ILRAD
1991 Annual Report

International Laboratory for Research on Animal Diseases

P O 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 African animal trypanosomiasis and tick-borne diseases, particularly East Coast fever, a virulent form of theileriosis.

ILRAD is one of 17 centres belonging to a global agricultural research network sponsored by the Consultative Group on International Agricultural Research, whose headquarters are located in the World Bank, Washington, D.C.

In 1991, ILRAD received funding from the African Development Bank, the Rockefeller Foundation, the United Nations Development Programme, the World Bank and the governments of Australia, Belgium, Canada, Denmark, Finland, France, Germany, India, Italy, Japan, the Netherlands, Norway, Sweden, Switzerland, the United Kingdom and the United States of America. ILRAD's total budget for 1991 was US $13.5 million.

ISBN 92-9055-091-0
The correct citation for this publication is: International Laboratory for Research on Animal Diseases. 1992. *ILRAD 1991: Annual Report of the International Laboratory for Research on Animal Diseases.* Nairobi: International Laboratory for Research on Animal Diseases.

CONTENTS

Research Support

TSETSE UNIT

TICK UNIT

LARGE ANIMAL PRODUCTION

LABORATORY ANIMAL PRODUCTION

BIOSTATISTICS AND COMPUTING SERVICES

Publications

Board of Directors

Senior Staff

Financial Statements

COSTS OF OPERATIONS

UNRESTRICTED AND RESTRICTED FUNDS

SOURCES AND APPLICATIONS OF FUNDS

ASSETS AND FUND BALANCES

THE INTERNATIONAL Laboratory for Research on Animal Diseases (ILRAD) was established in 1973 by the Consultative Group on International Agricultural Research (CGIAR), an association of donor agencies with headquarters in Washington, D.C. ILRAD was given a mandate to conduct intensive research leading to improved control of important livestock diseases in developing countries, particularly in Africa.

The work of the Laboratory focuses on immunological and related aspects of trypanosomiasis and tick-borne diseases. Throughout the tropics, where the climate is suitable for parasites and their insect vectors, these infectious protozoan diseases of livestock continue to hold back agricultural and rural development.

ILRAD's strategic research programs on tsetse-transmitted trypanosomiasis and tick-borne diseases, particularly the virulent form of theileriosis in Africa known as East Coast fever, are supported by a third program to assess the social, economic and environmental impacts of improved livestock disease control and by a fourth program that promotes cooperative research and technology transfer, conducts training activities and disseminates information and research results. ILRAD's facilities include a modern complex of research laboratories and supporting units at Kabete, located on the outskirts of Nairobi, Kenya, and a cattle-breeding ranch on the Kapiti Plains, about 70 kilometres southeast of the capital.

GOVERNANCE AND STAFF

THE LABORATORY is governed by an international Board of Directors with 12 members. This year, Professor N.O. Nielsen, from Canada, succeeded Professor I. Månsson (Sweden) as Board Chair and Dr. Florence Cheno-weth (Liberia) was elected to the Board on the retirement of Dr. Abdel Kadir (Nigeria). At the end of

1991, ILRAD staff in post comprised 48 senior scientific and administrative personnel, 10 long-term visiting scientists, 24 specialized technicians, 59 technical support staff and 289 general support staff. Turnover in core senior scientific staff was substantial, with five departures and three new arrivals; six long-term visiting scientists left in the year and two arrived; four vacancies were under recruitment at the year's end. Of a further ten approved international positions, in this year five were held by visiting scientists with external salary support and five remained vacant due to funding uncertainty.

ILRAD congratulates Professor I. Månsson on his appointment as Commander of the Royal Norwegian Order of Merit in May and Dr. Ivan Morrison, a longstanding ILRAD scientist now at the Compton Institute for Animal Health (UK), on being awarded this year the first Wellcome Trust Medal for Veterinary Research and the Keith Sinclair Prize. Seven ILRAD associated research fellows completed their studies and were awarded doctorate degrees during the year. Drs. Vinand Nantulya and Jim Lenahan left ILRAD having each given more than ten years of much-appreciated service to the institute.

CHANGES IN A YEAR OF REVIEWS

THIS WAS AN IMPORTANT year for ILRAD. First, an ILRAD-commissioned external management review was conducted in February. The review team recommended some changes in general management and a major strengthening of research management, which it proposed be conducted on a project basis. These recommendations were accepted. Positions for a Deputy Director General and an Information and Planning Officer were introduced. Heads of Programs reporting to the Director General were given full responsibility for managing ILRAD's four principal programs. The institute's former operational system, whereby scientists acted as Laboratory Coordinators, was replaced by research associates taking on the technical responsibilities of Unit Superintendents and reporting to a Laboratory Manager.

To obtain broad input into the development of future research strategies, major reviews of program activities were conducted and many strategy meetings held during the year. From these discussions, scientific staff developed project proposals and presented these to their colleagues and the Directors during the institute's annual Internal Review in October. Projects for future research were chosen according to revised research objectives, likely budgetary constraints, ILRAD's comparative advantages, the resources available, project manageability, and possibilities for collaborative links. Individuals were then chosen to lead the projects.

These changes, and the processes that effected them, have prompted better communication among staff members and

Dr. Ross Gray *(left)*, Director General of ILRAD, and Dr. William Pritchard, of the University of California at Davis, who is chairing the Expert Advisory Committee of the 1991–1992 Winrock study 'Assessment of Animal Agriculture in Sub-Saharan Africa'.

have brought about greater accountability in use of staff, time and materials. As the new research system settles down and adjustments are made, these changes are expected to lead to more effective research programs and greater scientific productivity.

Another reason for the importance of 1991 is that this year brings to a close ILRAD's third quinquennium. Throughout the year, objectives and directions were reconsidered and plans developed for the coming quinquennium, in line with a rolling five-year system of strategic research adjustments. Part of this activity included preparations made for ILRAD's third major external review organized by the Technical Advisory Committee on behalf of the CGIAR. Preliminary visits to the Laboratory from the Chairman and members of the review team were made late in the year, with the main phase of the review to occur in March and April 1992.

The preparations made for this review included a re-evaluation and redrafting of the Laboratory's Long-Term Plan, the hosting of two international workshops to solicit external scientific expertise and opinion regarding future research needs and priorities in the areas of bovine genome mapping and trypanotolerance (held in April) and improved control of the tick-borne diseases anaplasmosis, babesiosis and cowdriosis (May). (A third planning workshop, on *Theileria annulata*, was held late in 1990.) In addition, a team of five scientific consultants commissioned by the Laboratory reviewed ILRAD's achievements and future plans for research on the epidemiology of trypanosomiasis and East Coast fever. Throughout the year, staff wrote papers and compiled documentation on the achievements and impacts of ILRAD's research programs. The investment in these additional review-related activities, including time spent away from the bench this year, should be well repaid in terms of clear and well-focused programs for the Laboratory in the next quinquennium.

ILRAD'S GLOBAL RESEARCH IMPACT

The documents prepared in this review year served to highlight the global relevance of much of ILRAD's work. The basic research conducted at ILRAD on the African forms of trypanosomiasis and tick-borne diseases have consistently over the years produced information and diagnostic materials of immediate utility and benefit to scientists conducting research on related forms of these diseases that occur in other regions. The Laboratory's longstanding inves-tigations of the immune system of ruminants have generated an expertise in this area as considerable as it is uncommon. Serological reagents, including many monoclonal antibodies produced at ILRAD, are dispatched worldwide to further the research of scientists working to protect many different kinds of domestic livestock against disease. In a collaborative effort to map the bovine

genome and locate the genes that control disease resistance and other desirable productivity traits, ILRAD is developing and tailoring state-of-the-art technologies to speed up the process of identifying bovine genetic `markers'. For this international project, ILRAD is also producing a valuable resource herd of closely related families of cattle whose DNA is being analysed in advanced laboratories from Texas to Queensland. The techniques the Laboratory is developing for immunizing animals with genetically altered organisms and the adjuvants it is preparing to boost the effectiveness of the novel vaccines have a wide potential application. Finally, ILRAD is a pioneer in use of geographical information systems—computer programs designed to man-ipulate and analyse large amounts of spatial data—to determine the epidemiology of a livestock disease in an area, country or region of the developing world and to assess the likely results of implementing control measures against it.

THE NEED FOR BETTER CONTROL OF TROPICAL LIVE-STOCK DISEASES

A MAJOR STUDY was conducted this year to assess the future of animal agriculture in sub-Saharan Africa. (A report of the study, conducted by Winrock International, USA, will be published in early 1992.) Topping the lists of animal health constraints on livestock productivity that emerged from these deliberations are ILRAD's target diseases: tsetse-transmitted trypanosomiasis and tick-borne theileriosis, anaplasmosis, babesiosis and cowdriosis. These diseases clearly remain major factors in inhibiting the integration of livestock into Africa's agricultural production systems.

Efforts to control trypanosomiasis and tick-borne diseases continue to occupy scientists of national agricultural research systems throughout Africa and other regions as evidenced by the proceedings of `The 21st Meeting of the International Scientific Council on Trypanosomiasis Research and Control', held in Yamoussoukro, Côte d'Ivoire, in October, under the auspices of the Organisation of African Unity (OAU), and a workshop on `Ticks and Tick-Borne Disease Control' held in September in Kampala, Uganda, and sponsored by the OAU, ILRAD and the Food and Agriculture Organisation of the United Nations (FAO).

Disease control activities continue to cost farmers, governments and aid agencies large sums of money annually in livestock productivity losses and deaths. The major reasons for this are continuing persistence and expansion of the distribution of tsetse flies and trypanosomiasis, outbreaks of non-tsetse-transmitted trypanosomiasis, development of drug-resistant parasites, and the inadequacy of cattle-dipping regimes to control the tick vectors of theileriosis, anaplasmosis, babesiosis and cowdriosis.

Stone carving from an Egyptian tomb depicting cattle of Africa's ancient, humpless, *Bos taurus* breed. Note that the cow's calf is tethered to her foreleg during milking, a method still practised today by African smallholder farmers (Cairo Museum).

Domestic livestock are a particularly important resource in the development of sustainable agricultural production systems on small-scale farms in developing countries. To help farmers make full potential use of livestock, ILRAD's research programs aim to develop improved disease control methods based on prophylactic vaccination. This approach to maximizing livestock productivity is demonstrably safe, cost effective, reliable, environmentally sound and sustainable over the long term. Research attention is also given at ILRAD to extending the usefulness of the few chemotherapeutic drugs now available for preventing or treating trypanosomiasis and to widening the use of indigenous breeds of African livestock that are innately resistant to trypanosomiasis.

The main text of this annual report is not intended to be an exhaustive account of all the research, training and information activities carried out at ILRAD in 1991 but rather to explain in lay terms the major advances made in the year towards solving critical research problems in tick-borne disease and trypanosomiasis research and the major outputs of the year's work. For further information, readers are directed to ILRAD's *1991 Highlights* and *1991 Annual Scientific Report*, published earlier in the year. The following summarizes some of this year's research achievements.

THEILERIOSIS RESEARCH PROGRAM

THE THEILERIOSIS Research Program continued in 1991 to support regional and national disease-control projects, particularly those in Kenya, Zambia, Zanzibar (Tanzania) and Zimbabwe. ILRAD assisted staff running these projects by employing an array of advanced laboratory tests to define and characterize parasites obtained from their field sites, by providing the projects with reagents for the accurate diagnosis of tick-borne diseases, and, in conjunction with ILRAD's Socioeconomics Program, by developing and using epidemiological models to help project leaders determine which livestock populations are most at risk from tick-borne diseases. ILRAD staff also contributed to international meetings and workshops held throughout the year to assess the current global distribution, control methods and economic importance of tick-borne diseases, to update scientists on the latest findings in research on the biology of the causative organisms, and to ensure the optimization of research efforts for the production of new diagnostics and vaccines.

The ILRAD program has continued to develop and refine antibody-based ELISA systems and DNA probes for precise identification of tick-borne disease organisms. In consultation with laboratories in Australia, the USA and Latin America, antigenic molecules of *Babesia* parasites and *Anaplasma marginale* have been identified for the development of more specific tests for distinguishing these organisms.

East Coast fever is a virulent and often fatal form of theileriosis. The disease is caused by the parasite *Theileria parva*, which is transmitted to livestock by ticks. ILRAD estimates that 24 of the 63 million cattle in eastern, central and southern Africa are at risk of infection with *T. parva*.

Experiments conducted in 1991 have shown that genetic recombination occurs among six stocks of *T. parva* parasites obtained from different regions of Africa and causing different disease syndromes. This demonstration was made feasible by the earlier completion at ILRAD of a physical map of the *T. parva* genome and allocation of antigens and other marker genes to specific chromosomes of the parasite. The prevalence of such genetic exchange in the field, and consequent reassortment of antigenic molecules of the parasite, has important implications for those developing improved vaccines against East Coast fever.

Those working in the program on development of such a vaccine were encouraged by results of this year's research. These offer further evidence that inoculation of cattle with a recombinant form of an antigen located on the surface of the *T. parva* parasite (p67), which has been produced in bacteria in large quantities using genetic engineering techniques, has in most cases protected the cattle against subsequent challenge with a lethal dose of parasites.

TRYPANOSOMIASIS RESEARCH PROGRAM

THE TRYPANOSOMIASIS Research Program has developed highly sensitive tests (monoclonal-antibody-based ELISAs) for distinguishing trypanosome species. The reliability of these tests, which detect antigenic molecules of the parasites, in diagnosing livestock infections was validated this year by researchers in eight laboratories in Africa whose work was sponsored by FAO and the International Atomic Energy Agency (Vienna). The tests were made available to scientists in Asia and Latin America. The utility of these tests in diagnosing human trypanosomiasis (sleeping sickness) is now being determined in trials conducted with the World Health Organization (Geneva).

Other ELISA systems were developed in the year in collaboration with the University of Glasgow for detection of a commonly used trypanocidal drug in samples taken from infected animals. The improved ability to detect both parasites and levels of trypanocides in livestock will enable disease control workers rapidly to identify parasite drug resistance in future epidemiological surveys.

Trypanosome genes that are expressed differently in different life cycle stages of the parasite were identified in 1991, including a gene that controls the parasite's division cycle. Techniques for the transfer, or transfection, of foreign or mutated DNA into trypanosomes were established by the program this year. These transfection techniques are being used to identify the genes that enable trypanosomes to survive drug treatment and the genes that control the organism's infectivity.

Work conducted in 1991 showed that trypanotolerant

Animal trypanosomiasis is found in over a third of the African continent. Of a total population of some 160 million cattle in Africa, about 45 million are located in areas infested with the tsetse fly, the main transmitter of trypanosomiasis.

The tsetse belt occurs across Africa's humid and subhumid regions, the very ecological areas of the continent with the greatest potential for farming and ranching. It is estimated that control of this disease would make it possible to raise a further 120 million cattle in this region without additional environmental stress.

cattle, which are innately resistant to trypanosomiasis, make effective immune responses (humoral and cellular) to a parasite enzyme, a cysteine protease, and that macrophages from infected trypanotolerant cattle release greater quantities of cytokines, a chemical messenger of the immune system, than macrophages from infected animals that are susceptible to trypanosomiasis.

The Trypanosomiasis Program recently undertook research aimed at understanding the genetic basis of this resistance to trypanosomiasis. In 1991 the program made major contributions to worldwide efforts to map the bovine genome. A workshop held at ILRAD in May on this subject brought together scientists from advanced laboratories in Australia, Canada, France, Ireland, Israel, Switzerland, the UK and the USA as well as scientists from the International Livestock Centre for Africa (ILCA, Addis Ababa). The discussions and proceedings of the workshop have alerted the bovine gene mapping community to the importance of using the linkage map being developed to locate the trypanotolerance trait(s) and to coordinate efforts towards this goal.

Research at ILRAD is geared towards developing vaccines that will lastingly protect livestock from theileriosis and trypanosomiasis.

Immunization has proved the most cost-effective way of controlling infectious diseases. Because vaccination is based on natural processes, it is also the most sustainable disease control measure.

Vaccine development, however, requires considerable technical expertise. For this reason, much of ILRAD's research involves state-of-the-art biotechnology.

SOCIOECONOMICS RESEARCH PROGRAM

ILRAD'S SOCIOECONOMICS program continued in 1991 to develop methodologies for evaluating the efficacy of various control measures for tick-borne diseases and to refine computer-based models being developed to help decision-makers determine which livestock-keeping communities in the endemic regions of eastern, central and southern Africa will be best served by the introduction of immunization against East Coast fever. The models incorporate geographic information systems and data on farming systems and household economies as well as on livestock production and diseases. The models are being developed with assistance from staff of the International Centre of Insect Physiology and Ecology (Nairobi), ILCA and the Kenya Agricultural Research Institute. A smallholder farm model built to predict the economic impacts of implementing various livestock disease control methods in given areas, developed in collaboration with Texas A & M University, was completed this year; its accuracy is now being assessed.

ILRAD participated in a workshop held in 1991 on `Increased Sustainable Agricultural Productivity in Africa through the Use of Intelligent Geographic Information Systems with the Consultative Group for International Agricultural Research'. The workshop was sponsored jointly by ILRAD, the Rockefeller Foundation and the United Nations Environment Program (Nairobi). It was attended by members of CGIAR centres with programs in Africa and by experts from programs and institutions within and without the United Nations system. The meeting helped

SUSTAINABLE AGRICULTURE

Managing resources for agriculture to satisfy changing human needs while conserving natural resources and maintaining or enhancing the quality of the environment.

to determine common interests in the development, analysis and maintenance of biological and abiotic databases, development of data extra-polation systems, and development of simulation and expert systems models for advancing research and control of crop and livestock diseases.

OUTREACH PROGRAM

ILRAD'S OUTREACH PROGRAM in 1991 provided 36 scientists and technicians, most of them from African institutes, with individual training at the Laboratory for periods of one week to six months. A total of 21 laboratory technicians attended ILRAD courses this year on use of advanced techniques for diagnosing trypanosomiasis and tick-borne diseases in livestock. ILRAD also provided lecturers and financial support for participants of courses organized jointly with other international agencies that were held during 1991 in Burkina Faso (in French), in Côte d'Ivoire and in Uganda. Thirty graduate students, most of them from Africa and supported by ILRAD fellowships, undertook research work at ILRAD in 1991 leading to graduate degrees. In addition, ILRAD provided three senior African scientists with fellowships to conduct post-doctoral studies at ILRAD.

The heart of any program of basic research lies in the publication of its results in internationally recognized and refereed scientific journals. ILRAD's publication output this year retained its high standards in quality and numbers: 61 of 99 total publications published appeared in refereed scientific journals and 57 papers were presented at international meetings. As can be judged by the nature of these publications, ILRAD's research findings are of direct relevance not only to scientists conducting strategic research on specific animal diseases and their control but also to those conducting basic research in a wide variety of biological disciplines. (A bibliographic list of the year's publications appears at the end of this annual report).

VISITORS

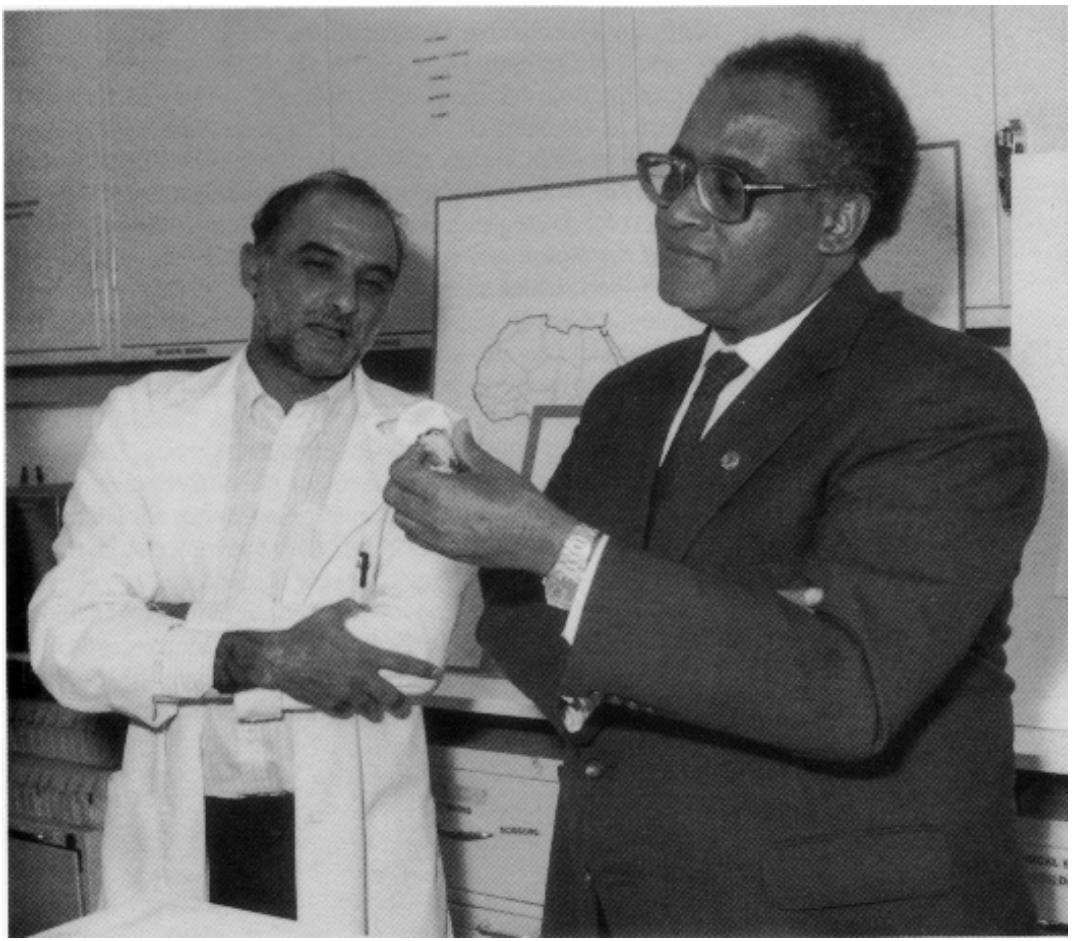
THE IMPORTANCE OF ILRAD's work to alleviate the severe restrictions in food production imposed by trypanosomiasis and tick-borne diseases attracts visits to the Laboratory by professionals who have made outstanding contributions in their fields. Among the distinguished guests who paid ILRAD visits in 1991 were the following: His Excellency Salim Ahmed Salim, Secretary General of the Organization of African Unity (Addis Ababa); Dr. Charles R. Cantor, Director of the Human Genome Centre at the University of California at Berkeley; Dr. Alain Provost and Dr. Georges Tacher, heads of the Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux (France) (Dr. Tacher this year succeeded Dr. Provost as

Director General); Dr. William R. Pritchard, ILRAD Board Chair Emeritus (USA); Prof. F.E.G. Cox, eminent parasitologist of Kings College, London; Dr. E.P. Cunningham, Chief of FAO's Animal Production and Health Division and Dr. Paul McKosker, also of FAO (Italy); Dr. Marilyn Scott, Director of the Institute of Parasitology at McGill University (Canada); Dr. Martin Kyomo, Director of the Southern Africa Centre for Cooperation in Agricultural Research (Botswana); Dr. Svein Kvaloy, Secretary General of the Norwegian Veterinary Association; Prof. Horchner, of the Free University of Berlin; Dr. Fowden Maxwell, of Texas A & M University; and Dr. Robert Howells, of the Wellcome Trust (UK).

ILRAD was also honoured to receive visits from the following senior representatives of ILRAD's donor organizations: Dr. Geoffrey Hawtin, Director of Agriculture at the International Development Research Centre (Canada); Dr. Cees de Haan and Dr. John Peberdy, specialists in livestock production at the World Bank (USA); Dr. Robert Bertram,

His Excellency Dr. Salim Ahmed Salim *(right),* Secretary General of the Organization of African Unity (Addis Ababa), was one of many distinguished visitors at ILRAD in 1991.

Dr. Salim was briefed by Dr. Subhash Morzaria, a senior scientist at ILRAD, on progress the Laboratory is making in research to develop a novel, broadly effective vaccine against East Coast fever.



The Honourable J. Nyagah, Kenya Minister for Livestock Development, on a tour of ILRAD's laboratories in 1991.

Research Advisor in the United States Agency for International Development; Dr. Paul Egger, Head of Agricultural Service at the Swiss Development Corporation and Humanitarian Aid; Drs. Klaus Winkel and Ebbe Schioler, Heads of the Research Section at the Danish International Development Agency; Prof. L. Holm-Nielsen, of the Royal Danish Agricultural University; and Dr. Stein W. Bie, Director of the Norwegian Center for International Agricultural Development.

Many important persons involved in the work of the CGIAR visited ILRAD this year, including members of the Technical Advisory Committee of the CGIAR and Directors, Trustees and members of external review teams of four other centres belonging to the CGIAR: ILCA, the International Potato Center (Peru), the International Service for National Agricultural Research (Netherlands) and the International Plant Genetic Resources Institute (Italy).

Important local visitors included the Honourable J. Nyagah, Minister for Livestock Development, Mr. Simeon Lesrima, Permanent Secretary, Dr. A.M. Mutai, Director of Livestock Production, and Dr. J.P.O. Wamukoya, Director of Veterinary Services, all of the Ministry of Livestock Development; the Honourable G. Aluoch, Assistant Minister for Research, Science and Technology; Dr. M.S. Abdallah, Chairman of the Board of the Kenya Medical Research Institute; Dr. J. Omuse, Director of the Kenya Trypanosomiasis Research Institute; and Dr. Walter Masiga, Director of the Inter-African Bureau for Animal Resources of the Organisation of African Unity. ILRAD also welcomed visits from the following members of the diplomatic corps who represent donor nations: Mr. Peter Eigen, Chief of Mission at the World Bank; Dr. John Westley, Chief of Mission of the United States Agency for International Development, and Mr. Fred C. Fischer, Director of USAID's Regional Economic Services Office for Eastern and Southern Africa; Mr. Masaru Morimoto, Chief of Mission of the Japanese International Cooperation Agency; His Excellency Dr. Christian Fellens, Ambassador of Belgium; and the High Commissioners of Britain, His Excellency Sir Roger and Lady Tomkys; Canada, His Excellency Lawrence A.H. Smith; and Uganda, His Excellency Joseph Tomusange.

I present the following report on the activities and achievements of ILRAD in 1991 for your attention as a record of the Laboratory's progress towards the goals of improved control of livestock diseases and improved livestock productivity.

A.R. Gray
Director General


Theileria parva

THEILERIOSES are debilitating, often fatal, diseases of domestic livestock. The organisms that cause these diseases are protozoan parasites belonging to the genus *Theileria*. Two species of this genus, *Theileria parva* and *Theileria annulata*, cause clinical disease in cattle that severely impedes dairy and beef farming and its improvement in countries in Africa, the Middle East and much of Asia.

The most economically important species in Africa is *Theileria parva*. This parasite causes East Coast fever (also known as Corridor disease and January disease) in eastern, central and southern Africa. Most cattle that are susceptible to the disease die from infection if not treated. Of the 63 million cattle raised in this region, over 24 million are at risk from East Coast fever.

The brown ear tick, *Rhipicephalus appendiculatus*, is the organism that transmits *Theileria parva* parasites between cattle as the tick feeds. This tick also takes up parasites from infected African Cape buffalo and passes them on to domestic cattle. Like several other wild species that are hosts to *Theileria* parasites, buffalo normally show no signs of clinical disease while infected.

Most domestic cattle, on the other hand, cannot tolerate infection with *T. parva*. The cattle most severely affected are highly productive taurine *(Bos taurus)* breeds from Europe, animals crossbred with these, and genetically improved indigenous Zebu *(Bos indicus)* cattle moved from areas free of East Coast fever into endemic areas. Farmers are often unable to crossbreed their relatively small indigenous animals with more productive cattle because the latter are highly susceptible to tick-borne diseases. Among indigenous cattle where the disease is endemically stable, up to 50% of the calves exposed to *T. Parva* die; in areas where the disease is endemically unstable, such as in

(Opposite) Reported distribution of the protozoan parasite *Theileria parva*. This parasite causes disease in cattle in eleven countries of eastern, central and southern Africa. (Antibodies to *T. parva* are still found in animal hosts in South Africa and Swaziland, but the disease has been fully under control in those countries for many years.)

A male brown ear tick. While talking a blood meal from cattle, ticks infected with *Theileria parva* transmit these parasites to the animals, causing theileriosis, commonly known as East Coast fever, January disease and Corridor disease.

livestock herds first introduced to the parasite, 80–100% of animals of all age groups may die. ILRAD scientists have estimated that East Coast fever in 1989 killed 1.1 million head of cattle and caused US $168 million in losses.

