TICKS AND TICK-BORNE DISEASE CONTROL

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A Contraction



PROCEEDINGS OF A JOINT OAU, FAO AND ILRAD WORKSHOP HELD IN KAMPALA, UGANDA 12–14 SEPTEMBER 1991

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PROCEEDINGS OF A JOINT OAU, FAO AND ILRAD WORKSHOP HELD IN KAMPALA, UGANDA 12–14 SEPTEMBER 1991

> Edited by T.T. Dolan

THE INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES BOX 30709 • NAIROBI • KENYA The International Laboratory for Research on Animal Diseases (ILRAD) was established in 1973 with a global mandate to develop effective control measures for livestock diseases that seriously limit world food production. ILRAD's research program focuses on animal trypanosomiasis and tick-borne diseases, particularly theileriosis (East Coast fever).

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Foreword

A workshop on 'Ticks and Tick-borne Disease Control' was held in Kampala, Uganda, from 12-14 September 1991. This was the fourth workshop in a series designed to bring together scientists and those responsible for tick-borne disease control in countries of eastern, central and southern Africa to discuss recent research findings applicable to these diseases and to address problems encountered in their control. The first two workshops in the series were held in Nairobi, Kenya, in 1984 and 1985 and were organized jointly by the International Laboratory for Research on Animal Diseases (ILRAD) and the Food and Agriculture Organization of the United Nations (FAO). The third workshop was held in Lilongwe, Malawi, in September 1988 and, together with this Kampala workshop, was organized jointly by the Inter-African Bureau of Animal Resources of the Organization of African Unity (OAU-IBAR), FAO and ILRAD. The Kampala workshop was timed to coincide with the annual review meeting of the FAO national and regional projects. The Government of Uganda convened an additional meeting on this occasion to address the specific tick and tick-borne disease problems in Uganda. Many of those who participated in the OAU/FAO/ILRAD workshop also participated in the Uganda meeting that followed.

The Government of Uganda gave the workshop and related meetings high priority and considerable support. We were honoured by the attendance of Dr. Samson Kisseka, the Vice President, Mrs. Victoria Sekitoleko, Minister for Agriculture, Animal Industry and Fisheries, and Dr. Speciosa Kazibwe, Minister for Women, Youth and Culture, an introductory address from His Excellency President Y.K. Museveni and a closing address from Mr. J.O. Bwangamoi, Deputy Minister for Agriculture, Animal Industry and Fisheries. The workshop was organized by a committee composed of Drs. Tom Dolan (ILRAD), Fred Musisi (FAO, Malawi), Jamlack Mutugi (FAO, Uganda), Charles Otim (Uganda) and Jotam Musime (OAU-IBAR). The Government of Uganda was extremely helpful in facilitating the arrangements for the workshop and meetings. Dr. Musime coordinated the workshop through the OAU-IBAR office in Nairobi, while Drs. Mutugi and Otim made arrangements for facilities and accommodation in Kampala. I am particularly grateful to Dr. Tony Musoke who facilitated the early interactions with different ministries, Makerere University and research workers in Uganda, and to Mr. Kepher Nguli for providing logistical support from Nairobi and for making travel arrangements for ILRAD-supported participants.

The Lilongwe workshop recommended that topics to be discussed at the next workshop should include tick-borne diseases other than theileriosis, in addition to country reports. The program reflected this recommendation, with papers presented on current and future methods of diagnosis, novel vaccines for tick-borne diseases and vaccines against ticks. Papers were also given on computer models and the assessment of impact and economics of disease control. Papers were given by representatives from OAU, FAO, the Southern African Development Co-ordination Conference (SADCC) and ILRAD on strategies for tick and tick-borne disease control and interactions within the region. The country reports are in general short summaries of the work done since the previous workshop. A detailed report is presented on the work in Eastern Zambia (by Lynen, Makala and Pas) because of the large-scale immunization conducted, the approach to vaccine delivery used, and the analysis and interpretation of results. A new element of the workshop was the selection of a special topic for focused discussion. Diapause was chosen because it had been the subject of recent publications in which its nature and importance in the epidemiology of theileriosis were addressed. A panel composed of Drs. Dirk Berkvens, Andy Norval, Rupert Pegram and Alan Young led the discussion and some 25 participants listened to or joined in the debate. Each session was followed by a discussion, and recommendations were drafted and agreed in the final session.

The recommendations made by the Lilongwe workshop relating to parasite nomenclature and definitions of disease reactions have been widely referred to and adopted. At this workshop the most significant recommendation was that of setting up a committee with responsibility to draft new standards for live vaccines for tick-borne diseases. The committee's responsibility will be to submit draft standards for consideration by OAU, FAO and ILRAD. If approved, they will be forwarded through appropriate channels for ultimate approval by the Standards Committee and member countries of the Office International des Epizooties (OIE).

Participation at the workshop was sponsored by the governments of Belgium and Uganda, the Overseas Development Administration of the United Kingdom, FAO, the International Centre of Insect Physiology and Ecology (ICIPE), ILRAD and OAU. Social functions were organized by the Government of Uganda, FAO and ILRAD and the series of meetings was given considerable attention on television, radio and in the Press.

Initial reports and summaries of the proceedings were typed during the workshop by the meeting secretariat. The corrected and edited versions were typed by Ms. Susan Nduta at ILRAD. Mr. Peter Werehire, ILRAD, prepared the camera-ready pages for publication.

> Thomas T. Dolan International Laboratory for Research on Animal Diseases Nairobi, April 1993

Opening address

His Excellency President Y.K. Museveni

President of Uganda

It is my great pleasure to be with you this morning to address you at the opening ceremony of the Government of Uganda workshop on the control of ticks and tick-borne diseases.

Ladies and gentlemen, I am particularly happy to see that you are gathered here to address yourselves most seriously to issues relating to the control of ticks and the most economically important group of diseases transmitted by them, namely East Coast fever, babesiosis, anaplasmosis and heartwater.

The importance of ticks and tick-borne diseases, and indeed of the meeting itself, is clearly demonstrated by the presence of a rich mixture of scientists with a deep knowledge and wide and invaluable experience in their areas of specialization who are here to identify, discuss and finally recommend yet newer approaches in the continuing effort in the fight against ticks and tick-borne diseases.

It is therefore my strong conviction and belief that at the conclusion of your deliberations, you will have come up with ideas and strategies, based on scientific facts, for the drawing up of a master plan which should provide both policy makers and those who implement with clear guidelines and direction for the effective and sustainable control of ticks and, therefore, tick-borne diseases. Such guidelines and the measures which you are going to recommend should be efficient, cost-effective and, above all, practical in the context of the economic, climatic and ecological factors prevailing in Uganda.

I would also like to advise further that your discussions should equally take into consideration the diverse characteristics, practices or behaviour of the farming community to which the recommendations apply and that the farmers themselves are by and large resource-poor.

The sole effective method of controlling ticks in this country has been, for a long time, the employment of acaricides by direct application to the animal and may remain so for at least some years in the foreseeable future. Associated with this method however are a number of problems, namely (i) poor sustainability, as a result of the ever rising prices for chemicals and the high cost of labour and inputs; (ii) unwanted chemical residues in animal products; (iii) environment pollution; and (iv) tick resistance.

The problem of sustainable livestock husbandry, however, is not new, but the biggest dilemma is its huge dimensions. To maintain an economic livestock production level therefore, in the presence of the endless challenges and constraints such as are posed by the problem of control of ticks and tick-borne diseases, requires a dedicated and tireless effort by a community of scientists like these gathered here, to constantly review the situation and exchange ideas with the objective of introducing new and innovative methods of control of, for instance in our situation, East Coast fever.

I should therefore say that the present workshop is being conducted at the right time. It is the right time because the need is high to review and update or modify our approaches and strategies for the control of tick-borne diseases to put them in line with the latest developments in knowledge.

I understand that a vaccine against East Coast fever was developed at the East African Veterinary Research Organization laboratory at Muguga, Kenya, and that presently it is being evaluated through field trials by many countries in East, Central and Southern Africa. I also learnt that a similar exercise to establish the efficacy of the vaccine against our own strains of the causal organisms of East Coast fever is being undertaken by scientists at the Animal Health Research Centre, under the joint financial support of the Danish and Ugandan governments. We are eagerly looking forward to the outcome and conclusions from the study but also hope that any of the findings and observations which may be available so far shall be adequately discussed in the present workshop. I must express my thanks to the Danish Government for the support, and the Food and Agriculture Organization for their executing role in this project.

Ladies and gendemen, you will know that the key figure in the transmission of East Coast fever, babesiosis, anaplasmosis and cowdriosis is of course the tick itself. It is therefore pertinent that, in your deliberations, due attention be given to the relevant aspects of controlling the tick. Even with the availability of prophylactic treatment for some of the tick-transmitted diseases, tick infestation, on cattle will still have to be controlled, or kept at low levels, perhaps using a strategic dipping approach.

It should also be our ultimate wish and desire that a vaccine to protect cattle against ticks, or at least against some of those ticks which are responsible for the major diseases, may also be found by our sister laboratories with greater research capacities and facilities.

