattle isolates. It appears that the *T. taurotragi* originating from wildlife may become adapted to cattle and that populations of these parasites are being maintained within cattle.

Serological tests to distinguish *T. taurotragi* from *T. parva* are unsatisfactory. Good positive control sera are not available and the IFA test is most reliable using piroplasm antigen. However, this stage generally does not occur at a sufficiently high parasitaemia to make suitable antigens. It would be preferable to develop a test making use of schizont antigen, which can be propagated in culture. Once better methods for the identification of this parasite are developed, the distribution of this *Theileria* species can be established. It is important to be able to recognize this parasite in the course of immunization trials, because vaccination against *T. parva* cannot prevent infection with *T. taurotragi*. Because of misdiagnosis, expensive drugs may be wasted when treatment is unnecessary. It is also important to be able to distinguish *T. taurotragi* from *T. parva* in ticks because these species are morphologically identical and therefore cause confusion in this epidemiological parameter.

THEILERIA FROM OTHER WILD BOVIDAE

Attempts have been made to transmit *Theileria* species detected in impala (*Aepyceros melampus*), wildebeest (*Connochaetes taurinus*) and waterbuck (*Kobus defassa*). Transmission from impala and wildebeest to cattle was unsuccessful, although the parasites could be transmitted between animals of the same species (Purnell et al., 1973; Grootenhuis et al., 1975). This is likely to be the situation with many of the wild Bovidae. The waterbuck can be infected with *T. p.*

lawrencei (Stagg, Young and Grootenhuis, unpublished results), but its importance as a reservoir of this parasite remains to be established. The waterbuck also carries its own *Theileria* and *Babesia* piroplasms (Fawcett et al., 1987).

The genus *Cytauxzoon*, in which *T. taurotragi* was placed originally, appears to be pathogenic only for certain wild Bovidae and was not considered in this presentation.

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Epidemiology and decision-making in theileriosis control

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Several methodologies are currently available for the control of theileriosis (reviewed by Young et al., 1988). Immunization by the infection-and-treatment method (Radley et al., 1975) is the latest to be added to the list. The choice of a particular strategy by a farmer or a government should depend on the prevailing epidemiological and socioeconomic circumstances in a given area. Although infection and treatment offers the prospect of more widespread control of the disease, no single method is cost-effective or appropriate in all parts of a region in which the disease occurs. The severity of theileriosis, and hence the justification for controlling it, varies with differences in epidemiology, livestock management and farming systems. Young et al. (1988) have recently reviewed the methodologies available for tick-borne disease control and proposed an integrated approach to their application in Africa. This paper examines circumstances under which three basic theileriosis control methods might be used and proposes a conceptual framework with which a decision might be made on the best method to use in a given area.

CHOICE OF CONTROL METHOD

For the purpose of this discussion, disease control interventions and the characteristics of cattle populations have been simplified. Four mutually exclusive control options are considered, although in reality combinations of these would probably be used in many circumstances. These options are: immunization using the infection-and-treatment method, treatment of clinically affected animals with antitheilerial drugs (Dolan, 1981), the control of *Rhipicephalus appendiculatus* with acaricides, and no action at all. The consequences of these options (in this paper no action is considered an option) are measured in terms of disease incidence (the number of new cases in a given time period) and case-fatality rate (the proportion of new cases that die). It is assumed that immunization and vector control will influence disease incidence, whereas treatment will influence the case-fatality rate. Table 1 indicates the possible result of these simplified control

actions in terms of disease incidence and case fatality rates in cattle populations of differing susceptibility. For the purposes of this discussion, it is also considered that immunization, treatment and vector control can be either effective or ineffective. The circumstances likely to govern the efficacy of each action are technical, logistic and socioeconomic.

Table 1. Possible effects of different theileriosis control actions

Action		Cattle population status		
		Very susceptible	Partially susceptible	Low susceptibility
Immunize	Effective	Incidence low***	Incidence v. low***	Incidence v. low
	Ineffective	Incidence medium-high*	Incidence low-medium*	Incidence low
Treat	Effective	Case-fatality low***	Case-fatality low***	Case-fatality low
	Ineffective	Case-fatality medium-high*	Case-fatality medium-high*	Case-fatality low
Vector control	Effective	Incidence low***	Incidence v. low**	Incidence v. low
	Ineffective	Incidence medium-high*	Incidence low-medium*	Incidence low
Do nothing		Incidence high Case-fatality high	Incidence medium Case-fatality high	Incidence low Case-fatality low

* Indicates incremental change in status from "do nothing".

Immunization

A knowledge of the *Theileria parva* strains occurring in the area is necessary and there should be demonstrated protection against field challenge by the immunizing stock. There should be long-term availability of both the immunizing stock and the chosen antitheilerial drug. The vaccine should be affordable and there should be an effective and accessible veterinary service to administer and monitor the immunization. The lack of one or more of these requirements will result in ineffective immunization.

Treatment

Clinical cases of theileriosis must be promptly identified, because to be effective treatment must be applied early in the course of the disease (Dolan et al., 1984; Chema et al., 1986). Such prompt action will require a good diagnostic ability and high standards of management by the farmer as well as proximity to (or

good communications with) effective veterinary services. Antitheilerial drugs should be available on a long-term basis and the treatment should be affordable. Again, the lack of one or more of these criteria will result in ineffective treatment.

Vector control

For controlling theileriosis by controlling *R. appendiculatus* with acaricides, the dipping or spraying should be carried out twice a week during periods of nymphal and adult tick activity. This generally requires a high level of management to be successful. Acaricide and water must always be available and the strength and efficacy of acaricide at delivery should be regularly monitored. The acaricide should also be affordable. Where these rather stringent requirements cannot be met, the vector, and hence disease, control will be ineffective.

ASSESSMENT OF POPULATION SUSCEPTIBILITY

A knowledge of cattle population susceptibility is clearly vital before choosing a disease control action. In general, this susceptibility will depend on the breed/type of animals present and on the challenge, particularly to young stock, by *T. parva*-infected ticks. A simple model of the effect of these parameters on disease incidence, case fatality and serum antibody prevalence rates is given in Figure 1. In circumstances of fairly constant challenge of indigenous Zebu cattle with infected ticks throughout the year, situations of endemic stability may exist where little or no clinical disease is observed (e.g., Moll et al., 1984; Young et al., 1986; Morzaria et al., in press). At the other extreme, exotic and grade livestock kept under even low *R. appendiculatus* challenge will be highly susceptible. The variety of situations between these extremes are determined by variations in genetic susceptibility of certain breeds or types and their crosses (Young, 1981; Dolan et al., 1982) and endemic instability in indigenous Zebu breeds where infected tick challenge is low or markedly seasonal (Moorhouse et al., 1986; D.L. Berkvens, personal communication). Situations are further complicated where livestock populations comprise both exotic and indigenous breeds and where different management systems (affecting tick challenge) are used within the same herd.

DECISION-MAKING

In the preliminary model presented, the selection of a control option and the susceptibility status of the target population provide an indication of expected

outcomes. The assessment of prevailing circumstances, such as availability of veterinary services and standard of management, to evaluate expected efficacy of the intervention further defines the possible outcome. Table 1 shows incremental changes in incidences and case-fatalities that may result from the intervention when compared with doing nothing. This simple deterministic model demonstrates that control options should be tailored for given sets of circumstances and that the range of efficient disease control actions available is considerably narrower than at first appears.

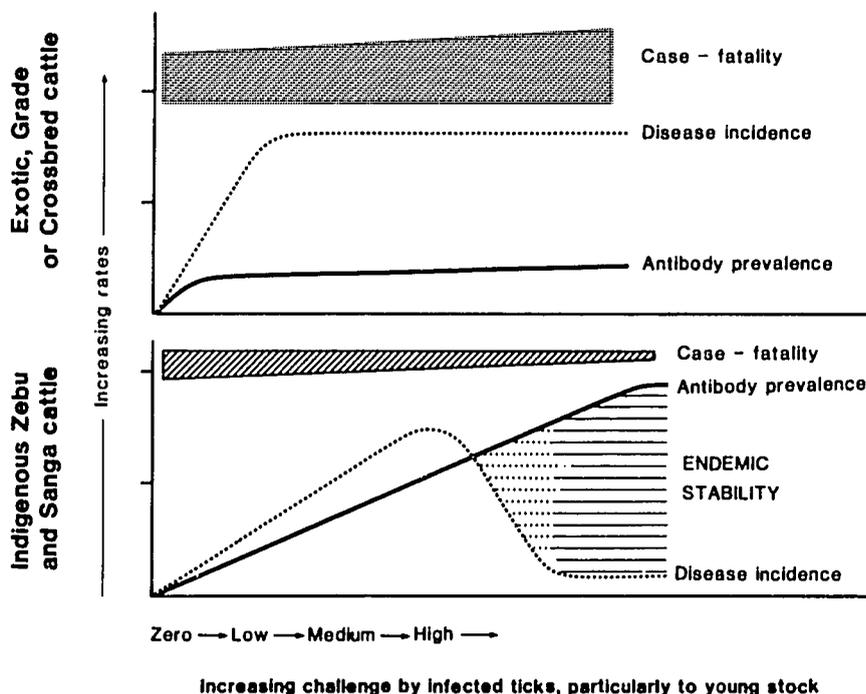


Figure 1. Hypothesized relationship between disease incidence, antibody prevalence and case-fatality under increasing infected-tick challenge in two groups of cattle with different susceptibilities.

