ILRAD REPORTS JULY 1983

Published by the International Laboratory for Research on Animal Diseases
Volume **1** Number **1**

Towards the development of an ECF vaccine

East Coast fever (ECF) is a particularly virulent form of theileriosis, a complex of diseases caused by single-celled *Theileria* parasites. ECF is caused by the parasite *T parva parva.* It is an important constraint on livestock development in Kenya, Uganda, Tanzania, Mozambique, Malawi, Zambia, Rwanda, Burundi and Zaire.

ECF may occur anywhere in eastern or central Africa where the altitude is below 2300 metres and rainfall is over 50 cm a year. These conditions are necessary for the survival of the brown ear tick *Rhipicephalus appendiculatus* which transmits the parasite to cattle. According to the Kenyan Minister for Livestock Development, Mr Paul Ngei, up to 70 000 cattle die every year of ECF in Kenya alone. In the region as a whole, the disease affects an estimated half a million cattle annually.

ECF is controlled primarily by dipping or spraying susceptible animals with acaricides, chemicals which kill the tick vector. Good pasture management and stall feeding systems help to keep cattle relatively free of ticks, and fencing and movement controls keep susceptible cattle away from animals which might carry the disease. But frequent dipping or spraying with acaricides is still necessary, and this is expensive and requires a high level of management. Also, cattle treated in this way do not build up any resistance to ECF, so any breakdown in the treatment regime can lead to serious losses. Thus there is an obvious need for an ECF vaccine.

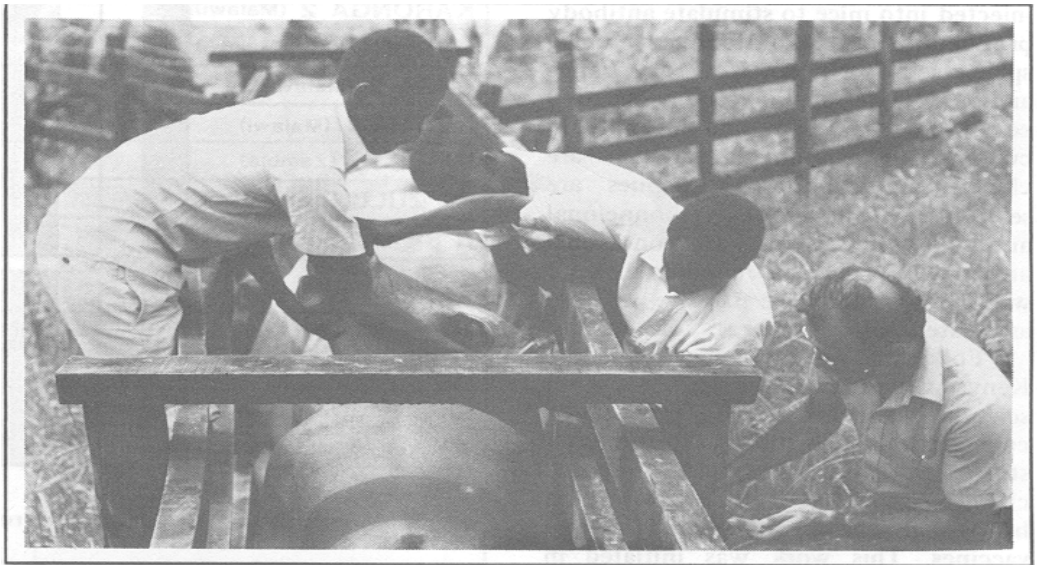
Parvaquone, a drug which kills the parasites, has been produced recently by Wellcome Kenya Ltd and is now marketed under the name Clexon. This is an important development, but effective treatment with parvaquone requires early diagnosis and a level of veterinary supervision which is not always available to farmers. The need for an ECF vaccine is as urgent as ever.

Early research on ECF

In areas where cattle are continuously exposed to low levels of ECF from birth, some undergo a mild infection and recover. Afterwards, they show long-lasting immunity to the

same or similar disease strains. This acquired resistance to reinfection suggests that prospects are promising for the development of an effective vaccine.