East Coast fever is controlled principally by immersing cattle in or spraying them with acaricides, chemical compounds that keep the animals free of ticks for periods of days to weeks. This control regime, however, has several shortcomings. Acaricides are costly and must be bought with hard currency, a scarce commodity in most developing countries. In areas heavily infested with ticks, cattle herds are walked to acaricide dips or spray races as often as twice a week for treatment; this frequency erodes the land, pollutes the environment with toxic residues and may be accelerating the development of tick resistance to the acaricides. In addition, proper acaricide treatment is difficult to administer in rural areas where there is little expertise in maintaining cattle dips and spray races, where water is often in short supply and where illegal cattle movements or civil unrest may periodically occur. Moreover, because cattle regularly treated with acaricides are not exposed to *T. parva*, they develop no immunity and thus have no protection against tick-borne parasites if acaricide treatment is interrupted or stopped altogether.

Cattle being dipped on a ranch in Kenya to keep them free of ticks. This is the most common tick control method used in Africa, but problems encountered in maintaining dips and spray races and the high costs of importing acaricides are forcing many governments to investigate other methods for controling ticks and the diseases they spread.

Curative drugs are relatively new tools for theileriosis control. For chemotherapy to be effective, the disease must be diagnosed accurately and early so that treatment can be given at the start of clinical disease. But early diagnosis of East Coast fever is difficult because the disease becomes clinically apparent only when it has reached an advanced stage.

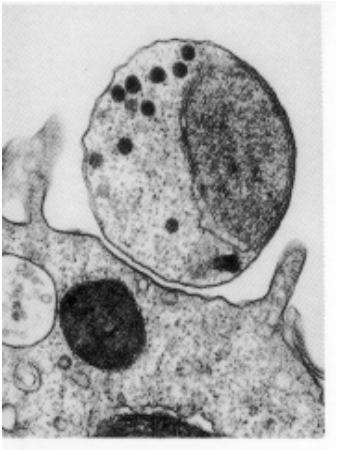
For all of these reasons, alternative and improved methods of controlling East Coast fever are urgently needed.

The life cycle of the single-celled *T. parva* parasite is complex. In both the tick vector and the mammalian host, the

parasite develops through a series of different stages. When feeding on an infected animal, the *R. appendiculatus* tick may ingest parasites of a form known as piroplasms, which infect bovine red blood cells. In the gut of the tick, the piroplasms undergo differentiation to sexual forms that fuse to form zygotes, which then differentiate into kinete forms. The kinetes migrate to the tick's salivary glands, where they differentiate into sporozoite forms. As the tick prepares to take a blood meal, these sporozoites are injected into the cattle along with tick saliva. In the animal host, the sporozoites attach to and enter lymphocytes, a type of white blood cell of the bovine immune system. Within two to three days of invading the lymphocytes, the sporozoites develop into forms called schizonts. The infected lymphocytes grow larger and begin to divide. As each lymphocyte divides, the schizont inside it also divides, ensuring infection of each of the two daughter cells produced by the lymphocyte.

In this manner, the lymphocytes that were initially parasitized expand rapidly and spread throughout the lymphoid system of the animal, giving rise to widespread destruction of cells of the host animal. In untreated, susceptible cattle, this usually results in an overwhelming infection of the lymphoid system and death within three to four weeks of infection.

In the later stages of infection, some of the schizonts differentiate into merozoite forms. Upon rupture of the lymphocytes, the merozoites are released into the bloodstream, where they invade red blood cells. In the red cells, the parasites change into piroplasms, which are able to infect ticks, and this completes the life cycle of *T. parva.*

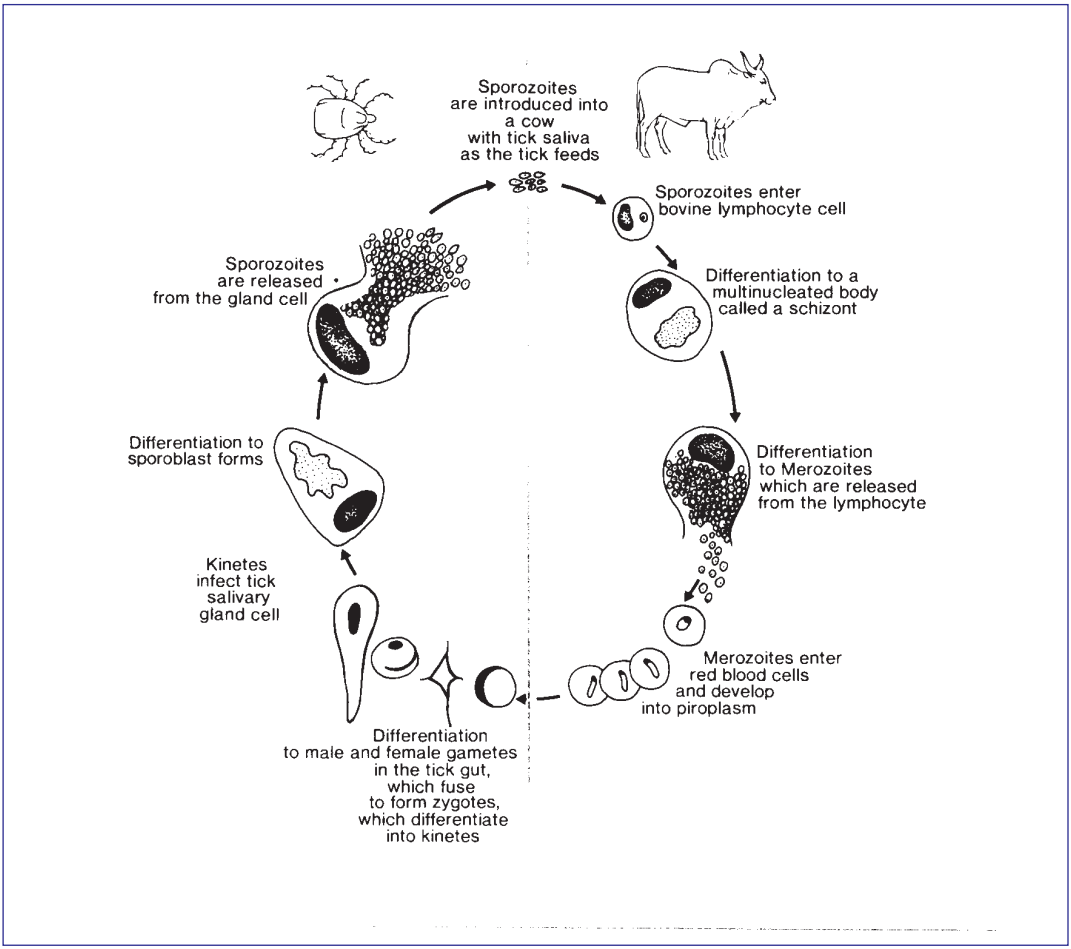


This electron micrograph shows a sporozoite form of the parasite *Theileria parva* (top cell) attaching itself to the outer membrane of a white blood cell of cattle, which the parasite will infect by invading the cell.

EPIDEMIOLOGY AND BIOLOGY

THOSE CATTLE that survive infection with *T. parva* are thereafter immune to East Coast fever. This has indicated the possibility of controlling the disease by immunizing livestock against the parasite, and, indeed, an immunization method using live parasites has existed for many years. Protection against challenge with a stock of *T. parva* can be induced in cattle by infecting them with the sporozoite form of the parasite while simultaneously treating the cattle with a tetracycline antibiotic drug to lessen the severity of the infection. After receiving this 'infection-and-treatment' immunization, cattle are protected against subsequent infection with that parasite stock.

This immunization method remains problematical, however, for several reasons, one of the most important being' antigenic diversity': different *T. parva* stocks occur in the field and these have different antigens—the specific molecules of the parasite that are recognized as foreign by the immune system of a mammalian host. Immunity produced in an animal against one stock of the parasite thus may not protect the



The life cycle of *Theileria parva*

Sporozoite forms of the parasite are transmitted from the tick to the bovine host in tick saliva as the infected tick feeds on an animal. In the animal host, the sporozoites attach to and enter lymphocytes, a type of white blood cell of the bovine immune system, where the sporozoites develop into forms called schizonts. The infected lymphocytes grow larger and begin to divide. As each enlarged lymphocyte divides, the schizont inside also divides, ensuring infection of each of two daughter cells produced.

The infected lymphocytes expand rapidly and spread throughout the lymphoid system of the animal, giving rise to widespread destruction of host cells. Some of the schizonts develop into merozoite forms, which are released from the lymphocytes into the bloodstream, where they invade red blood cells (erythrocytes). In the red cells, the parasites develop into forms called piroplasms, which are able to infect ticks. As ticks feed on animals infected with the parasite, they ingest red blood cells containing the piroplasms.

Once inside the tick gut, the parasites differentiate into male and female gamonts, which fuse to form zygotes. The zygotes differentiate into kinetes, which move to the salivary gland and enter a particular cell type. Here the parasites form sporoblasts, each of which gives rise to 30,000 to 50,000 sporozoites, a parasite form able to infect animals. The sporozoites are introduced into a mammalian host along with tick saliva when the tick feeds. This initiates a new cycle of parasite development.

animal against challenge with another. To develop a broadly effective vaccine against East Coast fever, scientists must first determine the antigenic composition and prevalence of *T. parva* stocks obtained from given areas. This information is being compiled in epidemiological and biological studies at ILRAD, which complement other major areas in the Theileriosis Research Program directed at developing immunization strategies against the sporozoite and schizont forms of the parasite.

IDENTIFYING *THEILERIA PARVA* PARASITES

DIFFERENT SPECIES AND STOCKS of *Theileria* are often indistinguishable when viewed under a light microscope. Success in protecting cattle in a given area against East Coast fever by administering the infection-and-treatment method depends on accurately identifying the parasite stocks that predominate in that area. Furthermore, by determining what *T. parva* strains occur in what areas and how often the parasites infect their tick and mammalian hosts, epidemiologists are gaining a better understanding of how East Coast fever is spread and in what areas livestock are at risk. For these reasons, ILRAD scientists have spent considerable time over the last several years developing increasingly sophisticated laboratory procedures for identifying parasites.

The traditional—and highly reliable—way of identifying a parasite stock or stocks that might provide wide immunity to East Coast fever is by conducting a cross-immunity test. Cattle are immunized by the infection-and-treatment method using one stock of *T. parva* and subsequently challenged with different stocks to discover which stocks break through, and thus which stocks are immunologically distinct. These cross-immunity tests are reliable but expensive and time-consuming. Scientists are therefore working to develop *in vitro* tests that can be used to establish immunological types of *T. parva.*

Monoclonal antibodies raised against different *Theileria* parasites can be used to identify *Theileria* species. The binding (positive) or non-binding (negative) of whole parasites or parasite components to a monoclonal antibody creates a `monoclonal antibody profile'. ILRAD has developed a panel of 11 monoclonal antibodies to distinguish *T. parva* stocks from each other. Although the profiles obtained using this panel demonstrate antigenic diversity and are a useful way of characterizing stocks, the positive and negative reactions obtained with the panel cannot yet be correlated with cross-protection. That is to say, use of this method fails to disclose whether infection of animals with one parasite stock will protect the animals against another stock or other stocks, and—if so—which one(s).

Whereas monoclonal antibody profiles distinguish *physical* differences among *T. parva* stocks, use of new

GENES
The chemical units of heredity that, when expressed, determine an organism's traits.

MONOCLONAL ANTIBODY
A population of identical antibody molecules produced by a single B lymphocyte that can be made against any protein and can be used as a probe to locate and purify the protein to study its structure and function.

DNA PROBE
A molecule of DNA (deoxyribonucleic acid), anywhere from 15 to thousands of nucleotides long, used to detect the occurrence of a corresponding sequence of DNA (or RNA, ribonucleic acid) in a mixture of nucleic acids obtained from cells.

DNA probes have proved a valuable tool for distinguishing species, strains and stocks of pathogenic protozoan parasites that may be difficult or impossible to distinguish morphologically or serologically.

Understanding the genetic variation that occurs in parasite populations is particularly important to epidemiologists and disease control workers.

Populations of a species may look alike but exhibit marked differences in such important factors as host preferences, infectivity, virulence and susceptibility to drugs.

Closely related species can be distinguished from each other using molecular techniques because each organism is a `living fossil' and contains, in the sequence of nucleotides of its DNA, its own evolutionary record. A sequence unique to a particular parasite will precisely identify it.

molecular biology techniques is enabling scientists to synthesize DNA molecules and to clone DNA sequences for use as `molecular probes' with which they can identify *genomic* differences among parasite stocks as well. Several years of molecular biology research at ILRAD on the genetic material of *T. parva* led scientists to identify fragments of DNA each of which is characteristic of a single *T. parva* stock. Having determined the nucleic acid sequences of these fragments, the scientists then made synthetic DNA probes called oligonucleotides—single strands of the double-stranded parasite DNA fragment—each of which combines, or hybridizes, only with genetic material obtained from the selected stock of *T. parva*. Using the new DNA probes, ILRAD scientists are able to characterize parasite samples with unequalled precision. *Theileria* species are distinguished using small subunit ribosomal oligonucleotides; parasites within *T. parva* are differentiated using Southern blot analysis and cloned probes.

Use of the polymerase chain reaction technique continued in 1991 to improve the nucleic acid probes being made at ILRAD for epidemiological work. The polymerase chain reaction is a technique for copying a defined stretch of DNA in the laboratory with readily available reagents. Because the number of copies increases exponentially, billions of copies can be made in a few hours. By using appropriate primers, the polymerase chain reaction can amplify a variable sequence of DNA located between two conserved regions in the parasite genome so that this sequence may be characterized. ILRAD scientists used the polymerase chain reaction in several ways during the year to identify genomic differences among *Theileria* species and stocks.

In 1991, oligonucleotides derived from *T. parva* repetitive DNA sequences were developed and used to differentiate the *T. parva* Muguga and *T. parva* Uganda stocks on a positive/negative basis. These oligonucleotides have been used to screen sporozoite DNA amplified using the polymerase chain reaction for potential recombinants

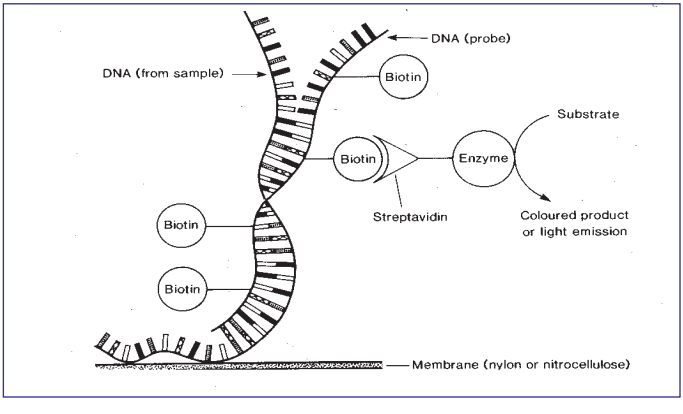


Diagram of the nucleic acid hybridization process used at ILRAD. Single-stranded DNA from an organism of interest is allowed to attach itself to a membrane. A single-stranded DNA probe binds to its immobilized complementary strand. This binding can be detected by labelling the probes with radioisotopes or with non-radioactive reporter molecules, such as the biotin-streptavidin-enzyme complex shown here.

between the two stocks. An oligonucleotide has also been developed that differentiates on a positive/negative basis DNA from the *T. parva* Marikebuni stock, which is being used in immunization trials in Kenya, from five other *T. parva* stocks.

Use of the *T. parva* ribosomal DNA probes to characterize parasite populations was evaluated during the year. Probing parasite DNA digested with the enzyme *EcoRI* with a *T. parva* small subunit ribosomal RNA gene splits *T. parva* stocks into two groups. Probing parasite DNA digested with *AccI* and *PvuII* enzymes reveals the existence of finer groupings among the stocks. A cloned telomeric DNA sequence was further characterized during the year and found to recognize seven or eight DNA fragments, several of which vary in size among *T. parva* stocks. Data obtained by probing elements of the Muguga and Uganda stocks with this telomeric probe were crucial in establishing the recombinant nature of parasites isolated from an animal that had been experimentally co-infected with these two stocks.

By determining the sequences of small subunit ribosomal RNA genes from different *Theileria* species, ILRAD scientists have discovered regions of the genetic material that are specific for each parasite species. Synthetic oligonucleotides derived from these regions were made to identify the *Theileria* species *parva, mutans, taurotragi, annulata, buffeli* (Marula) and an unknown *Theileria* species obtained from buffalo. The *T. buffeli* (Marula) parasite was isolated by scientists at the Kenya Agricultural Research Institute and identified using reagents provided by scientists from the National Institute of Animal Health, Japan. The oligonucleotides have been used to differentiate these species in a positive/negative manner by probing either ribosomal DNA amplified from whole genomic DNA using the polymerase chain reaction or parasite ribosomal RNA in schizont-infected lymphocytes. The probes were also used to detect *T. parva* and *T. taurotragi* ribosomal RNA in infected tick salivary glands.

Single clones have been generated from each of six selected stocks of *T. parva:* Muguga, Marikebuni, Mariakani, Uganda, Boleni and buffalo-derived 7014. The clones were characterized using monoclonal antibodies, DNA probes and pulsed-field gel electrophoresis. Each clone has a monoclonal antibody profile similar to that of its parent stock, although the DNA probes show some differences. The clones are now being further characterized *in vivo*. For immunological characterization, cattle are infected with a cloned parasite, allowed to recover, and then challenged with the clone's parent stock. If the immunized cattle are susceptible to a clone's parent stock, it is probable that the clone is not a major pathogenic or immunogenic component of the stock.

FLOW OF GENETIC INFORMATION

Genetic information is stored and replicated as DNA, interpreted by RNA and ultimately expressed as proteins.

The 'genetic code' is the relation between the sequence of the four kinds of bases in DNA and RNA and the sequence of the twenty kinds of amino acids in proteins.

The genetic code is beautiful in its simplicity and is nearly universal, that is, messenger RNA molecules are translated correctly into the same amino acid sequences by cells of very different species.

GENOME

The total genetic constitution of an organism. The DNA content of even a bacterial cell is very large; the much larger genome of a mammalian cell carries about three billion base pairs of information, most of which is arrayed along its chromosomal DNA.

The base sequences are arranged in discrete compartments of information: the individual genes. There are between 50,000 and 100,000 genes in the genome of a mammal; each one is responsible for specifying the structure of a particular gene product, usually a protein.

DEVELOPING IMPROVED TESTS TO DIAGNOSE INFECTIONS

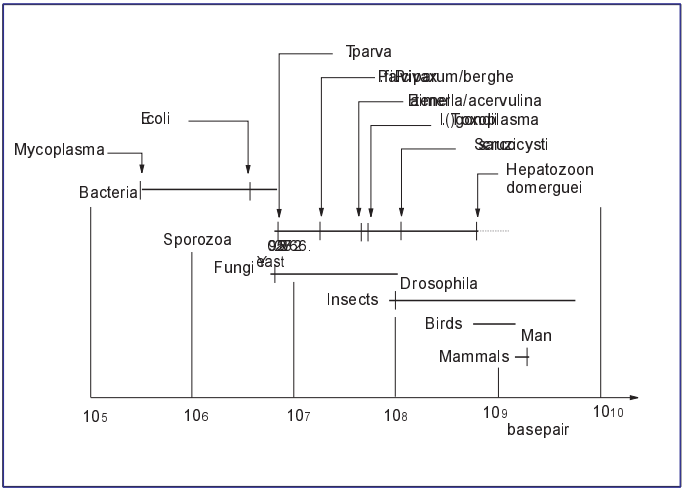
FURTHER PROGRESS was made in 1991 in the development of a range of tests based on enzyme-linked immunosorbent assays (ELISAs) for use in diagnosing infections with tick-borne disease pathogens. Antigen-capture ELISAs were developed for diagnosing infections with *Theileria mutans*, *Anaplasma marginale* and *Babesia bigemina*. These assays were evaluated using both laboratory and field sera from cattle. Antibody-detection ELISAs were also developed for diagnosing infection with these parasites. Several sporozoite and schizont antigens of *T. parva* are being evaluated for development of a diagnostic antibody ELISA.

The techniques for growing *B. bigemina* and *Babesia bovis* in laboratory cultures were transferred to ILRAD in 1991 by a visiting scientist from the Tick Fever Research Laboratory in Brisbane, Australia. A crude lysate-based ELISA for detecting antibodies to *B. bovis* in infected animals was also developed in the year.

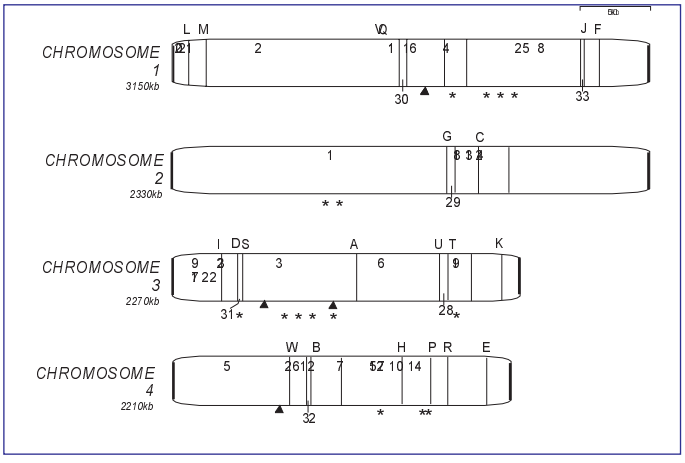
The development of a battery of standardized ELISAs for tick-borne diseases will greatly assist epidemiological studies and disease surveillance in tropical countries. Use of the assays will also assist ILRAD staff in defining disease challenge environments before testing novel vaccines in the field.

THE BIOLOGY OF *THEILERIA PARVA*

A MAJOR ACHIEVEMENT at ILRAD in 1991 was the completion of a physical map of the total genetic material of a stock of *T. parva* made using *Sfi* I linking clones. This is the first complete restriction map made of the genome of a protozoan parasite. A range of techniques comprising modern molecular biology and genetic manipulation were used in the mapping exercise. The project involved visits and collaboration between scientists at ILRAD



The sizes of the genomes of various animal groups. From *The Epidemiology of Theileriosis in Africa* by R.A.I. Norval, B.D. Perry and A.S. Young, London: Academic Press, 1991.



The restriction map of the *T. parva* genome, which was completed at ILRAD in 1991.

and the University of California at Berkeley's Human Genome Center at the Lawrence Berkeley Laboratory. The American scientists gave assistance and advice on use of state-of-the-art pulsed-field gel electrophoresis, a technique used to separate large DNA molecules according to size.

The results of this investigation show that *T. parva* has one of the smallest genomes known among nucleated (eukaryotic) cells and the smallest sporozoan genome reported to date. The four chromosomes of the genome comprise about 10 million base pairs, which is approximately twice the size of the common bacterium *Escherichia coli.*

The map locates all the genes isolated from *T. parva* to date. Several of these are of major significance because evidence suggests that they provoke the generation of protective immune responses in cattle, and thus may form the basis of an improved `subunit' vaccine against East Coast fever, which is based on antigenic components of the parasite rather than on live parasites (see below).

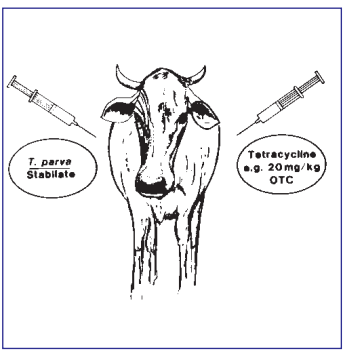
The map of the *T. parva* genome will be used in techniques to monitor at the molecular level the occurrence of genetic recombination in the life cycle of *T. parva*, which may help to generate the wide variety of parasite strains found in the field. Preliminary results obtained this year suggest that sexual recombination does occur, confirming much earlier morphological evidence of a sexual cycle in the parasite. Knowledge of the basis of this diversity will help researchers to develop an improved vaccine against East Coast fever that will work broadly and effectively against all the strains.

EPIDEMIOLOGY

STAFF MEMBERS conducting epidemiology studies in the theileriosis program continued to work with Africa's regional and national tick-borne disease control programs in determining the prevalence of East Coast fever in their countries, in establishing and conducting

Global research on the parasites that cause tick-borne diseases is conducted with three main objectives:

- to develop improved reagents to detect and identify disease-causing organisms in infected animals for epidemiological studies
- to identify molecules from infecting organisms for use in `subunit' vaccines that will replace unsatisfactory live vaccines in current use
- to develop vaccine technology for inducing immunity against the diseases



The infection-and-treatment method of immunizing livestock against East Coast fever. This method, using live parasites, has been refined over the years by scientists at ILRAD and the Kenya Agricultural Research Institute.

immunization programs based on the infection-and-treatment method and in training personnel to apply the method. ILRAD also provided national laboratories with reagents to characterize parasite types and to diagnose East Coast fever and other important tick-borne diseases, the latter caused by *Anaplasma*, *Babesia* and *Cowdria* parasites.

ILRAD's support has concentrated particularly on Pemba and Unguja islands of Zanzibar (Tanzania), on Zambia and on Zimbabwe, where good progress was made in implementing the infection-and-treatment method of immunization against theileriosis. Genetically improved smallholder cattle on Zanzibar were immunized in a program designed by the Zanzibari Government and implemented jointly with donor-funded projects. A Zanzibari staff member of this project was trained at ILRAD and in Zimbabwe (the latter with support from the International Development Research Centre, Canada) in methods for assessing the social and economic impacts of implementing East Coast fever immunization.

In Zambia, members of a Belgian-funded project with support from the government veterinary services have immunized over 50,000 indigenous cattle in an epidemic area using a locally isolated stock of *T. parva* in the infection-and-treatment method. Detailed epidemiological studies are being carried out. ILRAD scientists are supporting the project by characterizing the major *T. parva* parasites isolated in the area where the immunization trials are taking place.

In Zimbabwe, members of a project conducted jointly by the government and the Food and Agriculture Organization of the United Nations have completed a successful field immunization trial using a parasite isolate obtained from Zimbabwe. The isolate is being tested for its efficacy as a national immunizing stock for cattle-derived challenge. Isolates have also been produced from buffalo-derived parasites to provide an immunizing stock to complement the cattle-derived parasite should the government decide to use infection-and-treatment immunization as a disease control measure. Over 40 cell lines have been sent to ILRAD, where researchers are producing monoclonal antibody profiles of the parasite isolates and analysing the isolates using DNA probe techniques. ILRAD scientists have shown that the *T. parva* parasites derived from cattle in Zimbabwe are relatively homogeneous compared to those obtained in other parts of Africa. This suggests that the cattle-derived immunizing stock will be effective in protecting cattle against theileriosis throughout the country.

To help the institute formulate strategies for its research on tick-borne diseases in the coming decade, ILRAD convened a workshop in May on recent developments in research on anaplasmosis, babesiosis and cowdriosis. An estimated 975 million cattle worldwide are exposed to these

three major tick-borne diseases as well as to theileriosis. Cooperation among researchers working in advanced laboratories in Africa, Australia, Europe and the United States on better control of tick-borne diseases was strengthened at the workshop, during which arrangements were made for the exchange of promising diagnostic materials. This interchange will help speed up research progress, particularly the validation of new diagnostic tests to improve control of tick-borne diseases in the short term. The groundwork was also laid for future research collaborations.

In September, ILRAD scientists jointly with staff from the Organization of African Unity/Inter-African Bureau for Animal Resources and the Food and Agriculture Organization conducted the fourth in a series of workshops on control of ticks and tick-borne diseases. The workshop, held in Kampala, Uganda, was attended by representatives from 11 countries and many donor agencies. Committees were formed that are coordinating training courses for the region and defining standards for tick-borne disease vaccines.