Where substantial data are available, this decision process can be enhanced by the use of decision analysis (reviewed in an animal health context by Erb, 1988). This technique evaluates the effect of alternative interventions on predetermined parameters and incorporates an assessment of risk. In this context, risk is taken as an evaluation of the probability of the successful (and unsuccessful) application of a technique. Decision analysis is often confined to the technical parameters describing and quantifying the effects of successful application of an intervention and uses data that come from previous research (such as the effect of immunization on disease incidence), but which, where definitive data are available, may be broadened to incorporate key logistic and socioeconomic parameters. However, many of the logistic and socioeconomic parameters do not lend themselves to quantification.

Figure 2 is a decision tree illustrating the alternative actions emanating from a decision "node". Each strategy then encounters a probability node. At this point it is assumed that the strategy has a given probability of a favourable outcome (P) or a mutually exclusive unfavourable outcome (1 - P). The assignment of probability values is based on experimental evidence or expert opinion. Where probability values are in doubt, the selection of probable best and worst (that is, a sensitivity analysis) can be made. The indicators of favourability of the intervention will be predetermined parameters. These parameters may include disease incidence rate, case-fatality rate, seroconversion rate in a given age stratum of the target population, or productivity (such as weight gain, milk yield and ploughing efficiency). Parameter values may then be translated into economic terms. The values of favourable and unfavourable outcomes (such as incidence rate and economic effect) are then multiplied by their respective assigned probability values. The resultant values for favourable and unfavourable outcomes are then summed to provide a net effect of the intervention studied. The same process is then applied to the other interventions under consideration and the net effect of each intervention compared. At this stage the most favourable interventions should be assessed for their socioeconomic consequences before final decisions are made. This simple framework for decision analysis can be modified to assess a variety of different outcomes, depending on the availability of data and the quality of decision required.

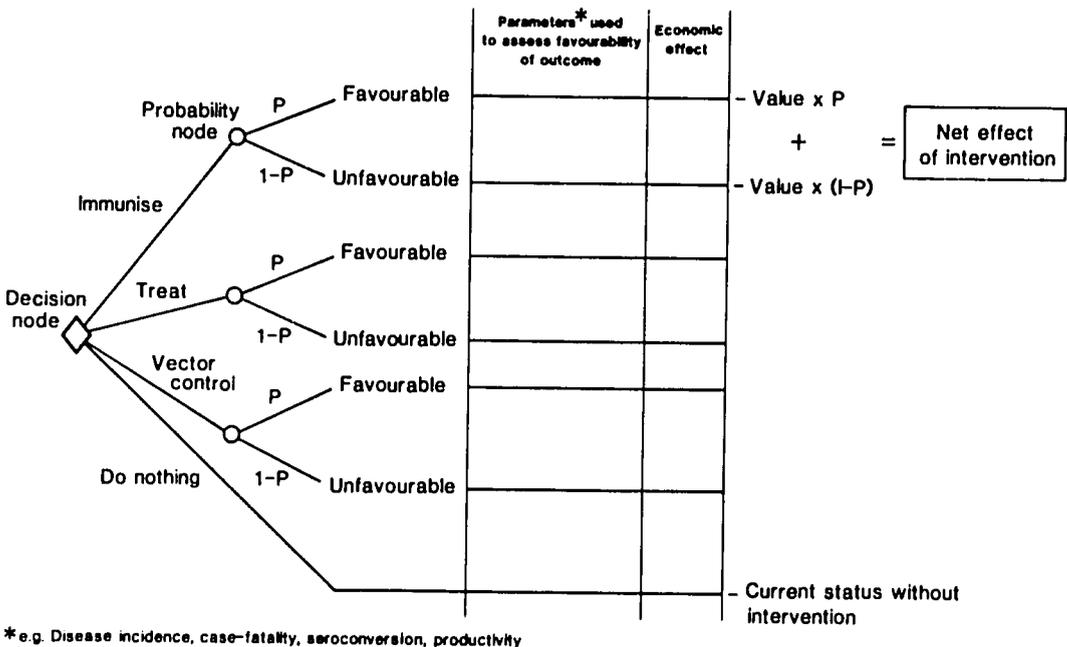


Figure 2. Simplified decision tree for estimating alternative East Coast fever control strategies.

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The risk of East Coast fever to livestock in Africa on a geographic basis

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East Coast fever (ECF) is a major cause of cattle mortality and loss of productivity in the eleven African countries in which it is known to exist. However, its impact varies considerably within these countries. This is due to differing virulence of *Theileria parva* strains, to differences in abundance and infectivity of the vector tick, *Rhipicephalus appendiculatus*, to the presence of other tick vectors and to differences in susceptibility to the tick and the parasite of the cattle breeds and types present. The impact of ECF control in a given area will depend on a number of factors. These include the incidence and severity of clinical disease experienced, the abundance of cattle, the role of cattle and their products in society, the relative importance of ECF compared with other diseases and the nutritional and management constraints to livestock production that are present. Before embarking on widespread ECF control programmes, it is thus essential to identify the different circumstances of disease risk prevailing within a target area so that such programmes can be tailored accordingly to permit optimum cost-effectiveness.

SELECTION OF EPIDEMIOLOGICAL PARAMETERS

Our initial approach was to acquire, collate and represent geographically data available from secondary sources (publications, reports) on seven of the major parameters influencing ECF epidemiology in the affected countries of eastern,

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central and southern Africa. The parameters, listed in Table 1, were chosen on the basis of their importance as determinants of theileriosis epidemiology and of their accessibility in the form of secondary source data. They were not intended to comprise a comprehensive list of the disease determinants. This approach is an extension of studies initiated by Irvin (1987) to collate information on the distribution of *R. appendiculatus* and *T. parva*.

Table 1. Epidemiological determinants of ECF chosen for geographic representation

Parameter	Quality	Data source
Host		
Cattle	Known distribution and distribution of major indigenous breeds/types	Inter-African Bureau of Animal Resources (IBAR) and published literature
Buffalo	Known distribution	Published literature
Restrictions to domestic host population		
Game parks	Known distribution and perceived level of security (i.e., exclusion of livestock)	Global Resources Information Database, United Nations Environmental Programme
Tsetse fly	Known distribution by species	IBAR
Vector		
<i>Rhipicephalus appendiculatus</i> ,	Recorded distribution	Published literature
<i>R. duttoni</i> ,	Probable distribution	Expert opinion
<i>R. nitens</i> ,		
<i>R. bergeoni</i> and		
<i>R. zambeziensis</i>		
Ecoclimatic suitability index (E.I.)	Distribution by index value	Climate: published literature, FAO, CIAT E.I.: CLIMEX (climate matching model), CSIRO
Disease		
Clinical theileriosis	Recorded distribution	Published literature, government reports
Antibodies to <i>Theileria parva</i>	Recorded distribution	Published literature, government reports

Data entry and analysis have been described by Lessard et al. (1988). In summary, data were entered into a computerized geographic information system

called ARC/INFO (Environmental Systems Research Institute, Redlands, California 92373, USA). This software programme was run on a Micro Vax 3 mainframe computer at the Global Resources Information Database (GRID) offices of the United Nations Environment Programme (UNEP). Some of the data, such as available cattle distribution maps, were digitized into the programme using a Calcomp 9100 digitizer. Other data, such as climatic variables, were entered in numerical form along with their qualifying latitude and longitude.

RESULTS AND DISCUSSION

Individual maps showing the distribution of each of the disease determinants studied have been produced (Figure 1) and are currently being prepared for publication (Lessard, P., L'Eplattenier, R., Norval, R.A.I., Perry, B.D., Dolan, T.T., Croze, H., Walker, J.B. and Irvin, A.D., in preparation). In addition, overlay maps of two or more parameters are being used to evaluate interactions between determinants and to define disease-risk zones (Figure 2).

Geographic information systems (GIS) provide a valuable method of collating and displaying data on a geographic basis. With accurate data and correct selection of the key parameters, GIS offer an extremely useful tool to the disease-control planner. Although still in the early stages of development, the systems have already allowed a considerable refinement of our understanding of the distribution and potential distribution of *R. appendiculatus* and the interaction of this tick with livestock. Improvements can be made to the system by improving data quality and increasing the number of determinants/parameters studied.

Improving data quality

The quality of data in the list of parameters studied is not uniform, and inherent differences in data collection techniques mean that there is a limit to potential improvements in quality. Continent-wide databases on cattle populations, for example, are collected on the basis of administrative boundaries, such as district and provincial, submitted by national livestock authorities. In many cases the distribution within the administrative unit may not be uniform, although it appears uniform on the distribution map. Tick distribution data, on the other hand, are often based on site-specific studies and are not necessarily representative of entire administrative regions. Analyses of interactions between two parameters collected under different circumstances are thus limited, but serve to identify areas where further data are required. A further limit to cattle population data is that they do not differentiate cattle breeds and types or management systems. Particularly important is the inclusion of data on the distribution of exotic and grade livestock, which are at greatest risk to ECF. These data are not widely available.