Madam Chairperson, let me take this opportunity to thank all the agencies who have at one time or another rendered to us assistance in the continuing effort to control livestock diseases both in the laboratory and in the performance of field programs. Special thanks in this respect, go to FAO, DANIDA, EEC, GTZ, UNDP, the World Bank, OAU-IBAR, WHO, IAEA and IFAD.

I would also like to recognize with deep appreciation the collaborative roles with our national livestock research institutions played by the International Laboratory for Research on Animal Diseases, the International Centre of Insect Physiology and Ecology and the Free University of Berlin.

I would like to thank all of you, and particularly our distinguished delegates from abroad, for having spared your time to come to this very important meeting. I also thank the organizers for the excellent arrangements which have made this meeting a success.

I hope the workshop will consider in detail all available but suitable alternatives of ticks and tick-borne diseases for Uganda, for the sustainable benefits of our livestock industry.

I wish you fruitful deliberations.

I now have the pleasure to declare the workshop open.

Thank you very much.

Address by the Food and Agriculture Organization Representative in Uganda

A.A. Khalil

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It gives me great pleasure to see such a large gathering of scientists, senior government workers and experts in the field of tick and tick-borne diseases. I understand 13 countries of the region are represented. Various directors of veterinary services represent their countries while the ticks and tick-borne disease projects are represented by the chief technical advisers (CTAs) and the national project directors. There are many other scientists and experts from a number of interested institutions like the International Laboratory for Animal Diseases (ILRAD). The Organization of African Unity's Inter-African Bureau for Animal Resources is represented by the Director, among others. We have also in the gathering senior technical and operation officers from the Food and Agriculture Organization's headquarters in Rome.

There is no doubt that this must surely be the most distinguished gathering of our animal and veterinary scientists ever seen. I am particularly pleased that the partnership of FAO, OAU-IBAR and ILRAD is almost complete in terms of having the critical human resources in tackling this tick-borne disease menace.

The Food and Agriculture Organization on its part has funded and executed many projects in the East, Central and Southern African region. Our experts were involved in the development of the only available method of East Coast fever immunization, some 25 years ago, and I am made to understand that some of you represented here worked and participated in the development and refinement of the method. My main purpose is not to praise your great effort but to challenge you to produce even better results. For this we can only turn to you and tell you that the member states of the United Nations view your deliberations over the next few days with great interest.

The implementation of the various projects can only happen, at least for now, through donor funding. Many agencies, both multinational and bilateral, have contributed in this area. I would, though, like to specifically thank the Kingdom of Denmark for funding so many of the projects in the region concerned with tick-borne diseases. Our own project in this country is part of this FAO/DANIDA partnership.

Finally, for all of us with an interest in enhancing food production and particularly animal protein, we wish the experts and policy makers gathered here the best of success and a very fruitful deliberation. Remember, we are seeking solutions for this problem not tomorrow, but today. Thank you.

COUNTRY REPORTS

- /

East Coast fever immunization in Burundi

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Immunization against East Coast fever (ECF) in Burundi began in 1981 with the preparation of local stabilates of *Theileria parva* at the Central Veterinary Laboratory, Bujumbura, with technical assistance from the German Agency for Technical Cooperation (GTZ) and the International Laboratory for Research on Animal Diseases (ILRAD). The original stabilates contained three local isolates—Gatumba, Gitega and Ngozi. The Ngozi isolate was later deleted due to its apparent association with ophthalmic problems. The present locally produced Burundi stabilates have been used on a small scale with satisfactory results since the early 1980s. The target group of animals has always been cross-bred (Ankole and exotic) calves and to a lesser extent pure exotic calves between 2 and 12 months of age.

The Burundi stabilates were evaluated from 1981 through 1985 and 402 calves were immunized during this time. Three government farms participated in this activity while only 10% of the calves were owned by private farmers. From 1986, the number of government farms and livestock projects on which ECF immunization has been used has increased to seven, while private cattle owners constituted 30 percent of total participation within the program. From 1986 through July 1991, 1781 calves were immunized.

The FAO/DANIDA project GCP/BOF/022/DEN evaluated the Malawi-produced trivalent *T. parva* (ECF) stabilate vaccine in Burundi during 1990 to test its ability to protect cross-bred calves against disease when challenged with the Burundi *T. parva* stocks. The results to date have been negative but not conclusive and a second immunization trial using the Malawi-prepared stabilates is proposed for early 1992. This is in an attempt to provide the Burundi Government with an alternative to their locally produced Burundi stabilates which could be of value in the future if local production of the Burundi stabilates has to be suspended for any reason.

The immediate livestock development objective for Burundi is to increase animal production by cross-breeding local cattle with higher producing exotic stock. This will require a much larger scale of ECF immunization in the immediate future. The present program of immunization must be expanded and improved to support this need. The Burundi Government recognizes that it will require outside help to support this activity which will involve further training of veterinary personnel, assistance with stabilate production and logistical support. Immediate plans must be made to provide this assistance to the variety of livestock improvement programs that are planned.

East Coast fever immunization in Malawi

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East Coast fever (ECF) immunization in the field started in 1984 soon after extensive field trials which confirmed the protection and safety of the 'Muguga Cocktail' against local Malawi strains of *Theileria parva*. Immunizations were limited to exotic cattle and their crosses in government and private farms and in the milk shed areas of Lilongwe and Mzuzu in the central and northern regions, respectively. Present knowledge suggests that local zebu cattle, which are under intensive tick control, develop enzootic instability. Therefore, it would be prudent to consider such herds for immunization. East Coast fever immunization is now being followed by immunization against the other tick-borne diseases, babesiosis, anaplasmosis and heartwater, as a routine.

The vaccine institute in Malawi has the capacity to satisfy the demands for Malawi and other ECF-affected countries. The institute has supplied vaccines and reagents to Burundi, Tanzania, Uganda and Zambia, and there is collaborative research with countries such as Zimbabwe and Zambia and with the International Laboratory for Research on Animal Diseases (ILRAD).

The real benefits from ECF immunization can only be assessed when the basic production losses attributed to ECF are known. Baseline data on production and fertility is being generated from areas where cattle have been immunized.

The production of the vaccines will continue while efforts are made to improve on quality and delivery. External assistance is being sought in support of the vaccine production laboratory.

Tick and tick-borne diseases: recent developments in Mozambique

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Mozambique is bordered by six countries: Tanzania, Zambia, Malawi, Zimbabwe, South Africa and Swaziland. Each of these countries has a different situation in terms of ticks and tick-borne diseases as compared to Mozambique. In Mozambique, ticks and tick-borne diseases are known as important constraints to livestock improvement. The most important tick vectors are: *Amblyomma hebraeum*, *A. variegatum*, *Boophilus micropius*, *B. decoloratus*, *Rhipicephalus appendiculatus* and *R. evertsi*. The diseases transmitted by these ticks which are present throughout the country are heartwater, babesiosis, anaplasmosis and theileriosis considered to be caused by *T. mutans*. East Coast fever however has only been identified clinically in the northwest Angonia District and once in cattle of Morrumbala District in Zambezia Province.

The Southern African Development Coordination Conference (SADCC) training course on major vector-borne livestock diseases (Swaziland, August 1990) formulated recommendations immediately relevant to the Mozambican situation, emphasizing the need for an epidemiological unit studying tick-borne diseases, their impact and different control methods.

In Mozambique a joint team from the National Veterinary Institute and the Veterinary Faculty, Eduardo Mondlane University, has established an indirect fluorescent antibody test (IFAT) for *Bahesia* and has gained some experience with the same test for *Cowdria* with antigen supplied by the SADCC research project in Harare. One veterinarian has been trained in Harare to culture *Cowdria* and the author will participate in a training course in October 1991. The same team is currently producing vaccines against *Bahesia* and *Cowdria* on a small scale.

Sera from cattle and goats from all provinces and types of management have been tested once. A card test for anaplasmosis has been applied on a limited scale using a commercial antigen. Serological results showed titres against *Bahesia* and *Cowdria* in all provinces and most types of management. However, large differences in the percentage of positive titres in different herds and/or provinces were found. This implies that movement or importation of cattle often represent hazards of tick-borne diseases.

In contrast to countries where ECF is endemic, as in some neighbouring countries, the situation in Mozambique is not so clear and needs to be investigated. The vector *R. appendiculatus* is found throughout the country, but the disease has been detected only in Angonia. However, in sera from the south, titres against *T. parva* were found in low numbers of cattle by Jacobsen (1985) using the IFAT and again using an ELISA (unpublished results).

In the near future cattle movement inside the country and importation from neighbouring countries can be expected and it is therefore urgent to define the real prevalence of T. parva in Mozambique and the possible carrier state of cattle to be introduced. Presently we do not have the capacity to perform these tests.

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Immunization against East Coast fever in Rwanda

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East Coast fever (ECF) is the major disease causing mortality in cattle in Rwanda, particularly in exotic pure breeds and their crosses with the indigenous Ankole breed. Immunization against ECF began during the FAO Tick Control Project (1977–1982) and a study was carried out to assess the distribution of the disease and to identify the species and strains of *Theileria* involved. *Theileria parva* and *T. mutans* were found to be responsible for 49% and 43% of theileriosis cases, respectively. The disease was diagnosed throughout the country, except in the northern (mountainous regions) where high altitude and low temperature limit the distribution of the theileriosis vectors, *Rhipicephalus appendiculatus* and *Amblyomma variegatum*. The local Ankole breed appears to be tolerant to these diseases.

