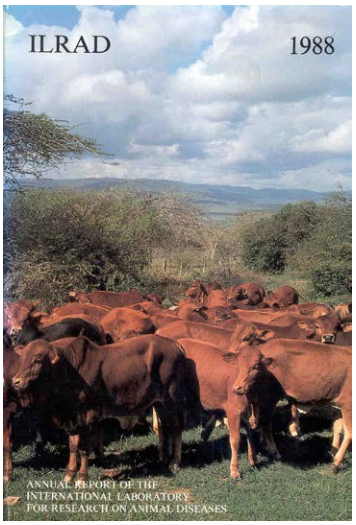
ILRAD

1988

ANNUAL REPORT OF THE
INTERNATIONAL LABORATORY

Annual Report of the International Laboratory for Research on Animal Diseases



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ILRAD 1988

P.O. BOX 30709 Nairobi, Kenya

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

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

The bulk of this year's annual report was compiled and written by Dr. Ivan Morrisson (theilerosis), Dr. Peter Gardiner (trypanosomiasis) and Dr. Brian Perry (epidemiology and socioeconomics).

The cover photograph was taken by Mr. David Elsworth. Photographs accompanying the text were taken by Mr. Elsworth, Mr. Paul Webster, Mr. Ian Gumm and Dr. Mike Shaw. Other graphics were produced by Mr. Joel Mwara and Mr. Francis Shikhubari.

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Foreword

The International Laboratory for Research on Animal Diseases (ILRAD) was established by the Consultative Group on International Agricultural Research (CGIAR) in 1973 with a mandate to conduct intensive research leading to improved control of livestock diseases. The Laboratory's research and training activities concentrate on immunological and related aspects of two diseases, theileriosis and trypanosomiasis, that seriously limit food production and rural development in Africa and other developing regions of the world. ILRAD's research is focused on two virulent forms of these diseases, East Coast fever and tsetse-transmitted trypanosomiasis.

ILRAD occupies a modern complex of research laboratories and supporting units at Kabete, on the outskirts of Nairobi, and has a cattle breeding ranch on the Kapiti Plains, about 50 kilometres from Nairobi. The Laboratory is governed by an International Board of Directors with 12 members. In April 1988 Professor I. Månsson (Sweden) was elected Chairman of the Board in succession to Professor H. Jahnke (West Germany). Dr. D. Chavanduka (Zimbabwe) and Professor E.N.W. Oppong (Ghana) retired by rotation after completing six years of service and were sums by Dr. L Mpelumbe (Tanzania) and Professor I. Abdulkadir (Nigeria).

In 1988 ILRAD was staffed by 59 senior scientific and administrative personnel, 25 specialized technicians, 50 advanced technical support staff and 282 general support staff. The nationalities of the staff comprise 15 countries, including Kenya. During this year ILRAD was particularly sorry to lose the services of Dr. R. Rowe, Director of Administration and Finance, and Ms. S. Westley, Information Services Editor, each of whom made major contributions to the development of the Laboratory over periods of more than seven years. The appointments of their successors, Mr. K. Geerts and Ms. S. MacMillan, respectively, and that of Dr. M. Tour, as Head of the restructured Department of Cooperative Programs, Training and Information, was warmly welcomed.

The Foreword to the *1987 Annual Report* summarized changes in the management of ILRAD's research programs that were introduced in response to recommendations made by the External Program Review and Management Review team, which completed their work in 1986. Program management was further strengthened in 1988 by the work of a Research Review Committee comprising the Director of Research and the Coordinators of the theileriosis and trypanosomiasis research programs.

The Research Review Committee oversees the general needs of ILRAD's two principal research programs, allocates resources, reviews research progress and works closely with the Program Committee of the Board of Directors on general research policy matters and with staff scientists who serve as Project Area Leaders on more applied aspects of the programs. Following approval by the Board of Directors, ILRAD published in the middle of the year an updated version of the Laboratory's long-term plan, entitled, *Meeting the Challenge of Livestock Diseases: ILRAD in the 1990s,* which gives an account of the structure and objectives of ILRAD's research program.

ILRAD instituted in this year a regular review of its research programs by panels of experts drawn from the international scientific community. One of the institute's research areas—trypanosome biology and biochemistry—was reviewed in November; in December the Program Committee of the Board of Directors examined the report and recommendations made by the Review Panel.

At the mid-year a new building donated by the Netherlands Government was completed. The building houses a much-expanded Department of Cooperative Programs, Training and Information in Africa and is already well-used. Towards the end of 1988 ILRAD signed an agreement with Swiss Development Cooperation for the construction of a laboratory in 1989 and 1990 to accommodate staff engaged in epidemiology and socioeconomics research. The completion of this laboratory will fulfil the recommendations of a recent publication of the Technical Advisory Committee of the CGIAR, *Review of Training in the CGIAR Institutes*, as well as recommendations of the External Program Review and Management Review teams, which were to reduce an increasing congestion in the laboratories and to provide better training facilities. We are most grateful to the donor nations which are providing these much-needed and much-welcomed facilities.

A full account of the activities of the research and training programs in 1988 comprises the bulk of this Annual Report. The comments that follow highlight the achievements and other matters regarding the year's work that warrant emphasis.

In the theileriosis research program, ILRAD's knowledge of the epidemiology of East Coast fever and its expertise in immunizing livestock by the infection-and-treatment method continued to be in demand locally and internationally. Good progress was made during the year in cloning *T. parva* stocks. This should greatly facilitate the development of diagnostic DNA probes to differentiate species and strains. The gene that carries a code for the production of a sporozoite antigen that induces immunity to East Coast fever has been sequenced. The gene for another antigen shared by both the sporozoite and the schizont forms of the parasite is being scrutinized. The project has built up an armoury of techniques for examining infection-related antigenic changes in lymphoblastoid cells and for using genes and other materials related to parasite antigens that might induce immunity. The techniques are being used to elucidate features of the cellular immune response to theileriosis.

The trypanosomiasis program is now testing and evaluating antigen-trapping technology using the enzyme-linked immunosorbent assay (ELISA) to diagnose tsetse-transmitted bovine trypanosome infections in the field. There appear to be valuable spin-off benefits of this procedure for improving the diagnosis of infections with human sleeping sickness and non-tsetse transmitted trypanosomiasis in Africa and other regions of the world. Research into treating animals with chemotherapeutic drugs to prevent or cure trypanosomiasis is being conducted in close collaboration with scientists at neighbouring institutes. This program is making use of trypanosome culture systems and advanced chromatographic and fluorometric technology to measure drug levels in bovine body fluids as well as drug-sensitivity in parasites. Research on natural livestock tolerance to trypanosomiasis has moved ahead both in the laboratory and in the field and 24 N'Dama calves have been produced using embryo-transfer techniques for in-depth studies. Results of ILRAD's basic studies on the biology and biochemistry of trypanosomes, including findings on the parasitic cell parts involved in the uptake of nutrients and in molecular sorting, were evaluated by experts in the field at an international workshop on protein traffic in parasites and mammalian cells, which ILRAD conducted at the Laboratory in September with financial support from the United Nations Development Program. A team working on humoral immune responses to trypanosomes was reorganized and expanded during the year and a group working on the severe anaemia produced in livestock suffering from trypanosomiasis produced interesting results in pioneering work that is being conducted on long-term bovine marrow cell culture.

The epidemiological and socioeconomics group has prepared papers on the impact of East Coast fever and trypanosomiass and the implications of their control in Africa, and has begun to monitor and map the distribution of these diseases in Africa and to assemble and evaluate epidemiological data in collaboration with the United Nations Environment Program. The group is also participating in cooperative field research studies with the Kenya Agricultural Research Institute. Part of the objectives of this relatively new research unit is to address aspects of

ILRAD's research programs that bear on environmental protection and sustainable agriculture, areas of special concern to members of the CGIAR.

ILRAD'S seven research support units—electron microscopy, tsetse and tick laboratories, central core, the experimental animal units, biostatistics and computing services, and training and information—continued in the year to provide high-quality materials and services to the research programs.

To enlist support for the research programs from as wide a spectrum of the scientific community as possible, ILRAD cooperates with many organizations. In 1988 ILRAD's research programs received major input from university groups in several countries outside Africa, notably, Berlin, on the chemistry of trypanosome antigens; Karlesruhe, on genomic events in *Theileria*-infected lymphocytes; Brussels and California, on genomic control of antigenic variation in trypanosomes; Antwerp, on diagnostic techniques for trypanosomiasis; Milan, on immunoglobulin types; Strathclyde, on statistic Edinburgh, on the bovine major histocompatability antigen complex; Cambridge, on DNA probes for *Theileria* strain characterization; Melbourne, on viral vectors for vaccine antigens; Canberra, on geographical information systems; and Basel, on trypanosome culture systems.

Within Africa, ILRAD increased its cooperative research with national scientists. It continued to participate in the African Trypanotolerant Livestock Network, along with the International Livestock Centre for Africa and scientists in Côte d'Ivoire, Ethiopia, Gabon, the Gambia, Senegal and Zaire. ILRAD also collaborated in work on the epidemiology and control of East Coast fever by running several joint projects, by conducting, along with the Food and Agriculture Organization of the United Nations (FAO) and the Organization of African Unity, an international workshop on this subject in Malawi, and by running a training course on the subject in Nairobi, with scientists participating from Burundi, Kenya, Malawi, Rwanda, Sudan, Tanzania, Uganda, Zaire, Zambia and Zimbabwe.

Field evaluations of ELISA, using monoclonal antibodies produced by ILRAD to diagnose trypanosome infections in livestock, were begun this year. This work is being carried out in collaboration with FAO, the International Atomic Energy Agency and scientists in the Gambia, Ghana, Kenya, Mali, Sudan, Tanzania, Uganda, Zambia and Zimbabwe. With guidance and cooperation from the World Health Organization, ILRAD has made available for testing in three African countries diagnostic materials with potential value for detecting human trypanosomiasis.

During the year scientists and research fellows from Kenya, Nigeria, Tanzania and Uganda worked directly with ILRAD staff members on projects of common research interests, such as the pathogenesis of anaemia, chemotherapeutic treatment for trypanosomiasis, and characterization of isolates of *Trypanosoma* and *Theileria* parasites. Many more scientists from Africa were able to broaden their professional expertise by attending one of the several continuing training programs run by ILRAD. A record number of 28 research fellows worked at ILRAD full or part time on degree-related training during 1988. A further 29 young scientists and technicians from Burundi, Ethiopia, the Gambia, Ghana, Kenya, Mali, Morocco, Senegal, Sudan, Tanzania, Zaire, Zambia and Zimbabwe participated in courses at the Laboratory conducted on ELISA, on the preparation of diagnostic reagents and on the control of East Coast fever.

The importance of theileriosis and animal trypanosomiasis in constraining rural economic development in Africa raises a high level of interest in the work of the Laboratory, particularly in donor nations, organizations working on related problems and the countries affected by the diseases. Members of the international staff continued to work as consultants, lecturers and advisers in response to calls from governments of African countries and donor agencies. It is a pleasure to note that one of ILRAD's Senior Scientists, Dr. O. ole-MoiYoi, was this year

appointed Vice-Chairman of Kenyatta University Council, in Nairobi.

In 1988 ILRAD received financial support from the African Development Bank, the European Economic Community, the Rockefeller Foundation, the United Nations Development Program, the World Bank, and the governments of Australia, Belgium, Canada, Denmark, France, India, Italy, Japan, the Netherlands, Norway, Sweden, Switzerland, the United Kingdom, the United States of America and West Germany. The Directors and staff of the Laboratory are most grateful for the continuing interest and support of these donor members of the CGIAR.

It is also a pleasure to acknowledge that this year Australia, Germany, Switzerland and the United Kingdom made additional funds available to laboratories in their own countries for work in ILRAD's research areas. In addition, Belgium, France, Italy, Japan, the FAO and the Rockefeller Foundation provided salary support for skilled personnel seconded to the institute. More informally, ILRAD greatly benefited from a visiting scientist program, which enabled scientists from advanced laboratories in donor countries to bring expertise and new technology to the Laboratory.

ILRAD continued to enjoy a productive relationship with the people and government of its host, Kenya, and maintained its important scientific links with the country's national research institutions and universities. To remain in touch with national needs and developments, ILRAD encouraged an increasing number of research fellows and visiting scientists from Africa to work at ILRAD during the year. The Laboratory also continued to sponsor researchers to attend ILRAD seminars and international scientific workshops. The inclusion of five eminent scientists from African countries on the Board of Directors this year ensured strong continental input in the formulation of the Laboratory's research policies. ILRAD participated in several continent-wide research networks and through its quarterly and annual scientific reports published in English and French, as well as annual proceedings of international conferences hosted by ILRAD, the institute ensured wide dissemination of its research findings.

ILRAD was honoured to receive visits from a number of eminent guests in 1988. These included Professor T. Scarascia-Mugnossa, Chancellor of the University of Tuscia (Italy); Professor M.M. Mahmoud, Vice-Chancellor of the University of Juba (Sudan); Dr. Hans Wyss, Director of the Africa Technical Department of the World Bank (Washington); Dr. Fritz Staehelin, Director of Swiss Development Cooperation (Bern); and Mr. Peter Goldmark, President of the Rockefeller Foundation (New York).

Several important visitors from the Kenya Government also visited ILRAD during the year. These included the Honourable Maina Wanjigi, Minister for Livestock Development; the Honourable George M. Ndotto, Minister for Research, Science and Technology; the Honourable Chris Kamunya, Member of Parliament for Dagoretti; Dr. T. Ogada, Director of Medical Services; and Mr. S.N. Arasa, Permanent Secretary in the Ministry of Research, Science and Technology. ILRAD also received visits from the Ambassadors of Belgium, Denmark, Finland, the Netherlands and Switzerland; the High Commissioner of Nigeria; Mr. S. Sinding, Director of the Kenyan Mission of the United States Agency for International Development; and Mr. K. Kumagishi, Resident Representative of the Japan International Cooperation Agency.

The main text of the following report and the long list of publications from the scientific staff that follows the report attest to the productivity of the Laboratory and the progress made towards its goal of improving the control of livestock diseases in Africa.

A.R. Gray
Director General
ILRAD

Abbreviations and Acronyms

AGRICOLA	Agricultural On-Line Access of the National Agriculture Library (Washington, D.C.)
AFRC	Agricultural and Food Research Council (UK)
cDNA	complementary DNA
CGIAR	Consultative Group for International Agricultural Research (Washington, D.C.)
cm	centimetres
CRD	cross-reacting determinant
DNA	deoxyribonucleic acid
ECF	East Coast fever
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations (Rome)
GPI-PLC	glycosyl-phosphatidylinositol phospholipase-C
IBAR	Inter-African Bureau of Animal Resources (Nairobi)
Ig	immunoglobulin
ILCA	International Livestock Centre for Africa (Addis Ababa)
ILRAD	International Laboratory for Research on Animal Diseases (Nairobi)
KARI	Kenya Agricultural Research Institute (Muguga, Kenya)
kDa	kilodalton
m	metre
MAb	monoclonal antibody
MHC	major histocompatibility complex
mfVSG	membrane-form variable surface glycoprotem
mg	milligram (0.001 of a gram)
ml	millilitre
µ	micro ($10^{-6}\times$)
µg	microgram (0.000,001 of a gram)
µM	micromolar
ng	nanogram (0.000,000,001 of a gram)
NDVI	normalized difference vegetation index
ODA	Overseas Development Administration (UK)
PCR	polymerase chain reaction
pg	picogram (0.000,000,000,001 of a gram)
PIM	polymorphic immunodominant molecule
UNEP	United Nations Environment Programme (Nairobi)
VAT	variable antigen type
VSG	variable surface glycoprotein

Theileriosis

Theileria is a genus of protozoan parasites transmitted by ticks predominantly to domestic and wild ruminants. *Theileria annulata* and *Theileria parva* are two species of this parasite that cause debilitating and often fatal diseases in cattle. *Theileria annulata* occurs over a broad area, extending from Asia to India, southern Russia, the Middle East and northern Africa. In eastern, central and southern Africa the most important species is *T*. *parva,* which restricts the distribution of cattle and hinders the development of beef and dairy production on the continent. The disease caused by *T*. *parva,* a virulent form of theileriosis, threatens the lives of about 25 million cattle in Burundi, Kenya, Malawi, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zaire, Zambia and Zimbabwe. The aim of ILRAD's theileriosis research program is to develop improved methods to control *T. parva* infections.

Three subtypes of *T*. *parva,* all of which are transmitted by the brown ear tick, *Rhipicephalus appendiculatus,* have been identified. *Theileria parva parva* and *Theileria parva bovis* are both transmitted between cattle: *T. p. parva* produces an acute, usually fatal disease called East Coast fever (ECF); *T. p*. *bovis* produces a milder form of theileriosis. *Theileria parva lawrencei* also causes an acute disease and is transmitted principally from Cape buffalo (*Syncerus*

caffer) to cattle. The buffalo acts as a carrier of the parasite and does not normally suffer from clinical disease, which is severe and usually fatal in cattle. No clear evidence exists that these three parasites represent true subspecies and *T*. *parva* is used in this report for all three subtypes.

All these parasites have a complex life cycle of development in the arthropod vector and mammalian host. *Rhipicephalus appendiculatus* is a three-host tick, feeding on an animal during each of the three stages of its life cycle—larva, nymph and adult. The larvae and nymphs that ingest parasites on one host are in turn infective as nymphs and adults, respectively, when they feed on another host during the next stage of their development. The parasites are transmitted most commonly when ticks feed on infected animals as nymphs and then on susceptible cattle as adults.

Theileria parva ingested by ticks first develops in the tick gut. The parasite then migrates to the salivary glands, where it develops further, forming infective sporozoites, which are injected into cattle in tick saliva when the tick feeds. Inside the host, the sporozoites attach to and enter lymphocytes, white blood cells of the bovine immune system (Figure 1). Within two to three days of invading the lymphocytes, the sporozoites begin to develop into multinucleate bodies called schizonts. The infected lymphocytes are transformed into enlarged lymphoblasts, which begin to multiply. As each lymphoblast divides, the parasite inside the cell also divides, so that both daughter cells produced by a dividing bovine lymphocyte are infected. This process causes the population of parasitized cells to increase rapidly and the infected cells spread throughout the lymphoid system of the animal. Virulent forms of the parasite eventually cause widespread destruction of the host's cells, which usually kills the animal within three to four weeks of its becoming infected.