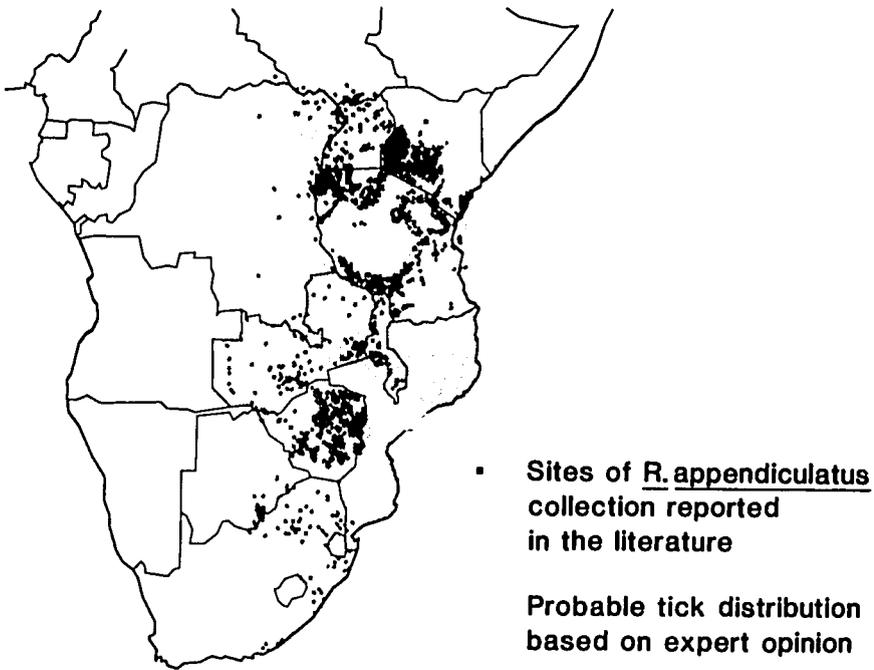


Figure 1. Probable distribution of *Rhipicephalus appendiculatus* in Africa.

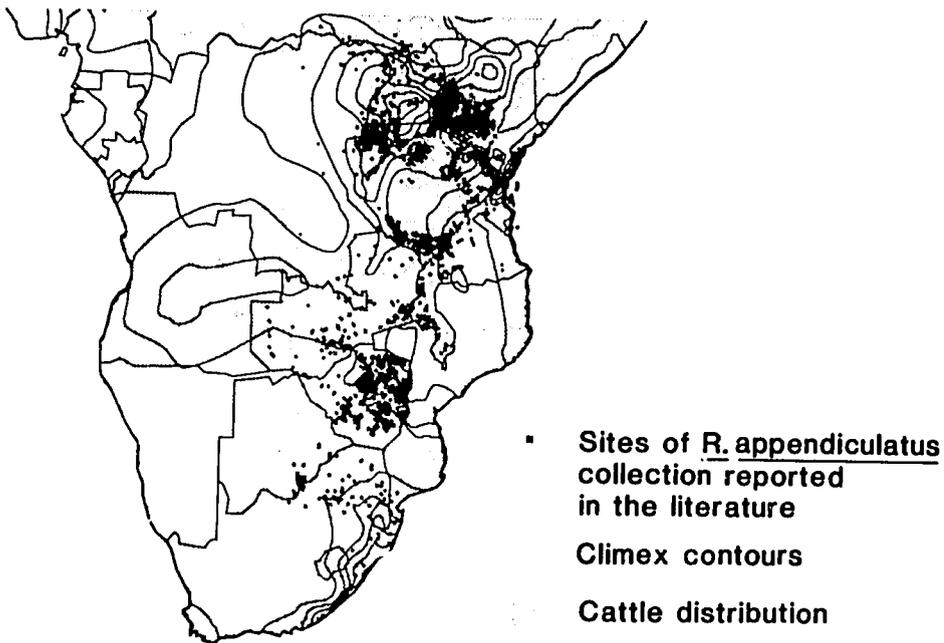


Figure 2. Combination overlay maps of recorded *Rhipicephalus appendiculatus* collections, reported cattle distribution and various ecoclimatic suitability index contours for *R. appendiculatus* development.

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Tick collection data often represent the results of a single survey. Further surveys carried out in districts or countries neighbouring that in which an original survey was made are often conducted many years after the original study. Distribution comparisons may thus be invalidated by marked differences in climate, host availability or acaricide use between surveys. Eventually, data from a given time period will be required from selected sites to validate predicted distribution changes.

For data on all parameters, higher resolution studies at specific sites should be carried out to assess the accuracy and validity of the databases.

Increasing the number of determinants/parameters studied

The list of determinants used in this study is far from exhaustive. Further studies to identify and evaluate the key parameters for geographic representation of ECF risk are warranted. A planned addition to the list is the normalized difference vegetation index (NDVI) acquired from satellite-derived Advanced Very High Resolution Radiometer databases at UNEP and the Regional Centre for Services in Surveying, Mapping and Remote Sensing, in Nairobi. This will provide seasonal and secular data on distribution of favourable habitats for *R. appendiculatus* as measured on the NDVI scale.

Certain other determinants likely to be important in assessing disease risk do not lend themselves to geographic display as yet, due to inadequate surveillance. Such determinants include infection rates of *R. appendiculatus* with *T. parva* and acaricide use policies and practices.

It is planned to supplement existing determinants with socioeconomic parameters in defined regions of the continent so as to assess the ability of these parameters to serve as predictors of disease impact on a geographical basis. Socioeconomic parameters include human population density/land use intensity, land tenure, livestock production system, livestock function (such as traction, milk) and the availability of veterinary services.

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Data handling for theileriosis immunization studies

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The development of infection and treatment as an immunization technique for the control of theileriosis continues to rely heavily on the interpretation of data from experimental and field studies. Experimental studies on immunization and related chemotherapy techniques have usually been designed to monitor small groups of animals intensively over short periods of time (for example, Dolan, 1985, 1986; Mutugi et al., 1988), the information collected being used to identify methods that are practical, effective and inexpensive and that produce few adverse reactions in experimental animals. In contrast, field studies have usually been undertaken to explore the difficulties in translating the techniques into the field and to measure production and economic effects of the disease or the control technique (see, for example, Dolan, 1985; Young, 1985; Morzaria et al., 1988). Should East Coast fever immunization become implemented more widely, follow-up surveillance studies will be necessary to measure the long-term consequences of the method.

WHAT CAN ANALYSIS OFFER?

Data from immunization studies reflect the relationships among animal, tick, parasite and environmental conditions. Each factor can contribute a different amount of variation to each measured parameter and so relationships which exist are not always obvious. Data must be analysed to understand these relationships. Data analysis does not always involve the rigorous application of statistical tests. A competent investigator will often be convinced of a claim from simply "eyeballing" data or plotting a simple graph. This is not to be discouraged, but the experimenter must be protected from prejudices. Statistical methods provide a scientific standard for planning studies, exploring data patterns and reporting scientific claims. In immunization and chemotherapy studies, statistical methods have generally involved testing for significant differences between treated and untreated (control) groups. However, an examination of unpublished and published immunization and chemotherapy studies shows that statistics in general,

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and the purpose of statistical tests in particular, are commonly misunderstood. As a result, certain inferences have been misleading and much time and effort have often been wasted.

SAMPLE SIZES TOO LARGE

Comparing groups and using unnecessarily large sample sizes can lead to “statistical significance”, which is not the same as “biological significance”. Given an indication of the variation normally associated with the parameter of interest, it is possible to design an experiment with the correct number of animals per group to be confident of detecting the order of difference considered to be biologically important.

SAMPLE SIZES TOO SMALL

Choosing sample sizes that are too small is a common error and frequently leads to a total waste of study resources. When it occurs, it can lead to claims of no significant difference between groups because the experiment was designed with inadequate data. Once again, this can be avoided by using simple calculations of the power of the statistical test based on biological expectation and parameter variation.

B I A S

Bias is another common error and can arise for many reasons. One treatment group may be advantaged simply because animals have not been randomized to ensure comparable groups at the start of the experiment. Alternatively, data from certain animals may be omitted in the belief that these animals produced “outliers”. This happens when the experimental protocol has not rigorously specified under what conditions animals can be excluded. Bias is not difficult to detect.

C O N F O U N D I N G

Detected differences among treatment groups may be confounded by other factors such as breed, genotype or age. Randomization helps to eliminate confounding, which frequently occurs when animals in different treatment groups are not managed similarly. Any observed differences among treatment groups cannot be attributed with certainty to treatment differences. Confounding is simple to avoid but may be difficult to detect.

TOO MANY STATISTICAL TESTS

When more than two treatment groups are compared, there is a temptation to test all possible pairs of groups. This can lead to misleading claims. For example, given 4 treatment groups, testing each group against each other involves 6 tests. If each test uses a 5% significance level, the chance of detecting a difference that does not really exist rises from 5% to 34%, that is, there is a 1 in 3 chance of claiming a non-existent difference. Excessive statistical testing arises in another context, when the same groups are tested repeatedly. These problems can be avoided using simple data handling methods, such as multiple range tests, and using the correct experimental design for repeated time measurements.

An immunization study should have clear objectives, one of which should be identified as the primary objective. The experimental procedure should be designed to achieve this objective. Past studies should be examined to give indications of expected variation in parameters. A protocol should be written with details of stabilate and chemotherapy administration, randomization of animals, legitimate reasons for excluding data from animals, the proposed method of data analysis and a justification for the number of animals to be used. Typically, a reasonable chance (80% power) of detecting (using a 5% test of significance) a 50% difference in successful immunization rates between two groups of animals receiving different immunization regimes would require at least 16 animals per group. Using similar test criteria and data from past chemotherapy trials (Dolan, 1986), we have calculated the approximate number of animals one would need to use in a trial in order to detect given time differences. Table 1 gives the numbers of animals required when comparing treated and untreated groups, Table 2 the numbers required when comparing one treated group with another treated group.