THEILERIA ANTIGENS

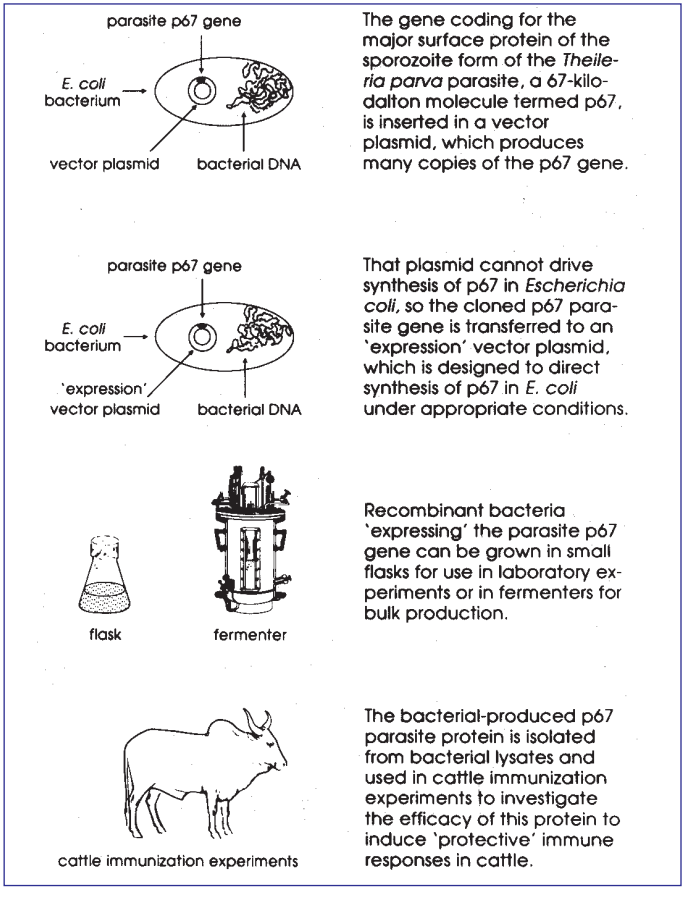
THE MAJOR DISADVANTAGE of the infection-and-treatment method—the only immunization method now available for East Coast fever—is its reliance on live parasites. These must be cryopreserved to remain infective and cattle immunized with the live parasites must be simultaneously treated with drugs to reduce the severity of disease. In addition, there is a risk that immunized cattle may remain carriers of infection. A subunit vaccine based on parasite antigens would therefore be a great improvement over the current live vaccines. Two forms of *T. parva* are of particular interest to ILRAD in this regard. The first are sporozoites, transmitted to cattle by the bite of infected ticks and thus the first form of the parasite encountered by an animal's immune system. The second are schizonts, which develop from sporozoites soon after the latter enter the host's white blood cells. ILRAD researchers have identified two proteins displayed on the surface of these forms that could be the bases of improved vaccines.

IMMUNIZATION AGAINST THE SPOROZOITE FORM OF THE PARASITE

OF THE TWO *T. parva* surface antigens ILRAD scientists found to be likely candidates for vaccine material, one, designated p67 because its molecular mass is about 67 kilodaltons, occurs only on the surface of sporozoites. This molecule has been found in all stocks of *T. parva* tested. It has been shown to induce antibodies in cattle that neutralize the ability of sporozoites to infect lymphocytes *in vitro*.

In 1989, ILRAD workers cloned the gene coding for p67 and filed a patent in the USA covering its potential use for vaccination against East Coast fever. In collaboration with researchers at SmithKline Beecham (Philadelphia), ILRAD produced large quantities of p67 using recombinant DNA technology. The recombinant protein has been shown to confer protection in almost 70% of animals immunized with it.

The antigen was further characterized in 1991. Oligonucleotide primers were synthesized to amplify the p67 gene from different stocks of the parasite using the polymerase chain reaction. The products obtained in the amplification process were cloned into plasmid vectors and subjected to DNA sequence analysis. Four stocks of *T. parva* have been analysed. Preliminary results suggest that the p67 gene is remarkably conserved among parasite stocks in both nucleotide and amino acid sequences. These results are encouraging to those working on the development of an anti-sporozoite vaccine because they suggest that the immunity engendered by p67 will cross-protect among different parasite stocks.



This figure gives a broad outline of ILRAD's use of genetic engineering to produce large quantities of a protein molecule identified at ILRAD that appears on the surface of *Theileria parva* parasites.

This molecule, termed p67, shows promise as a base for a vaccine to protect livestock against East Coast fever.

IMMUNIZATION AGAINST THE SCHIZONT FORM OF THE PARASITE

THE OTHER *T. parva* protein identified at ILRAD that will be explored for its potential as a vaccine or diagnostic antigen appears on both sporozoites and intracellular schizonts. The antigen is called the polymorphic immunodominant molecule, or PIM. The polymorphic nature of the molecule is reflected in a variation in its apparent size among different stocks of *T. parva* .The molecule is immunodominant because it is the major—and sometimes the only—schizont antigen recognized by serum taken from immune cattle. In addition, it is the predominant antigen recognized by monoclonal antibodies raised against the schizont.

Efforts this year concentrated on isolating the gene that codes for this antigen. Expression libraries were generated and probed with monoclonal antibodies. Identification of the gene will allow further characterization of the immunological significance of PIM.

The schizont is the pathogenic stage of *T. parva ;* as mentioned, it is this form that parasitizes host lymphocytes and causes them to proliferate excessively, which leads to the development of disease. The death or recovery of an animal depends greatly on its ability to control the rapidly dividing infected lymphocytes. Several features of immunity to *T. parva* infection suggest that protective host responses mounted against challenge are directed largely at the schizont stage of the parasite. Over the past decade, scientists at ILRAD have determined that these responses are likely to be mediated by a population of white blood cells known as T lymphocytes. Identifying the components of the parasite that induce these responses is a major area of research at the institute.

ILRAD scientists have determined that the main 'effector' in cell-mediated immune responses against *T. parva* is likely to be a subpopulation of T lymphocytes known as cytotoxic T lymphocytes. Parasite-specific cytotoxic Tlymphocytes cultured *in vitro* are capable of killing schizont-infected lymphocytes.

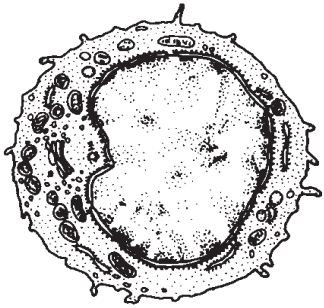
Results of an experiment conducted in 1990 provided evidence that cytotoxic T lymphocytes help protect immune cattle challenged with *T. parva* by killing schizont-infected cells. ILRAD's research in this area in 1991 focused on identifying the parasite molecules that serve as targets for both cytotoxic T cells and another subpopulation of T lymphocytes known as helper T cells. Scientists identified a 24-kilodalton parasite antigen that is recognized by immune helper T cells. Experiments are being conducted to decode the N-terminal sequence of this antigen with a view to designing oligonucleotide probes for screening parasite DNA libraries. Again, by identifying the gene for this protein, ILRAD scientists will be able to assess its immunological importance.

The traditional way to isolate a gene that encodes an antigen is to construct a gene library in which all the DNA

RECOMBINANT DNA TECHNOLOGY

Recombinant DNA basically is a tool that allows one to isolate, modify and move genes from one organism to another, usually into a "simple micro-organism" such as a bacterium or a yeast cell. This tool facilitates the evaluation of the structure and function of genes. This technology has been used to produce large amounts of gene products such as human insulin . . . and virus inhibiting interferons.'

— Lekh R. Batra, 'A Glossary of Recombinant DNA Technology and Genetic Engineering', in *Public Perception s of Biote chnolog y*, ed. L.R. Batra and W. Klassen, Bethesda, Maryland: Agricultural Research Institute, 1987.

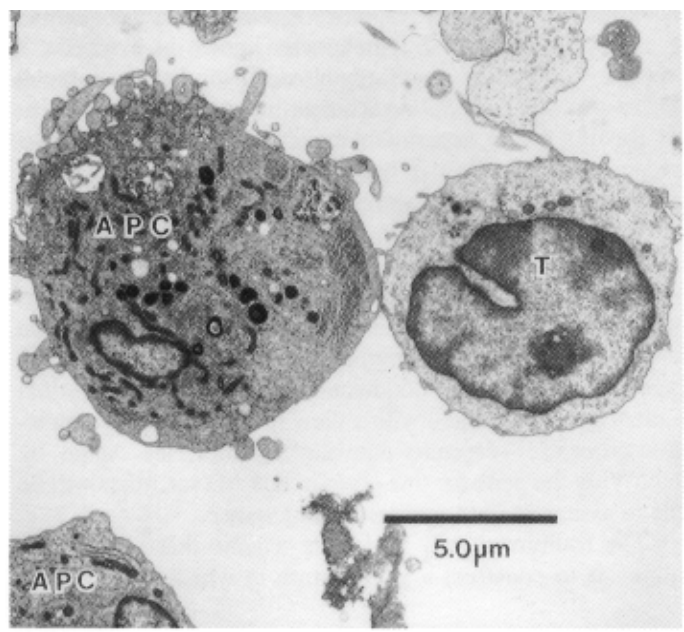


A bovine lymphocyte. These cells are infected by *Theileria parva* schizonts. The parasite takes over the reproductive machinery of these cells for its own benefit. The lymphocyte is influenced by the parasite to divide at frequent intervals. With each division, two parasite-infected daughter cells are produced.

expressed by an organism is cloned into plasmid DNA, which is then introduced into bacteria for expression of the gene product. In a recently developed variation of this approach, the gene library is transfected directly into mammalian cells. In 1991, scientists at ILRAD and the University Hospital in Utrecht (Netherlands) successfully employed this modified procedure and the expression COS cell system to isolate a gene that encodes an antigen produced by *T. parva* schizonts.

The scientists constructed a library of genes expressed by schizonts in plasmids specifically designed to express foreign genes in COS cells. These cells are derived from kidney cells of the African green monkey that have been infected with a mutant form of a simian virus (SV40) so as to produce large amounts of a large tumour antigen of SV40. When appropriate plasmids are introduced into COS cells, the tumour antigen causes the plasmids to replicate at high copy number. Any foreign gene that has been inserted into the plasmids is thus expressed at very high levels.

COS cells were transfected with the gene library and probed with antibodies that bind specifically to the *T. parva* schizont antigen. Using an enzyme-based detection system, researchers identified the COS cells containing the relevant plasmids, recovered the recombinant plasmids from the COS cells and isolated the gene encoding the schizont antigen. This gene is now being used to characterize the parasite antigen. The scientists working on this project particularly want to know if this *T. parva* antigen is recognized by cytotoxic T lymphocytes as well as by antibodies in immune cattle.



White blood cells of the mammalian immune system appear in this electron micrograph. The large macrophage-like antigen presenting cells (APC) scavenge and degrade foreign matter and present the foreign molecules on their surface to T lymphocytes (T).

There are two main kinds of T lymphocytes. Helper T cells secrete chemical messengers that help to fight the infection. Cytotoxic T cells directly attack and kill cells of the host animal that have become infected with parasites.

VACCINE DEVELOPMENT

THE SUCCESS of a vaccine against East Coast fever will depend on an effective means of delivering it to the bovine immune system. The aim of ILRAD's vaccine development research is to exploit the immunogenic and protective properties of candidate vaccine antigens. This program area includes studies to determine the inductive requirements of the bovine immune response and research on the development of antigen delivery systems.

ILRAD's approach to the development of new vaccines includes detailed studies of the bovine immune system. Information from these studies provides a rational basis for vaccine development. The protective immune response that has been identified and examined in most detail is the cell-mediated response to schizont-infected lymphocytes. These studies have contributed much to an understanding of the bovine immune response. Major areas of research on bovine immunology in 1991 were further characterizations of subpopulations of white blood cells (leucocytes), antigen-presenting cells, the T-cell receptors located on the surface of T lymphocytes, and glycoproteins known as MHC molecules.

IMMUNIZATION TRIALS

IN 1990, two cattle immunized at ILRAD with the recombinant p67 surface antigen were shown to be protected against challenge with homologous sporozoites. In experiments in 1991, 13 of 21 cattle immunized with the recombinant antigen were protected against homologous sporozoite challenge with *T. parva* Muguga and 6 of 11 cattle were protected against challenge with the heterologous parasite stock *T. parva* Marikebuni. The latter result provides further evidence that the ability of p67-specific monoclonal antibodies to cross-neutralize among stocks of *T. parva* is reflected in the ability of cattle immunized with the antigen to resist challenge with heterologous stocks.

The factors responsible for the varied responses to challenge among the immunized cattle are unclear. It seems likely, however, that those animals that did not respond to challenge were able to neutralize the sporozoite challenge sufficiently to prevent establishment of the schizont stage of the parasite. The animals that responded mildly and severely to infection, on the other hand, appeared to neutralize the sporozoites only partially or not at all. Thus, clinical signs of disease in the animals appear to reflect the degree to which the parasites are neutralized, the quantity of sporozoites that escape neutralization and the speed with which the animals cell-mediated responses are mobilized against schizont-infected cells. Indeed, no parasite establishment was detected in non-responder animals, even when their responses to parasite challenge were analysed using the polymerase chain reaction technique. The levels of

One of the Boran animals at ILRAD that was inoculated with a p67 surface molecule of the *Theileria parva* parasite and subsequently remained healthy when experimentally infected with a dose of the parasite that is normally fatal for these cattle.

VECTOR
Literally, a 'carrier'. The term is applied both to insects that carry disease-causing organisms and to plasmids or viruses that can carry genes across a cell wall barrier into a recipient cell, where the foreign DNA is expressed as proteins.

antigen-specific serum antibodies were similar in quantity and neutralizing ability in all the immunized animals and therefore did not appear to correlate with protection.

The vaccination strategy being developed at ILRAD using the p67 molecule does not depend on complete elimination of sporozoite challenge, but rather on a *limited* establishment of schizont parasitosis that will stimulate the generation of cell-mediated responses against schizont-infected cells. (The infection-and-treatment method of immunization is essentially based on this principle and has established its validity.) The success of a p67-based vaccination strategy is therefore likely to require a quantitative assessment of tick-transmitted challenge in the field and its reproduction under experimental conditions. This will enable scientists to determine the magnitude of neutralizing immune responses needed to limit parasite establishment sufficiently to allow the induction of cell-mediated responses.

PARASITE ANTIGEN DELIVERY SYSTEMS

A SUCCESSFUL VACCINE must deliver parasite antigens to the immune system of the animal host in such a way as to stimulate protective immune responses. Delivery vehicles based on recombinant organisms have several advantages over conventional antigen delivery systems. Recombinant vaccinia viruses have been shown in several experimental systems to give rise to potent antibody and cytotoxic T-cell responses. A recombinant vaccinia virus was constructed in 1989 that incorporates the gene encoding the p67 sporozoite antigen of *T. parva* .In 1990, the virus was shown to produce neutralizing responses in rabbits, guinea pigs and cattle. In 1991, recombinant vaccinia viruses continued to be evaluated as an antigen-delivery system for cattle. Experimental results showed that neither the virulence of the parent virus of the recombinant nor the virus dose significantly affected the outcome of immunizations.

CELL-MEDIATED IMMUNE RESPONSES OF CATTLE

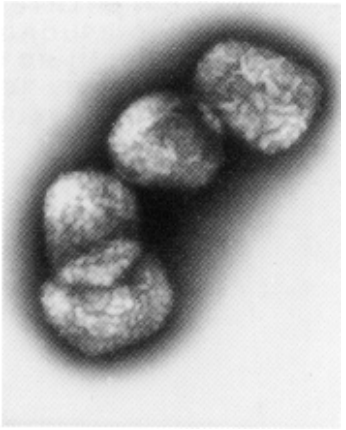
T LYMPHOCYTES recognize antigens only when these have been degraded by an antigen-presenting cell into peptide fragments, which are then transported to the cell surface. The parasite fragment is displayed on the surface in a binding cleft of a protein molecule of the major histocompatibility complex (MHC) (so-called because the protein is encoded by a family of genes known as the major histocompatibility complex). Cytotoxic T cells recognize foreign antigens that appear in the structural groove of class I MHC molecules on antigen-presenting cells. Helper T cells, in contrast, recognize antigens that are expressed on the cell surface in association with class II MHC molecules.

Antigen receptors on the surface of T cells recognize the combination of antigenic peptide and host MHC molecule. Cytotoxic T cells from an immune animal identify and kill only infected cells carrying MHC molecules identical to its own. This phenomenon is known as MHC restriction. Parasite antigens appear to associate preferentially with certain MHC molecules, suggesting that the MHC type of an animal may influence the specificity of its cytotoxic T-cell response to the parasite. The efficiency with which immune responses are induced is believed to correspond to the affinity of processed peptide for available MHC molecules.

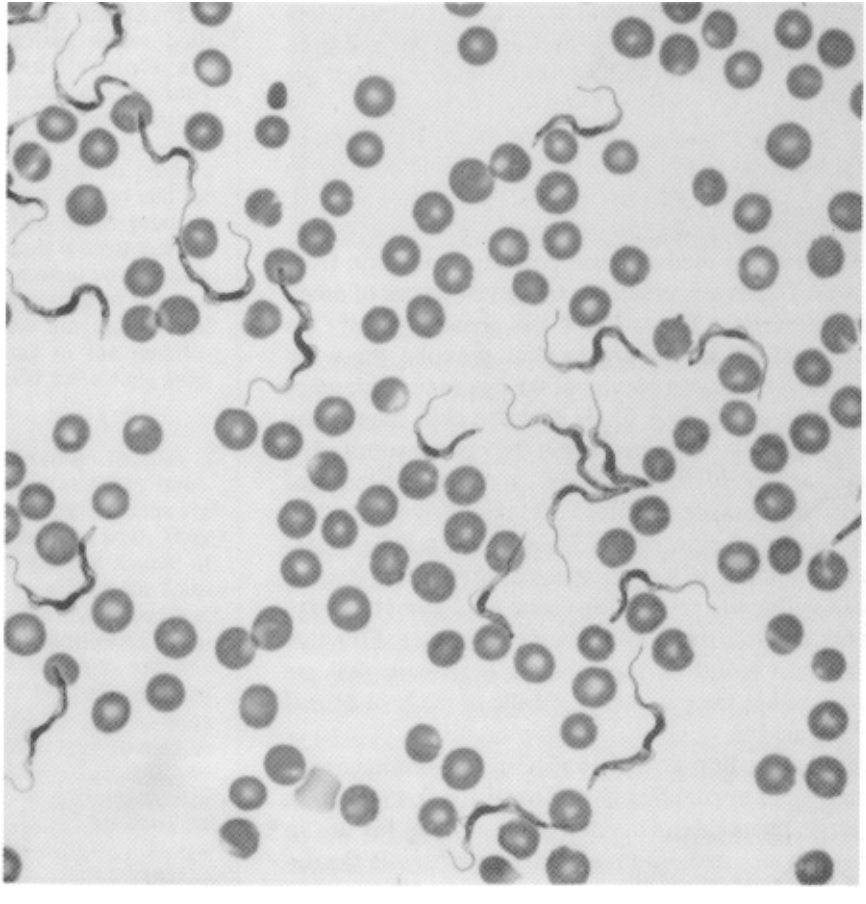
The central role played by MHC molecules in T-cell recognition makes characterization of these molecules in cattle a priority of ILRAD scientists. In experiments conducted in 1990, several non-related MHC-homozygous cattle that differed in class I MHC phenotype responded similarly to immunization with the native p67 antigen of *T. parva.* These results suggested that MHC phenotypes do not influence responses of cattle to entire protein antigens. To determine whether MHC-related factors affect the capacity of cattle to react to peptide fragments of parasite antigens, the responses of these cattle to synthetic peptides derived from p67 and from trypanosome VSG were measured. Only certain cattle in the group responded to the peptides. Although it remains unclear whether these observations related directly to MHC molecules, it was concluded that MHC factors are unlikely to compromise the efficacy of a vaccine based on entire antigen.

In summary, the apparent importance of cytotoxic T lymphocyte responses to recovery and protection of cattle from infection with *T. parva* is crucial to the development of novel vaccination strategies. The task that lies ahead is clearly the identification of parasite antigens that provoke these responses. In view of the complexity of the cytotoxic T lymphocyte recognition event, it is likely that this will require the production of parasite antigens within the cytoplasm of cells bearing appropriate MHC molecules, and the screening of these cells with parasite-specific cytotoxic T lymphocytes. Once suitable antigens have been identified, it will be necessary to define antigen delivery systems capable of expressing the antigens within the cells of the vaccinated animal in ways that will induce cytotoxic T lymphocyte responses. Effective delivery systems based on recombinant organisms that are able to grow within the cytoplasm of cells of the host animal are already available.

The genes encoding several promising antigens have now been cloned. Their availability will allow ILRAD scientists to generate large quantities of recombinant antigen for use in immunization trials, and—if these are successful—in the development of a prototype subunit vaccine against *T. parva.*



Four particles of a recombinant vaccinia virus negatively stained. These virus particles incorporate the gene that encodes the *Theileria parva* p67 antigen.



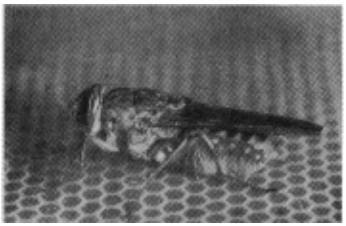
TRYPANOSOMIASIS occurs across more than a third of Africa. It is arguably the most important livestock disease on the continent. Like East Coast fever, trypanosomiasis is caused by infection with a protozoan parasite. In Africa, the single-celled trypanosomes are typically transmitted to domestic and wild animals, as well as to people, by tsetse flies as they feed on mammalian blood. The wide occurrence of this disease in people and their livestock continues to retard agricultural and economic development on the continent.

The tsetse fly (genus *Glossina*) vector of trypanosomiasis occurs only in Africa. Tsetse flies transmit two species of trypanosomes—*Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* that cause trypanosomiasis in people, commonly known as sleeping sickness. The fly also transmits four parasite species—*Trypanosoma brucei brucei, Trypanosoma congolense, Trypanosoma simiae and Trypanosoma vivax*–that cause serious forms of trypanosomiasis in cattle, sheep, goats, pigs and horses.

In parts of Africa and Asia, camels also suffer from trypanosomiasis. The camel disease is most commonly caused by *Trypanosoma evansi*, a species transmitted by biting flies other than tsetse. In large areas of Asia and South America, *T. evansi* poses a threat to other livestock as well, especially cattle, domestic buffalo and pigs. *Trypanosoma vivax*, although usually transmitted by the tsetse fly in Africa, also exists in the absence of this vector in the Caribbean and South America. Non-tsetse-transmitted trypanosomiasis is a potential threat to livestock in many parts of the world.

Tsetse flies occur in 36 countries and 10 million square kilometres of Africa. The risk of trypanosomiasis in much of this area precludes farmers from keeping cattle and small

(Opposite) Light micrograph of trypanosomes, protozoan parasites that cause animal trypanosomiasis, in mammalian blood.



A tsetse fly feeding on an artificial membrane at ILRAD. Africa is the only continent in which this fly—recognized by a long proboscis and the habit of resting with its wings folded scissor-like over its back—occurs.

The tsetse fly inhabits over a third of the continent, where it exposes some 30% of Africa's 150 million cattle to the risk of infection with trypanosomes. These parasites give rise to the debilitating and frequently fatal disease trypanosomiasis.

ruminants. This fact largely accounts for Africa's low livestock productivity: the animal protein produced per hectare on the continent is only one-seventieth of that produced in Europe. The impact of trypanosomiasis is even greater than these figures suggest because many of the areas inhabited by tsetse flies are potentially the most agriculturally productive in Africa. Thirty percent of Africa's cattle population, estimated to be 160 million, as well as comparable numbers of small ruminants, are at risk from the disease. Annual losses in meat production alone are estimated at US $5 billion. This economic deprivation is exacerbated by losses in milk yields, tractive power, waste products that provide natural fertilizer and fuel, and secondary products such as hides. In addition, 50 million people are exposed to the risk of contracting human trypanosomiasis.

The trypanosome parasites that cause disease in livestock and people also infect some wildlife species, which serve as a source, or reservoir, of infection for flies, which then infect domestic animals and people. Many kinds of wild animals tolerate infection with trypanosomes with no apparent ill effects. In humans and most domestic livestock, however, such a harmless relationship with the parasite has not evolved and the pathogenic effects of infection are severe.

For several days following infection with trypanosomes, animals show no signs of disease. One to two weeks later, susceptible animals develop intermittent fever and anaemia. In most endemic areas of Africa, cattle are bitten repeatedly by tsetse flies and are infected with different kinds of trypanosomes. Most livestock must forage daily for food and walk long distances for water. Under these stressful conditions, infected animals often continue to deteriorate for months before dying.

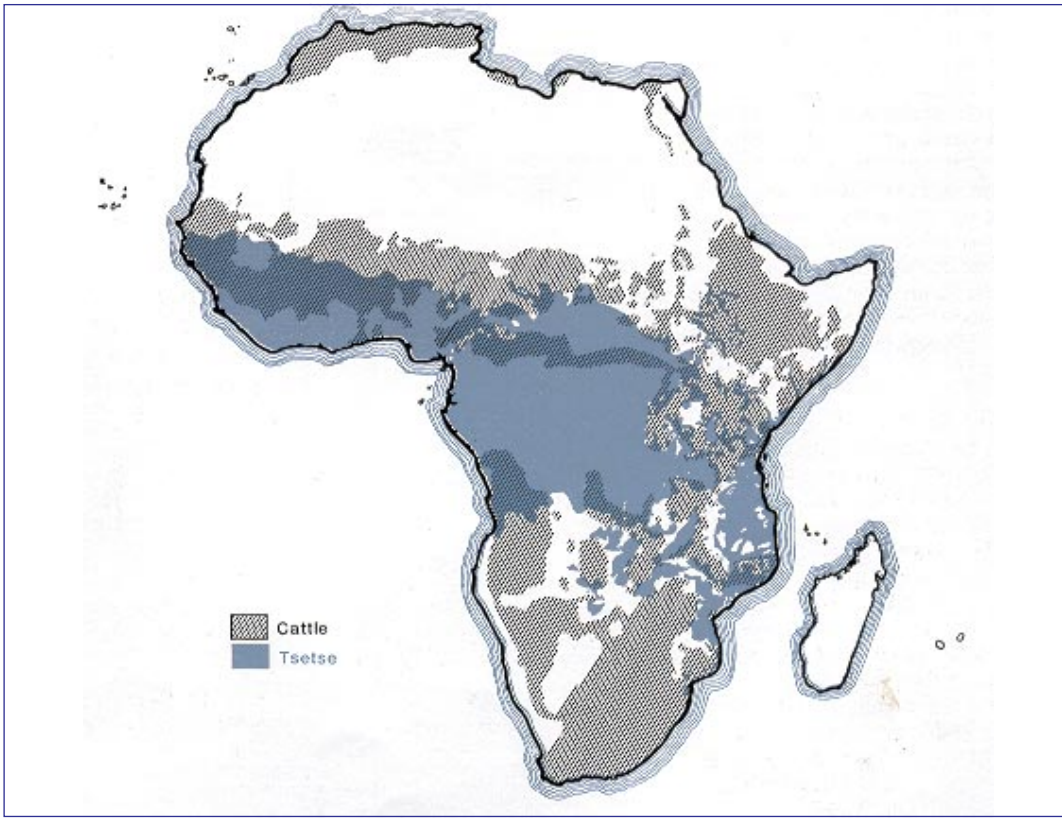
The life cycle of the trypanosome, like that of the Theileria parasite, is complex. In both the tsetse fly vector and the mammalian host, trypanosomes undergo a series of transformations into different forms. The tsetse fly ingests trypanosomes when it feeds on an animal infected by the parasite. In the fly, the parasites differentiate into several forms, culminating in the metacyclic form, which is able to infect mammalian hosts. When the infected fly next feeds, these metacyclic trypanosomes are injected into the skin of the host along with tsetse saliva. In the animal, the parasites differentiate into a form specially adapted to live in mammalian blood. These bloodstream parasites multiply by binary fission and enter the animal's lymphatic and blood circulation. As flies feed on animals infected with the parasite, the flies may take up blood containing trypanosomes, thus completing the parasite's life cycle.

CURRENT CONTROL METHODS

WIDELY DIFFERENT METHODS are used to control trypanosomiasis: administration of drugs to treat or prevent the disease, reduction of the fly populations that transmit the parasites and thus spread the disease, and keeping indigenous livestock breeds that resist the disease. Each of these methods is useful; indeed, without them, many livestock keepers would be out of business. None, however, is ideal and no single method or combination of methods is sufficiently efficacious and cost-effective to sustain small-scale livestock raising in much of the tsetse belt, which effectively seals off one-third of the continent otherwise suitable for grazing and mixed livestock/crop farming.