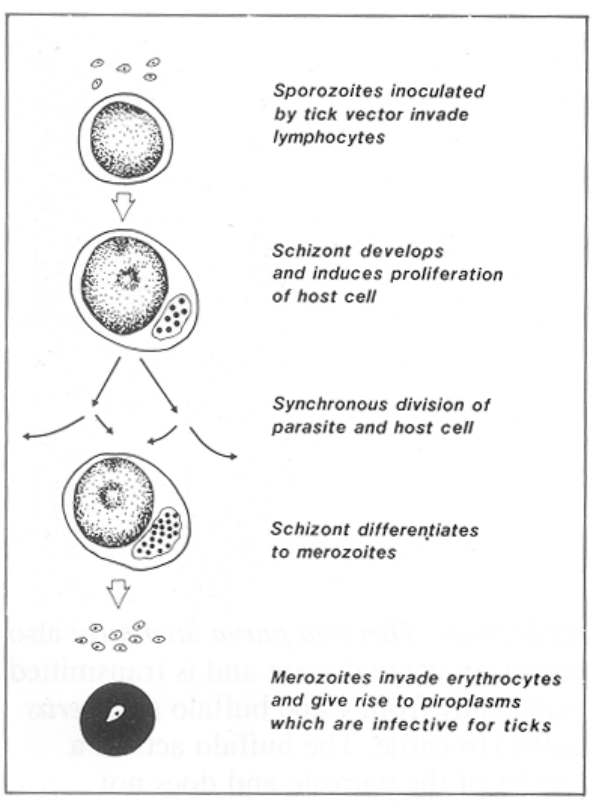


Figure 1. *Life cycle of* Theileria parva *in the bovine host.*

During the later stages of the infection, some of the *Theileria* schizonts differentiate to merozoite forms. The merozoites are released from the lymphocytes into the bloodstream, where they invade red blood cells, in which they develop into piroplasms (Figure 2). Ticks

become infected when they ingest red blood cells containing piroplasms as they feed, and this initiates a new cycle of parasite development.

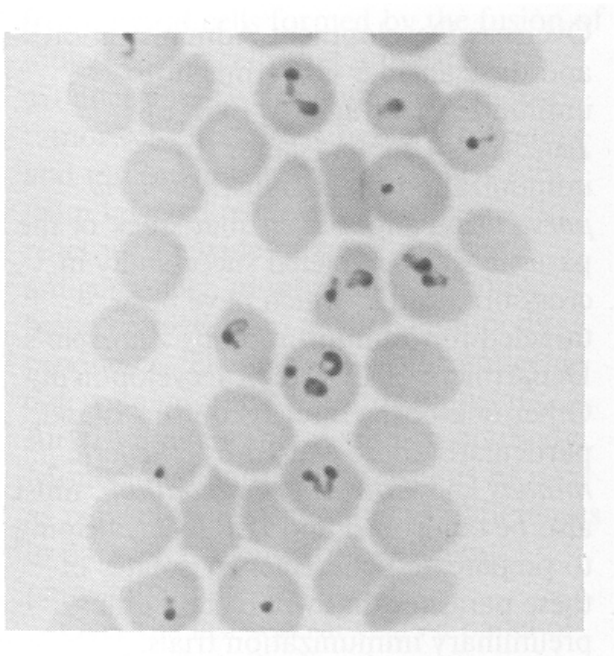


Figure 2. *Electron micrograph of bovine red blood cells parasitized by* Theileria parva.

East Coast fever is controlled principally by dipping or spraying cattle with substances that kill ticks, but this is expensive and the cattle remain fully susceptible to the disease; any interruption of the acaricide regime may be disastrous. Furthermore, ticks are developing resistance to the acaricides in use and most acaricides damage the environment. Two curative drugs have been developed to treat ECF, but they are relatively expensive and the disease must be diagnosed early for the treatment to be most effective. For these reasons alternative methods of ECF control are urgently needed.

Cattle that recover from ECF thereafter show long-lasting immunity to the disease, which suggests that the prospects of controlling ECF by immunization are good. However, antigenic diversity exists among strains of *Theileria* and immunity against one strain does not necessarily protect cattle against another. In view of the shortcomings of present control methods, new approaches must be developed to immunize livestock against ECF.

Epidemiology of East Coast fever

Cattle can be immunized against *T*. *parva* by infecting the animals with the sporozoite form of the parasite while at the same time treating the cattle with a long-acting formulation of the antibiotic drug oxytetracycline. This infection-and-treatment method is the only practical and effective form of immunization against *T. parva* in use today and is being tested in field trials in several countries in Africa. Because immunized cattle, as well as animals that recover naturally from infection or following treatment, are carriers of the infection, most field immunization trials are carried out using parasite populations that have been isolated from the regions in which the immunizations will be given.

ILRAD's epidemiology program helps national governments to evaluate the epidemiology and economic impact of theileriosis and to establish immunization programs based on the infection-and-treatment method. ILRAD advises national governments, trains personnel who will carry out the infection and treatment, provides reagents to national government laboratories for diagnosing the disease and characterizing the parasites and in some instances

helps to characterize in the laboratory the parasites that will be used in the immunization procedure. Immunological characterization of *T. parva* parasites still relies heavily on costly and time-consuming cross-protection experiments in cattle. An important part of ILRAD's epidemiology program, therefore, is research on the development of laboratory methods to characterize *Theileria* parasites.

Collaboration with national ECF projects

In 1988 the theileriosis program increased its technical and advisory help to national governments in the ECF endemic region. ILRAD scientists took part in discussions at a meeting organized by the Food and Agriculture Organization of the United Nations (FAO) that brought together representatives of five organizations that fund national and regional ECF immunization projects in Africa. ILRAD staff also participated in a national theileriosis seminar held in Zambia. ILRAD collaborated with FAO and the Organization of African Unity in organizing the third in a series of workshops on ECF immunization. At this meeting, held in Lilongwe, Malawi, participants from 12 countries discussed the epidemiology of theileriosis, the use of the infection-and-treatment method to control the disease, problems of infecting and treating animals in the field and methods used to assess the costs and benefits of immunizing cattle. ILRAD also conducted a four-week course on the control of tick-borne diseases with emphasis on immunization against theileriosis. The course was attended by veterinarians responsible for tick-borne disease control in six countries..

Staff from the theileriosis program worked directly with national governments during the year on conducting immunization trials in Zanzibar and Zimbabwe. An ILRAD team assisted a project funded by the Overseas Development Administration and the Zanzibar Government to immunize cattle against ECF on Zanzibar's Unguja Island. An immunization trial against *Theileria parva* using a local Zanzibar stock of the parasite was completed successfully in cross-bred cattle, which have been targeted for immunization by Zanzibar's Department of Livestock Development. Other tick-borne diseases on this island, particularly those caused by *Theileria mutans* and *Cowdria ruminantium*, must also be controlled and ILRAD is helping to prepare and characterize isolates of these parasites that will be tested in preliminary immunization trials.

The Zimbabwe Government, FAO and ILRAD have collaborated in conducting immunization trials in Zimbabwe in both the laboratory and the field using two stocks of *T. p. bovis.* Field trials using one of these stocks will be conducted during the next seasonal challenge, when the tick numbers increase after the rains. On a government experimental farm in Zimbabwe, studies are also being carried out on the transmission of *T. p. bovis* parasites from immunized carrier cattle through tick populations to susceptible cattle. ILRAD is studying the benefits and costs of various tick control strategies and the influence of these strategies on the endemic stability of tick-borne diseases in a variety of ecological zones to help policymakers in Zimbabwe choose the most appropriate tick control strategies to put into practice in given areas.

Characterizing parasite species and strains

Cattle that are immune to one population of *Theileria* may not resist infection with another, indicating that different, immunologically distinct, groups of parasites exist in the field. If a vaccine against ECF is to protect cattle against infection, it must stimulate immunity against the different populations of *T. parva* parasites the animals are likely to encounter. Practicable and reliable laboratory methods need to be developed with which to differentiate the antigenic groups of *Theileria* parasites. Regional and national veterinary and research institutes in Africa also need improved methods of characterization to define the *Theileria* parasites in their countries and to assess the results of their disease control programs. The epidemiology of

theileriosis is complicated by the presence of two other *Theileria* species, *T. mutans* and *T. taurotragi,* which infect cattle but are much less pathogenic than *T. parva*. Rapid and reliable methods to distinguish these *Theileria* species are also needed.

Two highly sensitive tools are being used at ILRAD to distinguish species and strains: parasite-specific monoclonal antibodies and DNA (deoxyribonucleic acid) probes. The DNA probes are sequences of DNA that can be used in hybridization procedures to detect the parasite-specific DNA sequences in DNA prepared from parasitized cells in the blood of infected cattle. Six *T. parva* stocks that have been well-characterized with respect to virulence, cross-protection and reactivity with existing monoclonal antibodies and DNA probes are being used to develop and refine new reagents for detecting antigenic differences that might characterize *Theileria* strains.

Cloning *T. parva* parasite populations

The occurrence of mixed parasite populations within the stocks of *T. parva* greatly complicates the interpretation of results of cross-protection experiments. To define immunological differences among parasite populations, it is essential to obtain parasite clones, which are cell populations each derived from a single ancestor parasite. Parasite clones that stimulate protection would provide more standardized material for cross-immunity trials. Such clones are also required to explore the possible role of genetic recombination in the antigenic diversity that occurs in *T. parva* parasites.

Cloned populations of the sporozoite form of the parasite are obtained by infecting bovine lymphocytes in the laboratory with *T. parva* sporozoites at ratios that ensure that the infection of each lymphocyte is initiated by a single sporozoite. Infected lymphocytes are then cloned from the resultant cultures and a cloned population of parasitized cells is inoculated into the animal from which the lymphocytes were taken. The infection established within the animal is then transmitted to ticks from which stabilates of sporozoites are prepared. Using this approach, cloned parasite populations have been derived from two Kenyan *T. parva* stocks (Mariakani and Boleni). Results of experiments with the two cloned populations indicate that the monoclonal antibody profiles of the clones resemble those of the parent stocks. Experiments are being carried out to determine whether these populations remain genetically stable after further passage through ticks; comparisons of the cross-protective properties of these populations with those of the parent stocks will also be made.

Detecting differences in parasite proteins

A great deal of work on the characterization of *Theileria* stocks *in vitro* has been based on the recognition of parasite antigens by monoclonal antibodies, detected by the indirect immunofluorescence antibody test. Monoclonal antibodies are produced from hybrid cells formed by the fusion of mouse spleen cells that are primed to produce specific antibodies and mouse tumor cells that are capable of growing and multiplying *in vitro*. Whereas sera from infected cattle can be expected to react with a wide range of parasite antigens, each monoclonal antibody, being derived from a cloned antibody-producing cell, detects a single antigenic determinant, the part of the antigen's surface that combines with an antibody. Monoclonal antibodies can thus be used as reagents to detect antigenic differences in parasite proteins from different parasite populations.

Over the last several years a panel of monoclonal antibodies that reacts with *T. parva* schizonts has been developed. When used in an indirect immunofluorescence antibody test, these antibodies detect antigenic differences among parasite stocks. Although no obvious correlation is observed between the antigenic differences and the crossprotective properties of the parasites, the monoclonal antibodies have nevertheless proved to be useful reagents with

which to characterize parasite populations. Both the reagents and the technology used at ILRAD in monoclonal antibody characterization were provided during the year to tick-borne disease control projects in Zimbabwe and Kenya.

Experiments to identify the parasite antigens recognized by the monoclonal antibodies show that most of the antibodies recognize different determinants on the same antigen. Moreover, when the antibodies were tested on lysates of parasitized cells, differences in the molecular mass of the antigen that these antibodies recognize were detected in different parasite populations. Much of the antibody response in mice and cattle to parasite schizonts is directed against the antigen that these monoclonal antibodies recognize. Efforts are being made to devise immunization procedures that will make it possible to produce monoclonal antibodies to other schizont proteins.

Parasite proteins have also been analysed using two-dimensional gel electrophoresis. This technique involves purifying schizonts that have been labelled with radioactive isotopes and separating solubilized schizont proteins into groups according to their molecular weight and electrical charge. Five protein spots observed in gels prepared from a *T. parva lawrencei* parasite were absent in gels prepared from several *T*. *parva parva* stocks. Attempts will be made to isolate these proteins with the aim of producing antibodies that can be used as reagents to type parasite strains in the field.

Detecting differences in parasite DNA

Studies were begun in 1985 to identify DNA sequences that could be used as cloned probes to identify *T*. *parva* subspecies and strains. Specific fragments of DNA from any source can be amplified by cloning. In 1986 and 1987 DNA sequences present as multiple copies in the *Theileria* genome were cloned from *Theileria parva* DNA. Used as radioactive probes, these repetitive sequences hybridized with DNA from *T*. *parva* but not with DNA from *T. mutans* or *T. tauratragi*. These sequences can thus be used as species-specific probes. In 1988 a repetitive DNA sequence specific for *T. mutans* was cloned from a *T. mutans* genomic library and the nucleotide sequence is being determined and tested against DNA from other *T*. *mutans* isolates. A genomic library has also been constructed from *T. taurotragi* and is being screened for species-specific DNA sequences.

Differences in DNA sequences that might be used to identify *Theileria* strains may also be detected by digesting the DNA with restriction enzymes and resolving the fragments by gel electrophoresis. Differences in the sizes of DNA fragments are known as restriction fragment length polymorphisms. When the *T. parva* repetitive DNA probes are hybridized with DNA from different stocks of *T. parva* and digested with various restriction enzymes, they detect restriction fragment length polymorphism. Similar differences in DNA have been detected in cloned parasitized cell lines derived from the same parasite stock, indicating that such stocks contain mixtures of genetically distinct parasites (Figure 3).

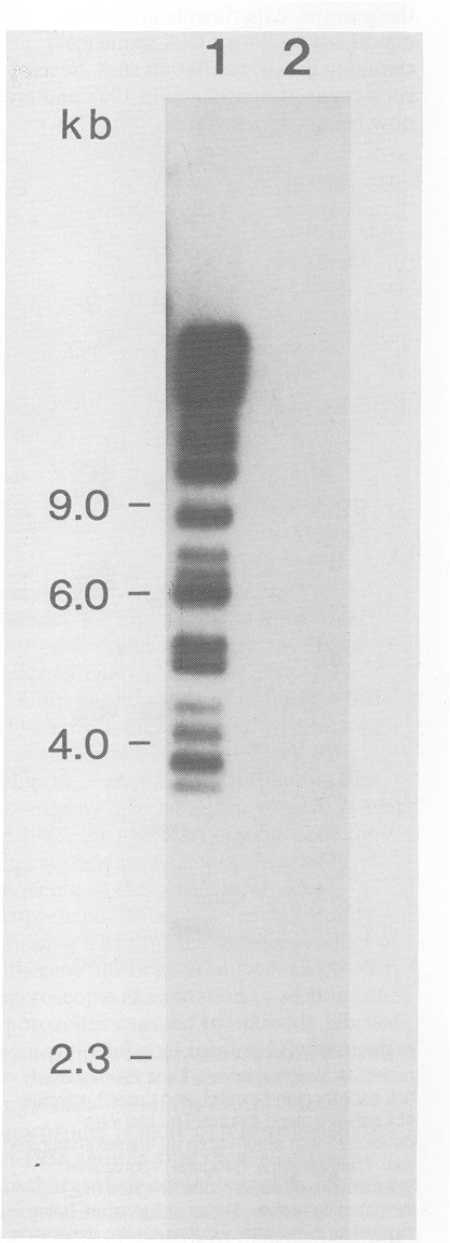


Figure 3. *Autoradiograph of a Southern blot of EcoRI digested* Theileria *DNAs probed with a repetitive DNA sequence specific for* T. mutans*: Track 1,* T. mutans *(Intona); Track 2,* T. parva *(Muguga). The probe shows no hybridization to* T. parva *DNA and recognizes at least 15 bands in* T. mutans *DNA.*

These DNA probes are proving to be valuable reagents for defining parasite stocks and characterizing cloned populations of *T. parva*. But because the probes are difficult to use in the field, there remains a need to develop DNA probes that will provide a positive or negative signal on blots of DNA from field isolates of *T. parva*.

Two approaches to the development of such probes are being pursued. The first involves further screening of *Theileria* DNA libraries for stock-specific sequences. This has been done with DNA from four stocks of *T. parva*. After carrying out several screenings, two useful sequences have been identified. Both were isolated from *T. p. bovis* (Boleni). When hybridized with parasite DNA, these sequences hybridized only with the Boleni parasite. The results of these experiments suggest that the potential of this approach for identifying stock-specific repetitive sequences may be limited.

A second approach to developing stock-specific DNA probes seeks to exploit the polymerase chain reaction. This involves identifying in the *Theileria* genome the short conserved sequences of DNA that do not change among parasite populations and that flank variable sequences. These conserved sequences can be used as primers in a polymerase chain reaction that is used to amplify the adjacent variable sequences of DNA. Oligonucleotides containing different variable regions can then be used as probes to identify particular variable sequences. This should be a highly sensitive method for detecting specific parasite populations. Work on this approach is being carried out in collaboration with scientists at the University of Cambridge and is initially concentrating on the repetitive DNA sequences that are already available.