During the FAO project, technicians were trained to perform ECF vaccination and monitoring. After the end of the project, the program of ECF immunization was taken over by the National Veterinary Laboratory at Rubirizi. To date, two strains of *T. parva*, *T. parva*, Nyakizu and *T. parva* Satinski, isolated in the southern and central parts of the country, are being used.

An average of 300 head of cattle are vaccinated each year on a voluntary basis. Even though the percentage of serious reactions after vaccination is not above five percent, many farmers are reluctant to vaccinate cattle because of the risk of reaction and mortality, the high cost of vaccination (approximately US\$ 10), the lack of trained staff to monitor vaccinated cattle in rural areas and the general unavailability, and expense, of long-acting tetracyclines, parvaquone (Clexon, Coopers) and halofuginone (Terit, Hoechst). Another constraint to the more widespread use of vaccination is that the National Veterinary Laboratory lacks qualified staff. The laboratory would benefit from support from specialized institutes, such as the International Laboratory for Research on Animat Diseases (ILRAD), and from the FAO/DANIDA projects working on tick-borne disease control in the region.

Ticks and tick-borne diseases in the Sudan

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In Sudan 72 tick species have been identified, 14 of which infest livestock and five are vectors of very common tick-borne diseases. *Hyalomma anatolicum* is the vector of *Theileria annulata* which causes a 30% calf mortality in exotic dairy herds, *T. hirci* which causes high mortality in sheep and *Bahesia equi* which causes equine babesiosis. *Rhipicephalus evertsi* transmits *T. hirci* and is a potential candidate for *T. annulata* transmission. *Boophilus* species transmit cattle babesiosis and anaplasmosis. *Amblyomma lepidum* and *A. variegatum* transmit heartwater in sheep. Recent outbreaks of heartwater in cattle have been reported. *Theileria parva*, transmitted by *Rhipicephalus appendiculatus*, occurs in the southern part of the country (see following paper by I.I. Julla).

Ticks and tick-borne diseases have become very significant problems in the modern production sector and in breed improvement programs. Tick infestation is also considered important in cattle and sheep because it causes debilitation, teat damage, hides and skin damage. Cases of camel paralysis have also been reported.

A strategy of chemical tick control will be adopted in the modern animal production sector, in the developing milk sector in irrigation schemes and in the nomadic cattle to reduce tick infestation to minimal levels.

Progress in research on theileriosis in Equatoria State, Sudan

I.I. Julla

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Epidemiological studies carried out in Equatoria State, Sudan, confirmed the presence of *Theileria parva*, the causative agent of East Coast fever (ECF) and the vector tick, *Rhipicephalus appendiculatus*. The disease affects the local breeds of cattle, especially the calf crop, causing a mortality of 80-100% (Morzaria *et al.*, 1981; Julla *et al.*, 1989). As a result, a joint project between the Directorate of Animal Resources, Equatoria State, and the University of Juba was established in 1987 with the following objectives: to develop a research program on theileriosis and related tick-borne diseases, babesiosis, cowdriosis and anaplasmosis and to develop means of control for *T. parva* through vaccination by the infection-and-treatment method.

The project activities have been divided into three phases.

- 1. Épidemiological studies.
- 2. Isolation and characterization of T. parva parasites present in the area.
- 3. Examination of the isolated and characterized parasites as immunizing strains using the infection-and-treatment method.

The project has established basic laboratory facilities at Juba University. The Norwegian Agency for Development Co-operation (NORAD) has assisted the project with funding for equipment and chemicals. The International Laboratory for Research on Animal Diseases (ILRAD) is supporting the project with technical advice, research collaboration and training. The project should allow Sudan (Equatoria State) to develop improved control measures for theileriosis and other tick-borne diseases.

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East Coast fever immunization trials in Uganda

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Three immunization trials against East Coast fever (ECF) were carried out in Lusenke, Mbarara and Sironko, representing central, western and eastern regions, respectively. The sites were in areas where ECF and other tick-borne diseases are a major problem. The primary objective of the trials was to evaluate the protection afforded by the trivalent *Theileria parva* stabilate vaccine produced in Malawi against challenge by *Theileria parva* parasites occurring in Uganda. The first trial in Lusenke gave broad indications that the trivalent vaccine was protecting cattle against the natural field *T. parva* challenge. However, a full and comprehensive assessment of this protection was complicated by the occurrence of other tick-borne diseases.

In the second trial in Mbarara District, Western Region, animals were immunized against *T. parva*, *Anaplasma marginale*, *Babesia bigemina*, *B. bovis* and *Cowdria ruminantium* before field exposure. The vaccinations were carried out in the following sequence:

- 1. Immunization of cattle using the trivalent stabilate vaccine stocks (22 January 1991). At the same time a number of cattle which were found to contain antibodies to *T. parva* following natural infection were challenged with the *T. parva* trivalent stabilate vaccine to test the protection against the vaccine stocks.
- 2. Homologous challenge of the immunized cattle to ascertain immunity (26 February 1991).
- 3. Vaccination of cattle against Cowdria ruminantium (9 April 1991).
- 4. Vaccination of cattle against Anaplasma and Babesia (21 May 1991).
- 5. Exposure of cattle to field challenge (15 July 1991).

A total of 67 cattle consisting of 18 pure-bred Friesian bulls and 49 cross-breds of exotic and Ankole types were selected for the trial.

- (a) Fifty cattle with no antibodies to *T. parva* in the indirect immunofluorescent antibody test were immunized using the trivalent stabilate stocks. These included the 18 Friesian bulls.
- (b) Seventeen cattle which had antibodies to *T. parva* from local *T. parva* infections were challenged directly with the trivalent vaccine parasites.

The results from the immunization, challenge and exposure phases of the trials were as follows:

1. Following immunization, a majority of the cattle, 37 out of 50 (74%), seroconverted to *T. parva* after inapparent reactions. Nine (18%) reacted mildly, and three (6%) had moderate to severe reactions. Seventeen out of the

17 antibody-positive cattle challenged directly with the trivalent vaccine stocks underwent inapparent reactions indicating cross-protection between the trivalent vaccine stocks and the local *T. parva* parasites to which the cattle had been exposed.

- 2. Homologous challenge confirmed immunity of the immunized cattle with 48 out of 50 cattle showing inapparent reaction to challenge.
- 3/4. The *Cowdria*, *Anaplasma*, *Babesia* vaccination produced few reactors with a small proportion of the immunized Friesian cattle showing a transient fever.
 - 5. The 67 cattle putatively protected against ECF by the trivalent vaccine and 10 susceptible controls were grazed in two large paddocks where ECF was known to be present. The cattle were not subjected to any form of tick control for over 60 days and the experiment is continuing.

The results to date show that tick challenge was moderate but *T. parva* infection was detected. Many cattle had detectable schizonts and all had a low piroplasm parasitaemia. One control animal died of acute *T. parva* infection. Two out of the 67 immunized cattle died of *T. parva* with very low schizont parasitoses, but with a post mortem picture indicative of buffalo-derived *T. parva* infection. Three other cattle, all Friesian, died of complications with Lumpy Skin Disease.

An analysis of results to date showed that 82% reacted inapparently, 14.9% had mild reactions, 2% had moderate reactions and 5.9% had severe reactions.

East Coast fever immunization in Tanzania Mainland

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Tanzania was included in the second phase of the FAO/DANIDA projects for East Coast fever (ECF) immunization in East and Central Africa and, after more than a ten-year interruption, ECF immunization field trials have been recommenced. Project activities started in June 1990 and have been restricted to the southern highlands in the Iringa and Mbeye regions.

East Coast fever is seen as the major hindrance to a rapid increase in the keeping of improved milk cattle in the small-holder sector. The government is supporting the small-holder sector by providing cross-bred heifers from livestock multiplication centres. In these centres, tick control is very strict. The in-calf heifers sold to the small-holders are therefore at high risk to ECF (and other tick-borne diseases) when they leave the centres and they are the main target group for immunization.

Three trials have been conducted each involving 20 animals immunized using the trivalent *Theileria parva* stabilate vaccine produced in Lilogwe. At the same time, approximately 650 heifers have been vaccinated and 120 of them have been distributed to small-holders.

The results of the trials have been positive. Monitoring of cattle following immunization using regular weighing has shown that production parameters are not affected by the immunization procedure. Clinically, transient fevers and lymph node swellings have been noted. Lymph node biopsies revealed few or no schizonts. Serum conversion rates have been satisfactory with over 90% developing antibodies in all three trials. In the first two trials, immunized and control animals were exposed to unlimited tick challenge. In the first trial, in which exposure took place in February 1991, the *Rhipicephalus appendiculatus* tick burden was very high, reaching a mean of 240 ticks per ear. The cattle reacted with fever and oedematous swelling in the head, ears and neck. *Anaplasma* and *Babesia* parasites were seen in blood smears 15 days after exposure. These parasites were thought to be of vaccine origin as the animals were immunized with these parasites and tick transmission was unlikely as the time was too short after vaccination. The result of the first trial was that five of the 20 immunized animals died of ECF. Eleven of the controls also died, the last one had to be sacrificed as it became blind due to a chronic ECF infection.