A concerted effort to develop an ECF vaccine was initiated in 1967 at the former East African Veterinary Research Organization (EAVRO), supported by the Food and Agriculture Organization (F.AO) of the United Nations and the United Nations Development Program (UNDP). Scientists demonstrated that it is possible to vaccinate cattle experimentally by infecting them with *Theileria* parasites and treating them at the same time with a long-lasting formulation of the antibiotic oxy-tetracycline (Terramycin LA-Pfizer). However, this 'infection and treatment' approach has a number of drawbacks. Originally concern centred on the fact that animals immunized in this way might retain live parasites in their blood stream and thus act as disease carriers. Also, it became clear that a number of different ECF strains occur in the field, and animals immune to one strain may be partly or fully susceptible to another. In addition, not all strains can be contained by oxytetracycline, so the initial infection with live parasites may be fatal.



Dr Subash Morzaria (on right) takes a blood sample from a steer exposed to ECF at a field site in Kenya's Coast Province.

Research on ECF declined in the mid-1970s with the termination of the FAO/UNDP project and the dissolution of the East African Community which supported EAVRO. This work has been taken up again in recent years by a group of scientists at ILRAD led by Dr Tony Irvin, in cooperation with EAVRO's successor, the Kenya Agricultural Research Institute (KARI), and the Ministry of Livestock Development.

It has now been shown that cattle which recover naturally from ECF commonly retain parasites in their bloodstream. Thus, the occasional retention of parasites in the blood of immunized animals may be acceptable since such animals will not add significantly to the pre-existing population of carriers. Dr Irvin and his team are tackling the other two problems encountered in the 'infection and treatment' approach — the identification and classification of different ECF strains and the development of safe and effective methods of infecting animals and treating them to confer immunity. Other promising immunization methods are also being investigated by ILRAD scientists.

Characterizing ECF strains

Different strains of *Theileria* parasites probably possess strain-specific antigens. These antigens are difficult to study directly, but scientists at ILRAD have produced antibodies which can be used to identify parasite strains in the laboratory according to their specific antigens.

First *Theileria* parasite material is injected into mice to stimulate antibody production Then

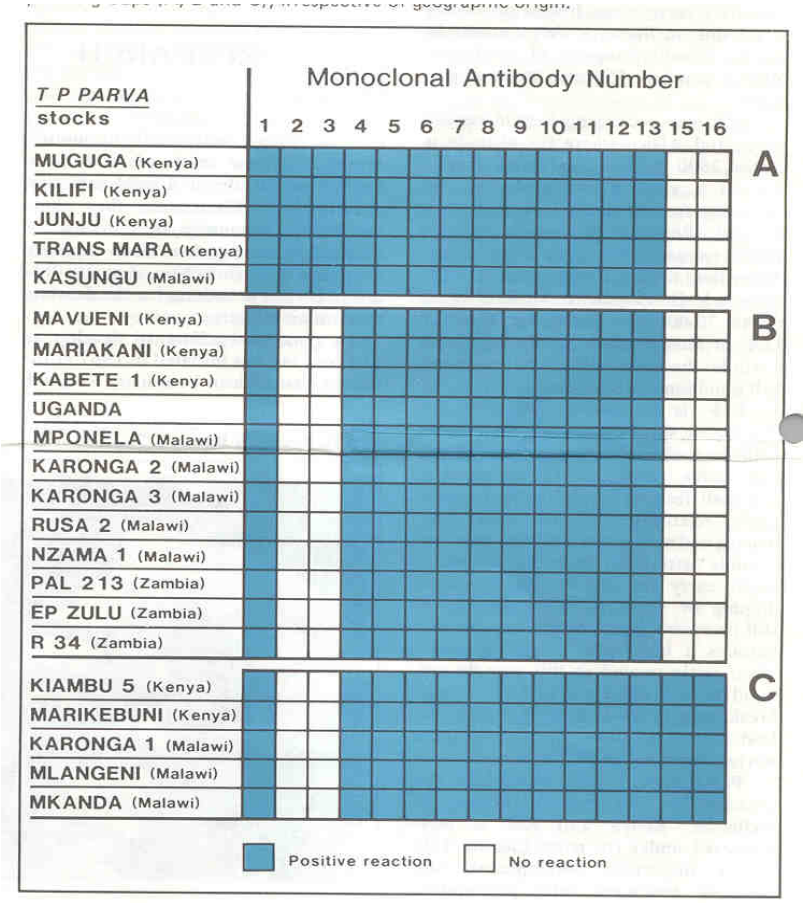
antibody-producing spleen cells are taken from these mice and fused with myeloma (bone marrow) cells which can grow and multiply in culture. The resulting hybrid cells are cloned and appropriate clones are selected which produce monoclonal antibodies. In this wavy, 16 monoclonal antibodies have been raised at ILRAD which respond to the parasite antigens associated with different ECF strains.