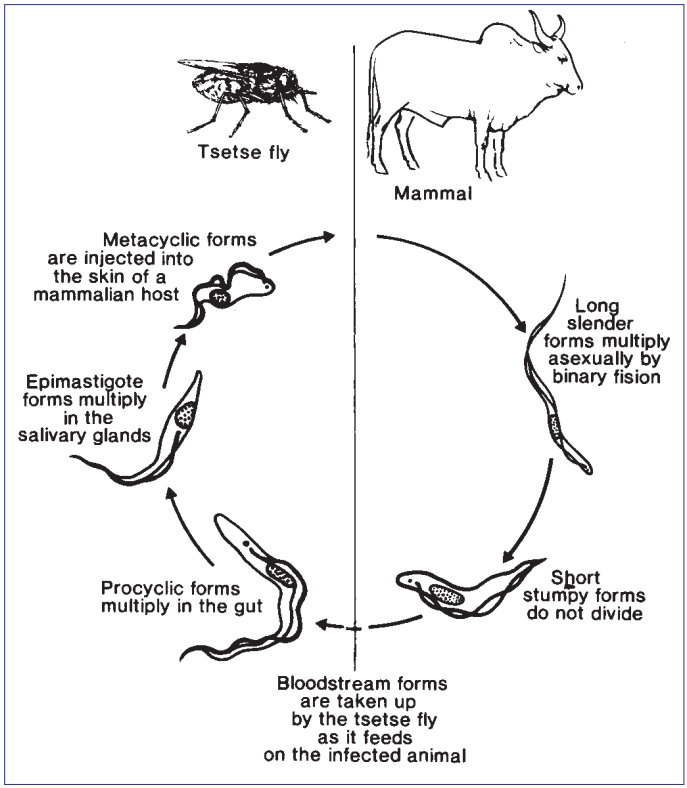
Drug treatment. Drug treatment is the most widely used means of controlling trypanosomiasis. The drugs available are effective but also relatively expensive. It is neither practical nor financially possible for most small farmers repeatedly to treat an animal that repeatedly becomes infected with trypanosomes. Moreover, widespread use of the few drug compounds developed for trypanosomiasis has led to increasing parasite resistance to the drugs and there are no signs that new and improved drugs for the animal disease will be produced in the near future.

The tsetse fly and the disease-causing trypanosome parasites it carries make 10 million square kilometres of Africa—an area as large as the continental USA—inhospitable for livestock. The tsetse belt occurs in Africa's humid and subhumid regions and these are the ecological areas with the greatest potential for farming and ranching on the continent. Note that little overlap exists between Africa's cattle-production and tsetse-infested areas except in regions of West and Central Africa where a few indigenous cattle breeds exist that are resistant to trypanosomiasis. Tsetse-infested areas are expanding in many parts of Africa.



Vector control. Three methods are used to control populations of the tsetse vector. Infested areas may be sprayed with low levels of modern insecticides to kill the flies, but application of such compounds over large areas is discouraged because of the pollution this causes the environment. Habitat hospitable to tsetse may be destroyed to rid areas of flies, but the felling of trees and clearance of bush is an unacceptable waste of diminishing natural resources. Most promising is the reduction of fly populations using tsetse targets and traps strategically placed in fly habitats. These targets and traps have been greatly improved over the last several years, and in some areas their deployment has reduced fly populations to tolerably low levels. To date, however, this control method has been demonstrated to work only in some types of areas and only for some species of fly.

Disease-resistant livestock. The third method used to control trypanosomiasis is to keep cattle of a few ancient African breeds, such as the West African N'Dama *(Bos taurus)*, which are innately able to tolerate infection with trypanosomes. The genetic ability to resist the pathogenic effects of infection is called 'trypanotolerance'. (Livestock that are susceptible to trypanosomiasis are known as 'trypanosusceptible' or, in this report, 'susceptible'.)



The life cycle of *Trypanosoma brucei.*

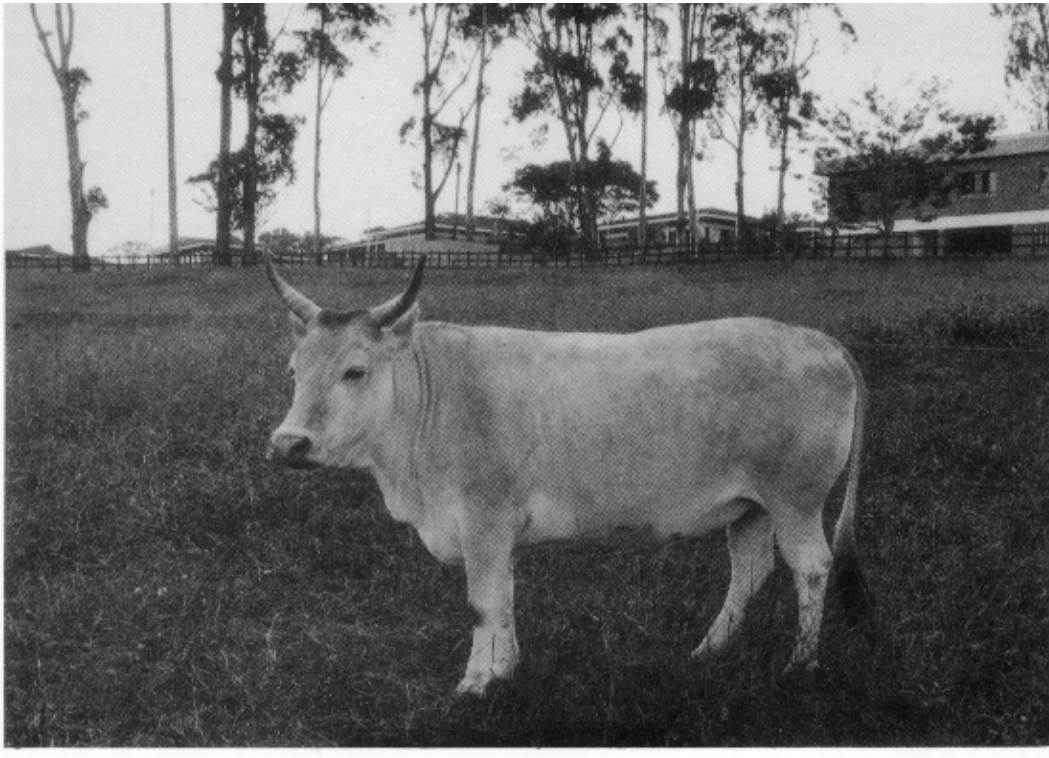
Infection begins when trypanosomes are injected into the blood of a mammal by a tsetse fly as it feeds on the animal. In the animal host, slender forms of the parasites multiply by binary fission until large parasite numbers build up in the blood. The trypanosomes then transform first into intermediate forms and then into stumpy forms, the latter being able to infect tsetse flies.

Stumpy forms of the parasites are ingested by a tsetse fly as it feeds on an infected animal. In the midgut of the tsetse fly, procyclic forms arise and undergo division, after which the parasites enter the proventriculus and later the salivary glands of the fly, where they become epimastigote forms and undergo further division. Finally, metacyclic forms arise in the salivary glands. Metacyclics are able to infect mammals, and the life cycle is repeated.

The mitochondrion is inactive in the slender forms, begins to become active in the stumpy forms and is fully active in forms that occur in the tsetse fly. Parasite forms that live in the mammalian bloodstream (slender, intermediate and stumpy) have a gly coprotein surface coat. This coat disappears in the procyclic forms that arise in the midgut of the tsetse fly and is later reformed in the metacyclic forms in the tsetse salivary glands.
(After Vickerman, 1979.)

Mechanisms to resist the disease may have evolved in wild animals and trypanotolerant cattle due to their long exposure to the parasite; the latter were probably introduced to the continent 7,000 years ago. The other, more common, breeds of cattle in Africa are relative newcomers. The widespread humped Zebu *(Bos indicus)* cattle, which are susceptible to trypanosomiasis, appeared in Africa in large numbers only about 1,300 years ago. Highly productive, genetically improved European *(Bos taurus)* cattle were introduced in this century; consequently, these 'grade' cattle are extremely susceptible to trypanosomiasis.

Use of trypanotolerant livestock is perhaps the most attractive of the control methods available and has made livestock rearing possible in tsetse-infested areas, but this method also has its drawbacks. First, the degree of disease resistance in an animal is not absolute; levels of trypanotolerance are reduced in animals under stress, particularly those with poor nutrition, a common condition of livestock raised in Africa's marginal farming areas. Second, although meat production of trypanotolerant livestock compares favourably with that of other breeds, the amount of milk trypanotolerant cattle produce is relatively low. Furthermore, trypanotolerant animals raised in traditional farming systems are typically small and are therefore not ideal for draught work. Finally, and most importantly, few such



The humpless N'Dama are an ancient African cattle breed, having arrived on the continent about 7,000 years ago. It is probable that the N'Dama's innate ability to resist trypanosomiasis evolved during the long association between these cattle and trypanosome parasites.

animals are available. In spite of their importance in tsetse-infested areas where other livestock cannot survive, trypanotolerant cattle constitute only 5% of the cattle raised in countries where tsetse flies occur. Numbers of these cattle are increasing, but slowly: merely to double the number of N'Dama cattle existing today, for example, will take 15 years at present breeding rates.

ANTIGENIC VARIATION IN TRYPANOSOMES

RESULTS OF CELL and molecular biology studies of trypanosomes conducted at ILRAD and other research institutes over several years have established that trypanosome populations avoid elimination by the mammalian immune system by employing a remarkable defence mechanism known as 'antigenic variation'. Most animal hosts make immune responses to the first wave of invading trypanosomes by producing antibodies against antigenic protein molecules displayed on the surface of the parasites. The antibodies bind to these antigens, and this initiates a series of immune events that eventually causes rupture (lysis) and death of the parasites.

However, before all the parasites can be eliminated, trypanosomes bearing a new surface molecule appear. While the immune system begins to produce antibodies against the new antigen, the second wave of trypanosomes multiplies rapidly, and before all parasites in the second wave can be destroyed, parasites appear displaying yet another molecular surface, and so the process continues, the parasite population always keeping a step ahead of the host's immune system. Unable to keep pace, the animal's immune system becomes exhausted. The animal becomes anaemic, loses weight and stops breeding and producing milk. After lingering for months, nearly useless, it dies.

Trypanosomes are able to express many kinds of surface proteins, known as 'variable surface glycoproteins', or VSGs. The VSGs are a major cell product of the parasite, accounting for 10% of all the proteins it synthesizes. (Some 300 to 1,000 trypanosome genes are estimated to encode VSGs.) Each of the many different strains of trypanosomes displays a

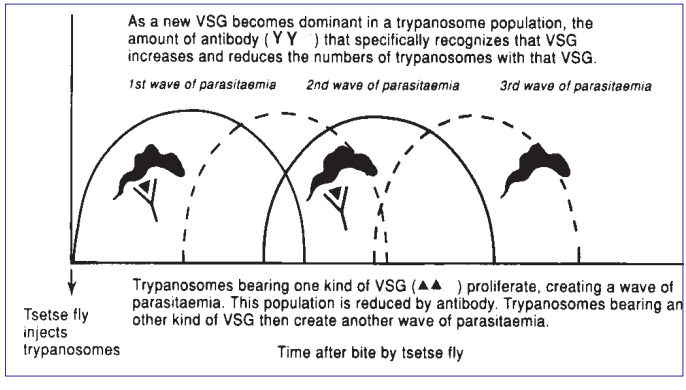


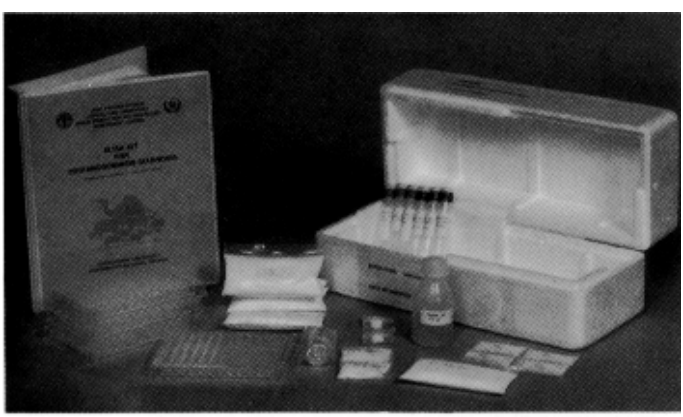
Diagram illustrating the process of antigenic variation, which enables trypanosomes to survive attack by the immune system of an animal host.

particular set of VSGs. Further complicating the matter, genetic recombination among trypanosome populations may occur in the field, increasing the potential for antigenic diversity. It therefore appears unlikely that a conventional vaccine, which primes an animal's immune system against just one or a few antigens, will be broadly effective if based on the trypanosome's variant surface proteins. With this in mind, ILRAD scientists are investigating other novel vaccine strategies for controlling the disease.

DIAGNOSIS AND EPIDEMIOLOGY

DISEASE CONTROL WORKERS and scientists need improved techniques for diagnosing trypanosomiasis that are not only accurate and reliable, but also sufficiently simple, robust and inexpensive for wide use in the field. Better diagnosis of trypanosomiasis would greatly improve treatment of individual animals, and better detection of infections in both mammalian hosts and tsetse vectors would help researchers to define the disease problem more precisely (several diseases manifest themselves in ways similar to trypanosomiasis and the latter may run a subclinical course), to better understand the epidemiology of trypanosomiasis (the behaviour of the disease in livestock populations) and to monitor and compare the impacts of implementing different trypanosomiasis control programs.

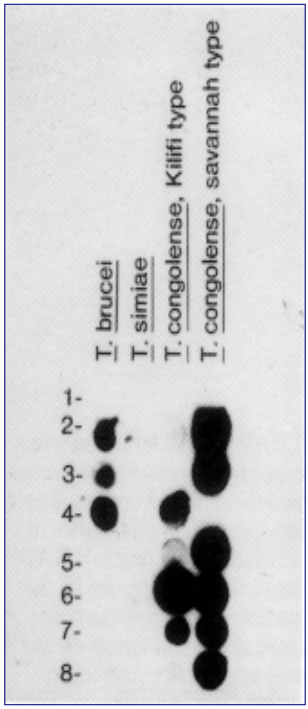
The first phase of a continent-wide evaluation of an ILRAD diagnostic test—the antigen-detection enzyme-linked immunosorbent assay, or 'ELISA' for short—was completed in 1991 and the results considered at a workshop held in Yamoussoukro, Côte d'Ivoire, in May. Using the antigen-detection ELISA, parasite components in blood samples are identified by the binding of monoclonal antibodies. Participants at the Yamoussoukro meeting judged the ELISA to be sensitive and reliable in detecting the three



ILRAD's ELISA kit (antigen-trapping enzyme-linked immunosorbent assay) used to detect and identify trypanosomes in infected animal hosts. ILRAD has developed tests for all the species of trypanosomes that cause disease in livestock as well as parasites that cause human sleeping sickness. Field evaluations of the assays were conducted with support from FAO and the International Atomic Energy Agency (Vienna).

major tsetse-transmitted trypanosome species that infect livestock as well as in detecting *T. evansi* infections in camels and pigs. In some areas where tsetse and trypanosomes were thought not to occur, trypanosome infections were detected with the ELISA. When sera were examined in these instances, antibodies to trypanosomes were also found, demonstrating both the usefulness of test and present shortcomings in epidemiological information.

A polyclonal serum to the antigen recognized by the monoclonal antibody used in the antigen-trapping ELISA for *T. congolense* was used to screen an expression library and several cDNA (copy DNA) clones were obtained. Following cloning of part of the gene encoding the *T. vivax* diagnostic antigen, the antigen was expressed in a bacterial system and the recombinant product found to be recognized by the appropriate monoclonal antibodies. The expressed products of these cloned 'diagnostic' genes will be used to provide standards (control antigens) for the antigen-trapping ELISA and the genes themselves will be examined for their usefulness as diagnostic tools at the DNA level.



ILRAD's probe panel used to detect trypanosome parasites.

A panel of species- and subspecies-specific DNA probes has been developed at ILRAD and used to detect and characterize trypanosome samples collected from experimental infections of tsetse flies and laboratory animals as well as from animals in the field. Rather than detecting the antigenic products of trypanosome genes, as the ELISA does, these probes detect the genes themselves. DNA probes are superior to the other available diagnostic tools in terms of specificity, sensitivity and ease of use when large numbers of samples need to be examined.

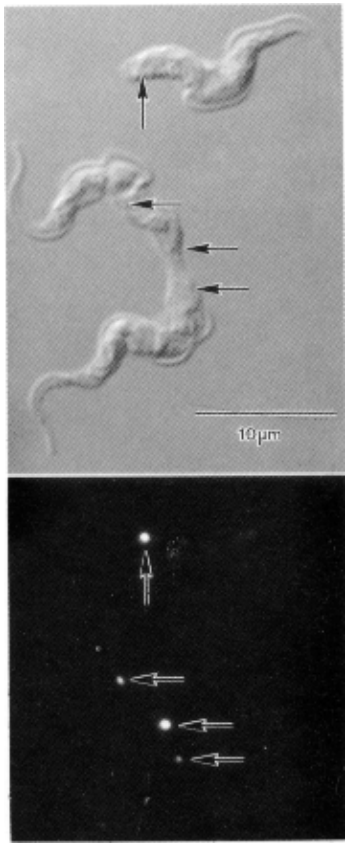
Most DNA probes are tagged with radioisotopes so that their hybridization with genetic material obtained from parasites can be visualized. This reliance on radioisotope labelling remains the greatest disadvantage of the technique. Because of the hazards, short shelf-lives and high costs and operational difficulties involved in use of radioisotopes, alternative ways of labelling DNA probes are now being explored. ILRAD staff aim to develop probes that are both safer than the radiolabelled probes now widely used in advanced laboratories and easier to use in the field.

DRUG RESISTANCE IN TRYPANOSOMES

DRUG TREATMENT for trypanosomiasis relies on three closely related compounds that have been widely used for more than 30 years: isometamidium, homidium and diminazene. Properly administered, these drugs have effectively controlled the disease, but parasite resistance to the compounds is increasing. In research aimed at maintaining the long-term efficacy of the compounds now in use, ILRAD scientists are looking for genetic markers of drug resistance in the parasites that could be used to inspect large numbers of parasite samples from cattle to determine quickly and easily if drug resistance is a problem in an area.

With scientists from the Kenya Trypanosomiasis Research Institute, ILRAD staff in 1991 developed an assay to detect drug resistance in T. vivax bloodstream form parasites. A calorimetric test to measure drug sensitivity in T. congolense bloodstream forms was also developed at ILRAD. Jointly with staff of the University of Glasgow, ILRAD scientists this year advanced work on an antibody-based ELISA to measure levels of isometamidium in mammalian blood.

Monoclonal antibodies raised against isometamidium will be used in cell biology studies to compare how the drug is taken up and compartmentalized in drug-sensitive and drug-resistant trypanosomes. Techniques to identify the parasite molecule with which isometamidium interacts were established this year in collaboration with a scientist from the Israel Institute of Technology (Haifa). With these techniques in place and the availability of resistant parasites raised at the Laboratory in simple culture systems, ILRAD is in a strong position to pursue physiological and membrane studies of drug uptake. The information obtained in these studies will lay the groundwork for devising better ways to use the drugs in the field and for developing treatment strategies that militate against the generation of drug-resistant parasite strains.



Two images produced of the same three trypanosomes after incubation for 20 hrs with isometamidium. The arrows point to areas of fluorescence, which indicate where the drug has concentrated in the parasites. Note that two of the parasites are in the process of dividing.

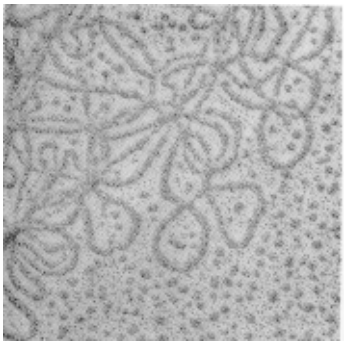
TRYPANOSOME ANTIGENS

SEVERAL TRYPANOSOME MOLECULES that can be examined for their potential to protect cattle from trypanosomiasis were isolated and characterized in 1991. Some of these antigens were revealed in studies of the endocytic system of trypanosomes, the process by which the parasites take up nutrients; others were discovered in empirical approaches taken to identify antigens. The gene for one of the most promising of the antigens, an enzyme (cysteine protease) of T. congolense associated with resistance to trypanosomiasis in cattle, has now been cloned.

MOLECULAR GENETICS OF TRYPANOSOMES

AN IMPORTANT GOAL of members of ILRAD's trypanosomiasis program is to improve understanding of the genetic basis of processes critical to the survival and development of the trypanosome. Of particular interest is the identification of genetic functions that could be interfered with and the identification of trypanosome genes and their protein products that are directly involved in drug resistance in parasites, degrees of immunity induced by parasite antigens, degrees of virulence in parasites and the development of disease in cattle.

Making use of technical breakthroughs in molecular biology, ILRAD scientists continued to refine technologies needed to insert foreign genes into trypanosomes and to identify which organisms internalize the erogenous genetic material. By transfecting total DNA from a virulent parasite, ILRAD staff in 1991 restored virulence to forms of *T. brucei* previously attenuated *in vitro*. Studies are now being conducted to identify the region of the genome responsible for the change in the transfected parasites. Results of these studies will help to identify the genes responsible for parasite virulence and to determine the nature of one of the main factors governing parasite growth.



Networks of coiled DNA that occur in a kinetoplast structure, outside the nucleus, of the trypanosome parasite.

Most DNA occurs in the cell nucleus of organisms. Along the threadlike length of DNA, the sequence of nucleotides carries the genetic code, which directs the formation of an entire organism and governs its biological functions.

The discrete functional units of DNA—the genes—direct protein synthesis.

Research is conducted at ILRAD on trypanosome genes that, because they are active only in certain stages of the parasite's life cycle, may control processes of differentiation and adaptation to mammalian or insect hosts. Genetic sequences normally associated with expressed RNA (ribonucleic acid) and a transposable genetic element widely distributed in the trypanosome genome were used to amplify DNA of different life cycle stages to identify differentially expressed parts of the genome.

Studies were also conducted in 1991 to identify genes and gene products involved in trypanosome cell division. Four classes of cell division cycle genes are being investigated and culture systems that synchronize the division of *T. congolense* are being developed. ILRAD scientists are collaborating in this work with scientists from the universities of Glasgow, Oxford and Brussels who are working on homologous genes in other kinetoplastids.

HOST IMMUNE RESPONSES TO INFECTION

LEARNING MORE ABOUT the complex interactions between hosts and parasites and their relationship to the development of disease will help scientists better understand how cattle generate immune responses to trypanosomes. This information may lead to the development of methods to manipulate those responses in ways that will block or inhibit parasite proliferation.

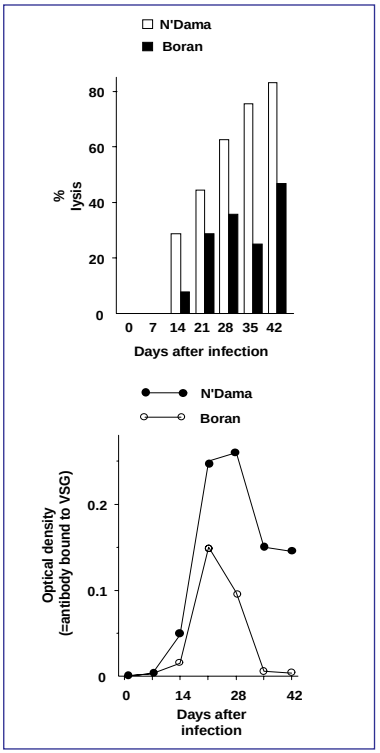
Studies were continued in 1991 of the responses to infection made by bovine B cells, which produce antibodies to trypanosomes, and T cells, which effect and regulate various immune mechanisms, including aspects of antibody production. Studies of macrophages were also continued. These cells ingest and destroy parasites of different kinds, and, like T cells, are involved in the regulation of bovine immune responses.

Past work at ILRAD has shown that antibodies directed against the surface of trypanosomes are able to neutralize parasites carrying the particular antigen used to induce them. Current research in this area is conducted into factors that induce antibody responses and the roles in immunity played by accessory cells and soluble factors. With increasing knowledge and availability of 'invariant' antigens, which comprise all the molecules of the parasite other than its variant surface glycoproteins, it may be possible to generate antibody responses that protect livestock against challenge with a broad range of trypanosome strains.

ILRAD has produced 50 trypanotolerant N'Dama cattle that now span three generations. ILRAD's original N'Dama were obtained as embryos from Gambian cows in 1983 and implanted in foster mothers of an East African Zebu breed called Boran. The institute thus has a unique opportunity to study under controlled circumstances the immune responses of the N'Dama to infection with trypanosomes and to define

A Boran bull. The Boran is an East African type of Zebu cattle.

Thoracic-humped cattle were introduced into Africa through Egypt in the fourth century B.C. Generally known as Zebu throughout the continent, these cattle now comprise most of Africa's indigenous cattle.



(Top) Results of a complement lysis assay demonstrated that when infected with trypanosomes, the trypanotolerant N'Dama cattle produce higher detectable levels of specific antibodies to trypanosome VSG antigens than the susceptible Boran.

(Bottom) Results of an ELISA demonstrated that both cattle breeds generate IgM class of antibodies to trypanosome VSGs, but that the N'Dama produce higher detectable levels of this antibody than the Boran.

the mechanisms that enable these animals to remain healthy while infected. A major area of ILRAD?s research is a comparison of the responses to infection made by the trypanotolerant N'Dama and the susceptible Boran.

Previous results of this research have shown that the N'Dama produce higher levels of detectable antibodies during infection and are also able to control infection better than their susceptible Zebu counterparts. This suggests that the immune system of trypanotolerant cattle may play a role in the superior ability of these cattle to control trypanosomiasis.

Recent work shows that the N'Dama produce higher detectable levels of predominantly IgM antibodies against parasite VSG than the Boran. In both Boran and N'Dama, levels of $CD5^+$ B cells increase dramatically following infection, and levels of total IgM also increase. The Boran produce higher levels of antibodies that react with irrelevant antigens than the N'Dama. Antibodies of the IgM isotype against invariant antigens are detectable in both breeds, but later in infection, more mature IgG^1 as well as IgM antibodies are detectable in the N'Dama.

The generation and maintenance of antibody responses usually requires the help of T cells. To compare T-cell responses of susceptible and trypanotolerant animals to trypanosome infection, T-cell subpopulations were prepared from peripheral blood obtained from Boran and N'Dama cattle undergoing primary infection with *T. congolense.* In the presence of parasite antigens, both Boran and N'Dama CD8 (cytotoxic) and, subsequently, WC1 (gamma/delta) T-cell populations proliferated. Proliferations of these T cells were more marked, however, among N'Dama than Boran cells. No T-cell responses were observed in either breed to appropriate VSG and there were low or absent responses of CD4 (helper) T cells in both breeds.

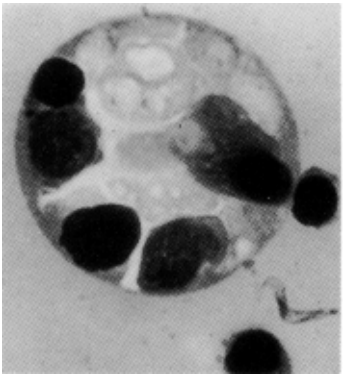
A striking feature of trypanosome infection in susceptible cattle is anaemia. Destruction of red blood cells is accelerated well above normal levels in infected animals. Past ILRAD work has shown that the ability to maintain normal levels of red blood cells during trypanosome challenge is a feature of trypanotolerant animals and that this trait is highly heritable. Elegant histological and electron microscopical studies indicate that macrophages are involved in the development of anaemia by phagocytosing (engulfing) red blood cells and other cell types, which contributes to the anaemia. Significantly, these studies have shown that the macrophages of infected Boran cattle are more destructive than those of infected N'Dama cattle. On the other hand, macrophages prepared from blood of N'Dama in the early stages of *T. congolense* infection release several times more co-stimulatory cytokines (IL1/IL6) in vitro than macrophages

from Boran cattle. Cytokines, such as tumor necrosis factor, gamma interferon and interleukins (abbreviated IL), are chemical messengers released by macrophages that have been activated by the presence of antigen.

These experimental results clearly show that macrophages are activated in both breeds of cattle when infected by trypanosomes but the functions of these activated scavenger cells differ between the breeds. It is not known how these different functions are induced, nor which trypanosome antigens directly or indirectly trigger macrophages to suppress immune responses, to engulf red blood cells and to produce cytokines. Answers to these questions are being sought because activities of the mononuclear phagocytic system appear to be pivotal in determining whether an animal will develop or resist disease.