The second trial exposure was to R. appendiculatus nymphs and six out of eight controls showed clinical ECF and three died in spite of treatment. The 20 immunized animals showed various degrees of fever, some of up to nine days' duration. Schizonts were detected in most animals. One of the 20 animals died of ECF.

COUNTRY REPORTS

In the third trial 20 animals were immunized and subjected to partial tick control. They are being monitored at regular intervals and the main herd is serving as control. No case of ECF has been detected in this herd and the trial is continuing.

Although results of the first trials do not show 100% protection, it is thought that the method is valuable and economically feasible for the target group of animals. Tanzania's need for vaccine in the future might approach 40,000 doses annually, provided that a realistic price can be found for the immunization.

East Coast fever immunization in Pemba, Tanzania

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Preliminary trials showed that immunization using a local isolate of *Theileria parva* was possible. A decision was taken to begin immunizing a specific group of animals, the F1 Jersey/zebu cross-breds, at as early an age as possible using the *T. parva* (Pemba/Mnarani) stabilate (2913) and treatment with buparvaquone (Butalex, Pitman Moore Ltd.). This drug was chosen because a single injection was likely to be effective in controlling the immunizing infection. As the cross-breds are owned by smallholder farmers on farms scattered over the whole island it would mean savings on travel expenses and time when compared to the two injections required if oxytetracycline was being used.

Approximately 100 animals of about two months of age were immunized with a 1/10 dilution of the stabilate and buparvaquone. Sera were collected on the day of immunization and on day 30. Seroconversion on day 30 was found to be low and variable, so it was assumed in most cases that immunization was unsuccessful. The cause of this was first thought to be the stabilate diluent which, due to erratic electricity supply, was often thawed and frozen inadvertently before use. It is likely that the stabilate diluent lost its buffering capacity and the altered pH had an adverse effect on the sporozoites.

In March 1991 fresh stabilate diluent was obtained and immunization was started using either buparvaquone on day 0 or two doses of oxytetracycline, on day 0 and day 3. Seroconversion was markedly better with oxytetracycline immunization of 25 calves, 75% were positive to *T. parva* schizont antigen in the indirect immunofluorescent antibody test at a dilution of 1/1000 or higher compared to less than 20% for the buparvaquone group. This suggests that buparvaquone with its schizontcidal activity is not suitable for immunization.

Field East Coast fever immunization in Ungunja, Tanzania

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Following a series of trials testing immunization against East Coast fever (ECF) using locally isolated parasites conducted jointly by the government, the International Laboratory for Research on Animal Diseases (ILRAD) and the Food and Agriculture Organization (FAO) project URT/86/022 between 1986 and 1990 on Unguja Island, Zanzibar, the government decided to introduce immunization as another method for the control of ECF in addition to acaricide application. Since the completion of the trials, a total of more than 300 calves and adult cattle have been immunized.

Serological examination, using the indirect fluorescent antibody test, of samples taken on day 28 after immunization showed that more than 98% of the immunized animals had developed antibody titres against *Theileria parva* schizonts ranging from 1:200 to more than 1:5000. The animals immunized since the completion of the trials did not suffer from anaemia which was a serious complication in the trials. The anaemia was thought to have been caused by *T. mutans* parasitaemia and other tick borne diseases because of unlimited exposure to ticks.

Cattle which were older than six months, including milking and pregnant cows, had lower numbers of microschizonts detected in lymph nodes compared to younger animals following immunization. Out of the 78 adult cows that were immunized in the field, 14 (21%) had to be treated with parvaquone (Clexon, Wellcome) while 38 out of the 94 (40%) young stock had to be treated for immunization reactions. East Coast fever immunization could be carried out safely at all stages of gestation and there was no loss in milk production in the 35 dairy animals that were immunized and studied.

The health status, plane of nutrition and absence of helminthiasis and intercurrent diseases must be strictly verified before a decision is made to immunize an animal. Further investigations are required on immunization of calves younger than six months, the effect of immunization on pregnancy and milk production and the reproductive performance. The assessment of the socioeconomic impact of immunization is necessary and the need for immunization against *Babesia bovis* and other tick-borne diseases, such as heartwater, *T. mutans* theileriosis and anaplasmosis, must be considered in the planning of the immunization program.

Theileriosis and other tick-borne diseases in North Kivu, Zaire

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The total cattle population in Zaire is estimated to be 1.4 million head, 45% are located in the east and the northeast where East Coast fever (ECF) is known to occur. Studies of the tick species and distribution in Zaire have shown that tick-borne disease vectors are widespread in the country.

The mortality rate of young calves due to ECF in North Kivu is about 15–20% per year, and the disease is severe, mainly in the low altitude areas (100–1800 m). The situation is similar in the northeastern part where the Nioka Research Station estimated that 30–70% of calves were dying from theileriosis. A trial using a form of immunization against ECF was conducted by the laboratory of Gabu in 1954–1956. The animals were artificially exposed to ticks infected with South African *Theileria parva* strains. Chlortracycline (Aureomycin, Cyanimid) at a dose of 10 mg/kg was administered intravenously to the animals on the day of tick application and treatment was repeated daily unt?! the pyrexia subsided. It was observed that it was necessary to use ticks infected with local strain combinations in order to obtain a solid immunity.

In North Kivu, local breeds of cattle are more resistant to tick-borne diseases than exotic cattle. The prevalence of tick-borne disease parasites estimated in 1980, by blood smears examination, was 29.7% for theileriosis, 21.9% for babesiosis and 10.6% for anaplasmosis. Using enzyme linked immunosorbent assays for the detection of antibodies and antigens of tick-borne disease pathogens, the following results were obtained from 256 sera screened.

Theileria mutans antibodies	9.7%
Theileria mutans antigens	37.3%
Anaplasma marginale antigens	55.4%
Theileria parva schizonts antibodies	21.0%
Babesia bigemina antibodies	22.5%

The titres were found to be higher in areas where strict tick control was not applied, compared to those practising efficient tick control. However that difference appeared less obvious for *A*. marginale.

Control is currently by acaricide application to the animals and treatment of clinical cases. The difficulties experienced in applying these measures are the high cost of acaricide, whereby 85% of the farmers cannot afford the expense, and the inability to exercise strict cattle movement control.

East Coast fever and other tick-borne diseases are the major factors limiting the productivity of livestock in eastern Zaire and improved strategies for control of these diseases are required.

East Coast fever immunization in Eastern Province, Zambia

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The Belgian Animal Disease Control Project (BADCP) started immunizations with a local field isolate, *Theileria parva* (Katete), in the Eastern Province in December 1987. The field situation had been studied in previous years, the *T. parva*, East Coast fever (ECF) incidence and tick activity were recorded, local strains were isolated and cross-immunity trials were carried out. The first immunization results were presented at the Workshop on Theileriosis held in Lilongwe in 1988 (Berkvens *et al.*, 1989).

PRESENT SITUATION

Tick population

Annual tick surveys during the months of January to March showed a slow westward spread of *Rhipicephalus appendiculatus*. However, since 1989, this extension has become less marked and the western boundary has remained at Tsitsi, about 50 km west of Sinda. It is felt that the arrest in the extension of the range is because ECF is now controlled and illegal cattle movement, in the wake of ECF epidemics, has ceased. In 1991 a new extension of the tick's range was noticed northeast of Sinda and a distinct southwest migration into the Nyanje area occurred. There have also been major extensions in the tick's range towards the southern (international) border of the province, as catt'e were moved back into the area after the cessation of hostilities.

The marginal areas of the tick's range still carry low tick numbers (<1 tick/animal) except in the areas where ECF epizootics occur. In the rest of the area, relatively high numbers of *R. appendiculatus* have been recorded over the past three years, featuring a second generation of adults in May/June and a second cohort of nymphs in August/September. Early rains in October 1989 resulted in abnormally high tick counts throughout 1990. Regular tick control at this moment is almost non-existent, except for some farmers who hand-spray their animals on a more or less regular basis. Most government dip tanks stop operating as early as April/May because of the high running costs, leaving susceptible animals fully exposed to the second wave of ECF in the province.

Local Theileria parva (Katete) isolate

The *T. parva* (Katete) field isolate as tick stabilate was sent to ILRAD for characterization. Monoclonal antibody profiles and DNA characterization placed the Katete isolate within the group of other Zambian *T. parva* (ECF type) isolates, and Katete-immunized cattle withstood subsequent challenge with the *T. parva* (Marikebuni) stock (R. Bishop, P.R. Spooner, S.P. Morzaria, and T.T. Dolan, personal communication).

Immunization

Immunization campaigns have been extended gradually to all ECF-affected districts of the Eastern Province. Serological and tick surveys preceded initiation of the immunization campaigns in order to establish the range of enzootic stability of the disease in the local cattle population. Immunization campaigns cover only the indigenous animals except in Chadiza District where a commercial farm (extensive ranching of Brahman cross-breds) has been included in the program.

To date 50,076 calves have been immunized (Table 1). Calves are immunized from three to four weeks up to one year of age at the start of the program and, afterwards, only calves up to four to five months are presented.