Following an agreement with the Kenya Government in 1979, ILRAD scientists launched a field program in collaboration with the Ministry of Livestock Development to isolate and characterize strains of ECF which could be used as the basis for experimental vaccines. This work was initiated in Kenya's Coast Province. Parasites isolated from infected cattle and ticks at the coast have been compared with strains identified earlier by scientists at EAVRO, and with isolates collected more recently in other parts of Kenya and in Uganda, Malawi and Zambia.

Twenty-two stocks of *T p parva* have been isolated from widely separated areas and screened using the battery of 16 monoclonal antibodies. The responses of the different antibodies are measured using the indirect fluorescent antibody (IFA) test. Based on comparisons of these responses, all the stocks tested so far fall into one of three groups, A, B or C, as shown in the figure. These groupings are not related to the areas from which the stocks were obtained.

Cattle have been immunized by infection and treatment using *Theileria* stocks identified by the antibody responses. These cattle were then challenged with different stocks from the same group as their original infection or with stocks from different groups. Animals challenged with a stock from the same group as their first infection showed very mild disease symptoms or none at all. Animals challenged with a stock from a different group often showed severe symptoms, and some died.

These results suggest that the characterization of stocks by monoclonal antibodies can provide information which predicts cross-protective patterns in cattle. Thus, it may be possible to produce vaccines for specific areas based on the isolation of local ECF strains. Eventually it may also be possible to develop hybrid strains which will protect animals over much wider regions.



Simplified diagram of the reactions of 15 monoclonal antibodies with macroschizonts of 22 T p parva *stocks, measured by the IFA test. Stocks from Kenya, Uganda, Malawi and Zambia have been screened. They all appear to fall into one of three profile groups (A, B and C), irrespective of geographic origin.*

Immunization trials at the Kenya coast

ILRAD scientists are now carrying out vaccination trials in Coast Province in cooperation with the Ministry of Livestock Development and the Agricultural Development Corporation. Two *T p parva* stocks were selected as the basis for these experiments, *T p parva* Kilifi from group A and *T p parva* Marikebuni from group C. Cross-immunity trials had suggested that cattle immunized with these stocks are protected against the other ECF stocks isolated at the coast.

Eleven Jersey calves between 3 and 12 months old were used for one field trial. This breed is highly susceptible to ECF. Eight calves were immunized by infection with Kilifi and Marikebuni parasites followed by two doses of oxytetracycline. After 25 days, four of these were infected and treated again and four were injected with parasites only, to boost their immunity. One of the second group reacted severely and was treated with parvaquone.

Fifty-four days after the booster immunization, the eight immunized calves and three non-immunized controls were taken to graze for 3 months in an endemic ECF area. Ticks were counted every second day; an average of over 50 ticks was found on each calf throughout the period. ECF parasites were observed in the lymph nodes of all the calves but the non-immunized calves showed much higher levels of parasitaemia and they all died of acute ECF. The immunized calves showed mild or inapparent symptoms and all survived.