Cytokines are essential components of immune responses, the making of blood cells and other physiological processes. They are normally active over short distances. Indeed, evidence suggests that cells can target release of cytokines directly onto other nearby cells. Abnormal levels of certain cytokines are thought to contribute to some diseases, and it is believed that some cytokines, such as IL1, IL6, tumour necrosis factor-alpha and gamma interferon, may be involved in the pathogenesis of trypanosomiasis. To find out if this is so, ILRAD scientists must compare the production of these cytokines in healthy and infected cattle and in trypanotolerant and susceptible animals. However, because these molecules generally are active only over short distances, can be absorbed rapidly by cells expressing their receptors and can be inhibited by a variety of serum proteins, they are difficult to detect in sera and plasma. An ILRAD group is therefore developing two methods for measuring cytokines at the cellular level. One assay uses monoclonal antibodies, saponin-permeabilized cells and the fluorescence-activated cell sorter to measure intracellular pools; the other assay uses the *in situ* hybridization technique to measure cytokine messenger RNA. Bovine GM-CSF, IL1, IL2, IL4 and gamma interferon probes are already available in the Laboratory. A probe for tumour necrosis factor-alpha is being made.

In other comparative immunological experiments conducted in 1991, four N'Dama and four Boran were infected with an unusual stock of *T. vivax* known to cause a particularly acute haemorrhagic disease in susceptible cattle. No differences were observed between the two breeds in the levels of parasitaemia or the pattern of recognition or type of antibody elicited. Members of both groups became anaemic. One of the N'Dama was treated when its packed cell volume fell to 13%. This experiment effectively demonstrated that the N'Dama, rather than being resistant to the pathogenic effects of infection with all trypanosomes, are as susceptible as the



Light micrograph of a large macrophage from the bone marrow of a calf infected with *Trypanosoma vivax*. Mature and immature red blood cells have been engulfed by the macrophage. A single trypanosome lies adjacent to the macrophage.

IMMUNOLOGY

'The life of every organism is constantly threatened by other organisms—this is the nature of the living world. In response, each species has evolved protective mechanisms, varying from camouflage colors, to poisons, to effective running muscles. From their continual battle with microorganisms, vertebrates have evolved an elaborate set of protective measures called, collectively, the *immune system.*

'The word immune (Latin *immunis,* meaning "exempt") implies freedom from a burden: an animal that is immune to a specific infecting agent will remain free of infection by that agent. The study of the immune system constitutes the discipline of *immunology.*'

— J. Darnell, H. Lodish and D. Baltimore, *Molecular Cell Biology,* 2nd ed., New York: Scientific American Books, 1990, p. 1003.

Boran to infection with this uncommonly haemorrhagic *T. vivax* stock from the coastal region of Kenya. This finding supports the view that trypanotolerance depends on the successful control of parasite populations. How trypanosome antigens are recognized by the immune system, how immune responses are induced and how the host genotype affects responses to infection are not yet known. The answers to these questions may be found in further studies of *T. vivax* infections in N'Dama cattle.

GENETICS OF HOST RESISTANCE TO TRYPANOSOMIASIS

THAT GENETIC RESISTANCE to trypanosomiasis exists in some cattle breeds is clear. As is also clear from the above discussion, however, neither the significance of immune mechanisms—such as the specific antibody, T-cell and cytokine responses made by infected N'Dama cattle—nor the genetic control of those mechanisms and of other potentially important non-immune mechanisms is yet understood. A direct way to investigate the nature of trypanotolerance is to follow its inheritance in families of cattle for the purpose of, first, finding characteristics—or 'markers'—that are inherited with the disease-resistance trait and second, finding the gene or genes that control it. The breeding herd of trypanotolerant N'Dama available at ILRAD and the institute's unique ability to assess the responses of these cattle to parasite challenge under highly controlled and experimental conditions make such a genetic marker and mapping project feasible.

ILRAD's bovine genome project was begun in 1990. The first two steps were to produce large families of cattle that segregate the trypanotolerance trait and to begin to identify some of the estimated 200 markers evenly spaced along the 60 chromosomes of cattle that will be needed to identify genetic markers associated with disease resistance. When developed, such markers will be a useful tool for breeders working to increase the numbers of trypanotolerant livestock and for researchers attempting to identify populations of animals to be targetted for germplasm conservation. Perhaps most importantly, the markers will also serve as starting points in a search of the bovine genome for the genes that control the trypanotolerance trait.

To produce the cattle needed for the mapping project, bulls from ILRAD's core herd of 10 trypanotolerant N'Dama were crossed with susceptible Boran cows in an accelerated breeding program that made use of embryo transfer expertise developed at ILRAD over the last several years. The cows were induced to produce more ova than normal. The ova were fertilized by artificial insemination and the embryos implanted in foster Boran mothers for gestation.

Two first-generation full-sibling families of N'Dama-Boran crossbred animals were produced in 1991. Each family consists of more than 30 calves. Thirty-eight of this first filial (F_1) generation and ten Boran control animals were challenged with *T. congolense* clone 1180 injected through the bite of infected tsetse flies. Three of the thirty-eight F_1s required treatment, compared with six of the ten Boran. The indications are that trypanotolerance is a typical quantitative trait.

Cross-breeding of the F_1s will begin in February 1992; the first group of second filial generation calves will be born in November 1992. Responses of the F_2 generation to challenge with *T. congolense*, including packed cell volume levels, parasitosis and aerological responses, will be recorded. The animals will be phenotyped for aspects of trypanotolerance and genotyped with DNA markers as these are identified at ILRAD and other advanced laboratories collaborating in this work.

To produce the genetic markers needed for the mapping project, ILRAD scientists are exploiting microsatellites—simple DNA nucleotide sequences that occur with a high frequency in the mammalian genome. Once identified, short, single-stranded synthetic DNA molecules that hybridize specifically with chromosome regions flanking the microsatellites are

The first Boran-N'Dama crossbred calves produced at ILRAD using embryo-transfer technology were born on ILRAD's Kapiti Ranch in 1990. By the end of 1991, 86 Boran-N'Dama crosses had been produced.

Large families of these cattle are needed for a new research project to locate the genes responsible for trypanotolerance, an ability of N'Dama and other livestock breeds indigenous in Africa to remain productive while infected with trypanosomes.

DNA SEQUENCING

Determining the precise order, or sequence, of the nucleotides that make up a gene once the gene has been located on one of several chromosomes.

'The nucleotides are arranged in a precise order like beads on a necklace. . . . One way to understand the difference between [genetic] mapping and sequencing is to think of a giant library. Mapping is like noting the titles and the order of all the books on the shelves; sequencing is reading the books.'

— Bruce M. Alberts, 'Making a Map of the Human Chromosomes', in *Headline News, Science Views,* ed. David Jarmul, Washington, D.C.: National Academy Press, 1991.

synthesized. These 'oligonucleotides' serve as primers in the polymerase chain reaction to produce labelled copies of the microsatellite regions. The length polymorphism of the copies can then be determined by reducing polyacryla me gel electrophoresis to give markers at known sites within the genome.

Applying this approach, ILRAD scientists identified nine sequence-tagged sites in 1991 for the bovine linkage map. Such work is labour-intensive, both in identifying markers and their locations and in determining the distribution of markers throughout the genome. However, the development of this linkage map of the bovine genome is a collaborative enterprise and the work involved in marker development is being shared among geneticists at ILRAD, the Commonwealth Scientific, Industrial and Research Organisation (Australia), the Hebrew University of Jerusalem and Texas A & M University (USA).

Furthermore, a breakthrough technology first reported in 1991 is speeding up and cutting the costs of the search for markers. The method is known as 'bulk segregant analysis' and employs random amplified polymorphic DNA markers, known by biologists as RAPDS. Used by themselves, RAPDs give imprecise information because each marker sample is associated with many pieces of DNA. However, by pooling and analysing all the DNA from the most resistant cattle in one test, and all the DNA from the least resistant cattle in another, markers linked to trypanotolerance are likely to stand out.

CONCLUSION

ILRAD'S TRYPANOSOMIASIS PROGRAM is working to fulfil two broad objectives. The first is to meet increasingly pressing requirements for immediate improvements in methods now used to control trypanosomiasis. This is being accomplished in two ways: by gaining a more complete understanding of the epidemiology of trypanosomiasis, through development of improved tests for detecting infection and identifying parasites, and by improving and making better use of current control methods, particularly the employment of drug therapy and trypanotolerant livestock.

The second objective of the program is to translate long-term research into practical and sustainable trypanosomiasis control. Research is being conducted towards the development of four novel control methods. (1) Identification of molecular mechanisms—parasite components or physical or chemical processes—that could be manipulated with new trypanocidal agents to disrupt parasite development or to make the parasites more vulnerable to the defences of the mammalian host. (2) Development of new therapeutics, other than trypanocides, on which to base treatment and disease management. (3) Identification of trypanosome antigens that, by inducing protective immune responses in cattle, are potential material on which to base vaccines. (4) Improvement of the responses of susceptible cattle to trypanosome infection through a better understanding of

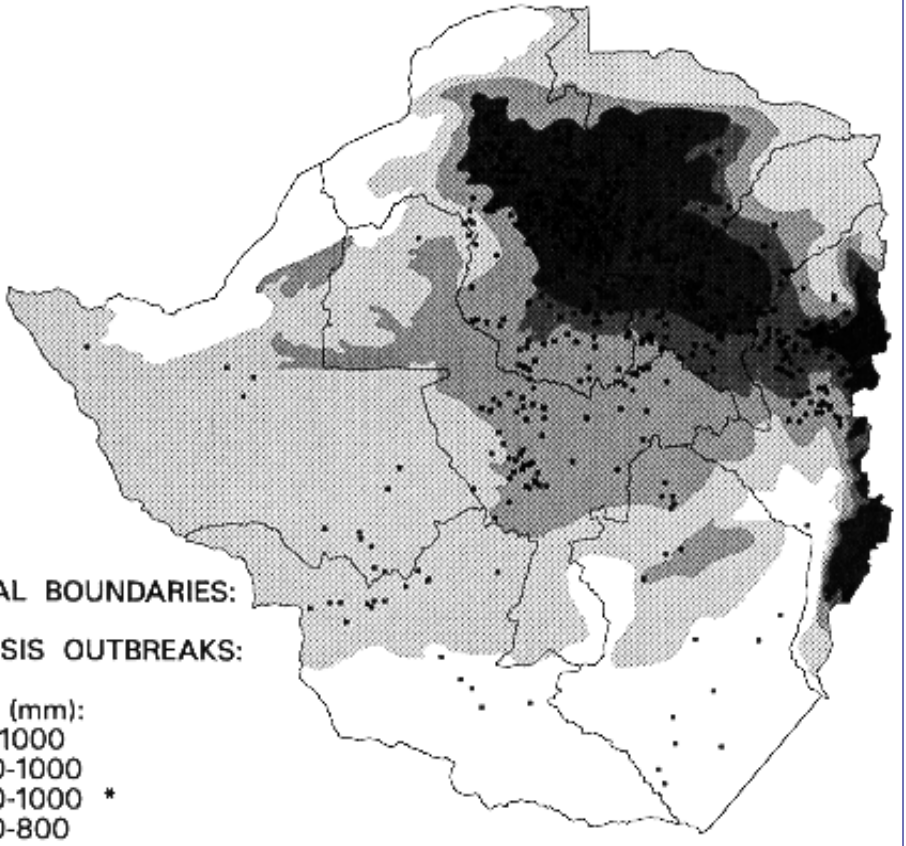
the innate resistance to trypanosomiasis displayed by trypanotolerant cattle. This research involves finding genetic markers that can be used to pick out animals highly resistant to trypanosomiasis for use in livestock breeding programs and to locate the gene or genes that control the trypanotolerance trait(s).

Any future methods developed to increase livestock resistance to trypanosomiasis and to decrease the pathogenic effects of infection would greatly alleviate the animal trypanosomiasis problem in Africa and in addition would help advance research of human trypanosomiasis and other trypanosome-caused diseases that occur in the tropics. Host resistance to the parasites might be increased in several ways. Vaccination to generate effective immune responses in livestock would, in addition to being of inestimable value in itself, constitute a major biological advance. An understanding of how trypanosome infection produces disease would lead to more effective treatment and would help further the development of livestock types able to resist the harmful effects of infection. An understanding of trypanotolerance would facilitate better use of trypanotolerant animals and, through genetic manipulations, might allow the transfer of trypanotolerant trait(s) to cattle breeds that, although susceptible to trypanosomiasis, possess other desirable characteristics.

An N'Dama and a Boran embryo were implanted at the same time into this recipient Boran cow, which subsequently produced these 'haemopoietic chimaeric' twins. The N'Dama calf, on the left, after sharing a placenta with its Boran twin in foetal life, has developed an essentially Boran bone marrow.

ILRAD scientists will challenge twins such as these with trypanosomes to determine which if any trypanotolerance traits have been transferred from the N'Dama to the Boran.

'Chimaeric': from *chimaera*, a mythological creature with the head of a lion, the body of a goat, and the tail of a serpent. '. . . A thing of immortal make, not human, lion-fronted and snake behind, a goat in the middle . . . .'
— *Iliad* (6. 179)


PROVINCIAL BOUNDARIES:
THEILERIOSIS OUTBREAKS:
RAINFALL (mm):
> 1000
750-1000
750-1000 *
650-800
450-650
< 600
* Severe dry spells in summer

A MULTIDISCIPLINARY TEAM of scientists supports ILRAD's disease research programs by investigating the factors that govern the successful application of improved livestock disease control, particularly through immunization, and the likely social, economic and environmental impacts of improved animal health. ILRAD's Socioeconomics Program, started in 1988, has focused its studies on East Coast fever in Africa and is now widening its scope to include investigations of African animal trypanosomiasis.

(Opposite) The distribution of reported outbreaks of theileriosis in Zimbabwe from 1979 to 1989 overlayed on the natural regions of the country. The map was made at ILRAD using a computer-based geographic information system.

EPIDEMIOLOGY OF LIVESTOCK DISEASE CONTROL

SCIENTISTS IN ILRAD's Socioeconomics Program have developed a methodology for conducting quantitative studies whose results will help to identify communities and cattle populations that would benefit most from the introduction of improved methods to control tick-borne diseases. In collaboration with scientists from the Kenya Agricultural Research Institute (KARI), the ILRAD staff applied these methods in studies conducted at two sites in Kenya, one in Kaloleni Division, Kilifi District, on the Kenya coast, and one in Uasin Gishu District, in the western highlands of the country. The staff also collaborated with researchers in Zimbabwe to study the effect of alternative disease control tactics in that country.

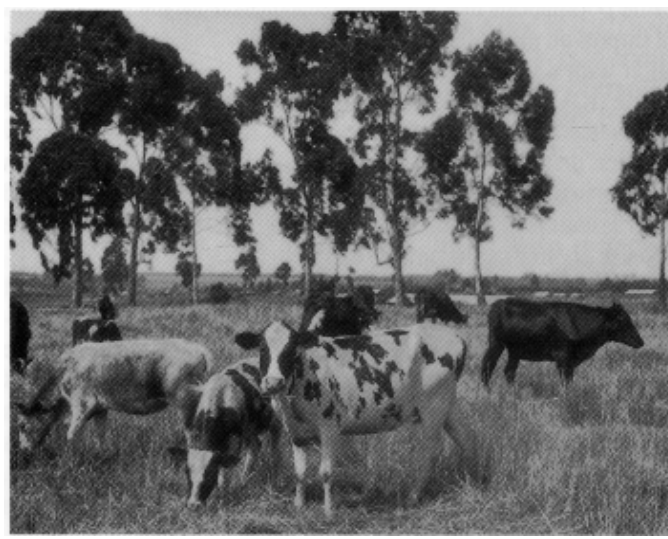
Epidemiological justification for implementing East Coast fever immunization at the Kenya study sites was obtained by conducting three basic studies. First, cattle in the target areas were surveyed to determine the prevalence of antibodies to *Theileria parva;* prevalence rates indicate levels of immunity that have developed in a population. The animals in this survey were chosen from strata according to their age, their type or breed, the type of grazing system in use, the size

of the herd and the agro-ecological zone of the target area. Prevalence rates specific for each strata were calculated with confidence intervals and comparisons were made among the strata. Low rates of antibody prevalence—which suggest that much of a cattle population is at risk of East Coast fever—were found in most strata of Uasin Gishu, in the genetically improved cattle in Kaloleni and in the Zebu cattle inhabiting low rainfall areas of Kaloleni.

The second component of ILRAD's epidemiological methodology was a study made to determine the best age at which to immunize cattle against East Coast fever. This study was carried out in groups of sentinel calves released at bimonthly intervals over one year in the coastal study site. The time to infection with *T. parva* ranged from 12 to 98 days, 88% of the calves developed East Coast fever and, in spite of treatment being administered, the case-fatality rate was 66%.

The third component of this methodology was a one-year longitundinal study of herds kept under different management systems. Among a group of 130 dairy cattle kept on 28 farms in the Kenya coastal study area, 22% contracted East Coast fever, with 73% of these cases occurring in calves; 22% of the 130 cattle died in total, 78% of these fatalities being due to East Coast fever.

The results of these collaborative studies suggest that immunization of crossbred dairy cattle in the wetter regions of Kenya's coastal lowlands is warranted and that immunization of Zebu calves in the drier coastal lowland areas may be economically justified as well. There appears to be a broader target population for immunization in the Kenya highlands study area. Farm-level economic studies will be made to assess the relative costs and benefits of vaccinating cattle in these areas.



Crossbred dairy cows in Kenya's highlands (Uasin Gishu District).

IDENTIFYING TARGET GROUPS OF FARMERS FOR IMPLEMENTATION OF LIVESTOCK DISEASE CONTROL

THE ABILITY TO IMPROVE livestock disease control in an area depends as much on a good understanding of the human environment as it does on accurate agro-ecological and biological assessments. Farmers make disease control and other livestock management decisions based on the goals of the household, the resources available and the prerequisites of other enterprises on the farm. Experiences over the last several decades among those developing improved agricultural technologies and transferring these to farmers through extension networks demonstrate that decision-making in farming communities is complex and—due to differences in the scale and priorities of production on the farms—highly variable.

By analysing the links between farmers and the livestock disease control system operating in their area researchers gain a better understanding of the different socioeconomic contexts of livestock disease and its control. The links between African small-scale farm management systems and local disease control infrastructures are poorly understood. Since 1989, ILRAD's Socioeconomics Program has collaborated with KARI and the International Livestock Centre for Africa (ILCA) to examine those links through use of a farming systems research strategy, an approach traditionally used to improve crop production. The ILRAD/KARI/ILCA team is developing profiles of the circumstances in which small farmers operate (farm income, human and livestock nutritional states, livestock disease prevalence, etc.) by characterizing farms and conducting diagnostic surveys. The data obtained are being used to identify the most appropriate farm groups in which to introduce improved disease control technologies.

To help target cattle populations for pilot immunization against East Coast fever, ILRAD scientists characterized farms in the Kilifi and Uasin Gishu study areas. In Kilifi, livestock production systems were classified according to agro-ecological zone, cattle type and grazing system. In the first two phases of farm characterization exercises being carried out in Uasin Gishu, farm size rather than agro-ecological zone and cattle type was found to be important in determining the choice of farming systems.

ECONOMIC IMPACT OF IMPROVED DISEASE CONTROL

BOTH FARM- AND HERD-LEVEL computer models have been developed by staff of ILRAD's Socioeconomics Program to improve the accuracy of estimates of the economic impacts of livestock diseases and their control. Data to validate the farm-level model have been collected jointly by ILRAD, KARI and ILCA scientists from the study area at the Kenya coast (Kaloleni Division, Kilifi District). These data were also used to assess the economic benefits of immunizing cattle in that area against East Coast fever.

Members of ILRAD's Socio-economics Program consult with staff from the International Livestock Centre for Africa and the Kenya Agricultural Research Institute and with farmers and extension workers in a collaborative research project being conducted to gain a better understanding of the cattle populations to be targetted for immunization against East Coast fever in Kaloleni Division, Kilifi District, on the coast of Kenya.

Most small-scale farmers in developing countries do not own or have access to motorized vehicles. Thus, on small farms in many parts of the continent where both crops and livestock are grown, cattle are the only means of power—other than human labour—for pulling ploughs to prepare land for cultivation and for transporting farm produce to market. It is clear that the draught animal will remain the main source of traction and power for agricultural operations in the developing world for a long time to come.

Cattle continue to play a central role in many traditional farming systems for other reasons as well. They are an important source of protein, mostly in the form of milk, for rural families, their manure helps to maintain and enhance soil fertility, and sales of cattle and cattle products are a main source of income. Indeed, in most African cultures, the number of cattle and other livestock a person owns directly indicates his or her wealth.

The data illustrate how much and in how many ways livestock contribute to the economy of African smallholders. On average, livestock of all types made up 70% of farm investment and 40% of farm-generated income, although these figures varied widely among agro-ecological zones, cattle types and grazing systems used. Cattle enterprise in the study area at the Kenya coast generated a mean annual gross profit of US $210 per cow in herds of taurine crossbreds and $84 per cow in Zebu herds. In taurine crosses, milk accounted for 74% of the economic output, animal disposal for 25%. In Zebu herds, milk accounted for 30% of the output, animal disposal 67%.

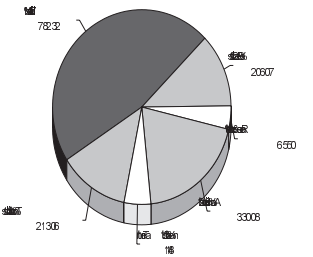
The farm model was developed in collaboration with scientists at Texas A & M University. It simulates annual farm production and consumption. It uses time increments of one year and runs for 10 years. Each year starts with the debts and assets accumulated by the end of the previous year. A prototype of the model and an accompanying database management system were tested and refined with data

obtained from selected farmers in the coastal and highland study areas as well as through discussions of preliminary results with these farmers. In addition to generating indicators of economic viability, such as net present values, benefit-cost ratios and internal rates of return, the model also calculates indicators of household nutritional sufficiency and quality.

The herd model takes into account herd size and structure, the estimated impact of a disease in terms of incidence, case-morbidity and case-fatality, and the effects of a given control method on the disease, on cattle productivity and on net income. Other indicators of financial impact such as marginal benefits, benefit-cost ratios and rates of return on capital can be calculated from the results of running the model for a particular area. The model is deterministic and runs for a study period of one year. It can be used to evaluate and compare different disease control strategies.

The herd model has been used to make preliminary estimates of the economic losses caused by East Coast fever and the *ex-ante* economics of its control using the infection-and-treatment method of immunization. These estimates were made for the region of 11 African countries where theileriosis caused by *T. parva* occurs, as well as for the country of Uganda and for Kenya's Kaloleni Division in Coast Province. When applied to the endemic region of eastern, central and southern Africa, total losses due to theileriosis in 1989 were estimated to be US $168 million. This figure includes an estimated mortality of 1.1 million cattle. An analysis of the economics of applying the infection-and-treatment immunization method in the region showed high economic returns, with benefit-cost ratios in the range of 9:1 to 17:1.

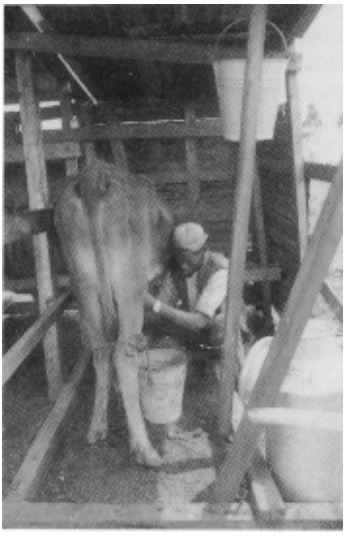
Calculated economic losses in 1989 due to *Theileria parva* infections in cattle in eleven African countries. The total economic loss was estimated to be US $168 million. (Figures are in US millions.)



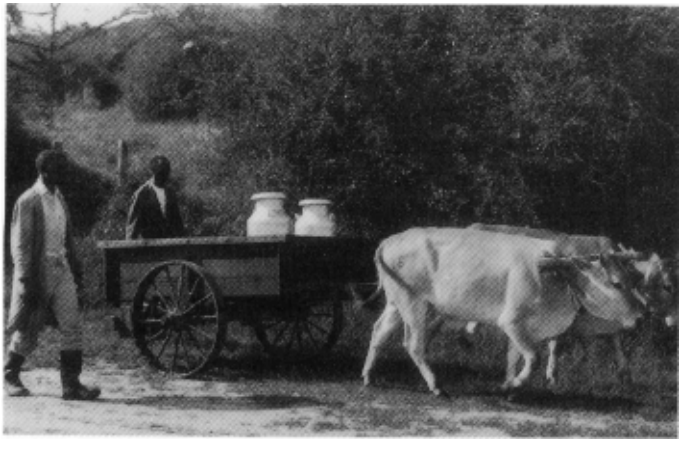
NUTRITIONAL IMPACT OF IMPROVED DISEASE CONTROL

DEVELOPMENT AGENCIES commonly equate changes in income levels with changes in people's welfare. Non-economic measures of the impacts of development, however, may be more pertinent indicators of a community's well-being. Measures of diet and nutrition, for example, are clearly linked both to food production and to health in rural households. But whereas the effects of cash cropping on household nutrition are often given due attention, the nutritional effects of changing livestock production systems have only recently begun to be examined.

In 1991, the ILRAD socioeconomics group began to investigate ways of incorporating into their program assessments of the nutritional as well as economic status of the livestock farmers their work is addressing. They developed a framework that determined the links between interventions in livestock management and household nutrition, identified methods for measuring nutritional impact and applied these in research at their study areas at the coast and in the highlands of Kenya.



A pilot survey of links between farm characteristics and food consumption patterns was conducted during the year among a sample of 72 households in Uasin Gishu District, Kenya. Large-scale farmers (those owning more than 100 acres) on average produced 18 times as much milk as small-scale farmers (with under 10 acres) and reserved for household use 4.5 times as much milk as small-scale farmers. The average daily incomes derived from milk sales ranged from KShs.10.15 on small farms to KShs.344.40 on large farms. Household milk consumption per consumer unit per day differed significantly between households on large (22 litres), medium (1.6 litres) and small farms (1 litre).



In 1991, the ILRAD Socioeconomics Program assessed the nutritional as well as economic status of small-scale farmers and their families.

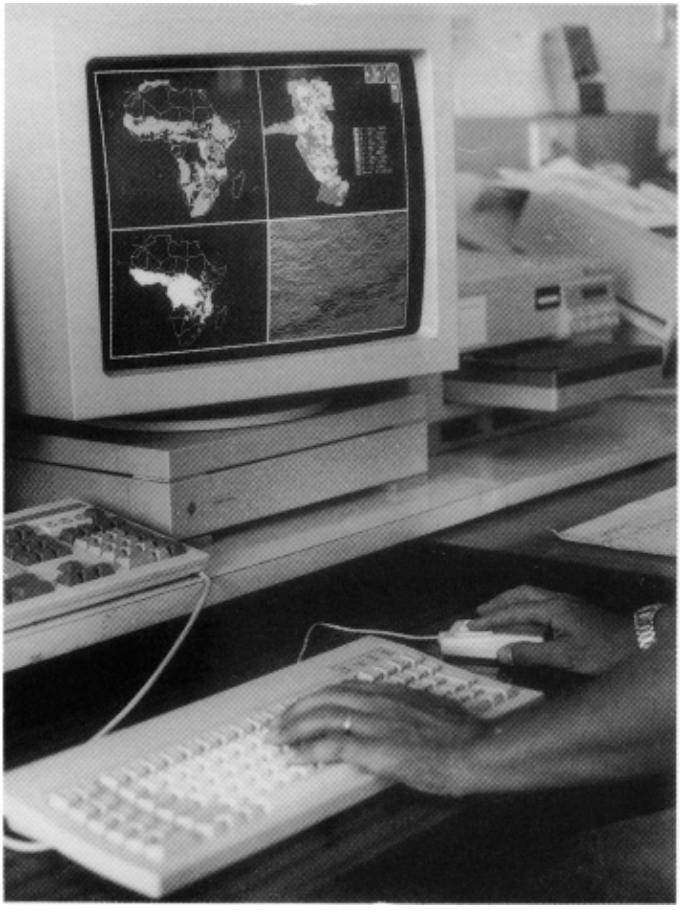
GEOGRAPHIC INFORMATION SYSTEMS

COMPUTER-BASED geographic information systems, designed to store, manipulate, analyse and display spatial data, have been tailored by ILRAD workers to help them predict the impact of livestock disease control in Africa. Program members are now using three geographic information systems: GRASS, a raster-based system running on SUN Graphics Workstations under the UNIX operating system, ARC/INFO, a vector-based system running on two IBM personal computers and a workstation, and IDRISI, a raster-based system running on an IBM personal computer. (Climatic surfaces represented by grids of data are best processed using a raster-based system; areas represented with discrete boundaries, such as farms, are best processed with a vector-based system.)