District	1987–89 calf crop	1990 calf crop	1991 calf crop	District total	Overall attendance %
Chipata	17,357	6,641	2,785*	26,783†	82
Chadiza	0	7,268	1.580*	8.848	84
Katete	0	6,466	3,161*	9.627	74
Lundazi	0	3,042	1,776*	4,818	81

TABLE 1. Number of calves immunized between December 1987 and August 1991 in East Coast fever-affected districts.

*Totals until August 1991.

[†]Represents 60% of the cattle population in the district.

Campaigns are carried out in three periods: the main campaign runs from late October until January for the main calf crop, with subsequent smaller campaigns conducted in April-May and June-July. At first, the intention was to carry out the campaigns before the highest field challenge which occurs in December-January and in May-June. However, experimental trials have shown that (i) immunized animals challenged with a known lethal dose as early as day 12 after immunization showed an even better response to the immunization (i.e. lower parasitaemias and earlier seroconversion) and (ii) animals infected with a known lethal dose could still be successfully immunized or treated as late as day 12 after initial infection, using the parotid gland swelling as the criterion for intervention (Belgian Animal Disease Control Project, unpublished data). The highest incidence of treated cases were found in the April-May campaigns.

The stabilate is produced at the Provincial Veterinary Laboratory at a 1/10 dilution to economize on storage facilities, i.e. liquid nitrogen and containers. At present, a total of 60,000 doses is stored in liquid nitrogen at the Provincial Veterinary Laboratory. The final 1/100 dilution (0.1 tick/dose) is prepared at the crush pen, producing an equivalent of either 20 or 60 doses, depending on the number of calves brought for immunization. A single dose of long-acting tetracycline (Terramycin LA, Pfizer) at a rate of 20 mg/kg liveweight is administered. All calves below 50 kg liveweight receive a minimum dose of 5 ml Terramycin LA. Since only newborn calves are presented in the June–July campaign, the cost of tetracycline is significantly lower compared to the other campaigns (Table 2).

District	Immunization campaign	Number immunized	Number treated	Percentage treated	Calves/100 ml Terra LA
Chipata	Nov-Jan	3,801	71	1.9	14
	April-May	1,145	43	3.7	15
	June–July	1,637	64	3.9	17
Chadiza	Nov–Jan	2,462	32	1.3	11
	April–May	552	47	8.5	11
	June–July	1,028	58	5.6	17
Lundazi	Nov–Jan	3,042	38	1.9	11
	April–May	914	50	3.7	13
	June-July	862	19	3.9	17
Katete	Nov–Jan	3,263	7	0.2	11
	April–May	1,523	13	0.9	11
	June-July	1,638	2	0.1	16
Overall		21,867	444	2.0	13

TABLE 2. Number of calves immunized and treated in the 1990–1991 immunization campaigns together with usage of Terramycin LA.

A cold chain has been established at district level. This entails deep freezers to store the medium used for dilution of the stabilate in the field and liquid nitrogen containers holding the stabilate. The latter are only in use during the actual campaigns. The campaigns are organized from the district veterinary offices by either the district veterinary officer or the district livestock officer, under supervision of a project member. All district laboratories have been furnished with basic diagnostic equipment. The veterinary assistants have been issued with a motorbike to assist them to carry out their duties.

All calves are ear-tagged prior to or at the time of the immunization to allow a proper evaluation of the exercise at the end of the season. Anthelmintics are given to calves in poor condition (highest incidence during the June–July campaign). Clinical

cases of ECF are treated with parvaquone (Clexon, Coopers) and this drug is provided to the veterinary assistants for follow-up treatments (Table 2). Cattle owners with calves below the age of three weeks that are treated for ECF or immunized are advised to present these calves again during the next campaign. Records are kept for all ear-tagged animals, indicating whether or not they were present at the time of the immunization and whether they were immunized or treated. A duplicate of this list is given to the veterinary assistant for each area. Meetings at district level are organized with all veterinary assistants after each campaign to discuss the exercise. After finishing the immunization season, a follow-up census is organized in August–September to record the fate of immunized and non-immunized calves.

RESULTS

Indigenous animals

Results are given for Chipata District as detailed records have been kept there over the last three years.

- To date no breakthrough of a field strain has been recorded in the immunized animals. In May 1988 a breakthrough was suspected, but isolation of the field strain and subsequent cross-immunity trials showed that full protection existed between the Katete stock and the field isolate.
- A follow-up of heifers from the 1987 immunization showed normal calving rates.
- Calf mortality has been reduced drastically from an average of 30–40% (figures from sentinel herds 1982–1988) to 2.5–3.5% for immunized calves. At the same time a distinct shift in causes of mortality was noted: ECF accounts for approximately 15% of the mortalities in immunized animals (compared to 85% in non-immunized calves), whereas mortalities caused by management-related problems increased from 12% in non-immunized calves to about 80% in immunized calves (Table 3). Other tick-borne diseases, sweating sickness and trypanosomiasis play a minor role in both immunized and non-immunized calves (Table 3).
- The reduction in calf mortality for the immunized population was accompanied by a steady decrease in calf mortality in the non-immunized population to only 14.7% in 1991 (Table 3). Results of the dissection of field ticks, to examine for any effect of immunization on tick infection rates, showed a similar steady decrease but these results are equivocal as mixed or other *Theileria* parasites cannot be discriminated.
- During the period 1987-1991 the total cattle population in the district increased by 38%, from 33,097 to 45,770 head, and the demand for work oxen was catered for. In cooperation with the Cold Storage Board (CSB) of Zambia, a joint venture was started in late 1990 allowing veterinary assistants to act as buyers in the field in order to increase the off-take of beef from the rurai areas. Cattle slaughter numbers (CSB and private butchers) increased from 1.5% in 1990 to 4.5% of the total population in 1991 (Table 4).

TABLE 3. Percentage mortality in immunized and non-immunized calves and causes of mortality (August 1991 census).

Percentage mortality

	1989	1990	1991
Non-immunized calves	29.4%	17.9%	14.7%
Immunized calves	3.2%	2.7%	3.6%

Causes of mortality 1990-91 season

	Non-immunized	Immunized
ECF	544 (85.5%)	36 (15.1%)
Other TBD	13 (2.0%)	2 (0.8%)
Sweating sickness	0(0%)	4 (1.7%)
Trypanosomiasis	3 (0.5%)	2 (0.8%)
Helminthiasis*	42 (6.6%)	94 (39.4%)
Management*	20 (3.1%)	72 (30.1%)
Accidents*	15 (2.3%)	29 (12.1%)
	637	239
Unconfirmed	15	27
Total	652	266
Census Population	4,438	7.435
% mortality overall	14.7%	3.6%
% ECF mortality overall	2.3%	0.5%

*Combined as management-related problems.

Commercial farm

The commercial farm practices extensive ranching with about 1500 head of Brahman cross-breds, producing 500 calves annually. Ticks and tick-borne diseases have been controlled by conventional dipping but increasing cost of acaricides made the farmer consider alternative methods of control.

The objective of this trial was to investigate the effects of relaxing conventional tick control on other tick-borne diseases after immunization against ECF. Frequent dipping using dioxathion (Altik), cypermethrin (Barricade), amitraz (Triatix) and recently chlorfenvinphos (Steladone), did not prevent clinical cases of theileriosis, babesiosis and occasionally heartwater occurring, especially at the beginning of the dry season. Resistance to cypermethrin resulted in a severe outbreak of babesiosis in 1987. Weekly tick collections showed the presence of low numbers of R. appendiculatus and the regular appearance, in higher numbers, of Boophilus microplus and Amblyomma variegatum.

TABLE 4. Annual changes in cattle population in Chipat	a District o	during t	he period
1985–91, together with numbers of animals slaughtered i	in 1990 and	1 1991 (January-
August).			

Year	1985	1986	1987*	1988	1989	1990	1991
Cattle population Percentage change Animals slaughtered Percentage increase (slaughtered animals in	33,534 ncluded)	32,258 -3.8	33,097 +2.6	35,677 +7.8	41,652 +16.7	44,199 +6.1 811 (8.0)	45,770 +3.6 1994 (8.0)

*Start of the immunization campaigns in December 1987.

In the experiment, 88 animals (44 calves of two to five months of age, with their dams) were immunized and herded separately. At the start of the experiment, animals had no antibodies to *T. parva* schizont antigen and moderate *T. mutans* and *Anaplasma marginale* titres. Only 3% of the animals showed antibody titres against *Babesia*. Dipping was stopped at the start of the trial and animals were given prophylactic treatments with imidocarb dipropionate (Imizol) every seven weeks over the next five months. *Theileria parva* (Katete) was used at a 1/100 dilution for immunization and the cattle were monitored weekly.

All except two animals developed antibodies. The non-reactors were a dam and a calf and it remains doubtful whether they were immunized. A very high number of both cows and calves remained positive for antibodies up to six months after immunization, despite the fact that no substantial field challenge was present. One adult animal required treatment for ECF four months after immunization. The calf that remained negative for *T. parva* died seven months later of ECF, its dam is still alive but has not developed antibodies. After stopping prophylactic coverage with imidocarb in August, no *Babesia* cases were recorded and serological results for November showed a high prevalence of antibody titres of both *B. bigemina* and *B. bovis*. One cow died in July 1991 from trypanosomiasis.