Table 1. Approximate number of animals required in groups to detect (80% power, 5% significance) the given time differences between treated and untreated groups, based on variability observed in past chemotherapy trials

Parameter	Estimated variance (days ²)	Order of difference expected (days)	Number of animals required per group
Time to appearance of schizonts	3.34	1	53
Time to febrile response	0.96	1	16
Time to recovery	80.1*	20	**
Time to death	22.3	5	11

* Animals recovering only.

** Untreated animals normally do not recover.

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Table 2. Approximate numbers of animals required in groups to detect (80% power, 5% significance) the given time differences between different treated groups, based on variability observed in past chemotherapy trials

Parameter	Estimated variance (days ²)	Order of difference expected (days)	Number of animals required per group
Time to appearance of macro-schizonts	3.34	1	53
Time to febrile response	0.96	1	16
Time to recovery	80.1*	1	1266
Time to death	22.3	2	88

* Animals recovering only.

Clearly, detecting differences between times to appearance of macroschizonts, recovery and death among treated groups requires large numbers of animals. The collection of such data is more likely to come from field studies and surveillance than from experimental studies. Differences between times to death between untreated and treated groups is less demanding on experimental subjects because a larger order of difference would normally be expected. Unfortunately, it is not possible to provide such tables specifically for immunization studies because published work does not provide sufficient data.

CAN COMPUTERS HELP?

Data collection requires data storage. Most experimental immunization studies generate only small databases and interest is restricted to the technical aspects of immunization. Typically, treatment factors are determined by stabilate and drug administrations. The following are commonly observed parameters:

- time to macroschizont detection
- time to febrile response
- time to x% parasitosis
- time to recovery or death outcome
- time to recovery
- time to death
- immunized or not immunized
- packed cell volume
- white blood cell count

For most purposes, these data can be recorded by hand and analysed manually using simple graphs and calculations.

Field and surveillance studies to measure the impact of immunization usually involve large groups of animals and more extensive data recording. To evaluate the impact, comprehensive attempts are made to record data associated with all possible influencing factors. Tick counts may be required to discover if animals are under challenge. Vector populations and pasture availability are driven by weather and thus climatic data may be required to explain events. Clinical interventions and herd health details are required to evaluate possible adverse reactions. Liveweight gains and milk offtake should be monitored to ensure no loss in productivity. All measurements should normally be repeated weekly or monthly over long periods of time.

As data recording becomes labour-intensive and analysis more demanding, computers offer an ideal way to handle the data. Computers require software to store and manipulate data. A wide range of software packages exists for data handling. Database packages enable researchers to design record structures for data and systematically to query the records. Spreadsheet packages enable one to perform calculations on numerical data without writing ad hoc programs. This is important in large data studies because statistical tests can be used to "trawl" the data routinely for significant differences and interactions. Once these have been identified, the investigator can focus on findings of interest. Alternatively, the data can be exported from the database to other statistical software packages for analysis. Graphics packages can be used to express trends in the data and produce visual images of the relationships among parameters. The fast rate of processing data stored electronically means that analyses can routinely take place during the course of the study and final analyses can be completed within days of the end of the study.

Recent developments in computer technology have opened up two important avenues for the further exploitation of data stored in databases. Geographical information systems (Burrough, 1986) software is now available on microcomputers and maps can be produced from databases. Expert systems (Waterman, 1986) software packages are available for storing knowledge bases. This means that statements from experts can be stored in a list and a user seeking an expert opinion can use the computer to interrogate the knowledge base. These developments will have implications for the long-term monitoring of East Coast fever immunization programmes.

Data stored electronically in databases also offer a convenient medium for the exchange of information. In the last few years most software packages have evolved towards an international standard for the export and import of data among software packages. It is therefore no longer necessary for researchers to use one recording system. What is important is a general consensus on what needs to be recorded so that the bases for a continental database can be established.

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Production and delivery costs of the infection-and-treatment vaccine against East Coast fever

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The infection-and-treatment method of immunization against *Theileria parva* has been tested in several field trials in different countries in East and Southern Africa, but no comprehensive assessment of its cost has been made. Radley (1981) estimated that it could cost US\$2.51 to immunize one animal, \$0.01 being the cost of producing one immunizing dose of the vaccine and \$2.50 the cost of buying the long-acting tetracycline used for treatment. It was estimated (Irvin, 1985) that in Malawi it costs between \$4 and \$5 to immunize one animal, \$0.10 being the cost of producing one dose of the stabilate and the balance being the cost of the long-acting tetracycline. Kiltz (1984) estimated the cost of immunizing one animal to be about \$20 in Burundi. These figures are estimates only: they do not take into account all cost components.

This paper summarizes the results of a study conducted in Kenya in 1988 on the best method for assessing the cost to a government of producing and delivering the infection-and-treatment vaccine in the field.

The costs of developing and delivering the vaccine were divided into five parts: isolation of immunizing stocks, *in vivo* characterization of laboratory stocks, *in vitro* characterization of laboratory stocks, preparation of bulk stabilate and vaccine delivery and monitoring. Costs were identified and estimated for each of these. Costs were estimated assuming a planning period of 30 years and an immunization capacity of 100 000 cattle per year.

Costs were grouped under capital or operating expenditures and were calculated in local and foreign currencies. The total cost of the method was finally expressed as the cost of immunization per animal. The cost of vaccine production was expressed as the cost per dose produced. A sensitivity analysis was made to provide probable ranges for the per-animal and per-dose costs.

* A complete report of the estimation procedure, *Immunization against East Coast Fever: Production and Delivery Costs of the Infection-and-Treatment Method*, by A.W. Mukhebi, S.P. Morzaria, B.D. Perry, T.T. Dolan and R.A.I. Norval, is in preparation and will be published by the International Laboratory for Research on Animal Diseases.

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The total cost of the method to the government over a 30-year period amounted to Kenya shillings (Kshs.) 118.7 million or \$7.0 million (Table 1). Of this, capital costs were Kshs.12.7 (\$0.7) and operating costs were Kshs.95.2 (\$5.6) million. A 10% contingency of Kshs.10.8 (\$0.6) million was added to the capital and operating costs to account for unforeseen costs. The cost that would be paid in local currency is Kshs.29.2 (\$1.7) million, or 25% of the total; the cost to be paid in foreign currency is Kshs.89.5 (\$5.3) million, or 75%.

Table 1. Cost of East Coast fever control by the infection-and-treatment method per animal immunized and per dose of vaccine based on Kenya circumstances, 1988

Item	Total cost over a 30-year period	
Capital costs	Kshs	12 699 379
Operating costs	Kshs	<u>95 229 502</u>
Total capital and operating costs	Kshs	107 928 881
10% contingency	Kshs	<u>10 792 888</u>
Total cost ^a	Kshs	<u>118 721 769</u>
Less remaining value of capital items ^b	Kshs	<u>1 675 355</u>
Net total cost	Kshs	<u>117 046 414</u>
No. of cattle immunized ^c		2 900 000
Cost per animal	Kshs	40.36
Cost per animal ^d	US\$	2.37
Net total cost	Kshs	117 046 414
Less field immunization cost ^e	Kshs	68 802 500
Less 75% operating transport cost ^f	Kshs	1 783 500
Less 10% of 75% operating transport cost	Kshs	<u>178 350</u>
Net vaccine production cost	Kshs	<u>46 282 064</u>
No. of vaccine doses produced ^g		3 045 000
Cost per dose	Kshs	15.20
Cost per dose ^d	US\$	0.89

a Total cost from study calculations.

b Remaining capital value at end of year 30.

c Assumed as: 25,000 animals in year 1 75 000 animals in year 2, and 100 000 animals per year in years 3-30.

d Exchange rate in January 1988: Kshs.17.00 to US\$1.00.

e Field immunization cost includes syringes, needles and antibiotics and excludes transport.

f Operating transport cost for field studies, vaccine delivery and monitoring; 75% for vaccine delivery.

g Assumed 5% more than number of cattle immunized above.

PRODUCTION AND DELIVERY COSTS

For the 30-year planning period, it was assumed 2.9 million cattle would be immunized. The cost of immunization per animal over the animal's life-time was estimated to be Kshs.40.36 (\$2.37), with a range of Kshs.36.31–50.45 (\$2.14–2.97). The cost of vaccine production per dose was estimated at Kshs.15.20 (\$0.89), with a range of Kshs.7.62–20.07 (\$0.45–1.18).

The cost of immunization to the livestock producer, assuming that the producer buys the vaccine from the government and that it is delivered at cost, would be the cost of taking the animal to and from the place of immunization. Such cost, whether an opportunity cost of family labour or a cash cost of hired labour, was not included in the above calculations. To assess the cost effectiveness of the infection-and-treatment method, the above costs of immunization plus the producer's labour cost and the cost of strategic acaricidal applications against ticks and tick-borne diseases should be compared to the costs of controlling ticks and tick-borne diseases by applying acaricides and using chemotherapeutic drugs.

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Buparvaquone, the new antitheilerial: a review of its efficacy and safety

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Buparvaquone is a second-generation hydroxynaphthoquinone related to parvaquone, with novel features that make it a promising compound for the therapy and prophylaxis of all forms of theileriosis. It has been tested extensively against *Theileria annulata*, *T. parva* and *T. sergenti*, both in laboratory studies and in field trials, and it has undergone a rigorous programme of toxicology and safety studies. Formulated as a solution for intramuscular injection (Butalex, Coopers Animal Health), it offers a safe and convenient alternative to existing antitheilerial products.