Spatial databases are being developed at the continental (Africa), regional (eastern Africa), country (Zimbabwe), district (Uasin Gishu, Kenya) and divisional (Kaloleni, Kenya) levels that will enable Program staff to integrate data

on the impacts of controlling animal diseases. To supplement existing databases, staff in 1991 digitized the best available data on continent-wide distributions of wildlife hosts, such as waterbuck and eland, and added reported distributions of the tick vector, *Amblyomma variegatum.*

In collaboration with staff from KARI and ILCA, much information was added to the regional and Kenya divisional geographical information system databases in 1991, including data on agro-ecological zones and transportation networks, and results of cattle censuses, serological surveys and dairy marketing studies. The country-level geographic information system for Zimbabwe being developed with the Zimbabwe Department of Veterinary Services now comprises an extensive database of over 60 layers of information. High-resolution satellite data are being compiled for the whole country for the purpose of evaluating the suitability of various habitats for tick survival. Zimbabwean experts are mapping the distributions of the wildlife hosts of various vector-borne livestock diseases for inclusion in the database.



African data displayed using ARC/INFO, a geographic information system.

ILRAD's Socioeconomics Program is tailoring computer-based geographic information systems and building mathematical models to help predict the impacts of improved livestock disease control.

Geographic information systems help researchers to analyse and compare spatial data of the many variables that impinge on livestock production and disease control. The mathematical models being developed will give decision makers continent-wide support in determining the most appropriate disease control and production policies to implement in given areas.

In recent years, ILRAD scientists have collaborated in modelling the dynamics of East Coast fever with scientists from the universities of London and Strathclyde in the UK as well as the Australian National University and the Commonwealth Scientific, Industrial and Research Organisation in Australia.

ILRAD is also collaborating with Texas A & M University (USA) in developing farm-level models of the economic and nutritional impacts of better control of East Coast fever.

With scientists at Imperial College, London, a model has been developed to investigate the transmission dynamics of *T. parva* infection by *R. appendiculatus* ticks to cattle; use of this model led to development of a method for estimating the rate at which cattle are infected by *T. parva* for the endemically stable states.

MODELS TO ASSESS THE IMPACT OF DISEASES AND THEIR CONTROL

THE VARIETY and complexity of the epidemiology of vector-borne diseases in Africa and the variety of livestock production systems in use on the continent necessitate development of mathematical models to formalize and synthesize the large amounts of empirical information needed to make rational decisions regarding livestock disease control. Given a sound understanding of the epidemiology of East Coast fever and other tick-borne diseases, models of these diseases will provide scientists, policymakers and disease control workers with powerful tools with which to examine and compare the merits of intervention strategies and policies.

Those assessing the impact of implementing a livestock disease control strategy must know the distribution of that disease. Disease reporting systems in Africa, however, can rarely provide data that are sufficiently detailed and accurate for this purpose. Fortunately, the distribution and abundance of the vectors of disease-causing parasites can serve as a valuable substitute for the distribution of vector-borne diseases. ILRAD's Socioeconomics Program staff has access to two models of vector distribution and two models of theileriosis occurrence and has collaborated with staff of the Nairobi-based United Nations Environment Program in developing appropriate databases for these models.

A study was begun in 1991 to validate predictions of the distribution in Africa of the tick vector of *T. parva, Rhipicephalus appendiculatus*, made using a climate-matching model called CLIMEX on a high-resolution (5-minute) interpolated climate database for Zimbabwe. CLIMEX calculates an eco-climatic suitability index for the tick on a scale of 0 to 100 so that the suitability of any particular area for *R. appendiculatus* can be accurately predicted. The distribution of the values of this index were compared with (1) reported tick and disease occurrence data, (2) natural regions (a rainfall-driven classification of agro-ecological zones) and (3) land-use categories, such as commercial, private or communal. Good correlations were found between the presence of an eco-climatic index value of 1 or greater and the recorded occurrence of *R. appendiculatus*. Correlations with theileriosis outbreaks were less precise, but were improved if known buffalo-associated outbreaks in the drier areas of the country in which the tick *Rhipicephalus zambeziensis* is reported to occur were excluded from the analysis.

`We now stand at a crossroads in theileriosis, and indeed in tick-borne disease, control. With new, more broadly immunogenic and safer vaccines on the horizon, it should be possible to adopt a totally new perspective on theileriosis control within a few years.'

The quote above and drawing below are taken from *The Epidemiology of Theileriosis in Africa*, a book written by two ILRAD scientists, B.D. Perry and A.S. Young, and a former ILRAD staff member, R.A.I. Norval, now working at the University of Florida. The drawing is also by an ILRAD scientist, D.J McKeever. The book was published in 1991 by Academic Press (UK), 481 pp.

COOPERATIVE PROGRAMS, TRAINING AND INFORMATION

ILRAD's OUTREACH ACTIVITIES fall under the Department of Cooperative Programs, Training and Information. The broad aim of ILRAD's cooperative activities is two-fold: (1) to advance our understanding of the nature of important tropical livestock diseases by employing biological, socioeconomic and environmental data, materials and expertise from the countries in which the diseases occur and (2) to enlarge the human and technical resources available for agricultural research and disease control in those countries.

In 1991, staff of this department and senior scientists from ILRAD's research programs paid visits to national agricultural research organizations in 11 African countries for the purpose of promoting collaborative research with ILRAD. Members of the missions consulted with national staff on research areas, personnel and institutions that would benefit from collaboration with ILRAD. A formal agreement was established this year with Sokoine University of Agriculture (Tanzania). This link will strengthen collaborative research between the two institutions on trypanosomiasis and theileriosis as well as on the socioeconomic changes that improved control of these diseases will bring about in livestock-keeping communities and their natural environments.

As discussed throughout this annual report, the many collaborative projects undertaken in 1991 fell in such diverse research areas as epidemiology, diagnosis, chemotherapy, drug resistance and the social, economic and environmental impacts of improved disease control. The collaborative projects served both as a channel for the transfer of technologies and as a means of accelerating research progress at ILRAD and the collaborating institutions.

ILRADs cooperative research activities are supported by high-quality service facilities, including the institute's

COOPERATIVE PROGRAMS

Major collaborative research at ILRAD includes the following.

- The African Trypanotolerant Livestock Network (Nairobi) and national agricultural research institutes in Côte d'Ivoire, Ethiopia, Gabon, Gambia, Kenya, Senegal and Zaire on aspects of genetic resistance to trypanosomiasis in cattle breeds.
- The Centre for Tropical Veterinary Medicine (UK), the Food and Agriculture Organization, the International Atomic Energy Agency (Vienna) and national agricultural research institutes in Egypt, Gambia, Ghana, Kenya, Mali, Senegal, Sudan, Tanzania, Zambia and Zimbabwe in evaluation of an ILRAD assay for diagnosing trypanosome infections.
- The Kenya Agricultural Research Institute (KARI) and the governments of Tanzania (Zanzibar), Zambia and Zimbabwe in characterizing parasite stocks for use in immunizing cattle against theileriosis.
- KARI, ILCA and Zimbabwe in socioeconomic studies made to determine the most appropriate strategies for controlling theileriosis.
- The Kenya Trypanosomiasis Research Institute on studies of drug resistance in trypanosomes.
- The International Centre of Insect Physiology and Ecology (Nairobi) on development of DNA probes to detect trypanosome infections in tsetse flies.

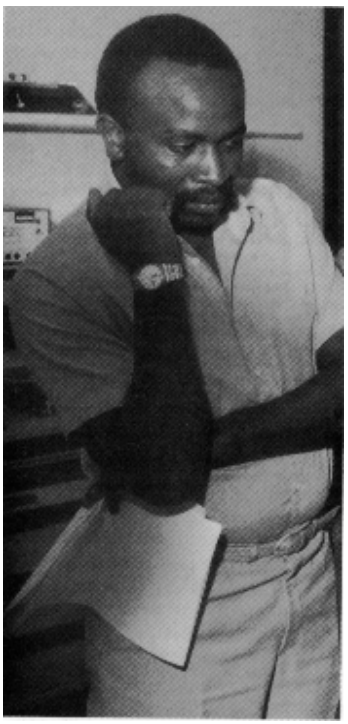
specialized library and its electron microscopy, vector, laboratory animal, graphic arts and biostatistics units. Cooperative research is also backstopped by a wide variety of training in the form of on-going reviews, lectures, seminars, meetings, workshops, conferences, individual instruction and special group courses that are held on all aspects of ILRAD's research, from bovine immune responses to recombinant organisms to biosafety standards to ethnoveterinary studies.

TRAINING

CHANGES ARE OCCURRING on millions of smallholder farms in Africa and other regions of the developing world due to a necessity to increase food production while conserving land and water resources. Many farmers are attempting to accomplish this dual task by integrating crop and livestock farming and by intensifying their livestock production. Guiding farmers, extension workers and veterinarians in how to manage these new farming systems will require a cadre of highly committed, trained and scientific personnel. It is the principal aim of ILRAD's Training Unit to help develop that human infrastructure.

In 1991, 36 scientists and senior laboratory technicians came to ILRAD for periods of one week to six months to receive individual training in techniques or areas specified by their research institute or disease control project. Three group courses with a total of 21 participants were held during the year on use of advanced techniques for diagnosing trypanosomiasis and tick-borne diseases. In addition, financial support was given to many researchers and disease control workers to attend courses run jointly by ILRAD and other international agencies in 1991 in Burkina Faso, Côte d'Ivoire and Uganda.

Alladin Kairo, a Kenyan Research Fellow at ILRAD studying the molecular biology of the trypanosome parasite for a Ph.D. degree.



Thirty-one students—most of them from Africa and supported by ILRAD fellowships—conducted research at ILRAD during all or part of 1991 towards Ph.D. and M.Sc. Degrees. Seven of these research fellows were awarded doctorate degrees this year ILRAD provided three additional fellowships to senior African scientists to do post-doctoral research at the institute.

ILRAD hosted two scientific workshops in 1991: 'Bovine Genome Mapping and Trypanotolerance', held in April, and 'Recent Developments in the Research and Control of Anaplasmosis, Babesiosis and Cowdriosis', held in May. Discussions at these workshops served both to update ILRAD staff on current research in these areas that is relevant to the institute and to inform scientists working in developed countries about the current state of livestock disease control and research in Africa.

ILRAD also helped to run three other workshops conducted in 1991: jointly with the Rockefeller Foundation

and the United Nations Environment Program, a workshop titled 'Increased Sustainable Agricultural Productivity in Africa through Use of Intelligent Geographic Information Systems' was held in Nairobi in January; with the Organisation of African Unity (OAU) and the Food and Agriculture Organization of the United Nations (FAO), a workshop on 'Ticks and Tick-Borne Disease Control' was held in Kampala, Uganda, in September; and with FAO and the World Health Organization, ILRAD supported the OAU'21st Meeting of the International Scientific Council for Trypanosomiasis Research and Control' and helped to run a training seminar held before the meeting in Yamoussoukro, Côte d'Ivoire, in October.

Country	TT	RF	SRF	PDF
From developing countries				
Benin		1		
Cameroon	2			1
Chad		1		
Ethiopia	1	1		
Gambia		1		
Ghana		1		
India	1			1
Kenya	17	15	1	1
Madagascar				1
Malawi	2			
Nigeria	1	3		
Senegal		1		
Somalia			1	
Tanzania	3	2		
Togo	1			
Uganda			1	
Zaire		2		
Zambia	3	1		
From developed countries				
Belgium				1
Canada	1	1		
France	2	1		
Italy		1		
Netherlands				1
Spain				1
UK				3
USA	1			1
Totals	*35*	*32*	*3*	*11*

Individual training at ILRAD in 1991; number of participants in different types of training by country of origin. Nearly 10% of ILRAD's budget is spent each year on training and related outreach activities.

TT = technical training
RF = Research Fellows
SRF = Senior Research Fellows
PDF = Post-Doctoral Fellows

'There is no evidence that any country or race is better than any other in scientific teachability: there is a good deal of evidence that all are much alike. Tradition and technical background seem to count for surprisingly little. . . .

'It is technically possible to carry out the scientific revolution in India, Africa, South-east Asia, Latin America, the Middle East, within fifty years. . . . Since the gap between the rich countries and the poor can be removed, it will be. If we are short-sighted, inept, incapable either of good-will or enlightened self-interest, then it may be removed to the accompaniment of war and starvation: but removed it will be. The questions are, how, and by whom.'

— C.P. Snow, *The Two Cultures and the Scientific Revolution,* Rede Lecture, 1959, Cambridge: Cambridge University Press, 1960, pp. 42–44.

THE GOAL OF ILRAD's Information Unit is to keep clients of the institute and agricultural policymakers, donor agencies, the media and the general public informed about progress ILRAD is making in its aim to improve the welfare of people in developing countries by improving their control of livestock diseases. Through its publication series, the unit also helps to transfer the products of this research to ILRAD's stakeholders, particularly to scientists in national agricultural research systems in developing countries.

The unit publishes an *ILRAD Annual Report*, an annual *ILRAD Highlights* and a quarterly scientific newsletter, *ILRAD Reports*, in French as well as English, and, in English only, an *ILRAD Annual Scientific Report*, an annual list of scientific publications produced by staff, one or two proceedings of international scientific meetings held at ILRAD, an annual *Program and Budget* document and a weekly *Internal Newsletter* distributed to all staff members and to the Board of Directors. All publications are written, edited, designed and electronically laid out to camera-ready stage in-house using a personal computer and laser printer. Translation and typesetting of French publications is contracted to a biomedical translation company in the USA.

The annual report, annual highlights and quarterly newsletter are intended for a wide range of readers, with emphasis on scientists and policymakers in national agricultural research systems. ILRAD's annual scientific report comprises abstracts disclosing the aims and results of each research project conducted at the Laboratory during the year. This book and the annual list of staff publications are intended for scientists and highly technical staff working in areas related to ILRAD's research programs as well as for new ILRAD staff.

Proceedings of the institute's international workshops are also intended mainly for a scientific readership. In 1991, ILRAD published summaries of papers presented at its May workshop on *Bovine Genome Mapping and Trypanotolerance.* This volume, edited by Alan Teale, who heads the ILRAD Trypanosomiasis Research Program, comprises reports from bovine gene mapping laboratories around the world and groups concerned directly with trypanotolerance research. Included are a summary of a final roundtable discussion and recommendations for the development of bovine genome mapping and the production and use of trypanotolerance resource material.

A new publication series was begun in 1991 to keep ILRAD's donors and stakeholders abreast of progress the Laboratory is making in developing better control of trypanosomiasis and tick-borne diseases. These *ILRAD Research Updates* are brief and nontechnical. Three issues were produced in 1991: 'Promising Results in Research on a

Research topics covered in the four 1991 issues of *ILRAD Reports:*

- ILRAD's use of recombinant DNA technology to improve identification of tick-borne and trypanosomal parasites.
- The role cytotoxic T cells play in protecting livestock against East Coast fever.
- The mechanisms by which cattle acquire immunity to trypanosomiasis.
- Current findings in research on *T. annulata*.
- Dysfunction of the pituitary gland in animals infected with trypanosomes.
- Current research and control of the tick-borne diseases anaplasmosis, babesiosis and cowdriosis.
- ILRAD's estimations of the losses due to theileriosis and the benefits of immunizing livestock against it.
- Recent progress in research on a vaccine against East Coast fever.
- The carrier state in cattle infected with *T. parva*.
- ILRAD's use of the polymerase chain reaction to synthesize large amounts of DNA for use in superior diagnostic tests and in experiments designed to gain a better understanding of the molecular mechanisms of trypanosomiasis and tick-borne diseases
- Identification of a trypanosome enzyme that prove a useful antigen in development of novel control methods for trypanosomiasis.

Vaccine against East Coast Fever' (August), 'Integration of Global Research on Tick-Borne Diseases' (October) and 'New Tests to Diagnose Trypanosomiasis' (December).

Highlighting other public awareness activities of the unit this year was participation in an 'International Centres Day' held in November at the International Centre for Research in Agroforestry (ICRAF). This all-day event was organized to celebrate ICRAF's recent entry into the Consultative Group on International Agricultural Research (CGIAR, Washington, D.C.) and to promote the work of international and national agricultural research programs in eastern Africa. Seven CGIAR institutes, the United Nations Environment Program (Nairobi) and two Kenyan agricultural research institutes took part. Over 500 invited guests from Kenyan government ministries, universities and research institutes, as well as donor representatives and media personnel, attended the celebrations, which included colourful exhibits in marquees, scientific demonstrations, short talks by the Directors General of ILRAD and ICRAF and scientists, and a catered lunch consisting of dishes made with the mandate agricultural products of the participating CGIAR centres.

LIBRARY

THE ILRAD LIBRARY acquires and disseminates scientific literature, most of it in the form of journals and books, to meet the information needs of staff members, visiting scientists and participants in the institute's training program. The emphasis of the collection is on the livestock diseases ILRAD focuses on, African animal trypanosomiasis and tick-borne diseases, as well as veterinary medicine and the biochemistry, chemotherapy, cytology, epidemiology, genetics, immunology, molecular and cell biology and pathology of these diseases and their causative parasites.

Journals form the main part of the Library's collection. In 1991, the Library subscribed to 250 specialized scientific journals, 25 monographic serials and 12 indexing or abstracting journals. The weekly *Current Contents* database is received on diskette as well as in printed form. A total of 267 books were purchased this year, bringing the total book stock to about 3,700 volumes. A *Weekly Alert* newsletter is produced and distributed to staff and other local libraries to highlight important new accessions in the Library.

The Library also introduced access to several international agricultural and biomedical databases stored electronically on CD-ROMs (compact disks-read only memory). These include Biological Abstracts, CAB International Abstracts, MEDLINE, Science Citation Index and SESAME. A local area computer network launched in the institute towards the end of the year made it possible for

UNIVERSAL BIBLIOGRAPHIC CONTROL

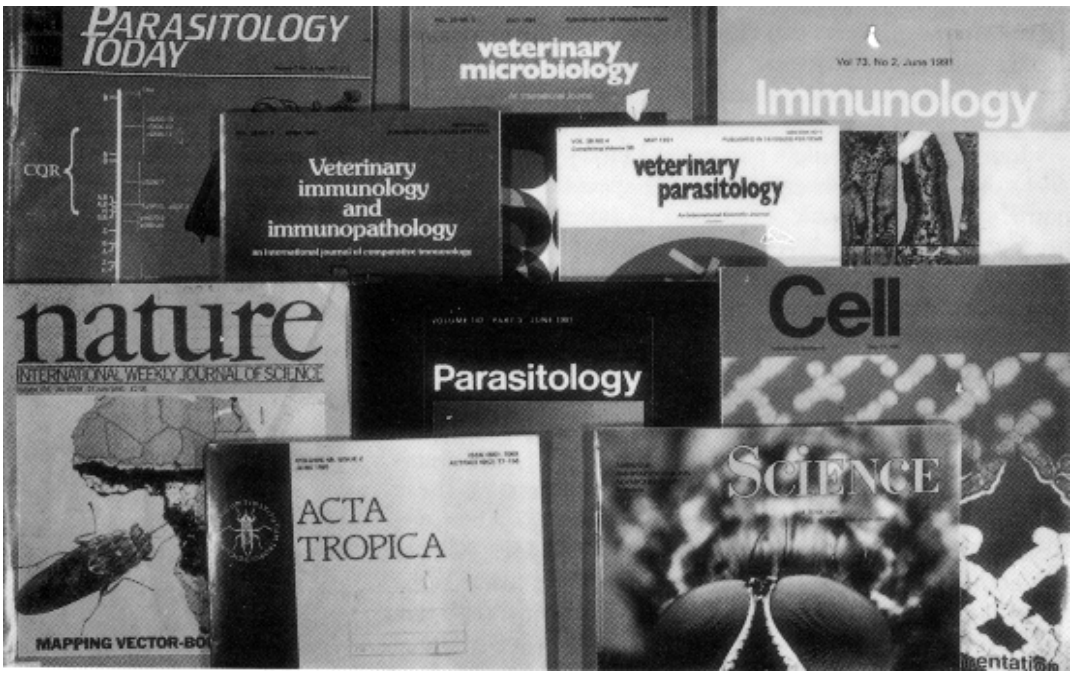
'With recent advances in technology, electronic databases come close to realizing the age-old ideal of a Universal Catalogue of Scientific Knowledge. Floppy diskettes, compact disks, and other storage and retrieval media yet to be developed are revolutionizing information discovery. These technologies are actually democratizing information—they bring to the individual's desktop computer the vast archive of published knowledge, and the ability to navigate effectively through it.'

Eugene Garfield, 'Systematic Serendipity and Universal Information Access', *CBE Views*, Vol. 15, No. 4, 1992, p. 81.

ILRAD scientists to begin conducting their own literature searches using office personal computers. Library staff gave a seminar this year to demonstrate how to use newly installed information technology, especially CD-ROMS, and how to access telecommunications services.

Because the Library's extensive, up-to-date and highly specialized journal collection is unique in Kenya, the Library extends its services to staff from a variety of other local research institutes and from government departments and universities. In 1991, the Library acquired over 700 reprints requested by researchers, made over 17,000 photocopies of journal articles for ILRAD staff and undertook over 160 literature searches for users. Staff helped strengthen the information services of national agricultural research systems through exchange of publications and interlibrary loans and by providing short-term practical training to ten library students from Kenyan universities and research institutes.

This year the Library borrowed 502 publications from libraries belonging to a cooperative research library network in Nairobi, loaned out 603 publications and donated over 800 copies of journals and over 1,000 books to the network. More than half the 160 literature searches undertaken during the year were conducted for external users from Kenya, Nigeria, Tanzania and Zambia. ILRAD also supplied over 1,000 reprints or photocopies of scientific papers to scientists from all over the world, notably from India, the USA and countries of sub-Saharan Africa.



TSETSE UNIT

ILRAD'S TSETSE UNIT develops and maintains colonies of tsetse flies for research on trypanosome parasites. Trypanosomes normally pass through several stages of their life cycle in the tsetse fly. Although it is possible to maintain trypanosomes *in vitro* and to infect laboratory animals and livestock by injecting parasites into the animals, trypanosomes maintained and transmitted artificially differ from those transmitted naturally through the bite of an infected tsetse fly. Thus, a great deal of the trypanosomiasis research conducted at ILRAD requires trypanosomes that have developed in tsetse flies.

Five tsetse breeding colonies were maintained at ILRAD in 1991. These were *Glossina morsitans centralis,* which originated from mainland Tanzania; *G. palpalis gambiensis* from Burkina Faso; and *G. pallidipes, G. brevipalpis* and *G. longipennis,* all from Kenya. These species represent the three taxonomic groups of tsetse—morsitans, palpalis and fusca.

All the colonies were maintained at 25 °C and fed five days a week on the ears of rabbits. *G. m. centralis* and *G. longipennis* were kept at 70% relative humidity, the other species at 80%. These colonies provided all the tsetse required for trypanosomiasis research at ILRAD in the year. The unit in addition supplied tsetse puparia to several research groups in Kenya and abroad.

As well as maintaining tsetse colonies, staff members of the unit conduct research on tsetse flies as vectors of African trypanosomiasis. Studies were undertaken in 1991 to compare the susceptibility of different tsetse populations to infection with trypanosomes. Two colonies of *G. pallidipes* were established using pupae deposited as larvae by wild female flies caught in two areas of Kenya: Shimba Hills, in Coast Province, and Nguruman, in Rift Valley Province. Both colonies were sufficiently productive for comparisons to be made of their vector competence for *T. congolense* stocks. Tsetse flies from Nguruman were

Mr. Jack Kabata, a technologist in ILRAD's Tsetse Unit, with tsetse flies in wire cages.

found to be significantly more susceptible than those from Shimba Hills to *T. congolense* stocks isolated from *G. pallidipes* from the two areas of Kenya. This result has important implications for those conducting research on the epidemiology trypanosomiasis.

TICK UNIT

THE TICK UNIT supplies scientists with ticks and tick salivary glands infected with the sporozoite form of *Theileria parva. Theileria* sporozoites can be obtained only by passage of the parasites between tick vectors and mammalian hosts. Nymphal ticks pick up the parasites from feeding on cattle infected with *Theileria* parva (Muguga) stabilate. After the adult ticks moult, they are fed for four days on rabbits and then dissected to harvest the infected salivary glands. Infection rates are determined for each batch of ticks.

Rhipicephalus appendiculatus is the most important tick species in East Africa, where it is the principal vector of *T. parva.* Stocks of this species maintained at ILRAD include those from Muguga, Kenya; O1 Pejeta, Kenya; McIwaine, Zimbabwe; Chipata, Zambia; and Entebbe, Uganda. Colonies of other tick species maintained at ILRAD include other *Rhipicephalus* species—*R. evertsi, R. pulchellus and R. zambeziensis*—as well as *Amblyomma variegatum* and *Amblyomma gemma,* vectors of *Cowdria ruminantium,* which causes heartwater; *Boophilus decoloratus,* vector of *Babesia* and *Anaplasma,* which cause redwater and gall sickness, respectively; and two relatively benign *Theileria* species, *T. mutans* and *T. velifera.*

A major research effort of the Tick Unit is to determine the factors that influence infection rates in ticks so as to maximize infection levels. A large database on tick infection rates has been assembled at ILRAD over the last four years. The data concern stabilate dose (*T. parva* Muguga 3087), disease response in cattle, infection rates in tick stocks, dexamethasone treatment of cattle, manipulation of the tick feeding site and modification of the temperature of the room where the ticks feed on cattle. ILRAD staff are collaborating with scientists from the University of Strathclyde on developing methods for interrogating the database.

Other studies were started in 1991 to examine the dynamics of cellular immune responses in the area of attachment of *T. parva*-infected and -uninfected *R. appendiculatus* in the lymph draining site. Histological studies show the layering of cement secreted by the tick to secure itself for feeding and the cellular infiltration of the area below the tick proboscis. Electromicrographic studies show the gradual release of single sporozoites from infected salivary gland cells. Immunological studies indicate that dermal dendritic cells are prominent in the infiltration during the first four days of feeding. Results of these studies will

'Proof that ticks were vectors of piroplasms was forthcoming almost one hundred years ago from studies of T. Smith and F.L. Kilborne in 1893 on Texas cattle fever.

'Smith identified *Piroplasma (Babesia) bigemina* as the causative organism of this disease while Kilborne, in elegant studies using experimental paddocks, demonstrated that *Boophilus annulatus* was the tick vector. This was the first time that an arthropod was shown to be a vector of disease. . . .

'C.P. Lounsbury (1904) was the first person to identify the vector of *Theileria parva* as the brown ear tick, *Rhipicephalus appendiculatus*.'

— B.D. Perry, A.S. Young (Head of ILRAD's Tick Unit) and R.A.I. Norval, *The Epidemiology of* Theileriosis in Africa, London: Academic Press, 1991, p. 131.

provide information on the nature of immune cell infiltration that may influence the success of infection.

LARGE ANIMAL PRODUCTION

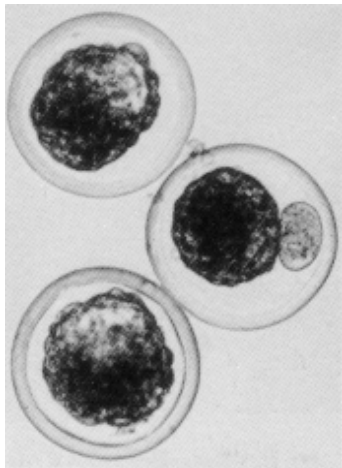
THE LARGE animal facilities on the ILRAD Farm, located next to ILRAD's laboratories, at Kabete, held about 500 head of cattle, 400 goats and 20 sheep during 1991 that were allocated for research projects at ILRAD. In addition to the experimental cattle, 361 calves were brought to the farm when a few days old to be raised under tick- and tsetse-free conditions.

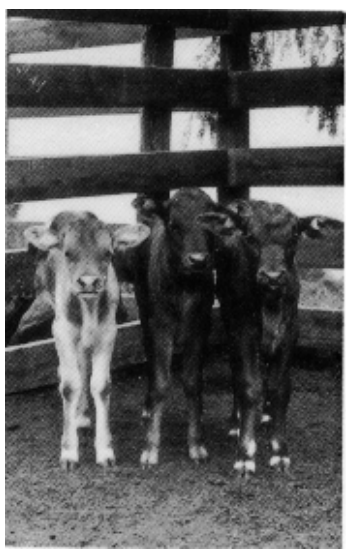
ILRAD's ranch, Kapiti Plains Estate, located about 70 km from Nairobi, carried an average of 2,800 head of cattle in 1991 and supplied 469 cattle to the ILRAD Farm and sold 607 cattle to local farmers and butchers. Animal health on both the ILRAD Farm and Kapiti Ranch was generally good. Altogether during the year, 1,061 calves were born on the ranch from a breeding herd of 1,151 cows, making a calving rate of 92.1%.