At another site, 16 Sahiwal/Red Poll calves were immunized against ECF by inoculation with Kilifi and Marikebuni parasites and treatment with oxytetracycline. Two controls were inoculated with the Kilifi parasites only and two with Marikebuni; five other controls were treated with oxytetracycline only. The two calves which were only inoculated with Marikebuni

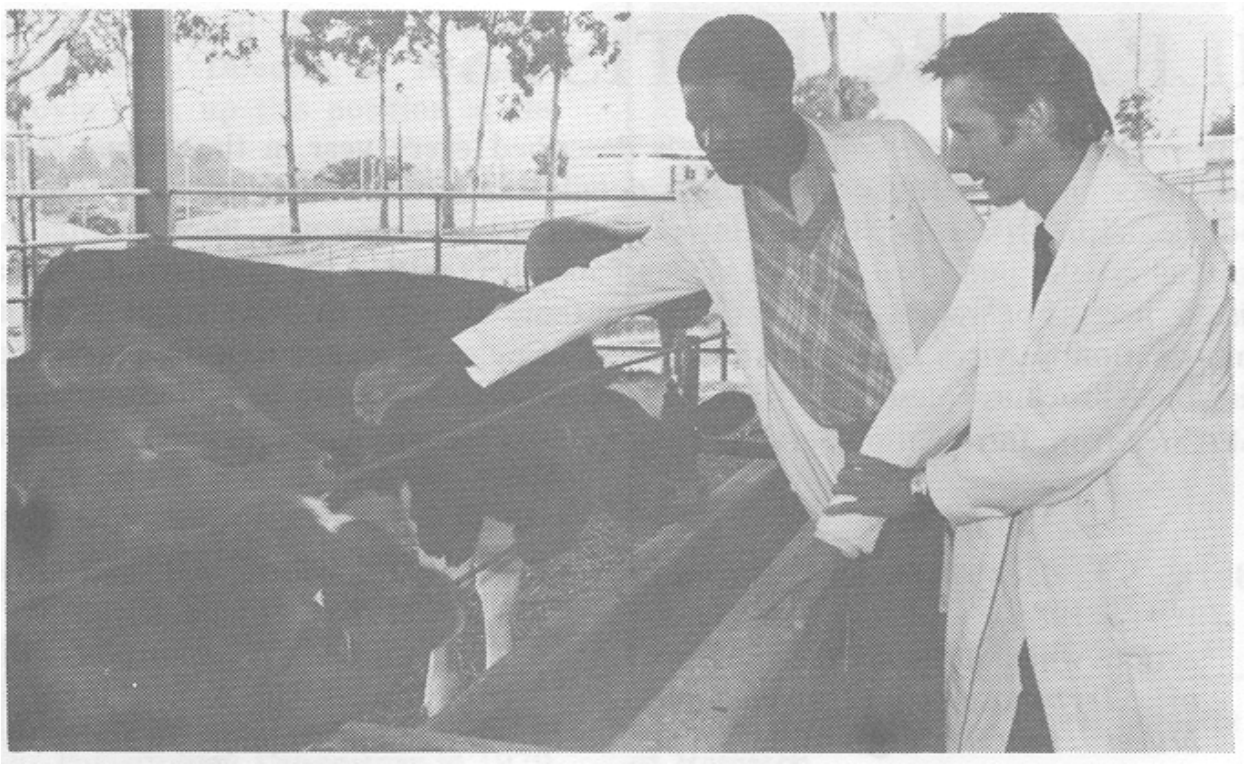
parasites developed severe parasitaemia and were treated with parvaquone. The two which were only inoculated with the Kilifi strain did not develop infection. This suggested that the Kilifi stabilate used may not have been viable, so these two calves were grouped with the five controls. The treated calves were thus probably only immunized against *T p parva* Marikebuni.

Forty-nine days after the initial immunization, all 25 calves were taken to graze for 3 months in fields infested with ticks. Parasites were observed in the lymph nodes of 8 out of the 18 immunized calves and all 7 controls. None of the immunized calves became seriously ill, while five of the controls suffered acute ECF and four died.