FEATURES OF BUPARVAQUONE

Buparvaquone was selected for development using the following three principal criteria:

- *In vitro* activity: EC₅₀ of 0.0003 mg/L (10^{-9} M) against *Theileria parva*
- Long plasma half-life (at least 7 days)
- Low toxicity: oral LD₅₀ in rats > 8000 mg/kg

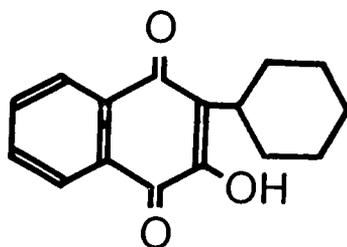
The high *in vitro* activity against *T. parva* (Muguga) E174 and the persistence in plasma following intramuscular injection may both be related to the tertiary-butyl moiety in the buparvaquone molecule, which results in far slower metabolism to the hydroxy derivative at the 4-position on the cyclohexyl ring (Figure 1). Parvaquone, which is much more readily metabolized, has an EC₅₀ of 0.006 mg/L (1.3×10^{-8} M) and persists in plasma for only about two days. A high-pressure liquid chromatography study (Kinabo and Bogan, 1988) confirms that buparvaquone persists longer in plasma than parvaquone. Acute oral toxicity in the rodent is similarly very low (> 8000 mg/kg) for both compounds.

EFFICACY AGAINST STABILATE-INDUCED THEILERIA INFECTIONS

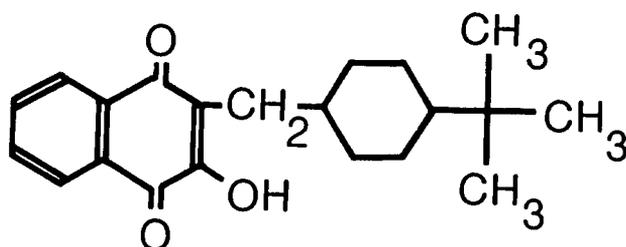
When formulated as a 5% solution for injection (Butalex) and injected by the intramuscular route, a dose of 2.5 mg/kg is highly effective against *T. parva* (McHardy et al., 1985), *T. annulata* (McHardy et al., 1985; Dhar et al., 1986)

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and *T. sergenti* (Minami et al., 1985). However, Linyoni et al. (in preparation) obtained less satisfactory results, especially with *T. parva lawrencei*.



parvaquone



buparvaquone

Figure 1. The structure of the antitheilerial compounds parvaquone and buparvaquone.

Dolan et al. (1988) showed that parvaquone (formulated as Clexon) is liable to eliminate stabilate infection of *T. parva* if infection and drug are injected simultaneously. Clexon therefore is unsuitable for use in a single-treatment infection-and-treatment system of immunization. McHardy and Wekesa (1985) reported that buparvaquone (2.5 mg/kg) injected at the same time as infection with *T. parva* suppressed but did not eliminate the infection at the schizont stage, and no piroplasms were detected in any of the 10 treated calves. All 10 untreated controls died of theileriosis (Table 1). Although treated animals were not challenge-infected, serology on five of them indicated homologous immunity.

Similarly encouraging results have been obtained since by various workers using *T. parva*. Mutugi et al. (1988) have reported good protection with *T. p. lawrencei*, and other such reports occur elsewhere in these proceedings. Similar results were obtained with *T. annulata* by Dhar et al. (1987).

In view of the prolonged plasma persistence of buparvaquone following intramuscular injection, McHardy and Wekesa (1985) also investigated the pro-

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phylactic effect of the compound by injecting livestock with 2.5 mg/kg buparvaquone seven days before infecting them with *T. parva* (Muguga) stabilate. Clinical disease was delayed significantly, and 7 of 10 calves survived to day 28, whereas all 10 untreated controls had died by day 23. U.V. Shastri (unpublished results) has demonstrated that in the face of heavy and consistent challenge with *T. annulata*, calves can be protected against clinical disease by administering buparvaquone. In a herd of calves in which theileriosis was endemic, he treated 18 clinical cases with 2.5 mg/kg buparvaquone. Ten were cured and of the seven that died, three also contracted enteritis. Ten in-contact but clinically normal calves were also treated and none developed clinical theileriosis. Of six similar in-contact calves, which were left untreated, four died of theileriosis (Table 2).

Table 1. Effect of simultaneous infection with *Theileria parva* (Muguga) and treatment with buparvaquone 2.5 mg/kg, injected intramuscularly

Parameter	Untreated controls	Buparvaquone 2.5 mg/kg at time of infection
Survival	0/10	10/10
Day of first schizont	8.2 ± 0.92	9.7 ± 1.6
Days of healthy schizonts	12.8 ± 1.3	0
Days of damaged schizonts	0	6.5 ± 1.9
Max. % piroplasms	57.7 ± 10.9	0

Table 2. Prophylactic trial of buparvaquone, 2.5 mg/kg in calves infected with *Theileria annulata* (U.V. Shastri, personal communication)

Clinical state	No.	No. treated	No. survived
Sick	17	17	10 *
Normal, in contact	10	10	10
Normal, in contact	6	0	2

* 3 deaths due to theileriosis and enteritis.

FIELD TRIALS WITH BUTALEX

Three major field trials have been conducted to observe the efficacy of Butalex in the therapy of theileriosis. Musisi et al. (unpublished results) tested a single dose of 2.5 mg/kg injected intramuscularly in 68 cattle diagnosed as infected with East Coast fever in Zambia. A cure rate of 91% was achieved (Table 3).

Butalex has also been tested against *T. annulata* infection in several countries. The largest trials have been in India and the Soviet Union. The Indian results are summarized in Table 4.

Table 3. Efficacy of buparvaquone at 2.5 mg/kg against field cases of *Theileria parva*, Zambia (F.L. Musisi, personal communication)

Severity of infection	No. treated	No. cured
Mild	20	20
Moderate	18	16 *
Severe	30	26
Total	68	62 (91%)

* 1 died of babesiosis.

Table 4. Efficacy of buparvaquone at 2.5 mg/kg against field cases of *Theileria annulata*, India 1987

Triallist	No. treated	No. cured	% cured
S. Dhar et al.	19	19	100.0
B.B. Verma	16	15 *	93.7
R.D. Sharma et al.	48	44	91.7
Total	83	78	94

* 1 animal died within 12 hours of treatment.

In all of these cases a single dose of Butalex was given. Dhar et al. (1986) have found that if haemoglobin is 6 g/100 ml or greater at the time of treatment, no supportive treatment is necessary to combat anaemia. If haemoglobin is 3–6 g/100 ml, treatment with iron and vitamins is advisable, but recovery can then be expected. When haemoglobin is below 3 g/100 ml, the prognosis is not good, even if supportive therapy is given.

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In the trials in the Soviet Union, conducted under the aegis of the All-Union Institute of Experimental Veterinary Medicine, in Moscow, a total of 402 cattle infected with *T. annulata* were treated, most with a single dose of Butalex (2.5 mg buparvaquone/kg), though some received up to three doses. An overall cure rate of 86% was achieved (Table 5).

Table 5. Efficacy of buparvaquone 2.5 mg/kg against field cases of *Theileria annulata* in the Soviet Union (V.T. Zablotsky, personal communication)

Clinical state	No. treated	No. cured	% cured
Mild (< 1% piroplasms)	97	96	98.9
Moderate (1–40% piroplasms)	293	244	83.3
Severe (> 40% piroplasms)	12	6	50.0
Total	402	346	86.1

In a further field trial in Egypt, Michael, el Refaii and McHardy (in press) treated dairy cattle known to be carrying low-grade infections of *T. annulata* and whose milk yields were very low. In a herd of 44, 22 were selected at random and injected with a single 2.5 mg/kg dose of buparvaquone. Over the next seven weeks the treated animals were clinically normal and their milk yield (though relatively low) was twice that of the untreated group of 22 cattle.

Thus, in addition to showing real promise as a therapeutic for theileriosis, particularly *T. annulata* infection and in the infection-and-treatment method of immunization, buparvaquone treatment may also improve the productivity of cattle carrying sub-clinical theilerial infections. Further research in all of these areas therefore seems justified.

MODE OF ACTION OF BUPARVAQUONE

The mode of action of buparvaquone on *Theileria* is not yet established, although studies by M. Fry et al. (unpublished) indicate an effect on energy generation, as demonstrated in coccidia (Fry et al., 1984). Electron microscopical studies on the effect of buparvaquone on *T. parva* schizonts in cultured lymphoid cells (N. McHardy and J. Beesley, unpublished results) show progressive vacuolation of the cytoplasm as the principal lesion. The surface membrane and the nuclear membrane remain apparently unaffected until the cytoplasmic disruption is very advanced. There is no apparent adverse effect on the structure of host lymphocytes, including mitochondria, which emphasizes the parasite-specificity of the mode of action of buparvaquone. It may also help to explain

the narrow spectrum of action of the compound, which is confined to certain sporozoan parasites (Table 6).

SAFETY STUDIES

An extensive range of safety studies has been conducted on buparvaquone and on the formulated injection, Butalex. Safety is established both for treated cattle and for humans consuming milk and meat from treated animals. The product is very safe. A milk-withholding period of two days is recommended, and cattle should not be slaughtered for human consumption within 42 days of treatment.