Genetic recombination, which involves the exchange of genetic material between parasite populations, may occur in *T. parva* populations during the parasite's development in the tick and contribute to the antigenic diversity that occurs in *T. parva* parasites. To study this, a series of DNA probes must be available that will hybridize with different sequences distributed throughout the parasite genome. The parasite genome has been analysed using pulsed-field gel electrophoresis, a technique that separates large fragments of DNA according to their size. When the restriction enzyme Sfi-1, which cuts DNA into large fragments, digests parasite DNA, 30 to 40 fragments are produced, some of which differ in size among parasite stocks, indicating that the sites of enzyme cleavage, and thus the sequences around these sites, differ among stocks. The existing repetitive DNA probes for *T. parva* hybridize with only a few of these fragments. To obtain probes that hybridize at different sites in the genome, experiments are being conducted to clone DNA sequences spanning the Sfi restriction sites. Several such clones were isolated in 1988 and are now being characterized.

Immunization against the sporozoite form of the parasite

Cattle that have been immunized against *T. parva* by simultaneous infection and treatment and then challenged with large numbers of sporozoites produce antisporozoite antibodies. When these antibodies are added to suspensions of sporozoites, the antibodies neutralize the ability of the sporozoites to infect lymphocytes. A characteristic of these antibodies that is important to ILRAD's sporozoite research is that the antibodies react with sporozoites from antigenically different strains. This indicates that it may be possible to induce broad protection against ECF by using sporozoite antigens.

The aims of the sporozoite research project are to identify the antigens on the surface of the sporozoite that induce the host's immune system to produce neutralizing antibodies, to clone the genes that code for these antigens and to test the ability of the gene products to induce immunity.

Sporozoite antigens recognized by sera of experimentally immunized animals

Sera from cattle immunized with sporozoites to produce high levels of antibodies have been used to probe Western blots of solubilized sporozoite proteins to identify sporozoite antigens that may induce immunity. These experiments led to the identification of four protein antigens against which most of the activity of the antibodies are directed (Figure 4). The molecular masses of the intact forms of these antigen molecules are approximately 104, 85, 67 and 43 kilodaltons (kDa). The 85-kDa antigen is also found in shizonts and in this stage has been shown to vary in molecular mass (69-104 kDa) among parasite populations. In the shizont, this antigen is immunodominant with regard to induction of antibody responses and is therefore referred to at ILRAD as the polymorphic immunodominant molecule (PIM). Mouse monoclonal antibodies have also been produced against sporozoites and all the mouse antibodies specifically recognize the 67-kDa antigen.

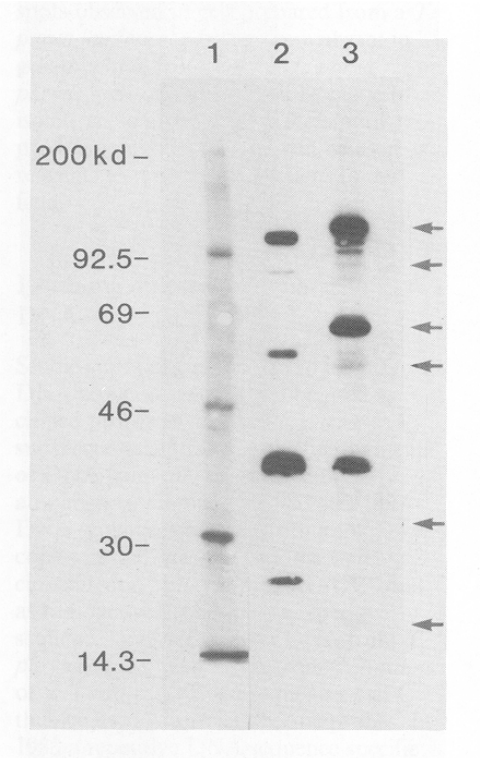


Figure 4. *Western blot analysis showing: Lane 1, molecular weight markers; Lane 2, uninfected tick salivary gland extract; and Lane 3, infected tick salivary gland extract. The blot was developed with serum from an animal immunized with* Theileria parva *(Muguga) sporozoites. Specific* T. parva *sporozoite antigens are indicated by arrows. These antigens are being studied as candidate antigens for the generation of protective immunity against East Coast fever in livestock.*

The 67-kDa molecule, which comprises a loose surface coat over the sporozoite surface, is involved in the initial binding of the sporozoite to a lymphocyte, after which the sporozoite enters the lymphocyte. Some of the monoclonal antibodies that specifically recognize this molecule strongly neutralize the ability of the sporozoites to infect lymphocytes. At least part of the antigen molecule is shed from the sporozoites as they enter the lymphocytes, and the specific monoclonal antibodies no longer react with sporozoites after they have been internalized.

Until recently the location of the 105-kDa antigen molecule in the sporozoite was not known because of lack of antisera or monoclonal antibodies that specifically recognized the antigen. Then, in 1987, the gene carrying the code for this molecule was cloned and the sequence of the gene's DNA was determined. In the last year a specific antiserum against the protein product of this gene has been produced. Electron microscopic examination of sections of sporozoites exposed to antibody labelled with colloidal gold localized the antigen to micronemes and rhoptries, cell organelles within the cytoplasm of sporozoites (Figure 5). On-going studies in 1988 showed that these structures discharge their contents shortly after the sporozoite invades a lymphocyte. Structural observations indicate that this event triggers the disintegration of the host cell membrane, which surrounds the parasite immediately after it invades the host lymphocyte. The antiserum to the 104-kDa antigen had no neutralizing effect, suggesting that this antigen is unlikely to be a target of antibody-mediated immunity.

The PIM antigen was first identified in schizonts using monoclonal antibodies produced against parasitized lymphocytes. The antigen was thought to be confined to the schizont stage, but experiments carried out in 1988 demonstrate that it is present in sporozoites as well (Figure 6). One of the specific monoclonal antibodies has been used to purify the antigen from

sporozoites and schizonts in order to produce antisera and provide material for amino acid sequencing.

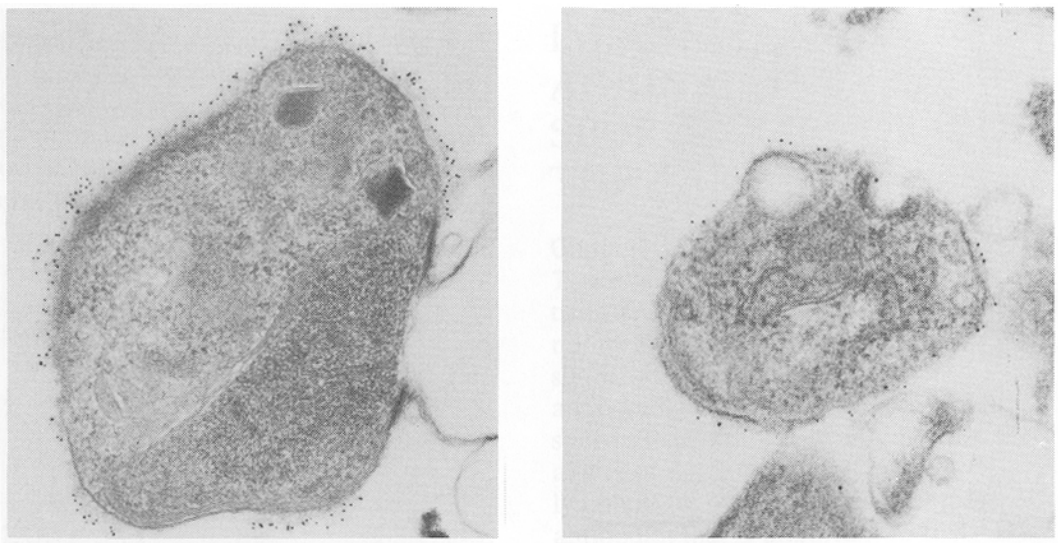


Figure 6. *Electron micrograph showing antibodies that recognize the 67-kDa protein binding to the surface of sporozoites (*left*); antibodies that recognize the 85-kDa protein in schizonts of* Theileria parva *also bound to the surface of sporozoites obtained from infected ticks (*right*). The surfaces of these sporozoites are labelled with the antibodies coupled to particles of protein A-gold, which appear as black dots. Magnification = × 70,000.*

Sporozoite antigens recognized by sera of animals in the field

Studies of *Theileria* antigens recognized by cattle sera have been carried out in collaboration with the international Centre of Insect Physiology and Ecology (Nairobi) on Rusinga Island, in Lake Victoria. Although ECF is endemic on this island, control of ticks and tick-borne diseases has not been practised there for the last ten years or more. Antibodies reactive with sporozoites, schizonts and piroplasms were found in sera from pregnant cows and from milk produced immediately after calving (colostrum). Of the calves born to these dams, at birth 56% had antibodies to sporozoites, 80% to schizonts and 84% to piroplasms. Approximately 60% of the sera from dams and calves showed neutralizing activity against sporozoites.

The major sporozoite antigens identified in experimental studies were also detected by sera from dams and their calves, which demonstrates that under conditions of natural challenge, cattle produce antibodies to these sporozoite antigens (Figure 7). The antibodies may contribute to an acquired immunity in these animals and may also provide protection in calves that take in colostrum containing the antibodies. These findings also imply that in cattle vaccinated in the field with these antigens, natural challenge would boost the specific antibody responses of the animals.

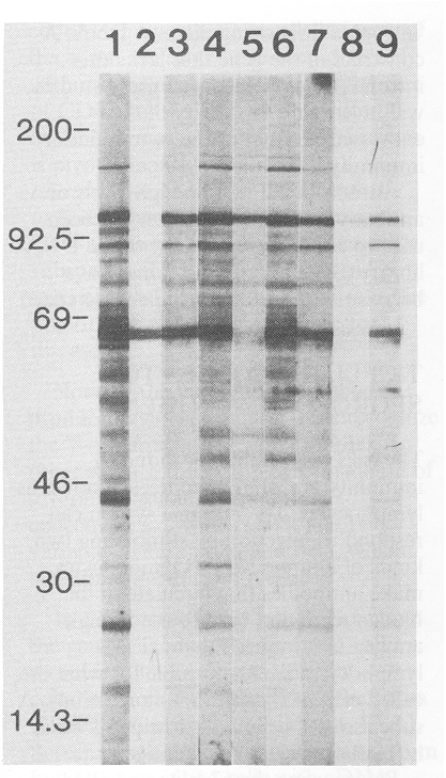


Figure 7. *Western blot analysis showing antigens recognized in* Theileria parva *Muguga sporozoites by: Lane 1, bovine anti-sporozoite serum C16; Lane 2, a sporozoite neutralizing monoclonal antibody; and Lanes 3-9, colostra of cows from an East Coast fever-endemic area. The colostra recognized the same major sporozoite antigens—105, 85 and 67 kDa—that were recognized by the monoclonal antibody and the hyperimmune cattle serum (C16) raised in the laboratory using lysates of sporozoites*.

Cloning genes that encode sporozoite antigens

The results obtained from studies of the sporozoite antigens recognized by sera of immune cattle suggest that both the 67-kDa and the PIM antigens are likely candidates for inducing protective immune responses, the latter antigen being of potential value against sporozoites and schizonts.

Efforts to clone genes encoding sporozoite antigens are thus focused on these two antigens. The isolation of a DNA copy (cDNA) clone that codes for a part of the 67-kDa molecule was reported in the *1987 Annual Report.* In an attempt to obtain a DNA clone containing the full-length gene, a genomic DNA library was prepared from *T. parva* piroplasms and screened by hybridization with the cDNA clone. A genomic clone 6.3 kilobases in length was isolated. This clone was sequenced and shown to contain an 'open reading frame—a length of DNA capable of encoding a protein—sufficient to encode about 50 kDa of protein. Two amino acid sequences, previously determined by sequencing fragments of the 67-kDa protein, were identified within the predicted amino acid sequence encoded by the clone. The DNA sequence of the gene has now been completely determined. The gene contains a short 'intron', a noncoding piece of DNA. A construct of the gene that lacks this intron is being prepared. Future studies will investigate the ability of the expressed product of the gene to induce immunity.

Antiserum containing high levels of antibody to the PIM antigen has been used to screen a *T. parva* genomic DNA library. Several genomic clones have been isolated and are being characterized.

T-cell responses to sporozoite antigens

The cells responsible for inducing immunity are white blood cells known as lymphocytes. The immune system can respond to infection by stimulating two kinds of lymphocytes. B lymphocytes make antibodies that circulate in the bloodstream and bind to the foreign antigen that induced them. T lymphocytes are responsible for what are called cell-mediated immune reactions. A subclass of T cells, called helper T cells, helps B cells respond to antigen.

Before using the 67-kDa and PIM antigens for immunization, it must be determined how effective the molecules are at inducing helper T-cell responses in cattle and to what extent genetic variation among individual animals determines the ability of the animals to respond to the antigens. The 67-kDa antigen is being used to search for answers to these questions.

Helper T-cell cultures were established by stimulating T cells from immunized cattle with antigen added to monocytes, white blood cells that ingest antigens. Helper T-cell clones specific for the 67-kDa antigen were then derived from these cultures. These cloned T cells will be used to map the sites on the antigen molecule that are recognized by T cells and to investigate possible genetic variation in these T-cell responses.

Immunization against the schizont form of the parasite

The third component of ILRAD's theileriosis research program, after studies of the epidemiology of ECF and immunization against the sporozoite form of the parasite, concerns the schizont stage of parasite development. When cattle develop immunity to ECF—following either natural infection and recovery or immunization by the infection-and-treatment method—evidence suggests that what helps to control the infection at the schizont stage are cell-mediated immune responses, which, as described above, involve T lymphocytes.

Unlike antibodies, T cells do not react with free antigens but rather recognize fragments of antigen that have been processed in host cells and presented on the surface of those cells. These antigenic fragments, or peptides, on the cell surface are associated with major histocompatibility molecules, a family of glycoproteins encoded by a complex of genes called the major histocompatibility complex (MHC). An antigen receptor on the surface of a T cell recognizes a foreign peptide on the surface of another cell only when the foreign peptide is associated with MHC glycoproteins.

Broadly speaking, there are two types of T cells. When exposed to antigens, regulatory and helper T cells produce soluble mediators that help generate antibody responses or other T cell responses, whereas cytolytic T cells are stimulated to kill cells expressing a specific antigen. Evidence suggests that both types of T-cell responses are induced by the schizont stage of *T. parva*.

The schizont research program aims to elucidate the role of T lymphocytes in bovine immune responses to *T. parva* and to identify parasite antigens that are recognized by T cells; these antigens could be used to immunize cattle against ECF. The program continues to study normal elements of the bovine immune system, which are important to understanding the immune mechanisms induced by *T. parva.*

Cell types responsible for immunity

T cells

To study the T-cell responses that play such an important role in immunity of cattle to *T. parva*, it is essential to be able to identify functionally important populations of T cells. The

method applied most commonly at ILRAD is to use mouse monoclonal antibodies specific for molecules on the surface of T cells. Over the last three years ILRAD has produced and characterized mouse monoclonal antibodies that react with bovine T-cell surface molecules analogous to CD2, CD4, CD5, CD6 and CD8 differentiation antigens in man. These mouse antibodies are now being used to define immature and mature bovine T cells, and to distinguish two major subpopulations of mature T cells based on expression of the CD4 (regulatory) or CD8 (cytolytic) T-cell antigens.

Experiments carried out in 1988 in collaboration with the Agricultural and Food Research Council (AFRC) Institute for Animal Health (UK) demonstrate that the CD4 and CD5 bovine T-cell antigens vary within a cattle population. In each instance, two codominantly expressed, allelic forms of the T-cell molecule have been detected with monoclonal antibodies. In the case of CD4, the two allelic forms differ in molecular mass. One of the CD5 alleles is predominantly associated with *Bos taurus* cattle and the other with *Bos indicus*, whereas the frequencies of the CD4 alleles are similar in the two cattle subspecies. These differences could be used as genetic markers in studies of the inheritance of genetic resistance to disease.

None of the monoclonal antibodies produced against bovine T cells recognize the T-cell antigen receptor complex. In other species, this complex is made up of several invariant polypeptides, collectively known as CD3, and two variable chains, alpha and beta, which constitute the antigen receptor.

In 1988 a study was initiated to clone the genes encoding the bovine T-cell receptor. This work was done in collaboration with scientists at the Dana Farber Cancer Institute (Boston, USA). Screening a cDNA library prepared from bovine white blood cells with cDNA probes for the human T-cell receptor proteins has resulted in the isolation of full-length cDNA clones for the gamma, delta and epsilon chains of CD3 and the variable alpha and beta receptor chains. Analyses of nucleotide sequences obtained for the 3' and 5' ends of the clones have demonstrated significant homology with sequences of the equivalent human and mouse genes. These cDNA clones will be used as probes to examine the expression of T-cell receptor components in different populations of T lymphocytes and to analyse T-cell receptor gene rearrangement in *Theileria*-specific T cells. Expressed products of the genes or selected synthetic peptides will also be used to produce specific monoclonal antibodies.

Cells that present antigen to T cells

Two main types of specialized cells are capable of ingesting foreign proteins and presenting processed antigen to T lymphocytes: monocytes, which in cattle are obtained from the blood, and dendritic cells, which are obtained from afferent lymph draining from various tissues into peripheral lymph nodes. Several monoclonal antibodies that react with monocytes and/or dendritic cells have been characterized (Figure 8). The dendritic cells can be distinguished from monocytes by their reactivity with these monoclonal antibodies. Dendritic cells have been shown to express much higher concentrations of MHC molecules on their surface than monocytes.