Breed and Treatment	Number	Level of Reaction to Challenge			
		None	Moderate	Severe	Death
Jersey					
Immunized	8	5	3	0	0
Controls	3	0	0	0	3
Sahiwal/Red Poll					
Immunized	18	10	8	0	0
Controls	7	0	2	1	4

Reactions of calves immunized experimentally against ECF and exposed to field challenge at two locations in Kenya's Coast Province.

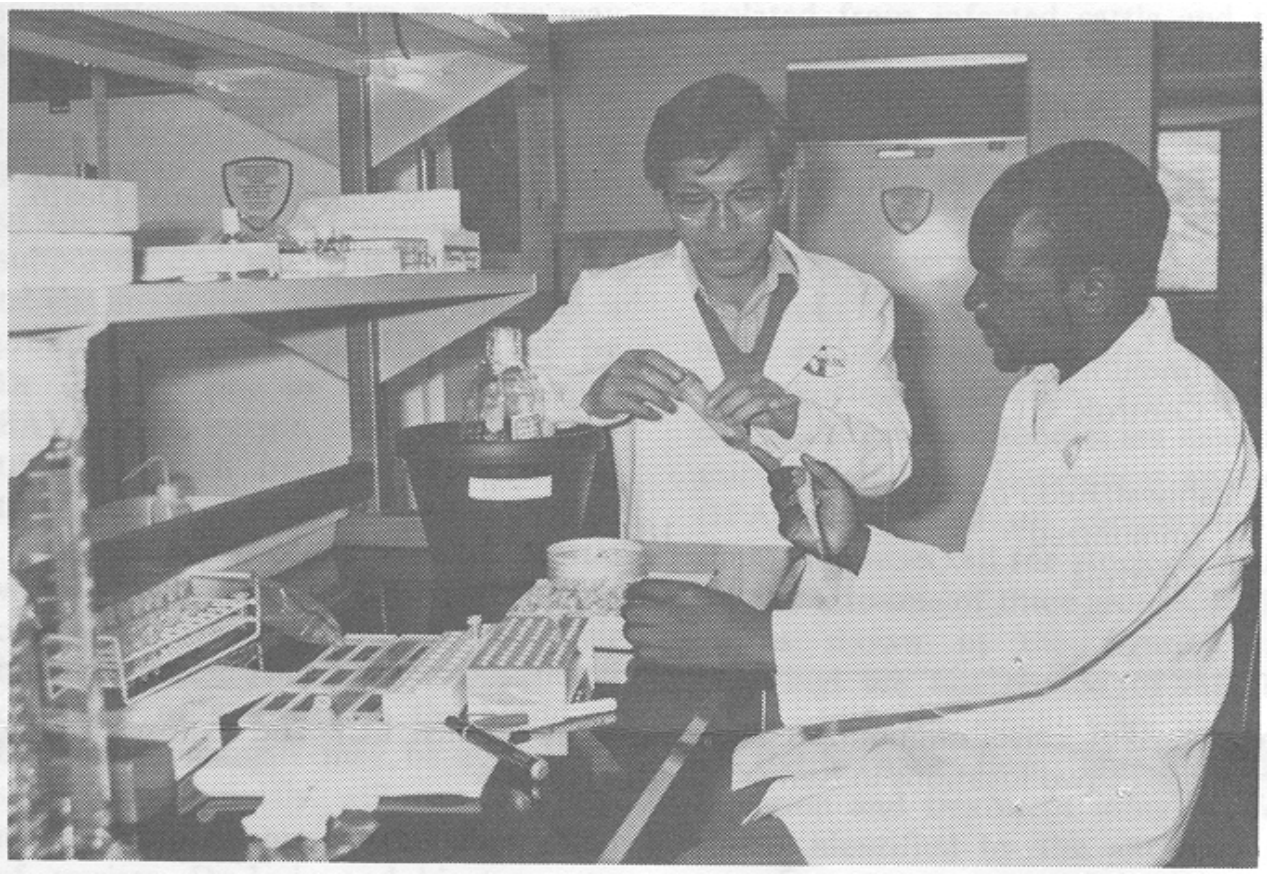
The results of these two trials are summarized in the table. Two other field trials are now in progress at different sites. Boran cattle have been immunized by infection with the Marikebuni and Kilifi strains or the Marikebuni strain only and treatment with oxytetracycline. They are now being exposed to infected ticks in two different field locations.