Thus, buparvaquone has a range of features that would justify further studies in the following three major areas for the control of theileriosis:

- a) therapy of clinical disease
- b) productivity improvement in carrier infections
- c) infection-and-treatment immunization

Table 6. Spectrum of activity of buparvaquone against selected protozoa and rickettsia

Efficacy	Source
Effective	
<i>Babesia equi</i>	G. Zaugg (in press)
<i>B. gibsoni</i>	Lu & Wong (personal communication)
<i>B. canis</i>	N. McHardy & P.K.I. Mackenzie (unpublished results)
<i>B. ovis</i>	N. McHardy & P.K.I. Mackenzie (unpublished results)
<i>Theileria sergenti</i>	T. Minami et al. (1985)
<i>T. buffeli</i>	A.J. de Vos (personal communication)
<i>T. hirci</i>	Z. Abbas and O.M. Osman (personal communication)
<i>Plasmodium</i> spp.	A.T. Hudson et al. (1986)
<i>Eimeria</i> spp.	A.T. Hudson et al. (1986)
Ineffective	
<i>Anaplasma marginale</i>	N. McHardy and P.K.I. Mackenzie (unpublished results)
<i>Cowdria ruminantium</i>	N. McHardy and P.K.I. Mackenzie (unpublished results)
<i>B. bigemina</i>	N. McHardy and P.K.I. Mackenzie (unpublished results)
<i>Cytauxoon felis</i>	E. Mitema (personal communication)

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ACKNOWLEDGEMENT

The involvement and enthusiasm of research workers around the world in studies on the efficacy and value of buparvaquone is gratefully acknowledged.

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The use of cypermethrin-impregnated ear-tags as an adjunct to East Coast fever immunization

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Clinical records maintained at the Ole District Livestock Extension Centre, Pemba, Tanzania, show that most cross-bred calves became infected with *Theileria parva* at a very early age, in spite of once weekly hand-spraying with coumaphos (Asuntol, Bayer). The mean age of detection of clinical infection with East Coast fever (ECF) in 50 cross-bred calves born in the Ole District between July 1985 and February 1986 was 63 days. The earliest infection was detected at 15 days. Approximately one-third of cross-bred calves treated with parvaquone (Clexon, Coopers Animal Health) at 10 mg/kg bodyweight suffered relapses or new patent infections, suggesting that although parvaquone is better than any previous chemotherapeutic agent, it is not entirely satisfactory. It is likely that artificially induced immunity using the infection-and-treatment method of immunization will produce a safer and more solid protective immunity.

It is not normally recommended to vaccinate calves under two months of age. Because of this and the evidence of early ECF infections in spite of once weekly hand-spraying with coumaphos, the efficacy of cypermethrin-impregnated ear-tags as an adjunct to tick control was investigated. It was thought that since the vector of ECF, *Rhipicephalus appendiculatus*, prefers to attach itself to the ears and head of livestock, this method of control might be particularly effective.

Cypermethrin-impregnated ear-tags (Decum, Fearing, USA) were placed in both ears of 20 East African shorthorn Zebu (*Bos indicus*) × Jersey (*Bos taurus*) calves at birth and removed at 8 weeks of age. The calves were hand-sprayed with coumaphos (Asuntol, Bayer) at weekly intervals. During the eight-week period of intensive tick control, no *T. parva* infections were recorded in this group of calves, either clinically or by blood and lymph gland biopsy smear examinations. Within three months of removal of the ear-tags, all 20 calves became infected with *T. parva*, which was confirmed microscopically.

The intensive tick control practised in this trial protected the calves against *T. parva* infection. If vaccination cannot be given until the calves have reached immunological maturity, the use of acaricide-impregnated ear-tags may be a

CYPERMETHRIN-IMPREGNATED EAR-TAGS

practical way of protecting calves until they can be immunized. However, if ticks are kept off calves completely for too long a period, the calves may not develop a natural immunity to *Anaplasma marginale* or *Babesia* species while still protected by natural age-related resistance.

ACKNOWLEDGEMENT

Cypermethrin-impregnated ear-tags were kindly supplied by Dr. A.S. Young, of the Kenya Agricultural Research Institute, Muguga, Kenya.

Classification of the Protozoa

Phylum III. **APICOMPLEXA** Levine, 1970

Class 2. **SPOROZOEAE** Leuckart, 1879

Subclass 3. **PIROPLASMIA** Levine, 1961

Order 1. **PIROPLASMIDA** Wenyon, 1926

Babesia

Dactylosoma

Theileria

From Levine et al (1980). A newly revised classification of the protozoa. *Journal of Protozoology* 27 p. 37–58

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PART 4

RECOMMENDATIONS AND INFORMATION EXCHANGE

Recommendations of the Committee on Co-ordination

- I. Regular meetings on tick-borne diseases and their vectors should be held.
 - A. These meetings should be attended by senior government veterinary policy-makers and research workers from countries within the East African region in which theileriosis occurs.
 - B. These meetings should be organized under the aegis of the Organization of African Unity in collaboration with international organizations directly involved in tick-borne disease control.
 - C. The topics for the meetings should be built around recent progress on theileriosis research and control and should include tick-borne diseases and their vectors.
- II. There should be a programme for tick-borne disease and vector control within the region which should:
 - A. be designed initially as a five-year programme of control and be subject to regular review
 - B. examine the needs of individual countries and recommend financial support from donors
 - C. harmonize national and/or regional programmes
 - D. adopt a standard disease-recording system to monitor the effects of control programmes
 - E. monitor and advise country projects so that the continuity of national programmes is not lost because of erratic donor support
- III. Training at both professional and technical levels should be encouraged by the following:
 - A. using the expertise that exists within the countries of the region
 - B. retaining staff trained in specialized areas within their ministries or departments and sustaining and encouraging their career developments
- IV. The long-term success of tick-borne disease control will depend on the existence of a cadre of skilled personnel within individual countries who have adequate resources to implement control programmes. Therefore:
 - A. in planning projects, expatriate input should be limited and directed towards training local manpower
 - B. funding for projects should be organized so that the projects are integrated into the national disease control programmes.

Recommendations of the Committee on Safety in the Preparation and Distribution of *Theileria* Stabilates

I. Safety in Handling Ticks

A. Field-Collected Ticks

Consideration should be given to the possible hazard to people from pathogens present in field-collected ticks. The most important pathogen recognized is Crimean Congo haemorrhagic fever virus, usually associated with ticks of the genus *Hyalomma* and widely prevalent within the geographic distribution of *Rhipicephalus appendiculatus*.

1. Persons handling field tick collections should be made aware of potential hazards.
2. Ticks of the *Hyalomma* species generally should not be removed from hosts; engorged or partially engorged ticks should not be crushed in the fingers.
3. If they must be removed, ticks should be handled with forceps.

B. Tick Handling Facilities

The handling of field-collected ticks in the laboratory must be controlled to avoid accidental attachment to personnel.

1. Field-collected ticks should be fed on rabbits and cattle in isolation facilities.
2. Animals on which ticks have fed should be destroyed following transmission and pick-up.
3. Following engorgement of field-collected ticks on laboratory animals, aliquots should be homogenized and tested for extraneous human pathogens by inoculation in neonatal mice and BHK and Vero cells. The effects of these inoculations should be studied through three passages.
4. Any unused ticks should be destroyed by chemical means or by incineration.

C. Stabilate Preparation

Care should be taken in the preparation of sporozoite stabilates to avoid aerosol infection of personnel with extraneous pathogens during the grinding of ticks.

1. Personnel grinding ticks should be educated in the potential hazards involved.
2. Access to areas where ticks are homogenized should be restricted to specified and informed personnel.
3. Personnel should wear protective clothing, including gloves and masks.
4. Tick grinding should be carried out under a bio-hazard hood with a negative air pressure.

D. Improving Awareness of Tick-Transmitted Zoonotic Pathogens

Safety for laboratory personnel can be improved if information is made more widely available and greater care is taken with identification of pathogens and diagnosis if personnel become ill.

1. Lists of potential human pathogens such as *Rickettsia conori*, *Borrelia* sp. (Lime disease), *Babesia divergens*, *B. microti*, *Ehrlichia sennetsu* and *E. canis* should be prepared, and known tick associations, distribution and human disease syndromes should be documented.
2. Methods for identifying extraneous pathogens in stabilates should be employed where possible.
3. Improved methods for identifying zoonotic viruses, rickettsia and bacteria in ticks should be developed.
4. Any suspected occurrence of human infection from ticks should be fully investigated and documented.

II. Safety for Cattle

A. Safety and Purity of Stabilates

Both ticks and experimental mammals are potential sources of contamination of stabilates with extraneous pathogens. In both cases, potential contaminants include *Ehrlichia bovis*, *Borrelia* sp., Oribi viruses, Bunya viruses and others.

1. Field ticks should not be used for the preparation of bulk stabilates. Well-characterized and pathogen-free laboratory colonies of ticks should be used for this purpose.
2. Only clean and healthy cattle and rabbits should be used for tick feeding.
3. Stabilates should be prepared in as sterile a manner as possible. Under some conditions, the use of antibiotics at concentrations appropriate for tissue culture may be indicated.
4. Prepared stabilates should be subjected to routine tests for safety by inoculation into neonatal mice and BHK and Vero cells, followed by three passages in these systems.

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5. Stabilates should be subjected to routine characterizations *in vivo*, which should involve infectivity testing in splenectomized and intact susceptible cattle, sensitivity to tetracyclines and other antitheilerial drugs and cross-immunity *in vivo*.

B. Contamination of Stabilates with other *Theileria parva* Parasites

Care must be taken to avoid contamination of the infection being used for preparation of stabilate in the laboratory with other *T. parva* stocks.

1. A set of rules should be established for handling infected ticks and the rules adhered to rigidly.
2. Tick unit facilities should allow for the strict separation of infected and uninfected ticks.
3. Tick unit personnel should use separate overalls for each batch of ticks used in stabilate preparation and the overalls should be sterilized daily.
4. Work should not be carried out on many different stocks simultaneously.
5. Stabilate storage systems should incorporate clear labelling of each stabilate tube.