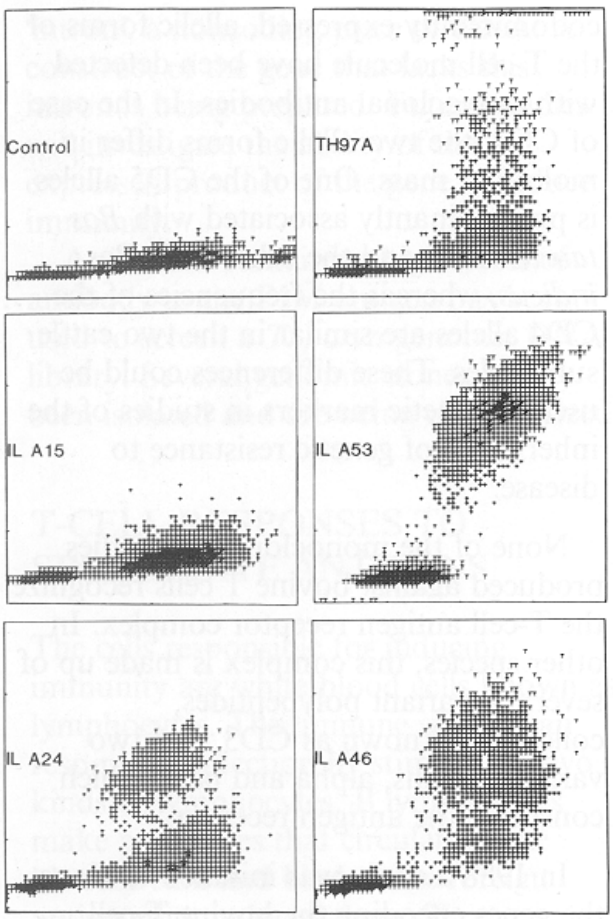


Figure 8. *Dot plot analysis of bovine afferent lymph dendritic cells stained with a panel of monoclonal antibodies that distinguishes the dendritic cells from blood monocytes. Vertical axis, intensity of fluorescence; horizontal axis, size of cells*.

These two cell types have been compared for their efficacy in presenting soluble antigen to helper T cells by using T cells from cattle immunized with purified *Trypanosoma brucei* variable surface glycoprotein. Dendritic cells were found to present antigen 10 to 100 times more efficiently than monocytes. These findings indicate that the dendritic cells play an important role in the induction of immune responses in cattle and suggest that antigen delivery systems used in vaccines should attempt to target the antigens to these cells.

The bovine major histocompatibility complex

The group of genes known as the major histocompatibility complex, or MHC, has been found in all mammalian species studied. These genes play an important role in the induction of both humoral and cellular immune responses. As mentioned above, both helper and cytolytic T lymphocytes can detect foreign antigens only in association with the glycoprotein products of host MHC genes. The MHC gene products fall within two major groups, called class I and class II, which are distinguished on the basis of their molecular nature, function and cellular distribution. These products—the MHC antigens—are integral components of cell membranes.

Major histocompatibility complex molecules that present antigenic peptides to T cells display marked variation among individuals of a species. The functional significance of this variation is that T cells that recognize antigen presented by the host's own cells do not recognize the same antigen presented by cells of a genetically unrelated animal. This phenomenon is known as MHC restriction. Antigens may also vary in their capacity to associate with different MHC

molecules. The variation in MHC molecules may thus have qualitative or quantitative influences on immune responses.

Class I MHC molecules present antigen to cytolytic T cells (CD8) and class II MHC molecules present antigen to helper T cells (CD4). Major histocompatibility complex molecules are encoded by a cluster of closely linked genes. In most species there are at least two gene loci each for class I and class II molecules, both genes at each locus being expressed. In view of the central role in immunization played by MHC gene products, it is important to be able to define different bovine MHC molecules and understand their function. Work on the bovine MHC at ILRAD falls into two areas: typing individual cattle with antibody reagents specific for MHC molecules and conducting experimental studies to define MHC genes and their products.

Cattle are serologically typed at ILRAD for MHC antigens using a panel of reagents consisting of 180 antisera and 20 monoclonal antibodies. The majority of these reagents react with class I MHC molecules. Serological typing continues to play an important role in the identification of animals with MHC phenotypes suitable for experimentation. An important component of this work is the use of embryo transfer techniques to produce full sibling families and identical twins of desired MHC phenotypes. The hormonal treatment regimes that successfully induce superovulations in *Bos taurus* cattle have been modified at ILRAD to optimize embryo transfer techniques in Boran (*Bos indicus*) cattle.

The organization of the bovine MHC gene region has been studied using the pulsed-field gel electrophoresis technique. This involves digesting bovine DNA with restriction enzymes that cut the DNA into large fragments and then separating the fragments in pulsed-field gels. Human class I and class II cDNA probes were then hybridized with the fragmented DNA. By using several restriction enzymes, it has been possible to construct a restriction map—a map that shows the location of each cutting (restriction) site in relation to its neighbours—of the class I and class II regions in one animal. From these data, the size of the class I region was estimated to be at least 750 kilobases and that of the class II region at least 300 kilobases. These are comparable to the sizes of equivalent regions in other species.

Studies to determine whether there is more than one functionally important class I gene locus in cattle were continued in 1988. Although serological typing of cattle appears to identify class I molecules encoded predominantly by one locus, biochemical evidence suggests that these molecules are encoded by at least two loci. During 1988 the existence of two distinct class I molecules encoded by the same MHC haplotype (the set of MHC genes inherited from one parent) has been confirmed using transfection technology.

The experiments were conducted using the offspring of a brother-sister mating. DNA from this animal, which is known to be homozygous for the MHC, was transfected along with a selectable marker into mouse fibroblasts. Cells expressing bovine class I MHC molecules were selected using a fluorescence-activated cell sorter following the staining of the cells with a fluoresceinated class I-specific monoclonal antibody. Positive cells were cloned and analysed with monoclonal antibodies and cytotoxic T-cell clones specific for the class I antigens of this animal. Two different types of transfected cell, expressing distinct bovine class I molecules, were identified (Figure 9). These findings provide strong evidence that there are at least two functionally important class I loci in cattle. One of the class I molecules identified in the transfectants is known to present *Theileria* antigens to specific cytolytic T cells. The transfectant expressing this antigen is therefore suitable for further transfection experiments involving *Theileria* genes to identify antigens of the schizont stage of the parasite that will induce protective immune responses.

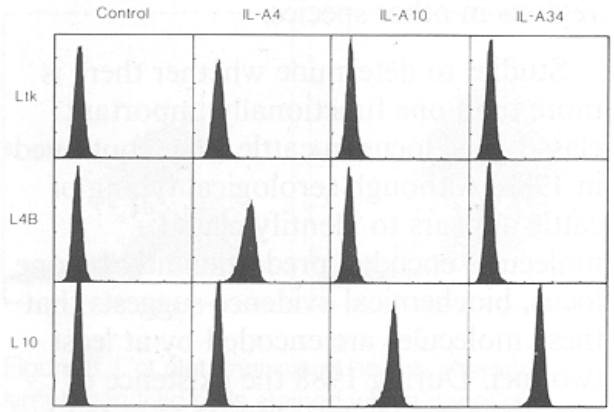


Figure 9. FACS (fluorescence-activated cell sorter) analysis of cells transfected with bovine class I MHC (major histocompatiblity complex) genes. The parent L cells (Ltk^-), and the cloned transfectants expressing either the KN104 (L4B) or the W10 (L10) MHC molecules, were stained with a KN104-specific monoclonal antibody (IL-A4), two W10-specific monoclonal antibodies (IL-A10 and IL-A34), or an unrelated monoclonal antibody (control). Vertical axis, number of cells; horizontal axis, intensity of fluorescence.

Few reagents exist to define different class II MHC molecules. For a variety of reasons, it is difficult to produce useful antisera or mouse monoclonal antibodies against the class II molecules. An alternative approach was explored in collaboration with the AFRC Institute of Animal Physiology and Genetics Research (Cambridge, UK) to produce bovine monoclonal antibodies. These were produced in 1988 by fusing bovine antibody-producing cells, obtained from the lymph nodes of immunized cattle, with a mouse tumor cell line and then selecting clones. Although on initial screening a large number of antibody-producing hybrids were identified, only one stable hybrid producing an anti-MHC monoclonal antibody was obtained following cloning. The monoclonal antibody produced by this hybrid specifically recognizes class I antigen. Further experiments will be carried out to explore this technique for producing monoclonal antibodies specific for class II molecules. Experiments aimed at cloning class II MHC genes were initiated in 1988 in collaboration with scientists at the AFRC Institute of Animal Physiology and Genetics Research (Edinburgh, UK).

The biology of *Theileria*-infected cells

An understanding of how *Theileria* parasites regulate the growth of host cells might lead to new methods for treating infected animals or reducing the pathogenicity of the parasites. Research in this area is aimed at defining the role of different types of lymphocytes in infections with *T*. *parva* and at identifying parasite molecules that may be responsible for activating host lymphocytes.

Past experiments show that the majority of parasitized cells in infected cattle are T lymphocytes and that most cell lines established from peripheral blood lymphocytes are T lymphocytes. Nevertheless, when purified populations of B cells are infected with *T parva*, they readily give rise to cell lines.

In 1988 cattle were inoculated with purified populations of their own T or B lymphocytes that had been exposed briefly in the laboratory to sporozoites. These experiments showed that T lymphocytes produced lethal infections whereas B lymphocytes gave rise to mild self-limiting infections. Purified populations of CD4 or CD8 T lymphocytes gave rise to lethal infections. On examination of the phenotype of infected cells from the lymph nodes of these animals, no transfer of the parasite to other cell types was detected. Further experiments are in progress to

determine whether the differences between T and B lymphocytes are due to a superior immunogenicity of the B cell or to differences in the way in which the growth of the cells is regulated.

Studies of factors that regulate the growth of parasitized cells are currently focused on protein kinases, enzymes that phosphorylate proteins. These enzymes modify proteins by catalyzing the transfer of a phosphate group to specific amino acids in host cell proteins. The phosphorylation in turn regulates the activity of these proteins.

Work carried out in 1986 and 1987 showed that several phosphorylated proteins were detected only in infected cells. The kinase activity has been shown to be specific for serine/threonine residues and has properties similar to casein kinases described in other mammalian cells and in insect cells.

Recent evidence suggests that part of the enzyme activity is derived from the parasite. A cDNA probe for the alpha subunit of *Drosophila* casein kinase has been used to identify and clone a homologous gene from a *Theileria* genomic DNA library. The gene, which is full length and free of introns, contains several short sequences characteristic of casein kinase genes. Attempts will be made to produce antibodies to the expressed product of this gene in order to determine whether it is associated with other polypeptides and where it is located in the infected cells.

Immunological responses to schizont-infected cells

The results of studies carried out over the last five years indicate that T lymphocyte responses against parasitized lymphoblasts are important in mediating immunity against *T*. *parva* Both class I restricted cytolytic T-cell responses and class II restricted helper T-cell responses have been detected and in some instances both types of T cell exhibit parasite strain specificity. These findings suggest that the T cells recognize processed parasite antigens on the surface of infected cells. Studies continued in 1988 to elucidate the role of these responses in immunity.

Parasite-specific cytolytic T cells

It is important to determine if the specificity of cytolytic T-cell responses correlates with patterns of cross-protection among parasite stocks. Work in this area is still focused on two Kenyan stocks of the parasite, *T. p*. *parva* (Muguga) and *T. p*. *parva* (Marikebuni). Cattle immunized with the Marikebuni stock are protected against challenge with Muguga, whereas some animals immunized with Muguga are susceptible to challenge with Marikebuni. It has become apparent that the analysis of strain specificity of cytolytic T-cell responses is complicated by the presence of mixtures of parasites within parasite stocks. Experiments in which cloned parasitized cell lines were examined with parasite-specific monoclonal antibodies and DNA probes revealed at least four distinct parasite populations within the Marikebuni stock. Cytolytic T-cell clones generated from animals immunized with *T. p. parva* (Muguga) have been tested for their capacity to kill cell lines infected with the different Marikebuni parasites. The clones were found to kill some Marikebuni-infected cell lines but not others, and clones derived from different cattle showed different patterns of killing of the different parasitized cell lines.

Similar differences in the pattern of killing were observed with cytolytic T -cell clones derived from the same animal but restricted by different class I molecules (Figure 10). These results indicate that the restricting MHC molecule exerts a strong influence on the antigenic specificity of the cytolytic T-cell response. Such variation in specificity may well explain the observed differences among individual cattle in the degree of cross protection induced by the Muguga

stock of the parasite. To answer this question, cloned parasites will be used in studies of immunization and cross-challenge. A limiting dilution analysis assay for cytolytic T cells has been developed for examining directly from the blood of immunized cattle the frequency and specificity of the cytolytic cells.

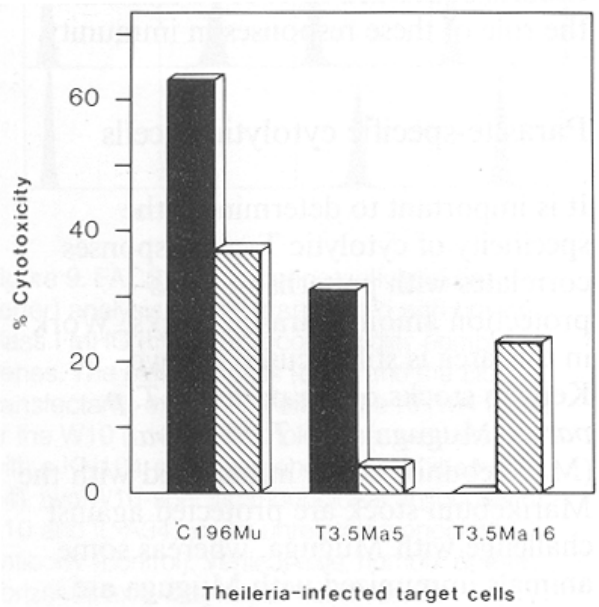


Figure 10. *Cytolytic activity of two T-cell clones generated from an animal immunized with* T. parva *(Muguga). The two clones were shown to be restricted by different class I MHC (major histocompatibility complex) molecules. They were tested on cells infected with Muguga (C196 Mu) and with two different Marikebuni parasites (T3.5 Ma5 and T3.5 Ma-l6). The pattern of killing of the two Marikebuni-infected lines by the clones is different, indicating that the MHC may influence the parasite strain-specificity of the T-cell response.*

Parasite-specific helper T cells

Experiments using helper T-cell clones specific for *Theileria*-infected cells have revealed two types of T-cell. Both types proliferate in response to stimulation with parasitized cells from the same animal. One type responds to glutaraldehyde-fixed parasitized cells, to purified schizonts and to membrane fractions of schizonts only when antigen-presenting cells are added to the cultures. The second type of T cell responds to fixed parasitized cells in the absence of antigen-presenting cells and to a soluble antigen fraction from parasitized cell lysates, in the presence of antigen-presenting cells. Helper T cells thus appear to recognize two different types of antigen in the infected cell. Since the latter type of T cell appears more efficient at recognizing antigen on the surface of infected cells, this type may be more important in immunity.

Bovine gamma-interferon

Although it is clear that parasite-specific T cells can kill *Theileria*-infected cells, it is possible that soluble mediators generated during the induction of T-cell responses also help to control the parasite. One such mediator, produced by activated T cells, is gamma-interferon. An assay for gamma-interferon using Semliki Forest virus and bovine fibroblasts has been established. Cultured *Theileria*-specific T cells of both the CD4 and CD8 subsets were found to produce readily detectable levels of gamma interferon. Moreover, production of gamma-interferon *in vivo* was demonstrated by examining supernatants of short-term cultures of lymph node cells derived from cattle undergoing immunization with *T. parva* by infection and treatment. However, recombinant bovine gamma-interferon, provided by Ciba-Geigy (Basel,

Switzerland), had no detectable effect either on the growth of established parasitized cell lines or on the establishment of infected cell lines.

Evidence exists, however, that soluble factors can act directly on the schizont. Anti-schizont activity, which is not antibody, has been detected in the serum of some cattle immediately after spontaneous recovery from infection with *T. parva*. This serum activity causes the intracellular death of schizonts in culture, apparently without damaging the host cell. Experiments are planned to fractionate these sera to identify the active component or components.

Identification of antigens recognized by parasite-specific t cells

In 1988 a major emphasis was placed on the identification of the antigens recognized by *Theileria*-specific *T* cells. Other studies of antigen processing and presentation indicate that soluble antigens internalized by antigen-presenting cells are readily recognized by helper T cells, whereas cytolytic T cells usually recognize only antigens that are being synthesized within presenting cells, such as a virus or intracellular parasite. Therefore, different strategies must be adopted for identifying antigens recognized by *Theileria*-specific helper and cytolytic T cells. Three approaches are being taken at ILRAD.

The first approach is to try to identify and purify from parasitized cells antigenic fractions that, in the presence of antigen-presenting cells, will stimulate parasite-specific T cells. This approach shows promise for antigens recognized by helper T cells but not for those recognized by cytotoxic T cells. A soluble antigenic fraction that stimulates parasite-specific helper T cells at concentrations of less than 10 ng/ml has been identified by column chromatography and high pressure liquid chromatography fractionation of infected cells (Figure 11). Efforts are being made to raise specific antibodies to this fraction with the aim of using them to screen parasite DNA libraries in order to identify the genes encoding these antigens.