Dr Evans Taracha and Dr Tony Irvin monitor cattle which have been immunized experimentally against ECF.

Other approaches to vaccine development

While field trials are being conducted in Coast Province, work is also in progress at ILRAD to develop new immunization techniques. Two possible starting points are being investigated as the basis for a vaccine which would stimulate effective immunity but would not require the level of veterinary supervision needed when animals are infected with live parasites. The two immunogens which are being purified and tested are antigens from *Theileria* sporozoites, the stage of the parasite which is transmitted to cattle from infected ticks, and antigens from *Theileria* schizonts, the stage which develops in the cells of infected cattle.



Dr Toru Fujinaga and Mr Reeves Njamunggeh check antigen preparations from ECF isolates before screening with monoclonal antibodies.

Another research effort concentrates on *Theileria parva lawrencei,* a subspecies of the parasite closely related to *T p parva. T p lawrencei* is normally carried by wild buffalo, but it can cause severe infections in cattle. The ECF situation appears much more complex in areas where cattle come into contact with infected buffalo. Scientists at ILRAD are working with colleagues at KARI and the Kenya Government's Veterinary Research Laboratories who are studying *Theileria* in buffalo and its transmission to domestic cattle. This project is supported by The Netherlands.

Using electron microscopy and other techniques, ILRAD scientists are also carrying out detailed studies of the lifecycle of ECF parasites to identify stages, which may be particularly vulnerable to control. The long-term goal of all ECF research at ILRAD is to produce safe and effective vaccines, which will protect cattle throughout the region where the disease is now endemic.

Recent articles by ILRAD scientists

The research conducted at ILRAD is described every year in the annual report. Scientists on the ILRAD staff publish detailed accounts of their research results in books and in articles written for international scientific journals. Book chapters and journal articles published by ILRAD staff members during the first part of 1983 are listed here. For reprints, contact the authors directly or send requests to the Information Services Department at ILRAD. Copies

of annual reports, publications lists and brochures describing ILRAD's research and training activities can also be obtained from Information Services. Single copies are sent without charge.

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Training opportunities at ILRAD—Steady advances since 1976

What is the role of training in an institute with a mandate to carry out basic scientific research? 'An important one', according to Dr Jim Lenahan, the Training Officer at ILRAD. 'But training activities must be carefully planned to complement, rather than compete with, the research program.'

The first annual report—for 1976—lists three graduate students who were carrying out research projects at ILRAD as part of their Ph.D. requirements. By the end of 1982, the scientific staff had more than doubled and training had become a full-fledged program at ILRAD. A total of 133 people had received training on an individual basis and over 600 had attended training curses, seminars and workshops.

Different types of individualized training are offered. Scientists and senior technicians come to ILRAD to learn specialized laboratory and field techniques. These are staff members

working in laboratories or field programs in developing countries, most often in Africa, and the specific training they receive depends on the needs of their home institutions. The goal is to identify individuals who are working in appropriate areas and to give them exactly the additional experience they need to do a better job.

Graduate students work at ILRAD for periods of 1 to 3 years to fulfil requirements for a higher degree. Most of these students are from Africa. Post-doctoral fellowships are also offered to young scientists who work within ILRAD's research program, normally for a period of 2 years. Candidates for post-doctoral positions are selected on an international basis, but preference is given to well-qualified scientists from African countries.