III. Effect of an Immunizing Stock

The introduction of an immunizing stock that does not originate from that area may result in that parasite or a component parasite(s) of that stock becoming established through a carrier state in cattle and ticks. The long-term effect of the introduction of new (and potentially lethal) parasites on the disease epidemiology should be monitored.

The characterization of parasites in target populations should be carried out before immunization and at intervals following immunization.

Recommendations of the Committee on Nomenclature

The committee originally chosen to discuss nomenclature was joined by a majority of the participants at this meeting. In addition to the recommendations listed below, it was proposed and adopted by the meeting that comments on nomenclature be included in the proceedings (see Appendix 1). It was further proposed and adopted that these comments be presented to the International Commission on Zoological Nomenclature.

I. The following definitions should be adopted.

- A. *Isolate*. Viable organisms, isolated on a single occasion from a field sample, in experimental hosts or culture systems, or prepared as a stabilate.
- B. *Stock*. All the populations of a parasite derived from an isolate without any implication of homogeneity or characterization. Populations comprising a single stock thus include infected cell lines and tick stabilates and subsequent parasite preparations derived from them.
- C. *Line*. A laboratory derivative of a stock maintained under defined physical conditions, for example, maintained as a culture of parasitized bovine lymphoid cells.
- D. *Parasite clone*. *Theileria* species line derived from a single parasite.
- E. *Cell clone*. *Theileria* species line derived from a single parasitized cell.
- F. *Strain*. A population of homogeneous organisms possessing a set of defined characters. Unambiguous characterization of a strain can be assured only if the population of organisms was initiated from a parasite clone.
- G. *Stabilate*. A sample of organisms preserved alive (usually in replicate) on a single occasion.

In accordance with these definitions, the term "strain" should be reserved for cloned parasite populations that have been precisely defined; where such definition has not been carried out, the terms "isolate" or "stock" should be used, according to the circumstances. (These definitions are essentially those from Irvin, A.D., Dobbelaere, D.A.E, Mwamachi, D.M., Minami, T., Spooner, P.R. and Ocama, J.G.R. [1983]. *Research in Veterinary Science* 35: 341–346.)

- ## II. Geographical and locational names should *not* be used for isolates or stocks and a code and numbering system should be adopted identifying:

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- A. parasite
 - B. originating laboratory
 - C. laboratory reference number
- III. The confusion in taxonomy must be resolved by consultation with expert opinion and within accepted international rules governing nomenclature.

Information exchange

Introduced by T.T. Dolan

The idea of a network for the exchange of information within the region affected by East Coast fever had been discussed at previous workshops. In the October 1984 workshop ten countries had expressed their interest in participating in such a network. The September 1985 workshop on collection, handling and analysis of performance and productivity data had the development of a network specifically in mind. At this latter meeting standardized data recording sheets were presented and analyses discussed and types of computer hardware and software that would allow the easy exchange of data were suggested. Despite the enthusiasm of some countries for this approach, no formal network has been established. However, informal exchanges have continued among workers in different countries and between these workers and others in international institutes or donor-funded projects in national institutes. The most consistent exchange has been between the various programmes of the Food and Agriculture Organization of the United Nations (FAO) and associated national programmes in the region. Some countries or workers within countries felt that they did not want their data handed around freely or they felt competent to handle their data independently. This was particularly so for countries with very active programmes. Countries, of course, must decide what they will do with their data and the aim of the network was not to use other people's data but to provide a framework within which ideas could be exchanged and data analysed using standardized methods.

One form of exchange is through workshops such as this, organized by ILRAD, FAO and OAU. They provide an opportunity to present recent research findings with a practical application and, in the case of this workshop, to highlight problems encountered with immunization. The formal presentations are complemented by informal exchanges, which may be more useful for individual workers.

The session was then opened for discussion and the following is a summary reported by Dr. J. Crees.

- 1) Dr. Dolan proposed that another meeting should be held. Dr. Masiga said that the Organization of African Unity (OAU) would be keen to support this meeting. Topics proposed included immunization against babesiosis and against ticks, in addition to theileriosis. Dr. Young proposed that more emphasis be given to chemotherapy and economics. Dr. McHardy said that the Co-ordination Committee's recommendation that requests for buparvaquone should be submitted through national authorities was acceptable to

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him only in so far as requests did not exceed supply. He did not wish to be personally responsible for allocating priorities among applicant countries.

- 2) Dr. Dolan referred back to his undertaking to co-ordinate the re-designing of the standard recording forms. Dr. Young drew attention to ILRAD's software for analysis of data from immunization trials. Dr. Gettinby advised that people do their own data analysis as far as possible and added that copies of the analytical programme could be made available by ILRAD. He suggested that potential users might be able to come to ILRAD for training in its use. He stressed that IBM-compatible hardware would be needed to run it.

Dr. Perry stressed that data on the economic aspects of ECF control should be collected.

- 3) Dr. Dolan recommended mapping confirmed outbreaks of tick-borne disease to give more accurate and detailed data on distribution. It will be essential to have better information on ticks and disease distribution if changes in tick or disease control policies are to be recommended. Dr. Masiga confirmed that disease reporting by member states of the OAU left much to be desired. It had been agreed that member states would submit copies of reports being prepared for the Office International des Epizooties, the Food and Agriculture Organization and the World Health Organization under pre-existing schemes to OAU/IBAR. A course has been held in Dakar for francophone countries on improving reporting standards and a course for anglophone countries is to be held in Tanzania. Dr. Perry proposed that the meeting should support the OAU's initiative, which was accepted.

Dr. Mkandawire drew attention to the poor state of within-country reporting, and suggested that every effort be made to improve national disease-reporting systems.

Dr. Norval proposed that maps that exist on the distribution of ticks and tick-borne diseases should be circulated among conference participants.

- 4) Dr. Kariuki stressed that there is a need for a test for the immunogenic nature of specific national parasite stocks for purposes of international certification, and asked when one might be available. Dr. Dolan said that work would continue on the characterization of stocks and clones from selected stocks of *Theileria parva*, but the process of developing characterization reagents for use *in vitro* would take time. Other institutions expected ILRAD to lead in such matters, but he noted that the characterization achieved so far, with monoclonal antibodies and other methods, did not correlate with the immunizing properties observed *in vivo*. These laboratory techniques provided reliable markers for individual stocks within the

laboratory, but no laboratory test of immunizing capacity is available.

- 5) Dr. Dolan proposed that a sub-committee be formed specifically to define criteria of severity of disease reactions so that control animals and breakthrough cases in immunization trials may be classified and salvaged by therapy rather than left to die. Dr. Young was selected as chairman, with Drs. Mutugi, Morzaria, Musisi and Gettinby as observers. (See Appendix 2.)

APPENDIX 1

NOMENCLATURE IN *THEILERIA*

The Oxford dictionary defines nomenclature as the terminology of a science. The brief of this committee was to consider the terminology being used by those working on theileriosis in Africa so as to achieve consistency among different laboratories. This should help communication between laboratories and veterinary services in different countries and provide consistency in scientific publication. Scientists working on theileriosis have discussed nomenclature over the years and believe that the new techniques and types of study provide the opportunity for a much tighter nomenclature than has been available previously.

The following recommendations have been assembled for further discussion with appropriate authorities on theileriosis and the International Commission on Zoological Nomenclature.

I. *Theileria* Species

There does not appear to be a problem in classifying *Theileria* species. The following species infecting cattle have been recognized in Africa:

Theileria annulata
Theileria mutans
Theileria orientalis
Theileria parva
Theileria taurotragi
Theileria velifera

There may be one or two more species infective to cattle that have yet to be described and further work is required to characterize *T. orientalis* fully.

Theileria taurotragi is an important parasite with a wide host range and a high prevalence in countries in the East African region. Serological tests may not be specific for a particular *Theileria* species because of shared antigens. Serological cross-reactions have been shown between *T. parva* and *T. annulata*, and *T. parva* and *T. taurotragi*. A panel of monoclonal antibodies against *Theileria* antigens are useful for differentiating species, as are DNA characteristics and probes. New diagnostic reagents would be very useful for differentiating *T. parva* from *T. taurotragi*.

* A copy of this Appendix has been sent to the International Commission on Zoological Nomenclature.

II. Sub-Species of *Theileria parva*

The sub-speciation of *T. parva* is an area in which change is urgently required. Recent results suggest that the sub-speciation of *Theileria parva* into *T. p. parva*, *T. p. lawrencei* and *T. p. bovis*, which was adopted for convenience rather than scientific accuracy, has no biological validity. It has been demonstrated that genetically these sub-species are not distinguishable. In one study it has been shown that the *T. p. bovis* parasite may be more closely related to one particular *T. p. parva* isolate than to other *T. p. bovis* isolates. In addition, it has been possible to transform *T. p. lawrencei* so that it behaves like *T. p. parva* in cattle. Further work is required to establish whether this change is due to a transformation in the behaviour of *T. p. lawrencei* in a different host or to selection of a sub-population of *T. p. lawrencei* that behaves like *T. p. parva*. Because many people working on *Theileria* adopted the trinomial system due to its convenience, this system became commonly used. It is recommended that the trinomial system be dropped. Descriptive terms, based on existing usage, could still be acceptable, such as "cattle-derived" and "buffalo-derived". This recommendation is believed to conform to international standards of nomenclature, but it is recognized that more discussion is needed to establish an acceptable system of suffixes to replace the present one, while retaining the convenience of its well understood shorthand.