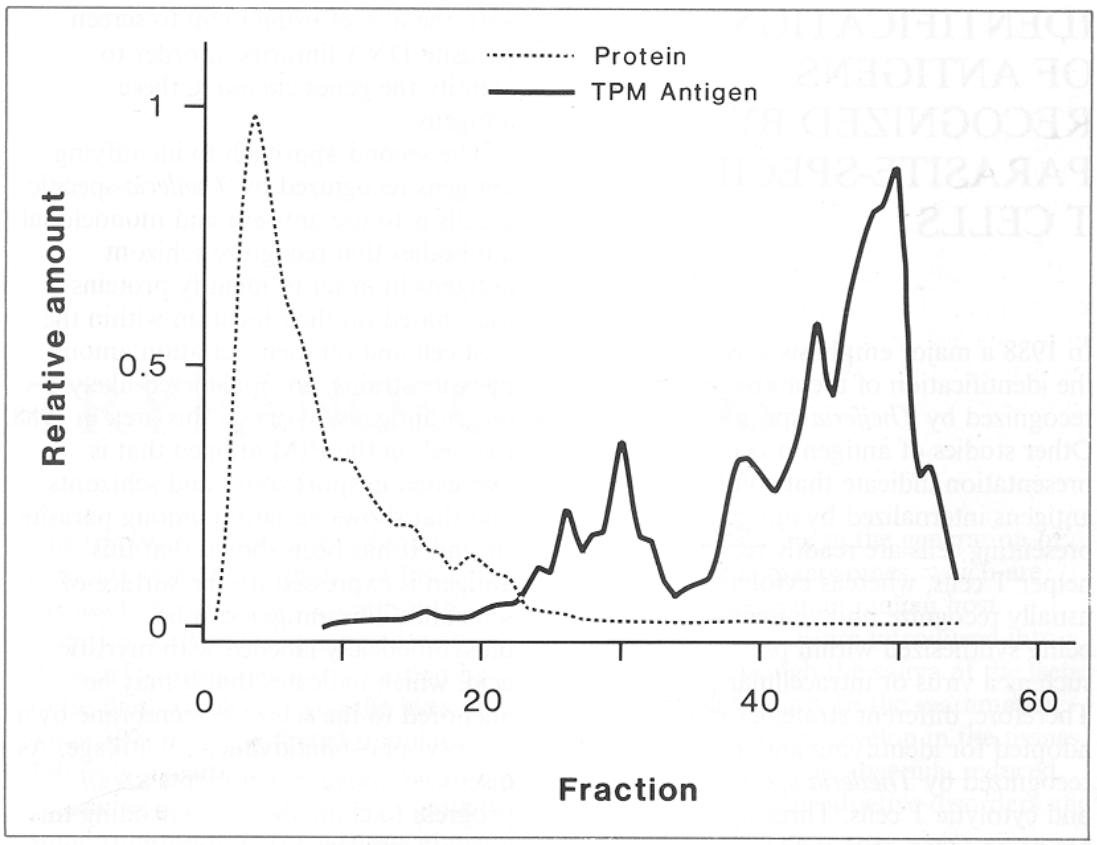


Figure 11. *Column and high-pressure liquid chromatography (HPLC) fractionation of a* Theileria*-specific cell surface antigen. The dotted line represents the protein content in each fraction. The solid line represents the proliferative response of T-cell clones that recognize the cell surface antigen. The protein fraction that induces greatest T-cell proliferation occurred with fractions coming off the HPLC column at the latest time. The stimulatory material has an approximate molecular mass of 10 kDa.*

The second approach to identifying antigens recognized by *Theileria*-specific T cells is to use antisera and monoclonal antibodies that recognize schizont antigens in order to identify proteins that, based on their location within the host cell and on their variation among parasite strains, are considered likely target antigens. Work in this area in 1988 focused on the PIM antigen that is expressed in sporozoites and schizonts and that shows variation among parasite strains. It has been shown that this antigen is expressed on the surface of schizonts. The antigen can be biosynthetically labelled with myristic acid, which indicates that it may be anchored to the schizont membrane by a glycosyl-phosphatidylinositol linkage. As discussed above, experiments are in progress to clone the gene encoding this parasite antigen. When fully characterized, this gene will be expressed in mammalian cells to determine if the product is recognized by parasite-specific T cells. Several other schizont antigens of potential interest are also being studied.

The third approach, initiated in 1988, is to screen parasite cDNA expression libraries with T cells. The technique for purifying schizonts has been refined and cDNA is being prepared from purified schizonts. Attempts will be made to screen the expressed products of a cDNA library in an 'expression vector', a vector in which the protein gene product is expressed, with parasite-specific helper T cells.

Trypanosomiasis

The trypanosomiases are a disease complex that affects man and his livestock in Africa, Asia and South America. ILRAD is concerned with those trypanosomiases transmitted by tsetse flies, which threaten the lives of some 30% of an estimated population of 150 million cattle in 37 countries of Africa, as well as comparable numbers of small ruminants. Annual losses in meat production alone are estimated at US$5 billion. This economic deprivation is exacerbated by losses in milk production, tractive power, waste products that provide natural fuel and fertilizer and secondary products such as clothing and hides. In addition, 50 million people are currently exposed to the risk of contracting human trypanosomiasis, known as sleeping sickness.

Trypanosomiasis is caused by blood-dwelling protozoan parasites called trypanosomes, which

infect man, cattle, sheep, goats, pigs, horses and camels. Wild animals can also be infected and serve as reservoirs of infection. Trypanosomes are transmitted by tsetse flies *(Glossinidae)* and other biting insects, which ingest the parasites in blood meals taken from infected animal hosts (Figure 12). In the tsetse fly the parasites multiply in the midgut or in the proboscis and undergo a developmental cycle culminating in the generation of metacyclic trypanosomes, which are infective to the mammalian host. Metacyclic forms are introduced into a new host through the saliva of the tsetse fly during feeding. In the mammalian host the parasites develop in the tissues and blood, causing anaemia, reduced productivity, reproductive disorders and death.

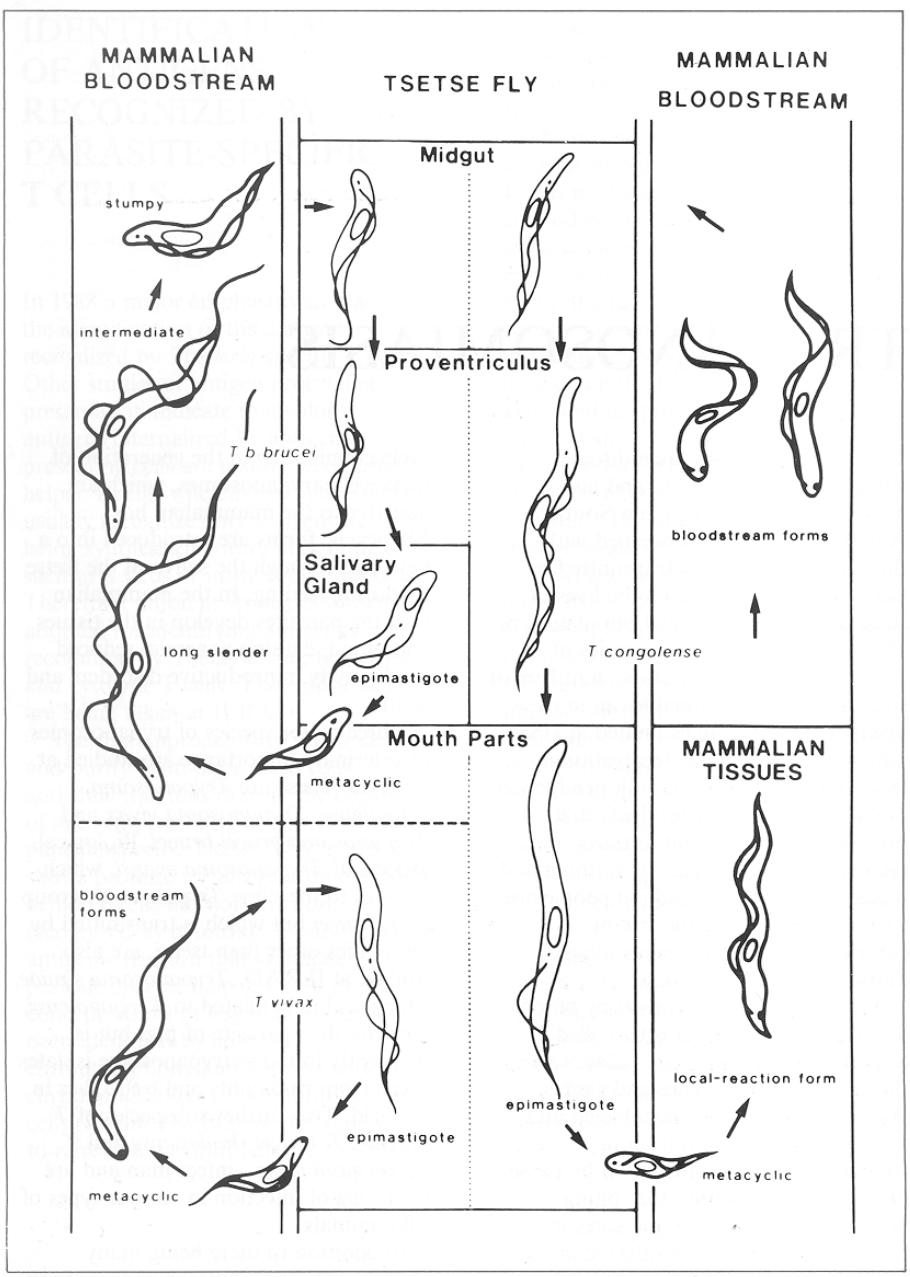


Figure 12. *Schematic diagram showing the life cycles of the three major tsetse-transmitted trypanosome species in Africa.*

Three major species of trypanosomes of veterinary importance are studied at ILRAD. These are *Trypanosoma congolense, Trypanosoma vivax* and *Trypanosoma brucei brucei.* Biological aspects of *Trypanosoma evansi,* which belongs to the same *Trypanozoon* group as *T. brucei* but which is transmitted by biting flies other than tsetse, are also studied at ILRAD.

Trypanosoma simiae, which is closely related to *T congolense,* is primarily a parasite of pigs but is frequently found in trypanosome isolates taken from ruminants and tsetse flies in the field. Two further subspecies of *T*. *brucei—T. brucei rhodesiense* and *T*. *brucei gambiense* —infect man and are reservoirs of infection in various types of wild animals.

In addition to there being many species of trypanosomes, a phenomenon called antigenic variation exhibited by each species helps to induce chronic infections in the hosts the parasites infect. Trypanosomes are covered by a dense coat made up of glycoprotein molecules, called variable surface glycoproteins (VSGs) (Figure 13). A trypanosome population causing infection in a single susceptible host animal can vary these surface antigens in a matter of a few days. Although the host usually generates a good antibody response to trypanosomes expressing a particular variable antigenic type (VAT), the large number of VATs that can be expressed within a single infection, estimated to be 300–1000 for *T*. *brucei,* causes chronic infections. Long-term infections in turn help ensure the transmission of the parasites to other animals and increase the severity of the pathogenesis, particularly anaemia, caused by trypanosome infection.

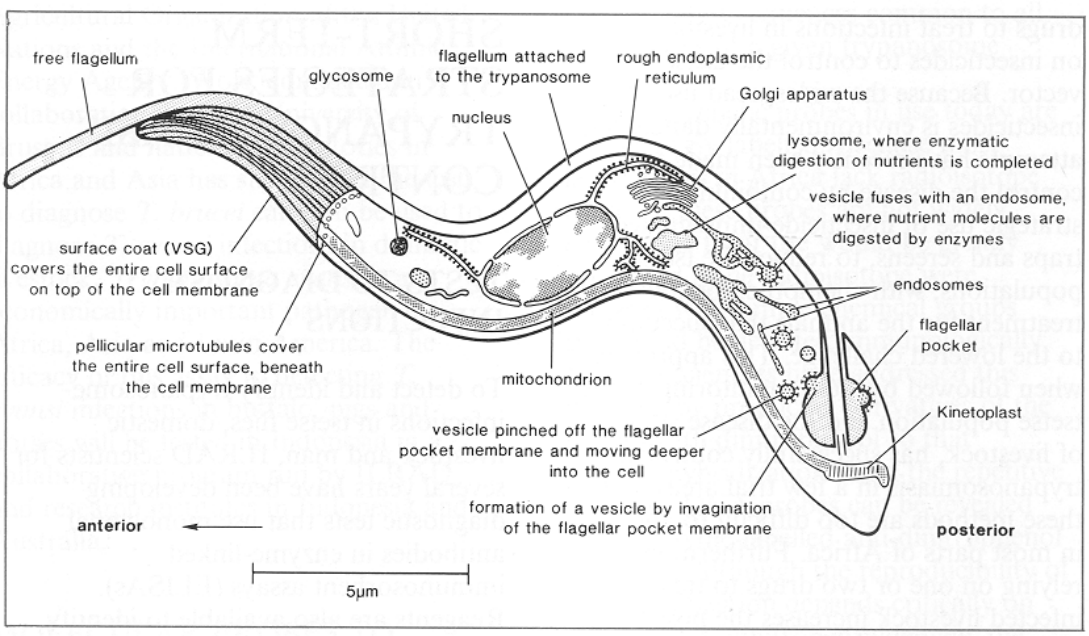


Figure 13. *Schematic diagram of a trypanosome of the* Trypanosoma brucei *group in its intermediate bloodstream form, illustrating the major organelles.*

It was initially believed that study of the antigenic variation displayed by trypanosomes would best elucidate how trypanosomes maintain themselves in the host animal. This information could then be used to develop vaccines to control or prevent the infection. But detailed studies of antigenic variation carried out at ILRAD and elsewhere over the last several years suggest that the number of VSGs expressed by trypanosomes is too great to make a vaccine based on the administration of a single, or even a few, VATs broadly effective. The total number of antigens expressed by any stock of trypanosomes, called a repertoire, is very large. These antigenic repertoires vary not only among but also within species: isolates in one area of Africa often differ in their antigenic repertoire from isolates in other areas. These distinct antigenic repertoires of a single species are known as serodemes. The existence of serodemes considerably enlarges the number of VATs encountered in the field.

Traditional management of trypanosomiasis has relied on chemotherapeutic drugs to treat infections in livestock and on insecticides to control the tsetse vector. Because the widespread

use of insecticides is environmentally damaging, attempts have recently been made to control the disease by combining the strategic use of insecticide-impregnated traps and screens, to reduce the tsetse populations, with chemotherapeutic treatment for the animals that succumb to the lowered challenge. This approach, when followed by close monitoring of the tsetse population and the disease status of livestock, has successfully controlled trypanosomiasis in a few trial areas, but these methods are too difficult to apply in most parts of Africa. Furthermore, relying on one or two drugs to treat infected livestock increases the possibility that the parasites will develop resistance to the drugs.

ILRAD is therefore taking an alternative approach and is seeking primarily immunological solutions to the problems posed by trypanosomiasis. The complexity of the antigenic variation exhibited by the parasite has necessitated that the research goals of the trypanosomiasis program be divided into short and long term. In the short term ILRAD is studying ways to develop more accurate tests to diagnose trypanosome infection in livestock, new ways with which to use the currently available trypanocides that will reduce the possibility of inducing parasite resistance to the drugs, and the reasons why some breeds of cattle are able to withstand trypanosome infection better than others. In the long term ILRAD is conducting research in two main areas. It is studying the responses ruminant hosts make to trypanosome infection, with the aim of enhancing normal mechanisms of resistance in livestock, and it is scrutinizing molecules and processes of the parasite in a search for key elements or activities that can be attacked with drugs without adversely affecting the host.

Short-term strategies for trypanosomiasis control

Tests to diagnose infections

To detect and identify trypanosome infections in tsetse flies, domestic livestock and man, ILRAD scientists for several years have been developing diagnostic tests that use monoclonal antibodies in enzyme-linked immunosorbent assays (ELISAs). Reagents are also available to identify trypanosomes of the subgenera *Trypanozoon, Nannomonas* and *Duttonella.* In 1988 new monoclonal antibodies were developed to identify protein rather than carbohydrate epitopes of *T. vivax* and *T*. *congolense* membrane proteins. These antibodies will reduce the likelihood of the ELISA giving false positive results. Furthermore, by detecting target proteins, the antibodies will enable scientists to identify fragments of DNA encoding the trypanosomal antigens in libraries of complementary DNA—DNA copied from messenger RNA—of the various trypanosome species. Recombinant antigens could then be synthesized in great quantity for use in inhibition ELISAs, which may be more sensitive in detecting infections than the technique presently used. In 1988 the trypanosomiasis program began to investigate the synthetic production of the diagnostic antigen for *T. brucei.*

The assays in their present form are now being tested for accuracy and sensitivity in national laboratories in ten African countries in collaboration with the World Health Organization and a joint division of the Food and Agricultural Organization of the United Nations and the International Atomic Energy Agency. Work conducted in collaboration with the University of Brussels and national laboratories in Africa and Asia has shown that the test to diagnose *T*. *brucei* can also be used to diagnose *T*. *evansi* infections in domestic livestock. *Trypanosoma evansi* is an economically important pathogen in Africa, Asia and Latin America. The efficacy of this assay in detecting *T*. *evansi* infections in buffalo, pigs and horses will be tested in Indonesia in a collaborative program run by ILRAD and research institutes in Indonesia and Australia.

Molecular probes to identify species

Our understanding of the epidemiology of both animal and human trypanosomiasis is

improved by an ability to identify more precisely the trypanosome species carried by tsetse flies. To this end ILRAD has produced DNA probes to detect differences among parasite species and subspecies at the genomic level. These highly sensitive probes can be used in the laboratory to distinguish repetitive DNA sequences that recognize specific species and subspecies of pathogenic trypanosomes. The probes also distinguish differences among trypanosome populations of the same species collected from different areas. When the probes were tested on field materials collected in Kenya in collaboration with the International Centre of Insect Physiology and Ecology (Nairobi), it was discovered that although the probes are species-specific and detect low numbers of trypanosomes in infected tsetse flies, the probes do not detect all isolates of a given species. These results demonstrate that genetic differences occur within species; attempts will now be made to identify DNA sequences common to all populations of a given trypanosome species.

All the genetic probes in use today are radioactively labelled. Because most laboratories in Africa lack radioisotope facilities, these probes are not widely employed. Their use would be greatly promoted if the radioisotope were replaced with simple chemical groups that could be detected immunologically. ILRAD scientists have addressed this problem by producing derivatives of the probes with dinitrophenol so that positive hybridization using the repetitive sequence DNA probes can be revealed by an enzyme-labelled anti-dinitrophenol antibody. Although the reproducibility of the derivatization depends critically on the purity of dinitrobenzaldehyde, the initial labelling agent, dinitrophenol-labelled probes can detect as little as 1-5 pg of purified DNA, which demonstrates a sensitivity similar to that of the radiolabelled probes and probably sufficient for detecting trypanosomes in tsetse.

Future work at ILRAD in this area will attempt to validate the diagnostic assays and to increase their sensitivity where that is desirable. ILRAD staff will also work with scientific organizations to develop forms of the tests that can be widely and easily used in the areas of the world affected by trypanosomiasis.