It has been agreed with the farmer to start a new trial using last year's calf crop (1990), based on immunization against ECF and imidocarb blanketing, to establish the length of prophylactic coverage (number of treatments) necessary to safeguard the animals against clinical babesiosis for their first year after immunization. The first experiment raises hopes that enzootic stability may be re-established in a short period of time.

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DIAGNOSIS AND CONTROL

23 '

Current methods for diagnosis of tick-borne diseases

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The methods of diagnosis of tick-borne diseases are described in a field manual published by FAO in 1984. The usual method of detection of tick-borne disease organisms is by microscopic examination of smears of blood, lymph node, spleen or brain stained with Giemsa's stain. Its advantages are that the specimens are easy to collect, transport and preserve, it requires no sophisticated equipment, other than a microscope, it can be carried out by relatively unskilled personnel and its accuracy can easily be monitored. The disadvantages are the difficulty in differentiating between a scanty parasitaemia/parasitosis in a clinically ill, dead or carrier animal and the difficulty in differentiating between species of parasites of different pathogenicity, especially *Theileria* species. Other methods used occasionally for detecting organisms are isolation by inoculation of animals or in cell cultures.

The standard system for the detection of antibodies to tick-borne diseases is by the indirect immunofluorescent antibody test (IFAT), using whole parasites in blood or from cell culture as antigen. Its advantages are that the antigens are easy to prepare, the test can be standardized for all tick-borne diseases, antibodies detected by the IFAT are very persistent after infection and the test is usually highly specific, although there may be some cross-reaction between related species. The major disadvantages are that it is very laborious and the interpretation is subjective. It is also difficult to achieve consistent results with *Anaplasma*. Other tests are available for *Anaplasma* but they too have disadvantages.

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New methods for diagnosis and characterization of tick-borne disease parasites

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Tick-borne diseases of cattle caused by the protozoal and rickettsial parasites of the genera Theileria, Babesia, Anaplasma and Cowdria are a great constraint to livestock development in Africa and other regions of the world. Accurate diagnosis of these organisms is essential in epidemiological studies and in planning control strategies. The currently available diagnostic methods are based on the identification of organisms in Giemsa-stained smears or detection of specific antibodies in serum. Both these methods are relatively insensitive and subjective. Therefore, newer tests, with higher sensitivity and specificity, using reagents produced by molecular biological techniques are being developed. For specific identification of parasites in cattle, DNA probes show great promise. For example, unique DNA sequences from the genomes of T. parva, T. mutans, T. taurotragi, T. buffeli, B. bigemina, B. bovis, A. marginale and C. ruminantium have been identified, cloned and used as probes to provide accurate identification of these parasites. The sensitivity of DNA probes can be greatly enhanced by use of the polymerase chain reaction (PCR) technique, which enables specific amplification of DNA by approximately a million-fold. It is expected that advances in PCR technology, and the use of non-radioactive probes, will allow wider application of the DNA-based diagnostics. DNA-based methods have been developed also for finer differentiation of parasite stocks and strains within a species. This has been exploited, particularly for T. parva, and the methodology developed for this parasite can be used to define and characterize other tick-borne parasites.

Rapid progress is also being made in improving the currently available serological tests. Improved enzyme-linked immunosorbent assays (ELISAs) based on conserved parasite-specific antigens offer the best option to replace the currently used tests. Parasite specific monoclonal antibodies have been utilized in antigen and antibody detection assays to identify both the presence of an infection as well as exposure to an infection. The ELISAs have higher specificity and sensitivity than the previously used serological tests. Genes encoding the conserved and specific antigens recognized by these monoclonal antibodies are being cloned and expressed, and the recombinant antigens provide well-defined antigens for the standardization of assays. These assays can then be utilized to provide reliable information on epidemiological studies within and between countries. It will also mean that the use of experimental animals for production of antigens and antibodies can be greatly reduced.

Current vaccines for tick-borne diseases

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There are four major tick-borne livestock diseases—anaplasmosis, babesiosis, theileriosis and heartwater. On recovery from a primary infection with any of the parasites causing these diseases, cattle become inimune to homologous challenge. Tick fever vaccines refer to Anaplasma centrale, Babesia bovis and B. bigemina blood vaccines. Anaplasma centrale (which provides protection against the pathogenic A. marginale) and B. bovis parasites are attenuated by rapid serial passage in splenectomized calves before starting vaccine production. Babesia bigemina parasites are attenuated by passage in intact calves every three months followed by splenectomy of the calf three months after infection and collection of the parasites for future vaccine production.

The two major theileriosis pathogens for which vaccines are prepared are *Theileria* annulata causing tropical theileriosis and *T. parva* causing the East Coast fever (ECF)/Corridor disease/Zimbabwean theileriosis complex. The tropical theileriosis vaccine is prepared from *T. annulata*-infected lymphoblastoid cells grown and attenuated by passage *in vitro*, while the vaccine for ECF/Corridor disease/Zimbabwean theileriosis consists of *T. parva* sporozoites from infected ticks prepared as stabilates and used simultaneously with tetracycline treatment. The vaccine in common use for heartwater is *Cowdria ruminantium*-infected blood from sheep but an alternative vaccine prepared from infected *Amb'yomma* nymphae can be used.

All these tick-borne disease vaccines are live and their ability to engender immunity depends on the establishment and multiplication of the parasites in the animal. Consequently, maintenance of infectivity during preparation and use is essential. Infectivity can be adversely affected by fluctuations in temperature and the chemical environment of the parasites. Precaution must be taken to avoid such fluctuations. All these vaccines are best stored at -196 °C in liquid nitrogen where they have an essentially infinite shelf-life. The tick fever vaccines in addition can be supplied as chilled vaccines with a usable life of seven days in the field.

Vaccine failure can occur as a result of either a failure to infect the animal or a lack of immunogenicity. Use of a highly infective dose, application of conservative limits on the shelf-life and education of the users would overcome failures due to a failure to infect. The choice of appropriate parasite stocks and attention to the number of passages a parasite has been subjected to would minimize failure due to lack of immunogenicity. A variety of non-cross-protecting immunogenic strains and possible antigenic drift in *T. parva* complicate decisions on cost-effective vaccine production. Also, with live vaccines there is always a potential risk of developing acute disease, dissemination of other infectious agents, such as bovine leucosis, and sensitization of animals to blood and tick components.

Novel vaccines against tick-borne diseases

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The vaccines available for the control of Anaplasma marginale, Babesia bigemina, B. bovis, Theileria parva, T. annulata and Cowdria ruminantium are all live. This presents logistical problems in terms of delivery because of the requirement for a cold chain to maintain viability and immunized animals become carriers of the vaccination parasites. It is therefore necessary to develop alternative vaccines to circumvent these disadvantages. To this end antigens which may induce protective immune responses are being isolated and characterized.

Antigens which provoke protective responses have been identified for B. bigemina and B. bovis and these are of 58 and 42 kDa, respectively. For A. marginale three antigens of 105, 86 and 36 kDa have been identified but research has concentrated on the 105 kDa antigen. The genes encoding the antigens of the three parasites have been expressed in various expression vector systems and recombinant antigens are presently being tested for their immunogenicity in cattle. Work on antigens evoking antibody responses to the two Theileria organisms has taken precedence over those inducing cellular responses because of the difficulties involved in identifying the latter antigens. Antigens identified so far include the 67 kDa and the polymorphic immunodominant (PIM) antigen for T. parva sporozoites and the 85 kDa complex for T. annulata. The gene encoding the T. annulata p85 complex has been expressed in the pGEX expression system and the recombinant antigen is presently being tested for immunogenicity in cattle. The gene encoding T. parva p67 has been isolated and characterized while the gene encoding PIM is yet to be identified. The p67 gene has been expressed in pMGI, a derivative of the pAS vector system, where it is fused to the first 85 amino acids of the non-structural protein of influenza virus A. The recombinant antigen induced high titre neutralizing antibodies in cattle and protected six of nine animals against homologous challenge and 6 of 11 against heterologous challenge.

Taken together the results are encouraging and pave the way for development of novel vaccines against these important tick-borne diseases. It is envisaged that when the genes encoding the relevant antigens are isolated and characterized, an attempt will be made to package them in one vector, such as *Salmonella*, for delivery to cattle.

Vaccines against ticks

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Although no commercial vaccines against ticks are available there are a number of active research programs investigating potentially protective antigens, particularly in Australia and Kenya. It is thought that tick vaccines would be useful measures to integrate into control programs for ticks and tick-borne diseases in Africa.

The possibility of developing tick vaccines is supported by observations from the field that individual cattle or breeds of cattle feed fewer ticks than others. Acquired resistance is an immunological phenomenon by which the animal develops an immunity after exposure to tick infestation and this is under genetic control. In Australia, it has been possible to breed cattle for resistance against *Boophilus microplus* infestation. In Africa, where several economically important tick vectors of disease occur in any locality, breeding such resistant cattle might be difficult.