ILRAD's Large Animal Unit has continued to refine the use of embryo transfer techniques. Staff members have tested and standardized methods for synchronizing the ovulation of Boran donor and recipient cows, for stimulating superovulation in Boran donors, for assessing the suitability of recipients and for handling and splitting embryos before implanting them in recipient cows. The program continued to do well throughout 1991, with 60 calves produced by embryo transfer, which included 21 pure Boran and six sets of identical Boran twins.

Embryo transfer is also being performed in N'Dama cattle, which come from West Africa, to produce N'Dama animals for experimental use in ILRAD's trypanosomiasis research program. Seven pure N'Dama calves were born in 1991, bringing ILRAD's total N'Dama production to 50. N'Dama and Boran haemopoetic chimeras can be produced by implanting one N'Dama embryo and one Boran embryo into the same recipient mother and allowing them to develop as twins. One set of chimaeric twins was born this year. The chimeras will be challenged with trypanosome parasites to investigate the transfer of trypanotolerance traits from the N'Dama to the Boran and the transfer of trypanosensitivity from the Boran to the N'Dama.

In 1989, ILRAD began a project to map the bovine genome. Large families of N'Dama/Boran crossbred cattle are needed for this project. Each family is produced at the Kapiti Ranch by superovulating a particular Boran cow several times a year and inseminating the cow with semen from the same N'Dama bull until 30 crossbred Boran/N'Dama calves are produced. Two families were completed in 1991; these consisted of 36 and 29 calves. Production of a further two families is in progress; by the end of 1991, these numbered 12 and 9 calves or confirmed pregnancies.





(Above) Seven-day old bovine embryos. *(Below)* Calves produced at ILRAD using embryo transfer techniques.

LABORATORY ANIMAL PRODUCTION

Mr. Bob King, Head of ILRAD's Laboratory Animal Production.

THE LABORATORY Animal Unit provides ILRAD's research and training programs with a regular supply of mice, rats and rabbits. In 1991, ILRAD was self-sufficient in the production of all of these laboratory animals. The Unit also maintains a small colony of guinea pigs, which are occasionally required for experimental work. All laboratory animals are housed and maintained according to international animal welfare conventions.

Colonies of two inbred mouse strain—BALB/c and C3H/He—were maintained. The C3H/He mice are used primarily for studies on host resistance to trypanosomiasis. The BALB/c mice and a colony of BALB/c × Swiss F_1 mice are maintained for the production of monoclonal antibodies. Random-bred Swiss mice are increasingly used for all other research work because they are more productive and grow faster than the inbred strains. The Beige × SCID strain is maintained for use in transfection experiments. The unit also maintains a colony of random-bred rats originating from the Sprague Dawley strain primarily for the production of trypanosomes. Rabbits are used mainly to produce antisera and to support the tsetse and tick colonies.

The total numbers of mice, rats and rabbits used at ILRAD in 1991 were 37,015, 11,818 and 1,253, respectively. In addition to supplying all the laboratory animals required by ILRAD scientists, the breeding unit provided a total of 1,265 mice, 280 rats and 4 rabbits to six other research organizations in Kenya.

BIOSTATISTICS AND COMPUTING SERVICES

ILRAD's BIOSTATISTICS and Computing Services Unit provides support and services to ILRAD's scientific and administrative staff. Scientists from Strathclyde University (UK) continued to provide support and expertise in statistics and mathematical modelling.

Several additional personal computers and peripherals were purchased in 1991, bringing the total number of microcomputers distributed throughout the institute to over 90. A local area network was installed during the year, which links most of the institute's computer users through an internal electronic mail service and enables them to access various commercial scientific databases.

ILRAD subscribes to CGNET, an international electronic mail service linking the CGIAR institutes. This service also gives ILRAD access to many other international electronic networks, such as BITNET (which comprises research institutes and universities around the world) and British Telecom Gold (British business). Electronic mail service was improved this year with the introduction in Kenya of KENPAC, a Kenyan public packet data network.

PUBLICATIONS

All the publications listed below were published in 1991. The numbers in parentheses are ILRAD Library accession numbers.

PUBLICATIONS PRODUCED BY THE INSTITUTE

*ILRAD 1990 Annual Report. Nai*robi, Kenya: International Laboratory for Research on Animal Diseases, 119 pp.

ILRAD 1990 Annual Scientific Report. Nairobi, Kenya: International Laboratory for Research on Animal Diseases, 118 pp.

ILRAD Highlights 1991. Nairobi, Kenya: International Laboratory for Research on Animal Diseases, 4 pp.

ILRAD Reports. Nairobi, Kenya: International Laboratory for Research on Animal Diseases.

JANUARY ISSUE: *Recombinant DNA Technology in the Diagnosis of African Livestock Disease*, 8 pp.

APRIL ISSUE: *The Mechanisms by which Cattle Acquire Immunity to* Trypanosomiasis, 6 pp.

JULY ISSUE: *Tick-Borne Diseases of Livestock*, 8 pp.

OCTOBER ISSUE: *Making Genes Visible*, 8 pp.

ILRAD Publications 1990. Nairobi, Kenya: International Laboratory for Research on Animal Diseases. 4 pp.

*ILRAD Research Updates.*I Nairobi, Kenya: International Laboratory for Research on Animal Diseases, 1 p. each.

NO. 1, AUGUST: *Promising Results in Research on a Vaccine against East Coast Fever.*

NO. 2, OCTOBER: *Integration of Global Research on Tick-Borne Diseases.*

NO. 3, DECEMBER: *New Tests to Diagnose Trypanosomiasis.*

ILRAD Program Plans and Funding Requirements 1988–1992: Funding Requirements for 1992. Nairobi, Kenya: International Laboratory for Research on Animal Diseases, 18 pp.

Teale, A.J. ed. *Bovine Genome Mapping and Trypanotolerance: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 9–11 April 1991.* Nairobi: International Laboratory for Research on Animal Diseases, 72 pp. (1045).

JOURNAL ARTICLES, PAPERS IN PROCEEDINGS AND BOOK CHAPTERS WRITTEN BY ILRAD STAFF

Aboagye-Kwarteng, T., Ole-MoiYoi, O.K. and Lonsdale-Eccles, J.D. Phosphorylation differences among proteins of bloodstream developmental stages of Trypanosoma brucei brucei. *The Biochemical Journal* 275: 7–14 (908).

Agyemang, K., Dwinger, R.H., Grieve, A.S. and Bah, M.L. Milk production characteristics of N'Dama cattle kept under village management in The Gambia. *Journal of Dairy Science* 74: 1599–1608 (1078).

Agyemang, K., Dwinger, R.H., Grieve, A.S. and Little, D.A. Studies on the effects of milking on calf growth and viability and on cow reproductive performance in traditionally managed N'Dama herds. *Animal Production* 53: 11–18 (1076).

Bajyana Songa, E., Hamers, R., Rickman, R., Nantulya, V.M., Mulla, A.F. and Magnus, E. 1991. Evidence for widespread asymptomatic *Trypanosoma rhodesiense* human infection in the Luangwa Valley (Zambia). *Tropical Medicine and Parasitology* 42: 389–393 (1081).

Baylis, H.A., Sohal, S.K., Carrington, M., Bishop, R.P. and Allsopp, B.A. An unusual repetitive gene family in *Theileria parva* which is stage specifically transcribed. *Molecular and Biochemical Parasitology* 49: 133–142 (1007).

Bensaid, A. and Hadam, M. Bovine CD4 (BoCD4). In: *Proceedings of the First International Workshop on Leukocyte Antigens in Cattle, Sheep and Goats, Hanover, Germany, 25 July 1989. Veterinary Immunology and Immunopathology*, Special Issue, Vol. 27, Nos. 1–3, pp. 51–54 (958).

Bensaid, A., Kaushal, A., Baldwin, C.L., Clevers, H., Young, J.R., Kemp, S.J., MacHugh, N.D., Toye, P.G. and Teale, A.J. Identification of expressed bovine class 1 MHC genes at two loci and demonstration of physical linkage. *Immunogenetics* 33: 247–254 (981).

Bensaid, A., Young, J.R., Kaushal, A. and Teale, A.J. Pulsed-field gel electrophoresis and its application in the physical analysis of the bovine major histocompatibility complex. In: Schook, L.B., Lewin, H.A. and McLaren, D.G. eds. *Gene-Mapping Techniques and Applications.* New York: Marcel Dekker, pp. 127–158 (835).

Bernoco, D., Lewin, H.A., Andersson, L., Arriens, M.A., Byrns, G., Cwik, S., Dayies, C.J., Hines, H.C., Leibold, W., Lie, O., Meggiolaro, D., Oliver, R.A., Ostergård, H., Spooner, R.L., Stewart-Haynes, J.A., Teale, A.J., Templeton, J.W. and Zanotti, M. Joint report of the Fourth International Bovine Lymphocyte Antigen (BOLA) Workshop, East Lansing, Michigan, USA, 25 August 1990. *Animal Genetics* 22: 477–496 (1051).

Bienen, E.J. and Shaw, M.K. Differential expression of the oligomycin-sensitive ATPase in bloodstream forms of *Trypanosoma brucei brucei*. *Molecular and Biochemical Parasitology* 48: 59–66 (932).

Bienen, E.J., Webster, P. and Fish, W.R. *Trypanosoma (Nannomonas) congolense:* changes in respiratory metabolism during the life cycle. *Experimental Parasitology* 73: 403–412 (910).

Chen, P.P., Conrad, P.A., ole-MoiYoi, O.K., Brown, W.C. and Dolan, T.T. DNA probes detect *Theileria parva* in the salivary glands of *Rhipicephalus appendiculatus* ticks. *Parasitology Research* 77: 590–594 (1006).

Coulibaly, L., Diarrasouba, I., d'Ieteren, G.D.M., Gnihan, N., Hecker, P., Itty, P., Kupper, W., Leak, S.G.A., Nagda, S.M., Rarieya, J.M., Schuetterle, A., Thorpe, W., Trail, J.C.M. and Traub, D. Effect of tsetse control by means of insecticide impregnated biconical traps on health and productivity of livestock in northern Côte d'Ivoire: preliminary results. In: *Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo, 1987.* Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, pp. 445–447 (1082).

DeMartini, J.C. and Baldwin, C.L. Effects of gamma interferon, tumor necrosis factor alpha and interleukin-2 on infection and proliferation of *Theileria parva*infected bovine lymphoblasts and production of interferon by parasitized cells. *Infection and Immunity 59: 4540–4546 (987).*

Dolan, T.T., Morzaria, S.P. and Katende, J. Theileriosis. In: Ozawa, Y., ed. *Manual of Recommended Diagnostic Techniques and Requirements for Biological Products for Lists A & B Diseases*, Vol. III, Part II. Paris: Office International des Epizooties, pp. 1–16 (935).

Doyle, J.J. Potential role of CGIAR centres in animal biotechnology transfer. In: Mailu, A.M., Mugah, J.O. and Fungoh, P.O., eds. *Biotechnology in Kenya: Proceedings of the National Conference on Plant and Animal Biotechnology, 25 February–3 March 1990, Nairobi, Kenya.* Nairobi: Initiatives Publishers for the Kenya Agricultural Research Institute, pp. 267–270 (1095).

Dwinger, R.H., Clifford, D.J., Agyemang, K., Grieve, A.S., Kora, S. and Jabang, B. Comparative studies on N'Dama and Zebu cattle following experimental infection with *Trypanosoma congolense. In: Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo, 1987.* Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, pp.394–401(1087).

Eirayah, I.E. and Kaminsky, R. The effect of diminazene aceturate and isometamidium chloride on cultured procyclic forms of susceptible and drug-resistant *Trypanosoma congolense.* Acta Tropica 49: 201–213 (923).

Flynn, J.N. and Sileghem, M. The role of the macrophage in induction of immunosuppression in *Trypanosoma congolense*-infected cattle. *Immunology* 74: 310–316 (950).

Fritsch, G., Nelson, R.T., Muiya, P., Naessens, J. and Black, S.J. Characterization of bovine haemopoietic progenitor cells using monoclonal antibodies and fluorocytometry. *Veterinary Immunology and Immunopathology* 27: 277–292 (796).

Goddeeris, B.M., Dunlap, S., Innes, E.A. and McKeever, D.J. A simple and efficient method for purifying and quantifying schizonts from *Theileria parva*-infected cells. *Parasitology Research* 77: 482–484 (982).

Goddeeris, B.M., Dunlap, S., Bensaid, A., MacHugh, N.D. and Morrison, W.I. Cell surface phenotype of two cloned populations of bovine lymphocytes displaying non-specific cytotoxic activity. In: *Proceedings of the First International Workshop on Leukocyte Antigens in Cattle, Sheep and Goats, Hanover, Germany, 25 July 1989. Veterinary Immunology and Immunopathology,* Special Issue, Vol. 27, Nos. 1–3, pp. 195–199 (963).

Gooding, R.H., Moloo, S.K. and Roiseth, B.M. Genetic variation in *Glossina brevipalpis, G. longipennis and G. pallidipes* and the phenetic relationships of *Glossina* species. Medical and Veterinary Entomology 5: 165–173 (994).

Gray, A.R. International Laboratory for Research on Animal Diseases: research achievements, problems and future planning. *Kenya Veterinarian* 11: 16–18 (1026).

Gumm, I.D. The axenic cultivation of insect forms of *Trypanosoma (Duttonelia) vivax* and development to the infective metacyclic stage. *Journal of Protozoology* 38: 163–171 (909).

Hirumi, H. and Hirumi, K. In vitro cultivation of T*rypanosoma congolense* bloodstream forms in the absence of feeder cell layers. *Parasitology* 102: 225–236 (907).

Hirumi, H., Hirumi, K., Moloo, S.K. and Shaw, M. In vitro cultivation of bloodstream trypomastigotes of *Trypanosoma vivax* without feeder cell layers. *Journal of Protozoology Research 1:1–12 (990).*

Howard, C.J., Sopp, P., Parsons, K.R., McKeever, D.J., Taracha, E.L.N., Jones, B.V., MacHugh, N.D. and Morrison, Wl. Distinction of naive and memory BoCD4 lymphocytes in calves with a monoclonal antibody, CC76, to a restricted determinant of the bovine leukocyte-common antigen, CD45. *European Journal of Immunology* 21: 2219–2226 (1032).

Howard, C.J., Morrison, W.I., Bensaid, A., Davis, W., Eskra, L., Gerdes, J., Hadam, M., Hurley, D., Leibold, W., Letesson, J.J., MacHugh, N.D., Naessens, J., O'Reilly, K.O., Parsons, K.R., Schlote, P., Sopp, P., Splitter, G. and Wilson, R. Summary of workshop findings for leukocyte antigens of cattle. In: *Proceedings of the First International Workshop on Leukocyte Antigens in Cattle, Sheep and Goats, Hanover, Germany, 25 July 1989. Veterinary Immunology and Immunopathology,* Special Issue, Vol. 27, Nos. 1–3, pp. 21–27 (956).

Kageruka, P., Mangus, E., Bajyana Songa, E., Nantulya, V.M., Jocherns, M., Hamers, R. and Mortelmans, J. Infectivity of *Trypanosoma (Trypanozoon) brucei gambiense for baboons (Papio hamadryas, Papio papio). Annales de la Société belge de Médicine tropicale* 71: 39–46 (978).

Kamanga-Sollo, E.I.P., Musoke, A.J., Nantulya, V.M., Rurangirwa, F.R. and Masake, R.A. Differences between N'Dama and Boran cattle in the ability of their peripheral blood leucocytes to bind antibody-coated trypanosomes. *Acta Tropica* 49: 109–117 (890).

Kaminsky, R. and Zweygarth. E. The effect of verapamil alone and in combination with trypanocides on multidrug-resistant *Trypanosoma brucei brucei. Acta Tropica* 49: 215–225 (895).

Kaminsky, R., Chuma, F., Gumm, I.D. and Zweygarth, E. *In vitro* assessing of isometamidium resistance in *Trypanosoma vivax*. In: *Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo, 1987.* Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, pp. 483–484 (1090).

Kaminsky, R., Chuma, F., Zweygarth, E., Kitosi, D.S. and Moloo, S.K. The effect of isometamidium chloride on insect forms of drug-sensitive and drug-resistant stocks of *Trypanosoma vivax:* studies in vitro and in tsetse flies. Parasitology Research 77: 13–17 (896).

Kemp, S.J. and Teale, A.J. Dinucleotide repeat polymorphism at the bovine locus *FSHB. Animal Genetics* 22: 435 (926).

Kemp, S.J. and Teale, A.J. International Laboratory for Research on Animal Diseases. In: Teale, A.J., ed. *Bovine Genome Mapping and Trypanotolerance: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 9–11 April 1991.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 29–30 (1047).

Kitungu, W.A.M. and Fish, W.R. The vacuolar ATPase of *Trypanosoma brucei brucei. FASEB Journal* 5: A750 (1104).

Kruska, R.L. and Perry, B.D. Evaluation of grazing lands in Zimbabwe using AVHRR normalised difference vegetation index. *Preventive Veterinary Medicine, Special Issue: Applications of Remote Sensing to Epidemiology and Parasitology,* Vol. 11, Nos. 3–4, pp. 361-363 (1018).

Leak, S.G.A., Colardelle, C., Coulibaly, L., d'leteren, G., Dumont, P., Feron, A., Hecker, P., Jeannin, P., Maloo, S., Minengu, M., Minja, S., Mulatu, W., Mulungu, M., Nankodaba, G., Ngamuna, S., Ordner, G., Sauveroche, B., Schuetterle, A., Tikubet, G., Touré, M., Trail, J.C.M., Tsotsi, E. and Yangari, G. Tsetse feeding habits and tsetse challenge at sites of the African Trypanotolerant Livestock Network. In: *Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo, 1987.* Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, pp. 523–528 (1083).

Leak, S.G.A., Colardelle, C., d'leteren, G., Dumont, P., Feron, A., Jeannin, P., Minengu, M., Mlungu, M., Ngamuna, S., Ordner, G., Sauveroche, B., Trail, J.C.M. and Yangari, G. *Glossina fusca* group tsetse as vectors of cattle trypanosomiasis in Gabon and Zaire. *Medical and Veterinary Entomology* 5: 111–120 (744).

Leegwater, P.A.J., Strating, M., Murphy, N.B., Kooy, R.F., van der Vliet, P.C. and Overdulve, J.P. The *Trypanosoma brucei* DNA polymerase alpha core subunit gene is developmentally regulated and linked to a constitutively expressed open reading frame. *Nucleic Acids Research* 19: 6441–6447 (983).

Letesson, J.J. and Bensaid, A. Bovine CD6 (BoCD6). In: *Proceedings of the First International Workshop on Leukocyte Antigens in Cattle, Sheep and Goats, Hanover, Germany, 25 July 1989. Veterinary Immunology and lmmunopathology,* Special Issue, Vol. 27, Nos. 1–3, pp. 61–64 (959),

Limo, M.K., Voigt, W.P., Tumbo-Oeri, A.G., Njogu, R.M. and ole-MoiYoi, O.K. Purification and characterization of an anticoagulant from the salivary glands of the ixodid tick *Rhipicephalus appendiculatus. Experimental Parasitology* 72: 418–429 (487).

Little, D.A., Dwinger, R.H., Agyemang, K., Kora, S. and Van der Grinten, P. Comparison of sesame cake and cottonseed as supplementary sources of protein to weaned N'Dama bull calves in the Gambia. *Tropical Animal Health and Production* 23: 126–132 (1077).

Logan, L.L., Anosa, V.O. and Shaw, M.K. Haemopoiesis in Ayrshire-Guernsey calves infected with the Galana stock of *Trypanosoma vivax.* In: *Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo, 1987.* Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, pp. 317–322 (785).

Lonsdale-Eccles, J.D. Proteinases of African trypanosomes. In: Coombs, G.H. and North, M.J. eds. *Biochemical Protozoology.* London: Taylor Francis, pp. 200–207 (922).

Lonsdale-Eccles, J.D., Mpimbaza, G.W.N., Mbawa, Z.M. and Kornblatt, J. Characterisation of the proteases of *Trypanosoma congolense and T. brucei. Journal of Cellular Biochemistry,* Supplement 15G: 149 (1092).

Lutje, V. and Black, S.J. Cellular interactions regulating the in vitro response of bovine lymphocytes to ovalbumin. *Veterinary Immunology and Immunopathology* 28: 275–288 (821).

MacHugh, N.D. and Sopp, P. 4.6 bovine CD8 (BoCD8). In: *Proceedings of the First International Workshop on Leukocyte Antigens in Cattle, Sheep and Goats, Hanover, Germany, 25 July 1989. Veterinary Immunology and Immunopathology,* Special Issue, Vol. 27, Nos. 1–3, pp. 65–69 (960).

MacHugh, N.D., Bensaid, A., Howard, C.J., Davis, W.C. and Morrison, W.I. Analysis of the reactivity of anti-bovine CD8 monoclonal antibodies with cloned T cell lines and mouse L-cells transfected with bovine CD8. In: *Proceedings of the First International Workshop on Leukocyte Antigens in Cattle, Sheep and Goats, Hanover, Germany, 25 July 1989. Veterinary Immunology and Immunopathology,* Special Issue, Vol. 27, Nos. 1–3, pp. 169–172 (962).

Maille, J.C., Authié, E., d'Ieteren, G.D.M., Leak, S.G.A., Maehl, J.H.H., Nagda, S.M., Rarieya, J.M., Rowlands, G.J., Sauveroche, B., Thorpe, W., Trail, J.C.M. and Yangari, G. Aspects of trypanotolerance and associations with growth rate in post-weaner N'Dama cattle under high trypanosomiasis risk. In: *Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo, 1987.* Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, pp. 391–393 (1089).

Makumi, J.N. and Moloo, S.K. *Trypanosoma vivax in Glossina palpalis gambiensis* do not appear to affect feeding behaviour, longevity or reproductive performance of the vector. Medical and Veterinary Entomology 5: 35–42 (779).

Maloo, S., Mining, S., Perry, B.D., Curry, J.J., Delehanty, J.M., Kruska, R.L., Mukhebi, A.W., Thorpe, W., Williamson, S.M. and Young, A.S. Determination of target populations of immunisation against East Coast fever in two contrasting areas using integrated epidemiological and socioeconomic variables. In: *Proceedings of the 6th International Symposium on Veterinary Epidemiology and Economics, Ottawa, Canada, 12–16 August 1991.* Guelph, Canada: Ontario Veterinary College, University of Guelph, pp. 217–220 (1107).

Masake, R.A. and Nantulya, V.M. The sensitivity of an antigen detection enzyme immunoassay for the diagnosis of *Trypanosoma congolense* infections in goats and cattle. *Journal of Parasitology* 77: 231–236 (911).

Mbawa, Z.R., Webster, P. and Lonsdale-Eccles, J.D. Immunolocalization of a cysteine protease within the lysosomal system of *Trypanosoma congolense. European Journal of Cell Biology* 56: 243–250 (984).

Mbawa, Z.R., Gumm, I.D., Fish, W.R. and Lonsdale-Eccles, J.D. Endopeptidase variations among different life cycle stages of African trypanosornes. *European Journal of Biochemistry* 195: 183–190 (906).

Mbawa, Z.R., Gumm, I.D., Fish, W.R. and Lonsdale-Eccles, J.D. Stage-specific expression of endopeptidolytic activity in African trypanosomes. In: *Keystone Symposium on Molecular and Cellular Biology: Abstracts of the 20th Annual Meeting, 6–25 April 1991, Colorado. Journal of Cellular Biochemistry,* Supplement 15G, p. 150 (1115).

McElwain, T.F., Perryman, L.E., Musoke, A.J. and McGuire, T.C. Molecular characterization and immunogenicity of neutralization-sensitive *Babesia bigemina* merozoite surface proteins. *Molecular and Biochemical Parasitology* 47: 213–222 (999).

McKeever, D.J., MacHugh, N.D., Goddeeris, B.M., Awino, E. and Morrison, W.I. Bovine afferent lymph veiled cells differ from blood monocytes in phenotype and accessory function. *Journal of Immunology* 147: 3703–3709 (995).

Moloo, S.K. Large-scale rearing of *Glossina longipennis* in the laboratory. *Acta Tropica* 48: 159–160 (815).

Morrison, W.I., Howard, C.J. and Hinson, C.A. Polymorphism of the CD4 and CD5 differentiation antigens in cattle. In: *Proceedings of the First International Workshop on Leukocyte Antigens in Cattle, Sheep and Goats, Hanover, Germany, 25 July 1989. Veterinary Immunology and Immunopathology,* Special Issue, Vol. 27, Nos. 1-3, pp. 236–238 (964).

Mulatu, W., d'Ieteren, G.D.M., Duffera, W., Ginna, T., Leak, S.G.A., Maehl, J.H.H., Nagda, S.M., Rarieya, J.M., Rowlands, G.J., Thorpe, W., Tikubet, G. and Trail, J.C.M. Factors affecting trypanosome prevalence in Zebu cattle exposed to high trypanosomiasis risk in south west Ethiopia. In: *Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo, 1987.* Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, pp. 507–510 (1085).

Musoke, A.J., Nene, V. and Iams, K. Progress towards a recombinant vaccine against East Coast fever (*Theileria parva* infection in cattle). In: Mailu, A.M., Mugah, J.O. and Fungoh, P.O., eds. *Biotechnology in Kenya: Proceedings of the National Conference on Plant and Animal Biotechnology, 25 February–3 March 1990, Nairobi, Kenya.* Nairobi: Initiatives Publishers for the Kenya Agricultural Research Institute, pp. 67–75 (891).

Mutugi, J.J., Young, A.S., Kariuki, D.P., ole Tameno, J.M. and Morzaria, S.P. Epidemiological observations on theileriosis following field immunisation using infection and treatment. *Tropical Animal Health and Production* 23: 75–82 (979).

Mwamachi, D.M., Rurangirwa, F.R., Musoke, A.J., and McGuire, T.C. Clone-specific immune colostrum induces increased resistance in goat kids challenged with *Trypanosoma congolense. Acta Tropica* 4: 27–36 (977).

Naessens, J. and Howard, C.J. 4.8 monoclonal antibodies reacting with bovine B cells (BoWC3, BoWC4 and BoWC5). In: *Proceedings of the First International Workshop on Leukocyte Antigens in Cattle, Sheep and Goats, Hanover, Germany, 25 July 1989. Veterinary Immunology and Immunopathology,* Special Issue, Vol. 27, Nos. 1–3, pp. 77–85 (927).

Nantulya, V.M. Molecular diagnosis of parasites. *Experientia* 47: 142–145 (985).

Nene, V., Iams, K.P. and Musoke, A.J. Molecular characterization of a *Theileria parva* surface sporozoite antigen. In: Vaccines 91. Cold Spring Harbor Laboratory, pp. 369–373 (918).

Norval, R.A.I., Perry. B.D., Gebreab, F. and Lessard, P. East Coast fever: a problem of the future for the horn of Africa? *Preventive Veterinary Medicine* 10: 163–172 (887).

Norval, R.A.I., Perry, B.D., Kruska, R. and Kundert, K. The use of climate data interpolation in estimating the distribution of *Amblyomma variegatum in Africa. Preventive Veterinary Medicine, Special Issue: Applications of Remote Sensing to Epidemiology and Parasitology,* Vol. 11, Nos. 34, pp. 365–366 (1029).

Norval, R.A.I., Lawrence, J.A., Young, A.S., Perry, B.D., Dolan, T.T. and Scott, J.R. *Theileria parva:* influence of vector, parasite and host relationships on the epidemiology of theileriosis in southern Africa. Parasitology 102: 347–356 (928).

Paling, R.W., Moloo, S.K., Scott, J.R., Gettinby, G., McOdimba, F.A. and Murray, M. Susceptibility of N'Dama and Boran cattle to sequential challenges with tsetse-transmitted clones of *Trypanosoma congolense. Parasite Immunology* 13: 427–445 (931).

Paling, R.W., Moloo, S.K., Scott, J.R., McOdimba, F.A., Logan-Henfrey, L.L., Murray, M. and Williams, D.J.L. Susceptibility of N'Dama and Boran cattle to tsetse-transmitted primary and rechallenge infections with a homologous serodeme of *Trypanosoma congolense. Parasite Immunology* 13: 413–425 (912).