Chemotherapy

One of the principal objectives of the work conducted at ILRAD on chemotherapy and chemoprophylaxis is to develop assays to quantify the levels of drugs in the blood and tissue fluids of treated livestock. Two kinds of such assays—chemical and biological—have been produced. The former, which requires sophisticated chemical techniques, will be used to quantify biological assays based on trypanosome culture systems. The simpler biological assays should prove useful to national laboratories.

In vitro assays of drug resistance

ILRAD scientists have shown that incorporating drugs in culture medium can inhibit the growth and development of trypanosomes *in vitro*, depending upon the sensitivity of the trypanosome population to the drugs used. Cultures of the bloodstream forms of the parasite were used in the first experiments in this area. Clear differences in Berenil-induced growth inhibition were observed (Figure 14). Bloodstream forms of fresh field isolates, however, are difficult to adapt to cultures. Cultures of procyclic forms of the trypanosome—the noninfective, multiplying forms of the parasite equivalent to the forms found in the tsetse midget—of *T. brucei* and *T. congolense* seem to be established more reliably, and tube assays have been developed for isolating and testing *T. congolense* and *T. brucei* for drug sensitivity.

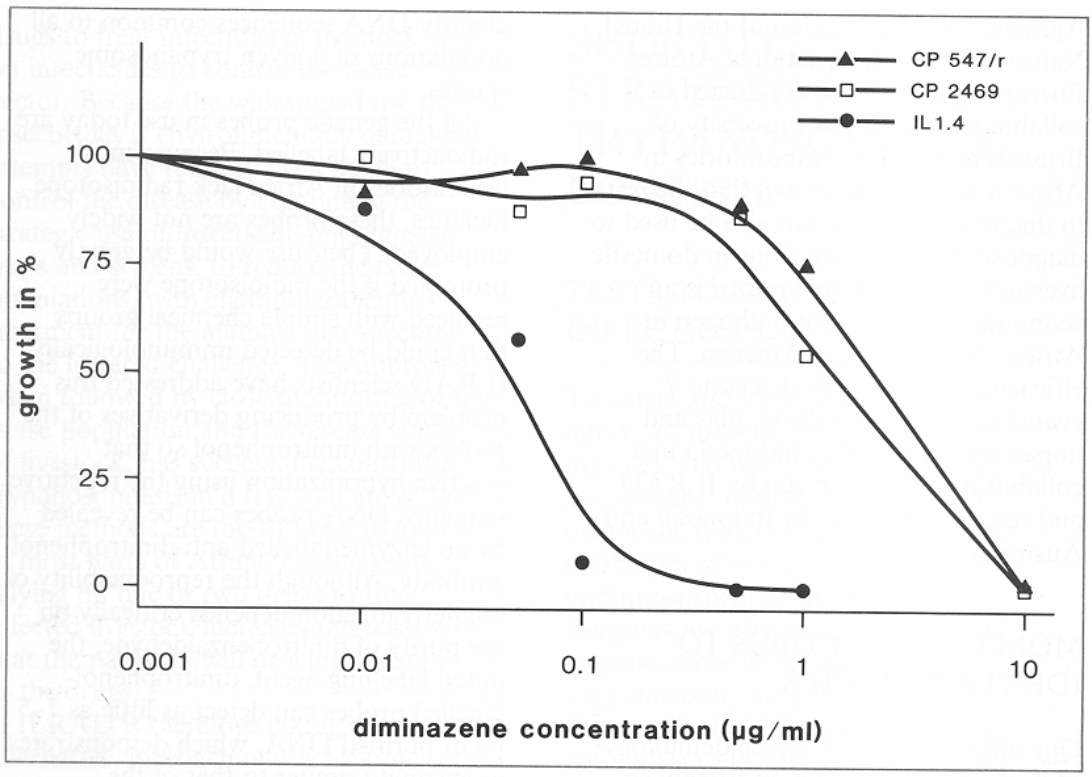


Figure 14. *Graph showing the comparative growth inhibition of drug-susceptible (IL 1.4) and drug-resistant (CP 547/r and CP 2469) trypanosomes in the presence of diminazene aceturate (Berenil). Trypanosomes were incubated at 37°C in 4% C02 in air for 24 hr in culture medium containing various drug concentrations. Growth was determined with a Coulter Counter and compared with control cultures.*

Blood samples containing trypanosomes are mixed with a culture medium and incubated overnight at 27°C in a plastic centrifuge tube. The trypanosomes in the supernatant are removed and transferred to culture flasks to allow the procyclic forms to develop and grow further. All trypanosome stocks were successfully isolated by this procedure in a medium based on the composition of tsetse haemolymph to which supplements had been added.

The growth of procyclic forms of drug-sensitive trypanosome stocks was inhibited when these were propagated for ten days in the presence of 1 ng/ ml of isometamidium chloride. In cultures of sensitive trypanosomes, the drug induced an increase in cell size, degradation of the kinetoplast and multinucleate forms to develop. Differences in growth inhibition of procyclic forms between resistant and sensitive stocks also occurred after incubation for 48 hours with 1–100 ng/ ml isometamidium chloride. Only minor differences, however, were observed when procyclic forms of resistant and sensitive *T*. *b. brucei* were cultivated with diminazene aceturate. Thus, whereas inhibition of the growth of procyclic forms can be used to test trypanosome stocks for their levels of resistance to isometamidium chloride, the same assay appears to be inappropriate for diminazene aceturate.

An alternative method of testing trypanosome populations for resistance to both isometamidium chloride and diminazene aceturate is the drug incubation infectivity test. Cultures of trypanosomes are incubated at 37° C in 4% carbon dioxide in air for 24 hours in the presence of a drug or plasma from drug-treated animals. Control cultures incubated with 1 % distilled water instead of a drug are incubated under the same conditions. After incubation, an aliquot from each culture is inoculated into mice. The mice are then screened for 30 days for trypanosome infections.

None of the drug-sensitive trypanosome stocks were able to infect the mice after incubation with 1 ng/ ml isometamidium chloride, but all resistant stocks were able to do so. Two of the isometamidium-resistant trypanosome stocks retained their infectivity after incubation with 10 or 50 ng/ ml of the drug. But when used at higher concentrations on resistant trypanosome populations, the drug increased the period between infection and the appearance of parasites in the blood of the mice.

Higher concentrations of diminazene aceturate were necessary to inhibit the infectivity of *T. b. brucei.* Differences in infectivity occurred when trypanosomes were incubated in the presence of 0.05–1.00 µg/ ml of diminazene aceturate. The drug-resistant trypanosome stocks retained infectivity after incubation with 1 µg/ ml of the drug. Thus, by using the drug incubation infectivity test it is possible to distinguish isometamidium-and diminazene-resistant trypanosome populations from those that are sensitive to these drugs. The test has been validated for *T. brucei, T. evansi* and a rodent-infective stock of *T. vivax.*

The stability of the drug-resistant trait has been further examined *in* vitro: a *T. brucei* stock resistant to diminazene, isometamidium, quinapyramine and mel B was grown *in vitro* and its sensitivity to these drugs was compared to that of a drug-sensitive trypanosome stock. There was little change in sensitivity after propagation for up to 275 days of bloodstream forms *in vitro* or after transformation of bloodstream forms into procyclic, epimastigote and, finally, metacyclic forms. These results suggest that drug resistance is a relatively stable characteristic even when trypanosomes are maintained *in vitro* in the absence of drugs.

Significant progress was made in cultivating certain stocks of the *Trypanozoon* group without feeder-layer cells. This has enabled researchers to conduct several new studies, including an evaluation of trypanocides in the absence of feeder-layer cells.

Drug resistance

The development of resistance in trypanosomes to the trypanocidal drugs currently available threatens our ability to continue to control trypanosomiasis. In collaboration with the departments of Veterinary Parasitology and Physiology at the University of Glasgow, ILRAD is conducting studies to determine new ways of treating livestock that are infected with trypanosomes known to be resistant to recommended doses of trypanocidal drugs. Reports from the field have suggested that intravenous administration of isometamidium chloride has a curative effect on cattle infected with trypanosome populations that are resistant to the drug when it is administered intramuscularly. In experiments at ILRAD, however, the intravenous administration of high levels of isometamidium chloride failed to cure cattle infected with cloned populations of *T. congolense* that were resistant to this drug when it was administered intramuscularly.

Using highly sensitive radiochemical and chemical techniques, a relay toxicity study, carried out in 1988 by the Department of Veterinary Pharmacology at the University of Glasgow in collaboration with ILRAD and the manufacturers of isometamidium chloride, Rhone-Poulenc (UK), showed that isometamidium chloride cannot be absorbed into blood and body fluids from the gastrointestinal tract of animals that were either dosed with the drug by mouth or fed with tissues of an animal given the drug intramuscularly one week previously. This makes it unlikely that human consumption of meat from animals treated with isometamidium chloride is a significant health hazard.

Tsetse biology

Male *Glossina* flies sexually sterilized by gamma-irradiation carry trypanosomiasis as efficiently as fertile males. However, when tsetse are given a blood meal containing 8–

12μg/ml of isometamidium chloride before a blood meal containing trypanosomes, infection of the flies by mature *T. vivax, T. congolense* and *T. b. brucei was* completely suppressed. It is therefore recommended that the flies used in the sterile insect release method of tsetse control be fed on blood containing isometamidium chloride.

Various other factors were investigated to determine their influence on the maturation of the three major species of trypanosomes and their transmission by tsetse. These parameters included the species of the wild mammalian hosts and tsetse vectors of the parasites and the occurrence of pre-existing trypanosome infections in the hosts and rickettsia-like organisms in the vectors (Figure 15). In ILRAD experiments, *G. m. centralis* appeared to be largely unaffected by these parameters and proved the most efficient vector of trypanosomiasis.

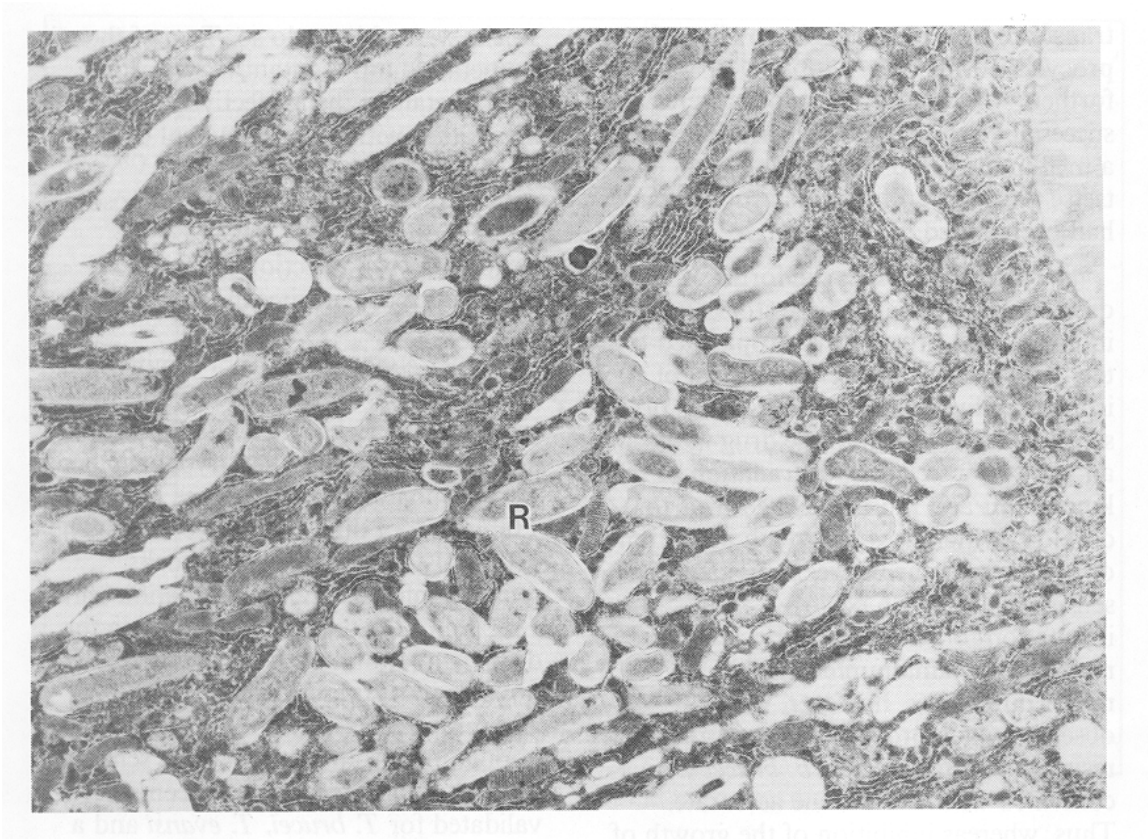


Figure 15. *Electron micrograph of part of a midgut epithelial cell from a 30-day-old non-teneral* Glossina morsitans centralis*, showing the presence of large numbers of rickettsia-like organisms (R) throughout the cytoplasm. Magnificatio*n = ×5,300.

Further entomological research at ILRAD shows that the developmental cycle of *T. vivax* in tsetse, thought to be confined to the proboscis of the fly, may extend to the cibarial-oesophageal region, the most anterior part of the digestive tract of a tsetse. Tsetse proboscides were excised at intervals beginning one hour after an infected feed and transferred to culture dishes. Parasite multiplication and full cyclical development were observed only in proboscides excised four or more hours after the infected blood meal. It thus appears that in some tsetse, development of *T. vivax* through its vector stages is initiated in the cibarial/ oesophageal region, from where the parasites migrate to the food canal of the proboscis, where maturation to the metacyclic forms completes the life cycle of the parasite.

Early in 1988 a tsetse control campaign conducted by the African Trypanotolerant Livestock Network was started in Côte d'Ivoire using biconical traps impregnated with cypermethrin insecticide. Initial results indicate that this control method can reduce tsetse populations by more than 98%.

A comparison of tsetse blood meals at two sites in Zaire and one site in Gabon demonstrated that *Glossina tabaniformis*—a species of tsetse belonging to the fusca, or forest-dwelling group—takes a considerable proportion of feeds from cattle in these areas when this species comes into contact with the cattle. Because the fusca group inhabits forest zones of West and Central Africa, most of which are distant from cattle grazing areas, it had previously been thought that *G. tabaniformis* contributed little to trypanosome transmission.

Trypanotolerance

Trypanotolerant livestock, principally the N'Dama breed of cattle from West Africa, offer another means of improving agricultural productivity in tsetse-infected areas. ILRAD collaborates with the International Livestock Centre for Africa (ILCA [Addis Ababa]) and national livestock ministries and development programs in West and Central Africa in the African Trypanotolerant Livestock Network. With this network and the International Trypanotolerance Centre (the Gambia), ILRAD is studying the productivity of trypanotolerant livestock under different levels of trypanosomiasis risk and is seeking economically viable ways to increase the productivity of these livestock.

ILRAD contributes to the network's activities by conducting collaborative programs on bovine genetics and trypanocide use and by helping national organizations in six countries apply standardized techniques in the collection of entomological and animal health data. A statistically significant relationship has been established at several sites between the monthly estimates of tsetse challenge and trypanosome prevalence in trypanotolerant cattle.

In a search for genetic markers of trypanotolerance, two polymorphic systems of bovine lymphocyte antigens were studied in 1988 in collaboration with ILCA. These systems are the major histocompatibility complex (MHC) and a more limited polymorphic system of common leucocyte antigens, which was detected in cattle only recently. The first objective of the study was to survey the MHC and common leucocyte antigen phenotypes of populations of N'Dama cattle in Zaire and the Gambia and to compare these phenotypes with corresponding profiles of trypanosensitive Boran cattle in Kenya. The second objective was to look for associations between these MHC and common leucocyte antigen phenotypes, trypanotolerance and the productivity of N'Dama cattle. Significant correlations have been found between the two classes of lymphocyte markers and the degree of resistance shown by trypanotolerant cattle exposed to trypanosomiasis by natural challenge. These provocative results, which suggest that there is a genetically selectable marker for the trypanotolerant trait, are being investigated further using larger numbers as well as family groups of cattle. The results also indicate a central role for immunity in the manifestation of the trypanotolerant trait.

ILRAD's embryo transfer experiments continued in 1988. N'Dama heifers produced at ILRAD from frozen N'Dama embryos brought in 1983 from the Gambia have since 1987 been regularly induced to superovulate using Folltropin or follicle-stimulating hormone derived from pigs. By implanting the best of the N'Dama embryos in Boran foster mothers, ILRAD has produced 24 N'Dama calves, which are used in studies of trypanotolerance and bovine genetics. ILRAD hopes to produce twin N'Dama calves by using these techniques so that research may be carried out on genetically matched animals.

The African Trypanotolerant Livestock Network will continue to evaluate the performance of trypanotolerant livestock in tsetse-infested areas of Africa, to seek ways to exploit the genetic resistance to trypanosomiasis in such livestock and to determine the most economic ways of improving the productivity of these livestock by nutritional and breeding strategies.

Long-term strategies for trypanosomiasis control

The biology of the trypanosome

The current methods used to control trypanosomiasis in cattle breeds other than N'Dama are highly susceptible to breakdown. The search for novel and more sustainable control measures for these breeds therefore forms the basis of ILRAD's long-term trypanosomiasis research program. Emphasis in this program is put on finding immunological ways to control the disease. Research is being conducted on the biology of the parasite in an attempt to discover processes unique to the parasite that could be the target of interventions. The aims of research on host-parasite interactions are to elucidate host mechanisms involved in controlling and destroying the parasite and then to enhance these mechanisms so as to reduce or alleviate the pathogenic manifestations of the disease. Research projects on basic parasite processes, such as the uptake of nutrients and the control of differentiation, were consolidated during the year and methods to cultivate trypanosomes were improved.

Antigenic variation

The variable surface glycoproteins (VSGs), which make up the surface coat of the trypanosome, bear a complex lipid-containing structure at their carboxyl end called the glycosylphosphatidylinositol, or GPI moiety. Part of this structure is responsible for anchoring the VSG molecules in the plasma membrane of the trypanosome. Unlike the protein part of VSG molecules, which differs from one VSG to another, the hydrophobic GPI anchor is similar in many of the surface coat molecules examined from *T*. *brucei* and *T*. *congolense.* Antibodies can be raised in rabbits that will react with virtually all VSGs from these trypanosome species because the antibodies bind to a cross-reacting determinant (CRD) within the hydrophobic anchor and not to the variable, protein part of the VSG molecule.