Current individual training activities

During the first 6 months of 1983, 11 post-doctoral fellows were conducting research at ILRAD and 17 graduate students were working in pursuit of higher degrees or completing their studies at universities overseas with ILRAD support. Nine scientists and senior technicians were receiving specialized training.

But numbers do not tell the whole story. Who are these people and what are they learning during their stay at ILRAD? The nine scientists and senior technicians who participated in the training program during the first half of 1983 are good examples. They came from 9 different African countries: Burundi, Kenya, Mali, Rwanda, Sudan, Tanzania, The Gambia, Zambia and Zimbabwe. Four are on the staff of national veterinary laboratories and one is in his country's national veterinary service. Two work for FAO animal disease projects, one is on the staff of a pest research institute and one works for a national livestock marketing board.

Each trainee is assigned to a member of ILRAD's scientific staff who has the primary responsibility for providing an appropriate training program. The nine trainees who were working at ILRAD during the first 6 months of 1983 stayed for periods ranging from 10 days to 7 months, depending on their specific training needs. Three learnt parasitological and immunological techniques related to trypanosomiasis. Three learnt advanced laboratory techniques required for East Coast fever research. Two learnt serological and diagnostic methods used in animal disease research in Africa, and one learnt improved methods of maintaining tsetse fly colonies. All are returning to their home institutions to put this training into practice.

Training Courses

Seventeen scientists and technicians participated in ILRAD training courses during the first 6 months of 1983. Thirteen participated in a Course on the Diagnosis of Haemotropic Cattle Diseases, with Emphasis on East Coast Fever, which was held from 14 February to 11 March. General sessions covered the pathology and epidemiology of ECF and the lifecycle of the *Theileria* parasite. Scientists then explained how to diagnose ECF and other haemotropic diseases of cattle and discussed current control methods and recent advances in the study of natural and induced immunity.

In addition to these discussions, the course participants learnt a number of laboratory techniques used in the study of ECF. These included:

- collection and examination of blood and lymph node biopsy smears from cattle
- recognition of ECF macrochizonts, microschizonts and piroplasms and differentiation of *Theileria* species
- recognition of *Rhipicephalus appendiculatus* and other important tick species
- dissection and preparation of tick salivary glands: recognition of different types of acini and different parasite forms
- indirect fluorescent antibody (IFA) test and other tests used for the diagnostic serology of ECF.

The 13 participants came from Kenya, Indonesia, Malawi, Mozambique, Somalia. Sudan, Tanzania, Uganda, Zambia and Zimbabwe. Four were veterinarians, eight were laboratory technicians and one was a veterinary assistant. Ten were staff members in national veterinary laboratories or livestock ministries and three were working in FAO projects. One participant from Zambia reported back to his laboratory director, 'The course included both theory and practical classes. It was set in such a way as to enable us to put into practice what we acquired when we go back to our respective countries.'

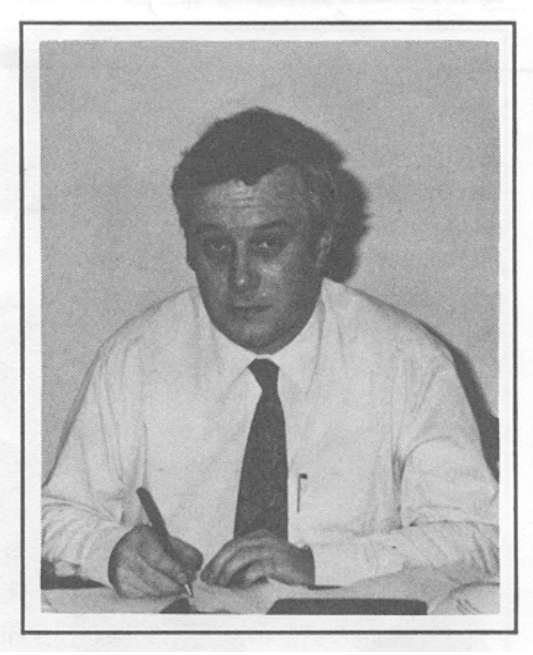
The third Training Course on Trypanosomiasis and other Parasitic Diseases was held at ILRAD from 31 May to 8 July. This series of courses is cosponsored by ILRAD and the International Livestock Centre for Africa (ILCA) to train field staff who are working in the ILCA/ILRAD livestock productivity and trypanotolerance research network. Eleven scientists have participated in training courses associated with the network.