Research has shown that animals can be immunized against tick infestation using crude tick antigens. These antigens can be divided into unconcealed antigens (antigens to which cattle are normally exposed during tick infestation) and concealed antigens (those to which cattle are not normally exposed during tick infestation). Unconcealed antigens include antigens from the cuticle, salivary glands or salivary gland secretions. Concealed antigens that have been used to date have been proteins located on the gut epithelium of the tick. Most progress has been made by two groups in Australia working on concealed antigens of *B. microplus* which have been purified and the genes responsible used to produce recombinant antigens. In laboratory experiments, immunization using these recombinant antigens can reduce *B. microplus* feeding success on cattle by 90%. However problems exist since not all stocks of *B. microplus* appear to have a particular antigen and so immunization is not universally successful. A further disadvantage of this approach is that the antigen is concealed so no natural boosting can occur.

The International Centre of Insect Physiology and Ecology (ICIPE) in Kenya is studying the effects of both unconcealed and concealed antigens for immunizing cattle against *Rhipicephalus appendiculatus* infestation with some success.

It is likely that a successful vaccine against ticks will have to contain several different antigens to be effective.

EPIDEMIOLOGY AND MODELLING

The ecology of the free-living stages of *Rhipicephalus appendiculatus* with reference to computer simulation modelling

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The developmental kinetics of *Rhipicephalus appendiculatus* are well understood and can be modelled satisfactorily. Simulating the phenology of this species, however, still presents formidable problems. The principal reason for the poor performance of most simulation models with respect to the situation in Southern Africa appears to be the failure to take into account behavioural diapause entered by adult ticks after moulting. In order to have any degree of success when attempting to understand the population dynamics of *R. appendiculatus*, the following parameters must be considered: (i) the environmental conditions that induce diapause, (ii) the conditions, both environmental and inherent to the tick, that maintain diapause and (iii) the relative benefits offered by the state of diapause.

Regular tick collections and behaviour studies under quasi-natural conditions in the Eastern Province of Zambia indicated that the phenology of *R. appendiculatus* was a result of an intricate interplay between the environmental conditions, the tick's diapausing behaviour and its body size. The behavioural studies demonstrated that a diapause is induced in newly moulted adults when the photoperiod falls below a threshold, provisionally identified as 11 hr 45 min. The results of these studies also indicated that, in the Eastern Province of Zambia, diapause was not terminated by means of a photoperiodic cue, but rather because the adults could not maintain their diapause due to their increasing (physiological) age. Given a rudimentary model of the diapausing behaviour, the species' phenology in the Eastern Province of Zambia can be understood by taking into account the developmental kinetics governed by the prevailing temperatures. However, at this point the overriding effect of the body size becomes obvious and the resulting simulation model quickly becomes prohibitively complex.

If computer simulation models are to be used to further our understanding of the phenology of R. appendiculatus, an effort will have to be made to obtain basic data concerning the tick's body size, its inheritance and its effect on popul ion dynamics. Furthermore, the exact mode of maintaining diapause and the relative benefits of this diapause must be elucidated. Even then it must be realized that 'real-life' simulations, beyond an attempt to understand observations made with regard to the species' ecology, may not be possible in the near future.

DISCUSSION ON TICKS, DIAPAUSE, MODELS AND CONTROL

The discussion was led by Drs. Dirk Berkvens, Andy Norval, Rupert Pegram and Alan Young and attended by about 25 participants to infection. The initial focus of the discussion was on two recent papers (Lawrence, 1991 and Norval *et al.*, 1991) on the epidemiology of theileriosis in Southern Africa. In these papers the case was argued for vector population dynamics and susceptibility of vector populations as major influences in the disease expression. It was postulated that vigorous tick control and unfavourable climatic conditions lead to the extinction of non-diapausing *Rhipicephalus appendiculatus* populations that may have been introduced from East Africa. The hypotheses were challenged by a case put forward by Pegram and Berkvens which was prepared for the workshop. The outcome of the discussion was a much better understanding by the participants of tick population dynamics and the role and nature of behavioural diapause (also from the preceding paper by Berkvens). A series of laboratory and field experiments was agreed to examine southern and eastern African (close to the equator) tick behaviour and survival in each others' environment to test some of the hypotheses.

The discussion then moved to the inheritance of size in *R. appendiculatus* and its influence on longevity of ticks and its role in *Theileria parva* transmission. This too will be the subject of some studies. The discussion finally turned to a general consideration of available models, their limitations and the ultimate possibility of a predictive model for use in determining national discase control strategies. It was generally felt that while some models in use for ticks were fairly efficient in defined circumstances, the few developed for tick-borne diseases were not good. It was agreed that much more work is required on existing models (Climex, T3, Lonestar, ECFXPERT, ICIPE and those of Berkvens and Medley) to 'mprove them and to develop new models that might also incorporate production data.

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Assessment of the impact of control measures against theileriosis

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Theileriosis caused by *Theileria parva* and transmitted by the tick *Rhipicephalus appendiculatus* is widely prevalent in 11 countries of eastern, central and southern Africa, where it presents a major constraint to the development of the livestock industry. The disease is of particular significance in taurine and taurine-zebu cross cattle and in more intensive livestock production systems that limit natural exposure of calves to infected ticks, and thus the development of endemic stability. With the availability of effective procedures for the immunization of cattle against theileriosis using broadly immunogenic stocks of *T. parva* in the infection-and-treatment method, and the prospect of effective sub-unit vaccines for all the major tick-borne diseases on the horizon, there is an increasing awareness of the importance of assessing the impact of controlling theileriosis and other tick-borne diseases for three main reasons:

- 1. To assist governments, farmers and donor agencies in decisions on the efficient allocation cf resources for tick-borne diseases control.
- 2. To define appropriate target populations for immunization and develop mechanisms for sustainable immunization programs.
- 3. To assess the long-term consequences of removing or reducing the constraint of tick-borne diseases from farming systems.

The Socioeconomics Program at ILRAD, in collaboration with scientists in national research organizations in the region, is carrying out research into the impact of theileriosis and its control in the following broad areas.

ECONOMIC IMPACT

Regional, national (Uganda) and farm-level (Kaloleni Division, Kenya) assessments of the cost of theileriosis and the economic benefits of applying infection-and-treatment immunization have been undertaken, and further studies (Kenya, Zimbabwe, Uasin Gishu District of Kenya) are under way or planned. The annual regional economic cost of theileriosis has been estimated to be US\$ 168 million, a figure which includes an estimated annual mortality of 1.1 million cattle (Mukhebi *et al.*, 1992). The benefits of theileriosis control by the infection-and-treatment method of immunization have been estimated to be high, particularly in taurine and taurine-zebu cross cattle, with benefit:cost ratios in the range of 2:1–17:1 (Mukhebi *et al.*, 1989; Mukhebi *et al.*, 1992; ILRAD, 1992). Further studies by ILRAD of financial and economic impacts of theileriosis control in different livestock production and theileriosis risk circumstances are under way in several countries of the region in collaboration with the relevant national research institutes.

SOCIAL AND ENVIRONMENTAL IMPACT

Methodologies for characterizing livestock production systems on the basis of the likely differential impact of disease control have been developed in Kenya. In Kilifi District of Coast Province, livestock production systems were classified according to agro-ecological zone, cattle type, and grazing system (ILRAD, 1991). In the highland Uasin Gishu District, farm size was found to be associated with cattle herd size and management practices, and was used as a grouping variable in order to examine inter-farm variation in access to economic and productive resources, in food distribution and in disease risk (Huss-Ashmore and Curry, 1992; Delehanty, 1991). Studies are also under way to evaluate the nutritional impact of disease control, and incorporate this assessment with economic impact. For small scale intensive dairy production at the Kenya Coast, the milk from an additional dairy cow surviving due to immunization against theileriosis was estimated to provide an average household with an additional 498 Keals per day, or Kshs 16.13 if the milk was sold. The annual profit from one dairy cow surviving could furnish 289-466 man-days of a balanced diet composed of commonly-eaten foods. (Huss-Ashmore, 1992). Environmental impact studies are in the planning stage. However, a conceptual model and hypotheses to be tested have been developed. Field work has begun at one site in eastern Africa, and is planned for other sites in southern, central and West Africa (Reid et al., 1993).

EPIDEMIOLOGICAL IMPACT

A combination of stratified serological studies and sentinel calf studies have been used in two districts of Kenya to identify the target populations for theileriosis immunization based on disease risk. In sentinel calf studies in Kilifi District of Coast Province, Kenya, the interval between first exposure to natural challenge and the acquisition of T. parva infection in a group of 15 taurine cross calves was found to range from 12–98 days. The incidence of ECF in this group was 88% and the case fatality 66% in spite of the availability of chemotherapy. This demonstrated the high priority for immunization of taurine cross calves and the narrow window of time within which inumunization should take place. In a cross sectional serological study of dairy cattle in the same distact, antibody prevalence rates to T. parva varied by agro-ecological zone, cattle type, age group and livestock management system from 85% in free-ranging zebu cattle in the wetter coastal lowland zone to less than 58% in zero-grazed dairy cattle in the same zone (S.H. Maloo, W. Thorpe, J.M. Katende, G. Kioo, V. Nantulya, S. Williamson, and B.D. Perry, in preparation). In a further study of zebu cattle in the district, antibody prevalence rates ranged from 22 to 85% across agro-ecological zones, suggesting differences in the justification for immunization against ECF in different zones (Deem et al., 1993).