It has been suggested that the lipid portion of the anchor must be removed from membrane-form VSG (mfVSG) by an endogenous VSG-specific phospholipase C (GPI-PLC) before antibodies will bind to the CRD. Using *T. congolense* metacyclic forms from culture and substantiating their findings with bloodstream forms of *T*. *congolense* and *T. vivax*, workers at ILRAD have shown that the method of sample preparation is extremely important: the anti-CRD antibodies will bind to suitably prepared VSGs without prior removal of the lipid. However, the addition of dithiothreitol, which causes the protein part of the VSG molecule to partially unfold, was crucial in these experiments (Figure 16). It would seem, therefore, that a conformational change in the protein part of the VSG molecule is needed to expose the CRD to the antibodies before the antibodies can bind to the CRD.

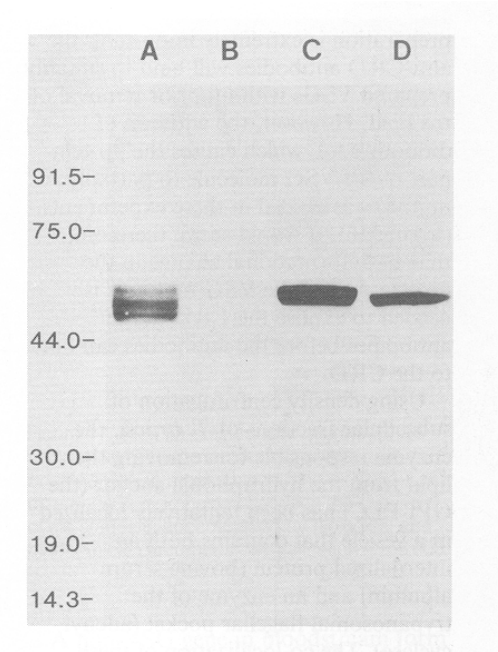


Figure 16. *Western blot probed with anti-CRD (cross-reacting determinant) antibodies illustrates that the binding of anti-CRD antibodies to VSG (variable surface glycoprotein) is dependent upon disulphide bond reduction: Lane A,* Trypanosoma congolense *metacyclics reduced with dithiothreitol; Lane B,* T. congolense *metacyclics without reduction; Lane C,* T. congolense *bloodstream forms reduced with dithiothreitol; Lane D,* T congolense *bloodstream forms without reduction. Metacyclic forms show a qualitative effect in anti-CRD antibody binding, bloodstream forms a quantitative effect.*

Using density centrifugation of subcellular fractions of *T*. *brucei,* the enzyme responsible for removing the lipid from the hydrophobic anchor (the GPI-PLC) has been tentatively localized in a vesicle that contains both an internalized protein (bovine serum albumin) and an enzyme of the trypanosomal flagellar pocket (adenyl cyclase). The co-localization of these three reagents suggests that the GPI-PLC may reside in a vesicle near the flagellar pocket and that it may play an important role in recycling VSG.

At the molecular genetic level, metacyclic VSG genes of *T*. *congolense* IL 3000 have been cloned and expressed in the bacterium *Escherichia coli.* This has provided nucleotide sequence data for the VSGs of genuine metacyclic trypanosomes. The only material previously available for examination comprised bloodstream-form trypanosomes expressing cross-reactive VSGs. The genetic context in which these metacyclic VSG genes are expressed is now being examined. This is important because metacyclic trypanosomes express a constant but smaller set of VSGs than bloodstream forms belonging to the same repertoire.

Differences have already been observed between the two metacyclic VSG genes that have been cloned, mVSG1 and mVSG2. Only one copy of mVSG1 is conserved in all the developmental stages examined; at least two copies of mVSG2 exist in the metacyclic trypanosomes. Each of the metacyclic VSG genes is expressed at a different locus on chromosomes that are 2 megabases in size. One gene, mVSG1, is activated *in situ*; the other, mVSG2, is rearranged when it is expressed.

Work is in progress to determine the amino acid sequence and ancillary moieties of two VSGs from rodent-infective *T. vivax* clones. No peptide homology between the two VSGs has been demonstrated by using serological techniques and little information on the amino acid sequence has been obtained from analyses of purified VSGs. However, the importance of this work was manifested in 1988 with the discovery that, unlike the little antigenic cross-reactivity

observed among geographically different populations of *T. brucei* or *T. congolense,* extensive antigenic cross-reactions occur among the variable antigen repertoires of cloned *T. vivax* populations derived from parasite populations isolated from widely different geographical locations across Africa. The cross-reactivity may be due to variable antigen genes shared among serodemes or to shared epitopes on the variable surface antigen molecules themselves. The assay used to analyse this cross-reactivity was immune lysis of bloodstream-form parasites. The assay demonstrates that the cross-reactivity occurs at the cell surface of living trypanosomes and not just between isolated molecules. Use of this assay has strengthened the view that the occurrence of cross-reactive antigens in *T. vivax* stocks contributes to the induction of immunity to this parasite species.

Differentiation

Although antigenic variation is the primary pathway used by the trypanosome to ensure its survival in the mammalian host, physiological mechanisms in the parasite that limit the numbers of parasites in the mammal also ensure trypanosomal survival by ensuring that some parasites are transmitted onward to the parasite's intermediate host, the tsetse fly. One of ILRAD's trypanosomiasis research areas focuses on genetic and biochemical work to identify the mechanisms in the parasite that control the parasite's proliferation and differentiation.

The change in bloodstream *T. brucei* trypanosomes from actively dividing slender forms to non-dividing stumpy forms (Figure 17) is of particular interest to ILRAD since this switch may provide a clue to the regulatory genes and sequences responsible for the differentiation. The aim of research in this area is first to understand the mechanisms involved in the differentiation process and then to design artificial mechanisms that mimic the process.

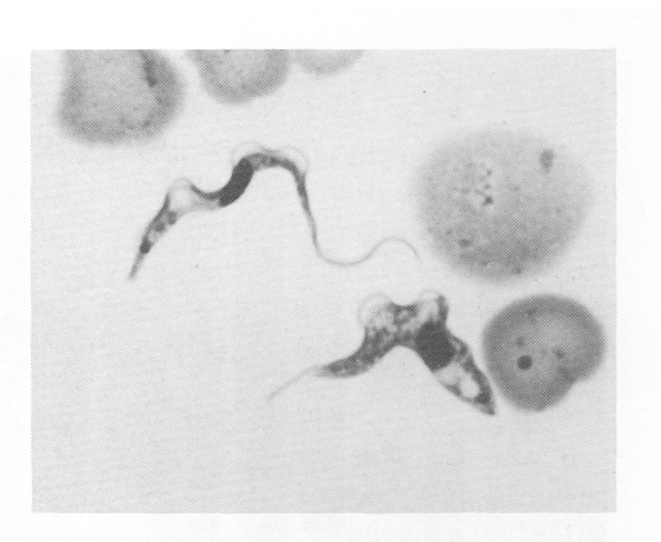


Figure 17. *Giemsa-stained light micrograph of slender and stumpy forms of* Trypanosoma brucei brucei *in mouse blood. The clear difference in morphology between the two life cycle stages is accompanied by biological and biochemical differences. Magnification = × 8,000.*

Libraries encoding complementary DNA (cDNA) from both slender and stumpy form trypanosomes have been constructed in lambda phage. Simple differential hybridization screening revealed no clones encoding differentially expressed sequences, indicating that the numbers and/or levels of differentially expressed sequences between the two forms are low.

A cDNA library enriched in stumpy specific sequences has been generated by subtractive hybridization selection of stage-specific sequences by copper chelate chromatography. Similar experiments to generate a library of slender enriched sequences failed. It is not yet clear whether this indicates that all sequences expressed in slender forms are also expressed in

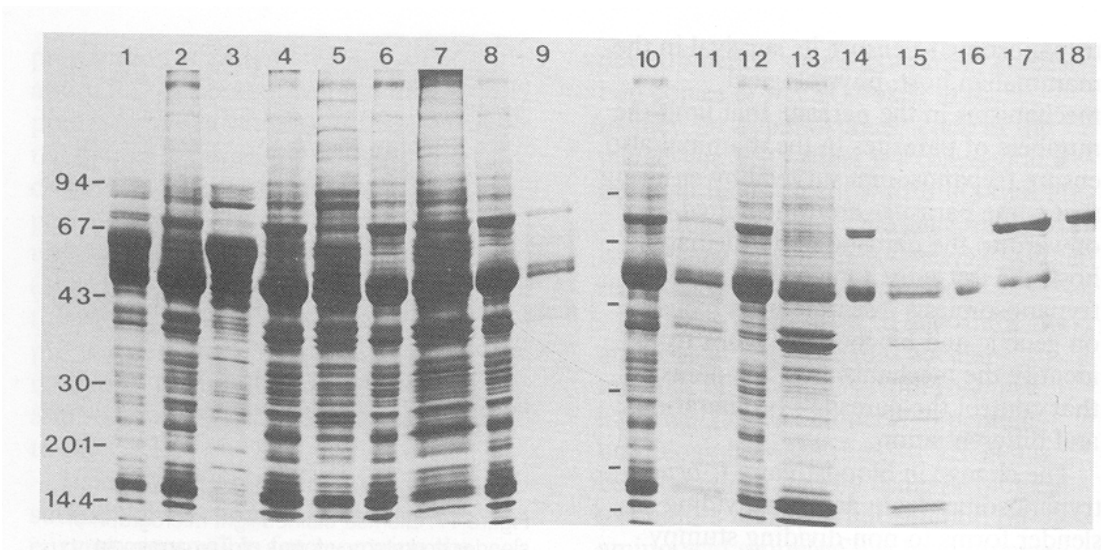
stumpy forms. ILRAD scientists intend to determine the function of the unique sequences by using the transfection systems now being developed for *T. brucei* (see below).

A non-VSG gene in bloodstream form *T. congolense,* which is amplified and developmentally regulated, has been identified. A detailed analysis of this gene may lead to the first identification of a trypanosomal promoter of transcription.

At the protein level, two *T*. *brucei* proteins, having molecular weights of 42 and 37 kDa, have been shown to increase their degree of phosphorylation during differentiation from long slender to stumpy forms. The two proteins appear to be phosphorylated by different enzymes, both enzymes being similar to casein kinase II but distinguished by their relative sensitivities to the inhibitors heparin and spermine. These enzymes, which differ from mammalian kinases in that they are inhibited rather than activated by polyamines and polycations, will be studied further.

Efforts were begun in 1988 to demonstrate whether metacyclic trypanosomes have a characteristic metabolism. Metacyclics of *T*. *congolense* derived axenically are, in terms of mitochondrial respiration, a stable metabolic intermediate between procyclic/epimastigote forms and bloodstream forms and may in some respects differ from metacyclics derived from tsetse. Preliminary evidence from *T*. *brucei* metacyclics, derived from feeder-layer cultures, suggested metabolic differences from *T*. *congolense* metacyclics.

ILRAD recently purified trypanosome intermediate filaments, a class of structural proteins, and then produced antibodies to the filaments so as to study both the distribution of this class of filaments in trypanosomes (Figure 18) and the possible developmental regulation of filament production and organization.



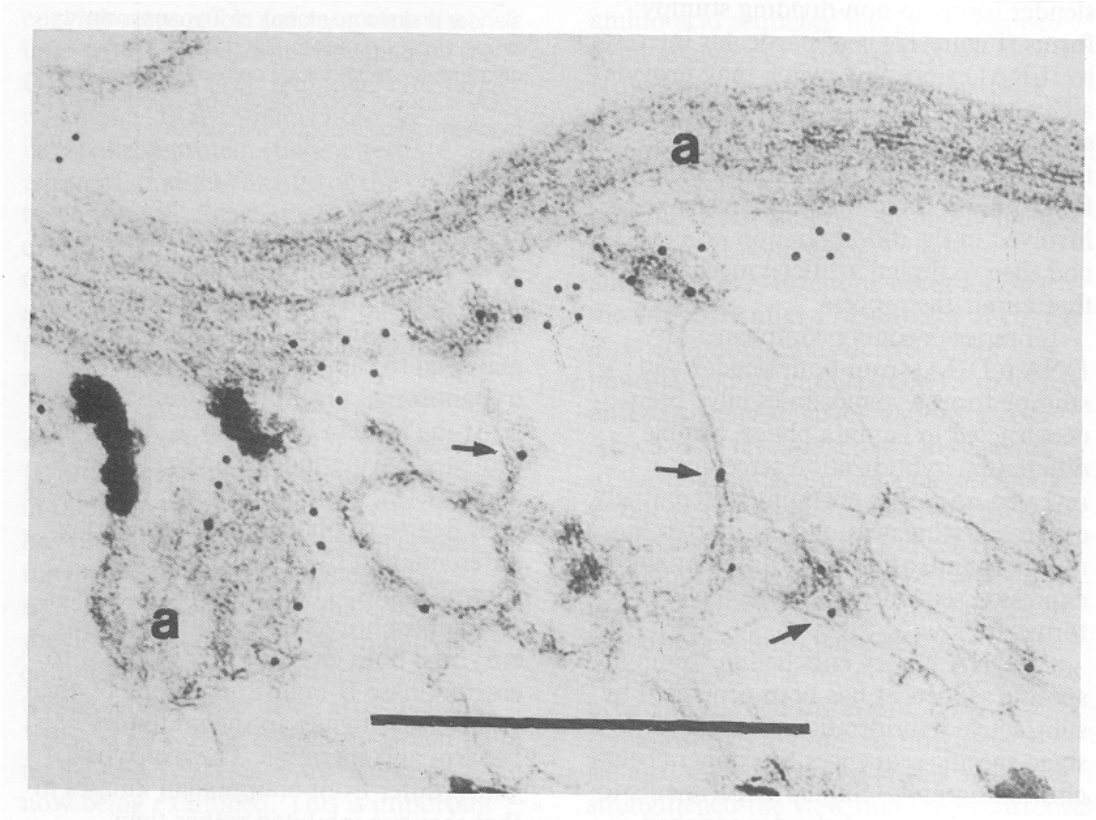


Figure 18. *(Top)* A Coomasie-stained SDS-PAGE (sodium dodecyl sulphate polyacrylamide-gel electrophoresis) analysis of Trypanosoma brucei ILTat 1.1 preparations at various stages of purification of the 80-kDa protein. A lysate of whole trypanosomes is shown in Lane 1. Lanes 3-17 show the gradual enrichment of Tp80 by extraction of lysates with high and low salt solutions, in the presence or absence of the detergent Triton ×-100. Purified Tp80 is shown in Lane 18. (Bottom) An electron micrograph showing the reactivity of antibodies to Tp80 with cytoskeletal ghosts of T. brucei. The distribution of bound antibodies was revealed by incubation with protein A-gold prior to embedding. The micrograph shows the flagellum and some associated filaments. The antibody labelled the paraflagellar rod and the filament-like structures (arrows) but not the axoneme (a) of the flagellum. Bar = 0.5µm.

Developing genetic systems

Encouraging progress has been made in developing a system for transfecting genetic material into trypanosomes in order to study the function of a given trypanosome gene. A series of vectors has been constructed to allow either transient or stable expression of the transfected DNA. Transient expression of transfected DNA has been detected and studies are under way to determine if stable expression of transfected materials has also been achieved.

Another powerful technique in the search for single-copy genes, or their transcripts in low number, is the polymerase chain reaction (PCR). This procedure makes it possible to amplify DNA present in very low copy number if information is available concerning the context of the gene, that is, the sequence data from the sites at either end of the gene. The PCR technique has been used successfully at ILRAD in trial experiments using VSG genes.The purchase of a PCR machine by ILRAD will greatly enhance investigations into the area of differentiation by facilitating the study of single-copy genes and their regulation.

Endocytosis

Host factors that trigger or support parasite multiplication or differentiation are being identified.

Host transferrin (an iron-carrying molecule in serum), high and low-density lipoproteins and a group of as yet unidentified factors in plasma have been shown to be essential for parasite multiplication.

Using horseradish peroxidase and colloidal gold coupled to serum proteins, the process by which cells take up nutrients or reagents—called endocytosis—can be demonstrated, and the organelles involved in the endocytotic pathway can be revealed. The uptake of nutrients by trypanosomes is of interest to ILRAD's researchers because in the flagellar pocket, through which nutrients enter the parasite, receptors may exist that specifically bind to nutrient molecules; if such receptors exist, they could perhaps be exploited in chemotherapeutic or immunological attacks on the parasite.

The uptake of lipids by trypanosomes from lipoproteins in their hosts was studied intensively during the year. Lipid uptake appears not to be mediated by receptors, as proposed by researchers in other laboratories. There is evidence, however, that trypanosomes ingest iron through a process of transferrin-receptor-mediated endocytosis. The amount of iron accumulated by cells at 37° C was quantified and the results suggest that the putative transferrin receptors either turn over extremely rapidly or shuttle back and forth from the cell surface to an intracellular location (Figure 19).