A full schedule of courses and conferences is planned for the months and years ahead. Activities for the second half of 1983 are listed in this newsletter. Increasing numbers of scientists and technicians will also be coming to ILRAD for individual training, always preserving the balance between opportunities for relevant training and basic research priorities.

ILRAD appoints research director

In December 1982, the Board of Directors appointed Dr Jack Doyle as ILRAD's first Director of Research. Dr Doyle took up this position at the beginning of 1983.

Dr Doyle comes from Scotland. He received his B.V.M.S. and Ph.D. degrees from the University of Glasgow and began his career as a lecturer in the Department of Veterinary Medicine, where he conducted research on the immunological aspects of fascioliasis. He then joined the staff of the World Health Organization (WHO) Immunology Laboratory at the University of Lausanne and worked on leischmaniasis immunology and antigenic variation in trypanosomiasis. He came to ILRAD in 1975 and was appointed Coordinator of the Parasitology Laboratory in 1979.



Dr Jack Doyle, ILRAD's new Director of Research.

Calendar notes for the second half of 1983

An *International Conference on the Application of Ruminant Immunology to the Control of Bovine Diseases* will be held at ILRAD from 26 to 30 September 1983. Specialists in the field

of ruminant immunology will meet participants from developing countries who are engaged in research on the immunological aspects of ruminant diseases. The murine, human and bovine immune systems will be reviewed and papers will be presented on current immunological research, with particular emphasis on bovine diseases occurring in Africa. The conference will be conducted in English and French.

An *Interregional Training Course on the Use of Nuclear Techniques in Animal Parasitology* will be held at ILRAD from 17 October to 11 November 1983. FAO and the International Atomic Energy Agency (IAEA) are cosponsors. The course, which is open to 20 participants, will be conducted in English. For more information on conferences and training activities at ILRAD, write to Dr Jim Lenahan, Training Officer, ILRAD, P O Box 30709, Nairobi, Kenya.

The International Centre of Insect Physiology and Ecology (ICIPE) is organizing an International Study *Workshop on Tsetse Behaviour and Population Ecology*, to be held in Nairobi from 23 to 29 October 1983. Topics proposed for discussion include: sensory physiology and reproductive aspects of tsetse behaviour, population ecology and sampling techniques, parasite-vector-host interactions and the impact of recent advances in the study of insect ecology and behaviour on tsetse control strategies. For further information on ICIPE courses and conferences, contact the Senior Communication and Information Officer, ICIPE, P O Box 30772, Nairobi, Kenya.

The *10th Executive Committee* :*Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)* will take place in Brazzaville, Congo, from 18 to 19 November 1983. The Committee will review research and training activities concerned with tsetse and trypanosomiasis control.

A *Training Seminar on Tsetse and Trypanasomiasis Control* will be held in Brazzaville from 21 to 30 November 1983, organized jointly by FAO, WHO and the Organization of African Unity (OAU). The seminar will cover environmental aspects of integrated tsetse-control programs, diagnosis and chemotherapy of animal trypanosomiasis, management of trypanosomiasis control campaigns, training, the role of trypanotolerant livestock and the economics of tsetse and trypanosomiasis control. The progress of the FAO trypanosomiasis control program will also be reviewed. More information on the Brazzaville meeting and training seminar can be obtained from the Director, OAU/IBAR, P O Box 30786. Nairobi, Kenya.

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International Laboratory for Research on Animal Diseases
P O Box 30709, Nairobi, Kenya
telephone: Nairobi 592311
telex: 22040 'ILRAD'
cable: ILRAD/Nairobi/Kenya