Data from these and other studies are being used to model the epidemiology of tick-borne diseases (Medley *et al.*, 1993) and the impact of control measures under

different circumstances (R.A.I. Norval, B.D. Perry, M.I. Meltzer, R. Kruska and T.H. Booth, in preparation; B.D. Perry, R.L. Kruska, R.A.I. Norval, S.E. Randolph, D.J. Rogers and U. Ushewokunze-Obatolu, in preparation).

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STRATEGIES



Perspective of the Organization of African Unity-Inter African Bureau for Animal Resources (OAU-IBAR)

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The Organization of African Unity-Inter African Bureau for Animal Resources (OAU-IBAR) is guided by the OAU member states when drawing up the priority list for the livestock diseases to be controlled on the continent. Until February 1989, the priority list included, in descending order, rinderpest, tsetse and trypanosomiasis and contagious bovine pleuro-pneumonia (CBPP). Since February 1989, tick and tick-borne disease control has been added to the list.

OAU-IBAR will collaborate very closely with FAO and ILRAD in the area of ticks and tick-borne disease control. IBAR's overall strategy is that the control of ticks and tick-borne diseases should be on a regional basis.

Perspective of the Southern African Development Coordination Conference (SADCC) Livestock Sector

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The Southern African Development Coordination Conference (SADCC) represents a grouping of the ten majority-ruled southern African states. The individual country disease priorities differ and even among the tick-borne diseases, individual country priorities are not the same. However SADCC considers ticks and tick-borne diseases as among the most important diseases in the region.

Ticks and tick-borne diseases are regional problems and there is in place a regional project to try and address these problems. This project is the Regional East Coast Fever and Heartwater Vaccines Production and Immunization Project based in Lilongwe, Malawi, and Harare, Zimbabwe. The main objective of the project is to create a self-supporting regional centre for the production of East Coast fever and heartwater vaccines to control these major diseases in the region.

Current funding of the ECF component of the project is due to end in December 1991. Discussions with the United Nations Development Program (UNDP) on funding the project beginning in January 1992 are ongoing. The UNDP is, however, only willing to consider funding the project if it is a priority project in SADCC, and if other donors agree to co-finar cing arrangements.

This is a priority project for the SADCC Livestock Sector and we would like to see it continue. Support is still required for the project to consolidate what has been achieved and for SADCC to prepare to take it over. SADCC is indeed committed to taking over the project, attaches great importance to it and would like to see it succeed. It is envisaged that it will be a commercially self-sustaining vaccine production unit selling vaccine to anyone willing to pay.

Perspective of the International Laboratory for Research on Animal Diseases (ILRAD)

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The International Laboratory for Research on Animal Diseases (ILRAD) was established by the Consultative Group on International Agricultural Research (CGIAR) in 1973 with a global mandate to improve livestock disease control. Since its inception it has worked on tsetse-transmitted trypanosomiasis and theileriosis caused by *Theileria parva*.

ILRAD has four programs: Trypanosomiasis, Theileriosis, Epidemiology and Socioeconomics and Cooperative Programs, Training and Information. ILRAD's Theileriosis Program is divided into three areas, epidemiology and biology, antigens and vaccine development. The involvement with tick-borne diseases in the eastern, central and southern Africa region is as a partner. ILRAD has been directly involved with the implementation of the infection-and-treatment method of control on Unguja and Pemba islands of Zanzibar and in Zimbabwe and has assisted national agricultural research systems in other countries of the region with training of staff, provision of diagnostic reagents and characterization of *Theileria parva* stocks. ILRAD's current research program is directed toward developing new vaccines against *T. parva* and improved diagnostic and characterization reagents for tick-borne diseases.

ILRAD's Epidemiology and Socioeconomics Program is developing methodologies for assessing the impact of disease control. It has selected two widely divergent areas for this work, Kenya and Zimbabwe, and has had an association with the Zanzibar *T. parva* immunization program in assessing the economic impact of the program. Once developed, these methodologies will be made available to assist in the assessment of the impact of control measures being implemented within the region.

The third ILRAD program that contributes to tick-borne disease control in the region is Cooperative Programs, Training and Information. This program is responsible for the formal courses run regularly on diagnosis of haemoprotozoan diseases, for specialist courses and for individual training. ILRAD's Training and Information Program will participate in discussions with national and regional projects to identify the training needs at different levels so that it complements the training offered or planned by the projects.

RECOMMENDATIONS

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Recommendations

- That a pre-investment phase be initiated for tick-borne disease control by immunization based upon quality controlled vaccines produced on a commercial, cost recovery basis and delivered in a sustainable manner.
- That a standing committee be formed to determine the standards for characterization, infectivity, viability and potency testing of stabilates to be used for *Theileria parva* vaccination and for blood vaccines for anaplasmosis, babesiosis and cowdriosis.
 - 1. The committee will meet to define its terms of reference which must be approved by OAU, FAO and ILRAD.
 - 2. The committee will have power to co-opt member(s) with specific technical expertise.
 - 3. The committee will have the power to review the vaccine production sy 'ems in use in the region and to ensure compliance with the standards laid down.
 - 4. The standards will be submitted to OIE for inclusion in the OIE Manual of Standards.
 - 5. The workshop nominated Drs. Chizyuka (Zambia), Dolan (ILRAD, Kenya), Irvin (ODA, Kenya), Pegram (FAO, Zimbabwe), Musime (OAU, Kenya) and Musisi (FAO, Malawi) to the committee.
- That existing models for ticks and tick-borne diseases should be more widely employed and evaluated, and that new models should be constructed as necessary, to assist in designing more effective control measures.
 - 1. The ultimate objective is predictive models that can be used by disease control planners in designing strategy.
 - 2. These models should be assembled, used and refined in Africa.
- That a network be established to provide effective information exchange on ticks and tick-borne diseases within the region.
 - 1. The network will be run by a committee and will be supported by FAO.
 - 2. The network be based in Malawi and will use the existing newsletter produced by the FAO-sponsored vaccine projects as a basis for its publications.
 - 3. The workshop participants nominated Drs. Mfitilodze, (University of Malawi), Lawrence (FAO, Malawi), Musime (OAU, Kenya) and Musisi (FAO, Malawi) to the committee.
- That the training needs of each country should be identified by the national and regional donor-supported projects in collaboration with NARS. The courses required to meet these needs should be discussed and harmonized between NARS, donor-funded projects, ILRAD and other IARCs.

Definition of technical or experimental death

The need for this definition is essentially humanitarian and is intended to apply in all experimental situations. There is no scientific justification for allowing unnecessary suffering in animals when immunization fails to prevent severe disease or when control animals are used to indicate that a stabilate, controlled tick or natural challenge is infective.

Technical or experimental death is defined as a state of severe disease in which it can be reasonably expected that an animal will die unless treated. For experimental purposes animals in this category will be recorded as having had a 'severe reaction' (Anon, 1989) and died but will be treated or euthanised.

In the laboratory, parameters such as parasitosis, fever, fall in total leucocyte count and the clinical condition of the animal may be used to determine severe reaction and therefore the state of technical or experimental death.

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CLOSING ADDRESS

Closing address

Hon. J.O. Bwangamoi

Deputy Minister of Agriculture, Animal Industry and Fisheries Government of Uganda Parliament Buildings Kampala, Uganda

I am very happy to be here this afternoon to officiate at this closing ceremony of the FAO/DANIDA review meeting and the OAU/ILRAD/FAO workshop on ticks and tick-borne diseases.

I understand from the organizers that these two meetings have been in session for six days. I have been informed that the participants at these meetings have involved experts in tick and tick-borne disease control, directors of veterinary services, donors and others interested in the tick and tick-borne disease problem.

I believe that the coming together of so many scientists like you has resulted in valuable exchange of knowledge and experiences and will greatly help to pave the way toward the establishment of practical, cost effective methods of controlling ticks and tick-borne diseases in East and Central Africa.

The Government of Uganda attaches great importance to increasing the output of animal products and livestock productivity in general through the improvement of animal health, nutrition, husbandry and management. Effective control of disease is a prerequisite to livestock development and the government therefore puts great emphasis on eliminating disease in order to create a favourable environment for production.

Ticks and tick-borne diseases are a major hindrance to livestock production in East and Central Africa and Uganda as a member of the region appreciates the efforts being made by the FAO and DANIDA program in combating these diseases. Your coming together therefore to discuss and find solutions to a common problem is very welcome.

I am informed that immunization trials against East Coast fever are going on in the various countries of the region.

I am happy to note that, among others, the meetings recommended that future projects should be designed to make available to national governments appropriate, safe and cost-effective technologies for control of ticks and tick-borne diseases.

I would like to take this opportunity to express the gratitude of the Government of Uganda to FAO and DANIDA for funding the program in tick and tick-borne disease control in East and Central Africa. I also wish to thank OAU-IBAR and ILRAD for the support they have given to Uganda in various aspects regarding the control of ticks and tick-borne diseases.

I now have much pleasure in declaring the FAO/DANIDA review meeting and the OAU-IBAR/ILRAD/FAO Ticks and Tick-borne Disease Control Workshop closed.

APPENDIX: LIST OF PARTICIPANTS

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