III. Terminology Recommended for Handling and Characterizing *Theileria* Parasites

- A. *Isolate*. Viable organisms, isolated on a single occasion from a field sample, in experimental hosts or culture systems, or prepared as a stabilate.
- B. *Stock*. All the populations of a parasite derived from an isolate without any implication of homogeneity or characterization. Populations comprising a single stock thus include cell lines and tick stabilates and subsequent parasite preparations derived from them.
- C. *Line*. A laboratory derivative of a stock maintained under defined physical conditions, such as a culture of parasitized bovine lymphoid cells.
- D. *Strain*. A population of homogeneous organisms possessing a set of defined characteristics. Unambiguous characterization of a strain can be assured only if the population of organisms was initiated from a parasite clone.
- E. *Stabilate*. A sample of organisms preserved alive (usually in replicate) on a single occasion.
- F. *Clone*. Genetically identical organisms derived from a single cell by asexual division.

G. *Parasite clone. Theileria* line derived from a single parasite.

H. *Cell clone. Theileria* line derived from a single parasitized cell.

IV. Classification of Stabilates

It is important to get away from the system of classifying stocks by geographical areas of origin. Instead, the nature of the stock, established using an increasing number of tests for their characterization, should be emphasized. Because a parasite stock is isolated from a certain geographical area, such as the Kenya coast, does not mean that the stock is different from those isolated in other parts of Kenya or in other countries.

Once adequate *in vitro* characterization techniques have been developed and validated, it will be possible to advise those responsible for national or international disease control on the safety of particular stocks for use in a country. The removal of geographical designations will assist this process. This has already been done for trypanosomes and malaria. This information can be easily included in the database concerning the stabilate.

The stabilate reference should contain data on the following:

A. Species of *Theileria*

For this purpose, *Theileria* should be abbreviated to *Th.* to distinguish it from *Trypanosoma*. Single-letter suffixes will denote species, for example, *Th.p.*, *Th.t.*, *Th.m.*, *Th.a.*, *Th.v.* and *Th.o.*

B. The Laboratory that Isolates a Stock

The second identifier would be an abbreviation of the laboratory that isolates the stock. In Kenya, for example, three institutes may be producing stabilates: the National Veterinary Research Centre (NVRC), Muguga; NVRC, Kabete; and ILRAD. These would be abbreviated:

Muguga	MU
Kabete	KA
ILRAD	IL

Hence, *Th.t.* IL would be used for an ILRAD *T. taurotragi* stabilate.

C. Heterogeneity of Stabilate

If there is evidence of a mixed *Theileria* species infection in the stabilate, such as *T. parva* and *T. taurotragi* at ILRAD, it should be identified as *Th.t./Th.p.* IL. If a *T. taurotragi* stabilate at NVRC, Kabete, was contaminated with *Erlichia bovis*, it should be identified as *Th.t./E.b.* KA.

D. Reference to Stabilate

If a parasite that we now refer to as *T. p. parva* (Marikebuni) was the first

stock isolated by ILRAD, it would be referred to as '1', for example, *Th.p.* IL 1. The first stabilate prepared from this stock would be referred to as *Th.p.* IL 1.1. It may be a reference stock and subsequent larger working stabilates would be referred to as *Th.p.* IL 1.2, and so on. If *Th.p.* IL 1.2 was cloned, it would then become *Th.p.* IL 1.2.1, and so on.

It is suggested that the use of the term "Muguga cocktail" be discouraged and be replaced by a trinomial designation of its three components. The removal of all references to the geographical origins of stabilates as mentioned above could bring the added benefit of facilitating the transfer of stabilates from one country to another. However, the movement of these stabilates must be with the authority of the governments involved and within the regulations on the movement of biologicals.

IV. Cloning Definitions

The following comments are included for consideration, but final recommendations must be made by a competent body of experts. This can be done only when studies have established which stages of the parasite are diploid and which haploid.

Cloning *Theileria* parasites is complicated because it is not known when meiosis occurs. Sexual reproduction occurs when micro- and macrogametes arising from piroplasms in erythrocytes fuse in the tick gut. Taking the example of *Plasmodium*, the parasite would continue through zygote and kinete stages as a diploid organism and reduction division would occur during sporogony, producing haploid sporozoites. Alternatively, but less likely, the sporozoite and schizont could remain diploid and reduction division might occur during merogony. Yet another possibility is that reduction division may occur at the zygote stage. Until this is known, care must be taken using the term "clone", remembering that a clone divides asexually and that the occurrence of meiosis may invalidate the genetic identity of a clone.

The possibilities for cloning at present are (1) inoculation of ticks with a single kinete, (2) selection of salivary glands with one infected acinus that by chance would be a product of one kinete, (3) the infection of cells with one sporozoite and (4) infected cell cloning. An ideal clone would be derived from a single kinete, a single sporozoite or a single schizont-infected cell. Any two of these methods used together are probably acceptable. The identification and stability of a clone can be checked by the methods of parasite characterization described earlier (see S.P. Morzaria, this meeting). An additional problem is that cloned theilerial parasites, as with malarial parasites, can produce both micro- and macrogametes and these parasites can be transmitted by ticks. This allows further genetic recombination.

Under these circumstances, the only "strains" that are likely to be produced from the heterogeneous stocks already isolated will have to be derived by

cloning and then fully characterized. In infection-and-treatment immunization it will be essential to continue using well-characterized stocks. It may be possible to manipulate the parasites by cloning and obtain individuals that provide a wide spectrum of protection and a low pathogenicity, so rendering unnecessary the use of drug treatments.

VI. Tick Vector Species

The currently accepted vectors of the important *Theileria* species are:

<i>T. annulata</i>	<i>Hyalomma</i> species
<i>T. parva</i>	<i>Rhipicephalus appendiculatus</i> complex
	<i>Rhipicephalus appendiculatus</i>
	<i>Rhipicephalus zambeziensis</i>
	<i>Rhipicephalus duttoni</i>
<i>T. taurotragi</i>	<i>Rhipicephalus appendiculatus</i>
	<i>Rhipicephalus pulchellus</i>
	<i>Rhipicephalus zambeziensis</i>
<i>T. velifera</i>	<i>Amblyomma</i> species
<i>T. mutans</i>	<i>Amblyomma</i> species
<i>T. orientalis</i>	<i>Amblyomma</i> species

In the last ten years some important revisions have been made in our knowledge of the ticks that transmit different *Theileria* species. Identification of tick vectors must be more critical than in the past and newer techniques, such as hydrocarbon profiles of cuticle and isoenzyme analysis of tick tissues, should be applied for the correct identification of closely related species.

It would be useful to assemble centrally any new data on the tick vectors of *Theileria*.

VII. Laboratory and Field Colonies of Tick Vectors

Interest in differences observed between laboratory raised ticks and field populations used in experiments has increased over the last few years. Enclosed and inbred laboratory populations may not represent the true type or behaviour of ticks in the field. Some laboratories use samples of local tick populations rather than laboratory stocks. Differences among laboratory populations have also been found. Studies of these differences should be encouraged and expanded. The word "strain" should not be used for a particular laboratory population unless it is fully characterized; "stock" should be used instead. In this way, consistency in terminology would be established between the *Theileria* parasites and their tick hosts. Thus, if the first field stock of *R. appendiculatus* established at Muguga was from the Trans-Mara, it would be referred to as *R.a.* MU.1.

APPENDIX 2

CLASSIFICATION OF *THEILERIA PARVA* REACTIONS IN CATTLE

It was decided during the discussion on Information Exchange that a small committee should draft a classification of *Theileria parva* reactions in cattle. This classification could then be used in reporting experiments or trials so that a generally accepted description of disease reactions is used and so that the interpretation of results from different studies by different authors is made easier.

The following clinical and parasitological parameters are used routinely in describing *T. parva* reactions:

Clinical

- fever, a rectal temperature greater than 39.4 °C associated with schizont parasitosis is considered a febrile reaction
- condition, general appearance of the animal, respiratory rate and consistency of faeces
- lymph node enlargement associated with hyperplasia, parasitosis and fever
- lacrimation and/or corneal opacity
- white blood cell count
- time to death or recovery

Parasitological

- prepatent period to detection of schizonts
- dissemination of schizonts
- degree of schizont parasitosis
- duration of schizont parasitosis
- prepatent period to detection of piroplasms
- piroplasm parasitaemia
- duration of parasitaemia

Broad classifications of reactions have been attempted using combinations of these parameters. A generally accepted and recommended classification is as follows:

- *No reaction or no apparent reaction:* no parasites are detected and no clinical signs are apparent.
- *Mild reaction:* few schizonts are detected, no fever occurs or fever persists for less than four days. The animal is otherwise clinically normal and recovers.

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- *Moderate reaction:* schizonts are detected, fever persists for longer than four but less than nine days. The animal shows mild and transient clinical signs and recovers.
- *Severe reaction and recovery or death:* schizonts are detected, fever persists for eight days or longer and the animal has obvious clinical signs of theileriosis. The animal may recover from a severe reaction but usually dies.

Schizont parasitosis should not be used alone to classify reactions because a direct correlation between parasitosis and the severity of disease is not always found. This is particularly true of *T. p. lawrencei* and *T. p. bovis* infections. Moreover, the estimation of schizont parasitosis is subjective and may also reflect a variation in the distribution of schizonts within and between lymph nodes.

Animals with a severe reaction should be treated to avoid unnecessary suffering or death. These cases can be recorded as severe reactions and/or as death.

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