Parsons, K.R. and MacHugh, N.D. Bovine CD1 (BoCD1). In: *Proceedings of the First International Workshop on Leukocyte Antigens in Cattle, Sheep and Goats, Hanover, Germany, 25 July 1989. Veterinary Immunology and Immunopathology,* Special Issue, Vol. 27, Nos. 1–3, pp. 37–41 (957).

Peregrine, A.S., Fasogbon, A.I., Scott, J.R., Knowles, G. and Moloo, S.K. *Trypanosoma congolense:* clonal variation in expression of resistance to isometamidium chloride and diminazene aceturate. In: *Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo,* 1987. Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, pp. 371–372 (782).

Peregrine, A.S., Knowles, G., Ibitayo, A.I., Scott, J.R., Moloo, S.K. and Murphy, N.B. Variation in resistance to isometamidium chloride and diminazene aceturate by clones derived from a stock of *Trypanosoma congolense. Parasitology* 102: 93–100 (905).

Peregrine, A.S., Moloo, S.K. and Whitelaw, D.D. Differences in sensitivity of Kenyan *Trypanosoma vivax* populations to the prophylactic and therapeutic actions of isometamidium chloride in Boran cattle. *Tropical Animal Health and Production* 23: 29–38 (784).

Perry, B.D., Kruska, R., Lessard, P., Norval, R.A.I. and Kundert, K. Estimating the distribution and abundance of *Rhipicephalus appendiculatus in Africa. Preventive Veterinary Medicine, Special Issue: Applications of Remote Sensing to Epidemiology and Parasitology,* Vol. 11, Nos. 3–4, pp. 261–268 (1019).

Perry, B.D., Norval, R.A.I., Kruska, R.L., Ushewookunze-Obatolu, U. and Booth, T. Predicting the epiderniology of tick-borne diseases of cattle in Zimbabwe using geographic information systems. In: *Proceedings of the 6th International Symposium on Veterinary Epidemiology and Economics, Ottawa, Canada, 12–16 August 1991.* Guelph, Canada: Ontario Veterinary College, University of Guelph, pp. 214–216 (1106).

Rawlings, P., Dwinger, R.H. and Snow, W.F. An analysis of survey measurements of tsetse challenge to trypanotolerant cattle in relation to aspects of analytical models of trypanosomiasis. *Parasitology* 102: 371–377 (1052).

Shaw, M.K. and Moloo, S.K. Comparative study on Rickettsia-like organisms in the midgut epithelial cells of different *Glossina* species. *Parasitology* 102: 193–199 (920).

Shaw, M.K., Tilney, L.G. and Musoke, A.J. The entry of *Theileria parva* sporozoites into bovine lymphocytes: evidence for MHC class 1 involvement. *Journal of Cell Biology* 113: 87–101 (921).

Splitter, G. and Morrison, W.I. 4.9 antigens expressed predominantly on monocytes and granulocytes: identification of bovine CD11b and CD11c. In: Howard, C.J. and Morrison, W.I., eds. *Proceedings of the First International Workshop on Leukocyte Antigens in Cattle, Sheep and Goats, Hanover, Germany, 25 July 1989. Veterinary Immunology and Immunopathology,* Special Issue, Vol. 27, Nos. 1–3, pp. 87–90 (961).

Sutherland, I.A., Moloo, S.K., Holmes, P.H. and Peregrine, A.S. Therapeutic and prophylactic activity of isometamidium chloride against a tsetse-transmitted drug-resistant clone of *Trypanosoma congolense* in Boran cattle. *Acta Tropica* 49: 57–64 (913).

Sutherland, I.A., Peregrine, A.S., Lonsdale-Eccles, J.D. and Holnies, P.H. Reduced accumulation of isometamidium by drug-resistant T*rypanosoma congolense. Parasitology* 103: 245–251 (925).

Teale, A.J. Biotechnologies applied to the diagnosis of disease, with special reference to trypanosomiasis and theileriosis of livestock. In: Hunter, A.G., ed. *Biotechnology in Livestock in Developing Countries: Proceedings of Biotechnology 1989, An International Conference on the Application of Biotechnology to Livestock in Developing Countries Held in the University of Edinburgh, 4–8 September 1989.* Edinburgh: Centre for Tropical Veterinary Medicine, pp. 272–285 (832).

Teale, A.J. ed. *Bovine Genome Mapping and Trypanotolerance: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 9–11 April 1991.* Nairobi: International Laboratory for Research on Animal Diseases, 72 pp. (1045).

Teale, A.J. Mapping and trypanotolerance. In: Teale, A.J., ed. *Bovine Genome Mapping and Trypanotolerance: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 9–11 April 1991.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 5–7 (1046).

Teale, A.J., Kemp, S.J. and Morrison, W.I. The major histocompatibility complex and disease resistance in cattle. In: Owen, J.B. and Axford, R.F.E., eds. *Breeding for Disease Resistance in Farm Animals: Proceedings of a Symposium Held at Bangor, United Kingdom, 13–14 September 1990.* Oxford: C.A.B. International, pp. 86–99 (997).

Toye, P.G., Goddeeris, B.M., Iams, K., Musoke, A.J. and Morrison, W.I. Characterization of a polymorphic immunodominant molecule in sporozoites and schizonts of *Theileria parva. Parasite Immunology* 13: 49–62 (840).

Trail, J.C.M., d'Ieteren, G.D.M., Maille, J.C., Yangari, G. and Nantulya, Y.M. Use of antigen-detection enzyme immunoassays in assessment of trypanotolerance in N'Dama cattle. *Acta Tropica* 50: 11–18 (1015).

van Winckel, F., d'Ieteren, G.D.M., Leak, S.G.A., Maehl, J.H.H., Minengu, M., Nagda, S.M., Ngamuna, S., Rarieya, J.M., Rowlands, G.J., Thorpe, W. and Trail, J.C.M. Preliminary results of a study of N'Dama cattle introduced in a metayage system in Idiofa District, Zaire. In: *Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo, 1987.* Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, pp. 520–522 (1086).

Waitumbi, J.N., Young, J.R. and Opiyo, E.A. Characterisation of brucei-type trypanosome isolates from camels in Marsabit, northern Kenya. In: *Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo, 1987.* Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, p. 127 (1091).

Williams, D.J.L., Naessens, J., Moloo, S.K. and Scott, J.R. Analysis of peripheral blood leukocyte populations in N'Dama and Boran cattle following *Glossina morsitans centralis*-transmitted *Trypanosoma congolense infection. In: Proceedings of the International Scientific Council for Trypanosomiasis Research and Control: 19th Meeting, Lomé, Togo, 1987.* Nairobi: Organization of African Unity/Scientific, Technical and Research Commission, pp. 152–161 (1088).

Williams, D.J.L., Naessens, J., Scott, J.R. and McOdimba, F.A. Analysis of peripheral leucocyte populations in N'Dama and Boran cattle following a rechallenge infection with *Trypanosoma congolense. Parasite Immunology* 13: 171–185 (870).

Young, J.R. and Morzaria, S.P. Agarose entrapment method for the production of S*fi* I linking library for *Theileria parva*. *Genetic Analysis Techniques and Applications* 8: 148–150 (848).

Zweygarth, E. and Karninsky, R. Evaluation of DL-α difluoromethylornithine against susceptible and drug-resistant *Trypanosoma brucei brucei*. *Acta Tropica* 48: 223–232 (850).

Zweygarth, E., Gray, M.A. and Kaminsky, R. Axenic *in vitro* cultivation of *Trypanosoma vivax* trypomastigote forms. *Tropical Medicine and Parasitology* 42: 45–48 (951).

Zweygarth, E., Kaminsky, R. and Gray, M.A. *In vitro* assessment of isometamidium chloride susceptibility of *Trypanosoma vivax* bloodstream forms. Parasitology Research 77: 714–716 (1013).

BOARD OF DIRECTORS

April 1991–March 1992

Dr. F.A. Chenoweth
(Liberia)
Agricultural Economist
United States Agency
for International Development
Lusaka, Zambia

Dr. P. Doherty
(Australia)
Immunologist
Department of Immunology
St. Jude Children's Research
Hospital
Memphis, Tennessee, USA

Dr. P.T. Englund
(USA)
Molecular Biologist
Department of Biological
Chemistry
Johns Hopkins University
School of Medicine
Baltimore, Maryland, USA

Dr. A.R. Gray
(UK)
Protozoologist
Director General
The International Laboratory
for Research on Animal
Diseases
Nairobi, Kenya

Professor I. Månsson
(Sweden)
Microbiologist
Department of Veterinary
Microbiology
College of Veterinary Medicine
Swedish University of
Agricultural Sciences
Uppsala, Sweden

Dr. I.S. Mpelumbe
(Tanzania)
Director of Veterinarian
Services (retired)
Director of the Veterinary
Consultancy Clinic
Dar es Salaam, Tanzania

Dr. W.K. Ngulo
(Kenya)
Director of Research/
Veterinarian
Ministry of Research, Science
and Technology
Nairobi, Kenya

Professor N.O. Nielsen
(Board Chairman)
(Canada)
Pathologist
Dean of Ontario Veterinary
College
University of Guelph
Ontario, Canada

Dr. A.S. Sidibé
(Mali)
Coordinator of the Pan African
Rinderpest Campaign/
Veterinarian
Organization of African Unity/
Inter-African Bureau of
Animal Resources
Bamako, Mali

Dr. G. Tacher
(France)
Epidemiologist/Veterinarian
Director of Institut d'Elevage et
de Médecine Vétérinaire des
Pays Tropicaux
Maisons-Alfort, France

Dr. J.P.O. Wamukoya
(Kenya)
Veterinarian/Epidemiologist
Director of Veterinary Services
Ministry of Livestock
Development
Nairobi, Kenya

Professor C.C. Wang
(USA)
Biochemist
Department of Pharmaceutical
Chemistry
University of California at
San Francisco
San Francisco, California, USA

SENIOR STAFF

ADMINISTRATION

A.R. Gray
Director General
J.J. Doyle
Deputy Director General
K.F. Geerts
Director of Administration
P.R. Gardiner
Information and Planning Officer
B.C. Lloyd
Financial Controller
G. Migwi
Chief Personnel Officer
M.A. Craig
Business Manager
M.W. Holt
Chief Engineer
O.M. Nthiwa
Chief Accountant
M.A. Lobo
Electronics Engineer
F.N. Matee
Purchasing Officer
G. Mzera
Stores Superintendent
M. Onyango
Security Officer
C. Ndungi
Transport Officer
F. Gicheru
Catering Officer
J. Owino
Housing Officer

LAB 1 BIOCHEMISTRY AND PARASITOLOGY OF TRYPANOSOMIASIS

D.J. Grab
Scientist
J.D. Lonsdale-Eccles
Scientist
A.S. Peregrine
Scientist
J. Wilkes
Scientist
T.W. Pearson
Visiting Scientist
D. Nandan
Post-Doctoral Fellow
G.W.N. Mpimbaza
Laboratory Superintendent
M. Dirie
Senior Research Fellow
J. Waitumbi
Senior Research Fellow
B. Burleigh
Research Fellow
V. Codjia
Research Fellow
A. Ibitayo
Research Fellow
M. Mamman
Research Fellow
B. Mboya
Research Fellow
Z. Nkhungulu
Research Fellow
O. Tosomba
Research Fellow
A. Adema
Laboratory Technician
S. arap Kemei
Laboratory Technician
R. Thatthi
Laboratory Technician
Y. Verjee
Laboratory Technician

LAB 2 BIOCHEMISTRY AND MOLECULAR BIOLOGY

O.K. ole-MoiYoi
Senior Scientist
K. Iams
Scientist
P.A.O. Majiwa
Scientist
N.B. Murphy
Scientist
V. Nene
Scientist
P. Leegwater
Visiting Scientist
B.C. Ricard
Visiting Scientist
R. Pele
Post-Doctoral Fellow
E. Gobright
Research Associate
M. Macklin
Research Associate
N. Doshi
Research Fellow
W. Endege
Research Fellow
H.R. Jamnadass
Research Fellow
A. Kairo
Research Fellow
M. Kibe
Research Fellow
G. Ndoutamia
Research Fellow
M. Maina
Laboratory Technician
E.O. Omolo
Laboratory Technician
J. Osaso
Laboratory Technician
P. Pandit
Laboratory Technician
S. Wasike
Laboratory Technician

LAB 3 CELL BIOLOGY

H. Hirumi
Senior Scientist
W.R. Fish
Scientist
R. Kaminsky
Scientist
Y. Yabu
Visiting Scientist
I.D. Gumm
Research Associate
K. Hirumi
Research Associate
W. Kitungu
Research Fellow
N. Wasike
Research Fellow
F. Chuma
Laboratory Technician
C. W. Muriuki
Laboratory Technician
A. M. Muthiani
Laboratory Technician
D. Ndegwa
Laboratory Technician
J.R. Wando
Laboratory Technician

LAB 4 IMMUNOBIOLOGY

D.J.L. Williams
Trypanosomiasis Program
Area Leader: Host Resistance
E. Authié
Scientist
L.L. Logan-Henfrey
Scientist
J.N. Flynn
Post-Doctoral Fellow
M. Sileghem
Scientist
B. Mertens
Visiting Scientist
J. Naessens
Visiting Scientist
A. Andrianarivo
Post-Doctoral Fellow
L. Gaidulis
Research Associate
J. Magondu
Research Associate
J. Newson
Research Associate
K.A. Taylor
Research Associate
A. Boulangé
Research Fellow
B. Buza
Research Fellow
D.C.W. Russo
Research Fellow
V. Taiwo
Research Fellow
F.A. McOdimba
Laboratory Technician
F. Mucheru
Laboratory Technician
B.G. Muchiri
Laboratory Technician
P. Muiya
Laboratory Technician
D.K. Muteti
Laboratory Technician
J. Nthale
Laboratory Technician
M.O. Opollo
Laboratory Technician
R. Saya
Laboratory Technician
K. Tikolo
Laboratory Technician

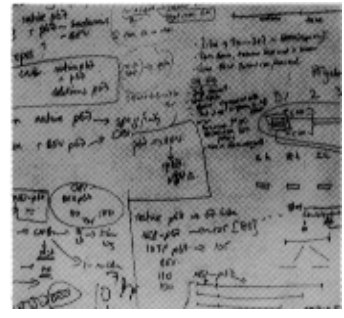
LAB 5 PARASITOLOGY AND EPIDEMIOLOGY OF THEILERIOSIS

T.T. Dolan
Theileriosis Program Head
A.J. Musoke
Theileriosis Program Area Leader: Antigens
S.B. Morzaria
Theileriosis Program Area Leader: Epidemiology
R. Bishop
Scientist .
T. Imada
Visiting Scientist
S. Williamson
Visiting scientist
Y. Yagi
Visiting Scientist
C. Nkonge
Research Associate
R.A. Skilton
Research Associate
P.R. Spooner
Research Associate
E.N. Kimbita
Research Fellow
B. Mushi
Research Fellow
J.N. Gachanja
Laboratory Technician
L. Gichuru
Laboratory Technician
L.K. Juma
Laboratory Technician
J. Kiarie
Laboratory Technician
D.O. Lugo
Laboratory Technician
T. Ndolo
Laboratory Technician
R. Njamunggeh
Laboratory Technician
S. Njuguna
Laboratory Technician
B. Sohanpal
Laboratory Technician
S. Wanyonyi
Laboratory Technician

nature
INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

LAB 6 CELLULAR IMMUNOLOGY AND PATHOLOGY

D.J. McKeever
Theileriosis Program Area Leader: Vaccine Developent
P.G. Toye
Scientist
E.A. Innes
Post-Doctoral Fellow
M.G. Juarrero
Post-Doctoral Fellow
E.L.N. Taracha
Post-Doctoral Fellow
A. Bensaid
Visiting Scientist
R. Kennedy
Research Associate
G. Lamb
Laboratory Superintendent
N.D. MacHugh
Research Associate
A. Diaite
Research Fellow
P. Malu
Research Fellow
H. Pereira
Research Fellow
E.O. Awino
Laboratory Technician
S.A. Kaushal
Laboratory Technician
J.K. Mburu
Laboratory Technician
F. Mbwika
Laboratory Technician
D.N. Ngugi
Laboratory Technician
J.K. Nyanjui
Laboratory Technician
M. Waithaka
Laboratory Technician



LAB 7 PARASITOLOGY AND EPIDEMIOLOGY OF TRYPANOSOMIASIS

A.J. Teale
Trypanosomiasis Program Head
V.M. Nantulya
Senior Scientist
S.J. Kemp
Scientist
R. Masake
Scientist
L. Brezinsky
Post-Doctoral Fellow
M. Eisler
Visiting Scientist
R. Suzuki
Visiting Scientist
J.M. Katende
Research Associate
S.G.A. Leak
Research Associate
K. Bosompen
Research Fellow
A. Jaye
Research Fellow
D. Kihurani
Research Fellow
R. Waihenya
Research Fellow
E. Waithanji
Research Fellow
H. Gathuo
Laboratory Technician
J. Makau
Laboratory Technician
J. Mwakaya
Laboratory Technician
J.E. Ngatti
Laboratory Technician
J.T. Njuguna
Laboratory Technician
M. Omenya
Laboratory Technician
J.A. Sore
Laboratory Technician
J.M. Wambugu
Laboratory Technician

LAB 8 EPIDEMIOLOGY AND SOCIOECONOMICS

B.D. Perry
Epidemiology and Socio-economics Program Head
J.J. Curry
Scientist
A.W. Mukhebi
Scientist
R. Huss-Ashmore
Visiting Scientist
R.L. Kruska
Research Associate
H. Ochanda
Research Fellow

TSETSE LABORATORY

S.K. Moloo
Unit Head
N.N. Gitire
Laboratory Technician
F. Kaaria
Laboratory Technician
J. Kabata
Laboratory Technician
N. Kuria
Laboratory Technician
C. Lugonzo
Laboratory Technician
I. Okumu
Laboratory Technician

ELECTRON MICROSCOPY

M.K. Shaw
Unit Head
C. Wells
Research Associate
L.G. Tilney
Visiting Scientist

TICK LABORATORY

A.S. Young
Unit Head
F.N. Mwakima
Research Associate
M. Baya
Laboratory Technician
S. Mwaura
Laboratory Technician
G. Njihia
Laboratory Technician
P. Theuri
Laboratory Technician
M. Waithaka
Laboratory Technician

CENTRAL CORE UNIT

C. Hinson
Laboratory Manager

EXPERIMENTAL ANIMAL UNITS

R.C. King
Unit Head
J.D. Kennedy
Scientist/Veterinarian
S.J. Kimani
Farm Manager/Kabete
P.K. Ng'eno
Ranch Manager/ Kapiti Plains Estate

BIOSTATISTICS AND COMPUTING SERVICES UNIT

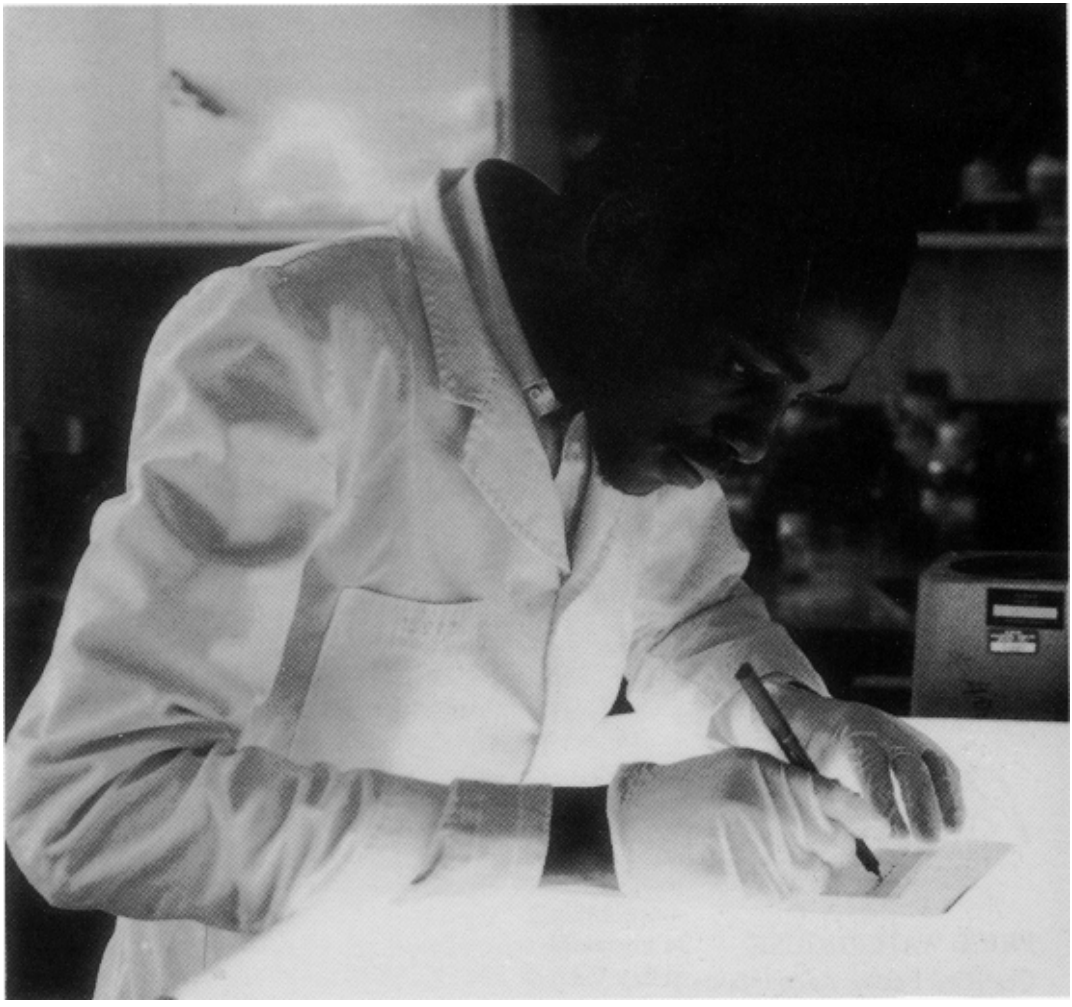
J.R. Scott
Research Associate

COOPERATIVE PROGRAMS, TRAINING AND INFORMATION DEPARTMENT

M. Touré
Department Head
J.K. Lenahan
Training and Outreach Officer
S. MacMillan
Science Writer
W. Umbima
Librarian
D. Elsworth
Scientific Photographer/ Graphic Arts Supervisor
K.N. Nguli
Assistant Training Officer

WILDLIFE DISEASES PROJECT

J.G. Grootenhuis
Senior Scientist



Certified Public Accountants

Rattansi Educational Trust Building
Koinanage Street
PO Box 41968
Nairobi Kenya

Telephone 221244
Telecopier (254-2)335937
Telex 22149 CHUNGA
Cables PRICEWATER

Price Waterhouse

REPORT TO THE DIRECTORS OF THE INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES (ILRAD)

We have reviewed the abridged financial statements set out in Tables A1 to A4, which contain information extracted from the accounting records of ILRAD for the years ended 31 December 1990 and 1991.

We confirm that the information set out in the abridged financial statements is consistent with that contained in the audited financial statements for the years ended 31 December 1990 and 1991, on which we expressed an unqualified opinion.

Price Waterhouse

PRICE WATERHOUSE
Certified Public Accountants

22 July 1992

Table A1. Summary costs by program and activity (US$ thousands)

COSTS OF OPERATIONS	1991	1990
Research		
Parasitology—Trypanosomiasis	734	579
Biochemistry	767	784
Cell Biology	564	562
Immunobiology	638	607
Parasitology—Theileriosis	752	667
Pathology	465	435
Immunoparasitology	854	758
Tsetse Laboratory	235	249
Tick Laboratory	203	115
Electron Microscopy	162	110
Epidemiology and Socioeconomics	439	382
International Trypanotolerance Centre, Gambia	—	146
Research subtotals	5,813	5,394
Research Support		
Office of Director of Research	706	823
Farm Animal Production	509	556
Laboratory Animal Production	156	148
Radioisotope and Central Core Services	433	520
Research Support subtotals	1,804	2,047
Training and Conferences	1,267	1,206
Library and Information Services	642	555
Administration		
Board of Directors	152	150
Office of the Director General	599	488
Finance	379	371
Personnel	120	154
Purchasing	509	532
Administration subtotals	1,759	1,695
General Operations		
Engineering	863	880
Transport	187	176
Services	342	333
Food and Housing	(18)	38
Stores	55	57
General Operations subtotals	1,429	1,484
TOTAL OPERATIONS BEFORE DEPRECIATION	12,714	12,381
DEPRECIATION	1,157	1,169
TOTAL OPERATIONS AFTER DEPRECIATION	**13,871**	**13,550**

Table A2. Summary of core operating funds from donors (US$ thousands)

UNRESTRICTED AND RESTRICTED FUNDS FROM DONORS	1991	1990
Unrestricted Funds from Donors		
World Bank	2,524	2,800
United States Agency for International Development	1,900	1,985
United Kingdom	1,322	1,086
Canadian International Development Agency	964	900
Germany	872	728
Switzerland	792	688
Sweden	536	470
Netherlands	491	410
Japan	473	434
Norway	305	302
Belgium	293	280
Finland	278	248
African Development Bank	250	250
France	197	192
Denmark	174	152
Italy	80	86
India	25	25
Unrestricted Funds from Donors subtotals	11,476	11,036
Restricted Funds from Donors		
United Nations Development Programme	899	797
Italy	350	350
Belgium	293	250
Rockefeller Foundation	175	334
Japan	150	150
European Economic Community for the ITC, Gambia	—	146
Australia	77	117
Restricted Funds from Donors subtotals	1,944	2,144
TOTAL UNRESTRICTED AND RESTRICTED FUNDS FROM DONORS	**13,420**	**13,180**

Table A3. Summary of sources and application of funds (US$ thousands)

	1991	1990
SOURCES OF FUNDS		
Core Operating Funds		
Unrestricted Funds from Donors	11,476	11,036
Restricted Funds from Donors	1,944	2,144
Earned Income Applied in the Year	359	516
Exchange Gain on Donations	100	414
Unexpended Balance from Previous Year	500	398
TOTAL SOURCES	**14,379**	**14,508**
APPLICATIONS OF FUNDS		
Core Operations before Depreciation	12,714	12,381
Capital Expenditure	236	1,257
Transfer to Working Funds	—	270
Transfer to Capital Replacement Fund	976	100
Unexpended Balance Carried Forward	453	500
TOTAL APPLICATIONS	**14,379**	**14,508**

Table A4. Balance sheet as at 31 December 1991 (US$ thousands)

	1991	1990
ASSETS		
Fixed Assets		
Land and Buildings	7,961	8,293
Research Equipment	1,894	2,755
Other Assets	1,228	978
Subsidiary Company		
Investment	1,786	1,786
Long-Term Loan	30	30
Fixed Assets subtotal	12,899	13,842
Revolving Fund Assets	100	100
Net Current Assets	4,534	3,605
TOTAL ASSETS EMPLOYED	**17,533**	**17,547**
FUND BALANCES		
Capital Fund	12,899	13,842
Working Capital	2,813	2,813
Unrestricted Core Surplus	453	500
Revolving Fund	100	100
Capital Replacement Fund	1,268	292
TOTAL FUNDS	**17,533**	**17,547**

Note: 1990 figures have been revised to account for a newly introduced depreciation policy.

CREDITS

Cover painting
Timothy Brooke, Nairobi, Kenya

Photographs
David Elsworth, ILRAD Graphics Unit
Francis Shikhubari, ILRAD Graphics Unit

Electron micrographs
Mike Shaw, ILRAD Electron Microscopy Unit

Maps
Russell Kruska, ILRAD Socioeconomics Unit

Figures
Joel Mwaura, ILRAD Graphics Unit
John Mayienga, University of Nairobi

Text, design and typesetting
Susan MacMillan, ILRAD Science Writer

With end-of-the-year reports and critical readings from ILRAD scientists, particularly: Tom Dolan, Head of the Theileriosis Research Program; Alan Teale, Head of the Trypanosomiasis Research Program, Brian Perry, Head of the Socioeconomics Research Program, and Ross Gray, Director General.

Printing
Majestic Printing Works Limited, Kijabe Street, Post Office Box 42466, Nairobi, Kenya

Produced by the ILRAD Information Unit in September 1992

The International Laboratory for Research on Animal Diseases
Post Office Box 30709, Nairobi, Kenya
Phone: (254-2) 632-311 • Fax: (254-2) 631-499 • Electronic mail: 157:CGI017