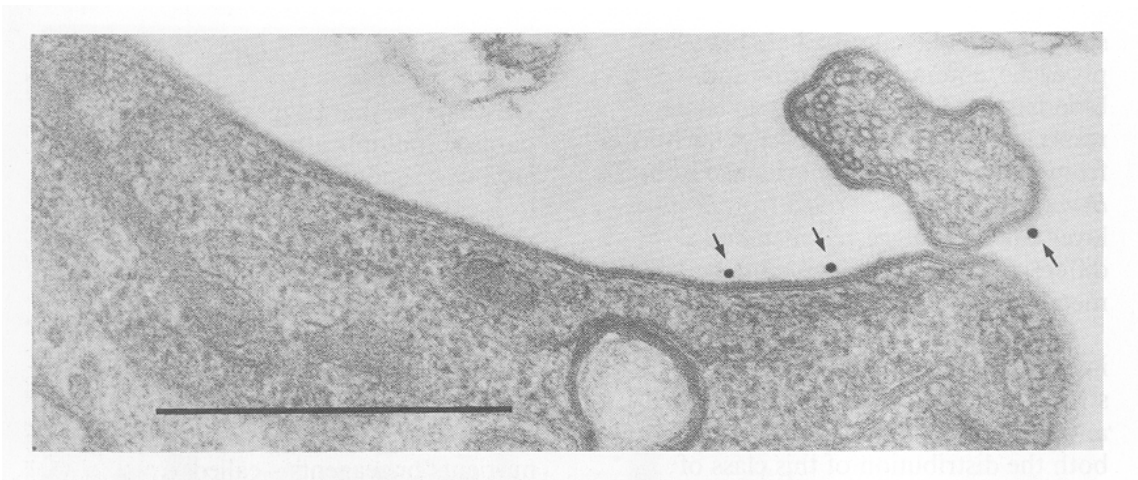


Figure 19. *Electron micrograph showing colloidal gold particles coupled to bovine transferrin (arrows) binding to the surface of living trypanosomes. The binding is inhibited in the presence of free transferrin (not shown). Bar = 0.5µm.*

Cells do not take up significant quantities of iron in the absence of transferrin. Morphological experiments show that iron-bound transferrin, but not transferrin lacking iron, binds to the cell surface at 0° C. Upon warming to 37° C, the iron-bound transferrin clusters on the flagellum and in the flagellar pocket, from where it is taken up into tubularvesicular organelles. Further experiments will be carried out in 1989 to attempt to isolate and characterize the transferrin receptor in trypanosomes.

Morphological studies have demonstrated that most reagents internalized by trypanosomes accumulate in a distinct region between the nucleus and the flagellar pocket. Vesicles that appear to be the first in a series of intracellular organelles involved in endocytosis have been purified from African trypanosomes, and antibodies to a 70-kDa protein appear to identify endosomes or lysosomes, two organelles in the endocytotic pathway (Figure 20). The demonstration of these structural markers has been complemented by advances in enzymatic characterization of the endocytotic process. Lysosomal thiol-dependent proteases have been identified and partially purified from both *T. brucei* and *T. congolense.* The *T. congolense* enzyme, which has been characterized in more detail, shows inhibitor and activator profiles suggestive of the cysteine class of proteases. These enzymes, as well as hydrolases in the

flagellar pocket, may thus identify compartments in the endocytotic pathway of trypanosomes as well as potential targets for inhibitors of nutrient processing in the parasites.

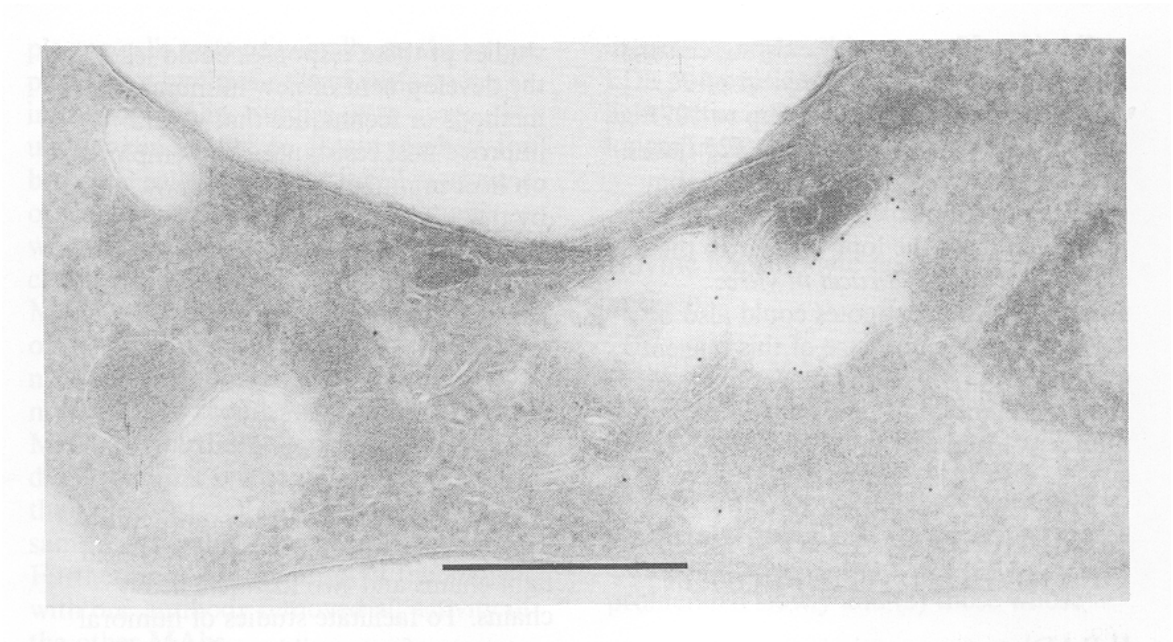


Figure 20. *Electron micrograph showing antibodies that recognize the 70-kDa protein binding to membranes of vesicles inside trypanosomes. The antibody binding is visualized with colloidal gold particles coupled to protein A. Bar = 0.5µm.*

The flagellar pocket and its associated enzyme-containing, intracellular, endocytotic structures will continue to be studied closely to determine the roles these structures play not only in the metabolism and turnover of variable surface antigens, but also in the metabolism and endocytosis of vital nutrients and other molecules required for trypanosome multiplication and differentiation.

Cultivating trypanosomes

The basic studies of the trypanosome undertaken at ILRAD are supported by refinements in the systems used to cultivate the three major parasite species through all stages of their life cycles. Good progress was made particularly in growing trypanosomes in simplified systems, without supporting feeder-layer cells or with a reduced concentration of added serum.

For several years cultures of the vector stages (epimastigote and metacylic) of various *T. vivax* stock and a West East African *T. vivax* stock and a West African *T. vivax* stock were established as vector-stage culturs in the above manner and then subcultured in Eagle's modified Minimum Essential Medium, supplemented with 20% foetal bovine serum, L-glutamine, hypoxanthine and non-essential amino acids, but without feeder-layer cells. More importantly, vector-stage cultures of another East African *T. vivax* stock were established directly from bloodstream forms and without feeder-layer cells (Figure 21). Metacyclics from these *T.vivax* cultures were shown to be infective for mammals.

After testing a number of commercial serum substitutes, it was found that serum substitutes, it was found that Serum Plus^{TM} (a medium supplement containing very low levels of serum proteins) adequately supports the growth of bloodstream forms of *T. brucei in vitro.* The medium supporting highest density of trypanosomes (2.2×10^6bloodstream forms/ml) used modified Minimum Essential Medium containing 20% Serum Plus, 500 µM bathocuproine disulphoric acid. This may prove useful in the development of a serum-free medium, but from

a practical point of view, Serum Plus is an economical alternative, reducing the cost to less than one-third of that of using foetal bovine serum.

A different system has been used to grow *T. congolense* IL 3000 *in vitro* for the production of metacyclic forms in numbers sufficient for research purposes. The introduction of Nunc Cell Factories, each 'factory' having a surface area of 600 cm^2, has led to single harvests of up to 5×10^9 metacyclic trypanosomes of this clone and has greatly facilitated the biochemical and molecular biological studies of the parasite.

Further refinements of existing culture systems have increased the yield of *T. brucei* metacyclic forms and up to 107 can now be harvested from 40 T25 flasks. Studies employing bovine haemoglobin have suggested a role for this reagent in the switch from the long slender to the stumpy form of *T. brucei in vitro*. Procyclic trypomastigotes could also be generated in the presence of this reagent even at 37°C, indicating that trypanosome differentiation may depend on a number of signals.

Immunological responses of the host to infection

ILRAD's trypanosomiasis research continues to focus on immune responses of ruminants to the disease because studies of these responses could lead to the development of new immunization methods or techniques that would improve host resistance. This emphasis on host immune responses is now aided by the wide range of reagents that ILRAD has developed over the last decade to define the elements of the ruminant immune system.

Reagents and assays for immunological studies

An antibody molecule is composed of four polypeptide chains-two identical light chains and two identical heavy chains. To facilitate studies of humoral responses of susceptible and tolerant breeds of cattle infected with trypanosomes, ILRAD has produced monoclonal antibodies (MAbs) that specifically detect bovine light chains of the following classes of immunoglobulins (Ig): IgM, IgG, IgG_1, IgG_2 and IgA. To determine whether any MAbs detected allotypic determinants, each pair of MAbs was tested on 50 to 100 *Bos taurus* and *Bos indicus* cattle. Only MAb ILA50 (speck for IgM) was shown to recognize an allotypic determinant.

These MAbs have been used to develop a sandwich ELISA to measure changes in total immunoglobulin levels and trypanosome-specific immunoglobulin levels during infection. An inhibition ELISA has also been developed to measure low amounts of immunoglobulin secreted during *in vitro* assays.

Markers for bovine B cells

ILRAD researchers are now well equipped with MAbs that can distinguish various bovine T-cell subsets, but our ability to recognize B cells and their maturation to antibody-producing plasma cells rests principally on the possession by these cells of surface immunoglobulin. Studies were undertaken in 1988 to obtain markers for bovine B cells. A mature B-cell marker, of 150 kDa under reducing conditions, was detected by three different monoclonal antibodies (Figure 22). All three MAbs stained the B-cell areas in sections of lymph node, tonsil and spleen and did not stain thymocytes, peripheral T cells, null cells and monocytes. Although the MAbs detected different epitopes (they did not inhibit binding of one another to the B-cell surface), they precipitated the same 150-kDa membrane molecule. Furthermore, precipitation of the antigen with one antibody removed all activity of the other MAbs.

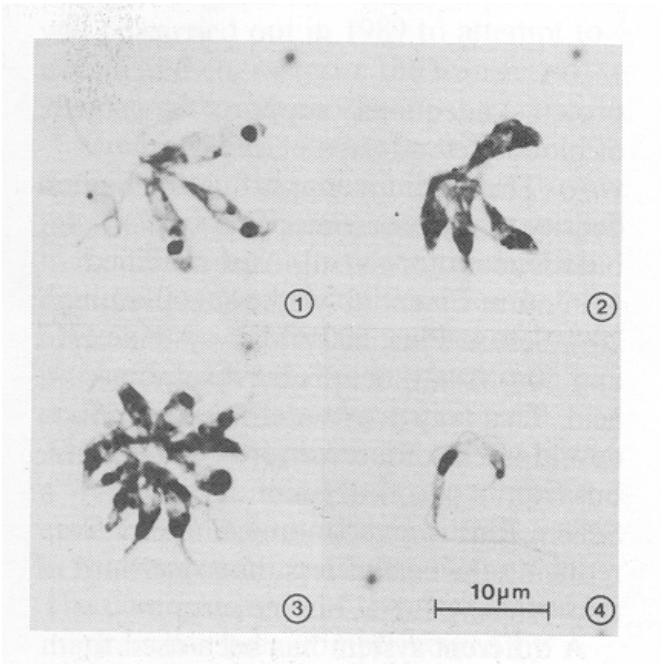


Figure 21. *Bright-field light micrographs of Giemsa-stained insect forms of* Trypanosoma vivax. *East African stock CP 2331, from axenic culture. All magnifications × 1800. Pictures 1 and 2 show rosette formation of non-infective epimastigotes attached to plastic. The kinetoplast is anterior to the nucleus. Picture 3 shows an increase in rosette size due to dividing epimastigotes. Picture 4 shows free-swimming infective metacyclics with a prominent undulating membrane and a terminal kinetoplast.*

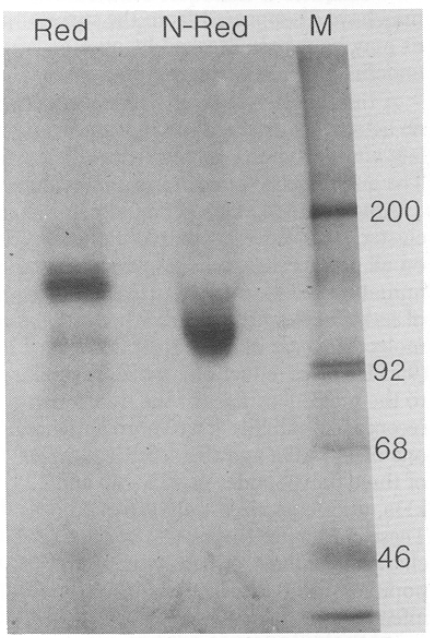


Figure 22. *The molecular weight of a B lymphocyte membrane antigen was determined by electrophoresis under reducing (Red: a band of 150 kDa) and non-reducing (N-Red: a band of 120 kDa) conditions. This molecular weight is similar to the human antigen CD21, which is the receptor for C3d, a complement fragment.*

A surprising observation was that expression of this antigen was correlated with membrane Ig but the MAbs did not bind to serum Ig or other serum proteins, nor were they inhibited by different anti-Ig antibodies. The marker may be homologous to the human CD21 antigen (a

complement receptor), which has a similar molecular mass and cell distribution, although it has not been reported whether the expression of CD21 is correlated with that of Ig.

There was no evidence for the existence of a δ chain on the surface of bovine lymphocytes. Precipitations of iodinated bovine peripheral blood monocytes and spleen cells with a MAb that detects Ig light chains (which precipitated all the Ig classes from serum) revealed only one heavy chain, identical in molecular mass to the μ chain of IgM. To eliminate the possibility that the bovine δ chain has the same molecular mass as the μ chain (80 kDa), lysates were preabsorbed for IgM. No additional Ig could be precipitated. Either bovine B cells do not express IgD on their surface or IgD does not possess light chains. It thus appears that through some mutational event the gene for the δ chain in cattle is replaced by a gene for the 150kDa polypeptide, which cannot bind light chains and whose expression is no longer independent of the μ gene.

Bovine lymphocyte activation antigens

A further 14 MAbs have been raised that specifically detect bovine T or B activation markers. These MAbs were divided into three groups: (1) those whose expression was highest when cells proliferated rapidly, (2) those whose expression was highest when cells proliferated slowly and (3) those whose expression was not influenced by the speed of proliferation.

Three antigens were characterized in the first group: a 100-kDa antigen found only on activated T cells and with properties similar to the human Tal marker, a 53-kDa antigen found on T and B cells and a third antigen that could not be precipitated but that was found on proliferating T and B cells.

In the second group, an antigen of 185 kDa (125 kDa after reduction) was recognized by at least three antibodies. This antigen was found neither on transformed nor on resting cells. However, it was present on IL-2-dependent T-cell lines, but sometimes varied in its expression, decreasing when cell multiplication increased. This antigen may have disappeared from the surface or may have been masked by an undefined factor during rapid division.

A third cluster of three MAbs defined an activation marker that appeared very late after lectin activation (2-5 weeks). The antigen was found only on T cells.

Among other MAbs that did not cluster, one MAb recognized a marker on all proliferating cells tested and on immature red blood cells up to the stage of reticulocytes. This marker had a molecular mass of more than 200 kDa (95 kDa after reduction), which is similar to the molecular mass of the transferrin receptor in humans. Two more antigens, one of 115 kDa and the other a complex of three polypeptides of 185, 160 and 125 kDa, also recognized only activated cells. These clusters of MAbs will be used to characterize the proliferative states of populations of cells in trypanosome-infected cattle.

Recirculating leucocyte populations in infected cattle

A group of five trypanosome-naive Boran cattle (group 1) were challenged using *Glossina morsitans centralis* infected with *T*. *congolense* clone 13E-8. They were treated when their packed cell volume, commonly used to measure the degree of anaemia, dropped to 15% between 36 and 54 days after infection. A second group of five Boran cattle (group 2) and a third group of five N'Dama cattle (group 3) were each similarly infected with *T congolense* clone 13E-8. Groups 2 and 3 had both 32 months previously been infected with the homologous serodeme. Each animal in groups 2 and 3 had serum antibodies capable of neutralizing the infecting metacyclics prior to infection, and the numbers of chancres that developed in these groups were lower than the numbers in group 1.

The rechallenged Borans (group 2) became parasitaemic and chronically anaemic but did not require treatment before day 70. Three of the five animals in this group eventually cured themselves. The N'Damas, on the other hand, controlled the level and duration of parasitaemia as well as the degree of anaemia and showed no clinical symptoms of the disease.

Low levels of reticulocytes were detected in all three groups of cattle until day 35, despite a greater decrease in the packed cell volume in the two Boran groups. The chronic anaemia in the rechallenged Boran (group 2) was accompanied by significantly increased levels of reticulocytes, although the overall packed cell volumes continued to drop.

Recirculating lymphocyte populations in infected animals were analysed by flow cytometry using MAbs. The levels of B cells increased in both the Borans in group 1 and the N'Damas in group 2. The CD5 antigen was expressed on 50-90% of the peripheral blood B cells in infected animals but on only 5-10% of peripheral blood B cells in uninfected animals. Qualitative and quantitative differences in antibody levels among the groups are being examined.

A decrease in the $CD8^+$ cell population was observed in all three groups of animals, but the N'Dama showed a small increase in $CD4^+$ cells in contrast to a decrease in these cells observed in both the naive and the rechallenged Boran groups. These interesting changes in T-cell subsets during infection are being analysed further.

Mechanisms of antigen presentation

Two *in vitro* systems have been used to study the role of macrophages and B lymphocytes as antigen-presenting cells in cattle and to compare the requirements for presentation of particulate and soluble antigens in cattle. Sheep erythrocytes were used as a particulate antigen in one *in vitro* system and ovalbumin as a soluble antigen in the other. In both systems, secondary *in vitro* cultures were derived from peripheral blood lymphocytes of cattle that had been primed *in vivo* and stimulated with optimal concentrations of antigen.

When antigen binds to antibody molecules on the surface of a resting B cell, it usually initiates a proliferation and differentiation of B cells to cells that secrete soluble antibody. The two cultures in this experiment were tested for cell proliferation after five days and for antibody production (by an antigen-specific ELISA of the culture supernatants) after ten days. The fluorescence-activated cell sorter was used to obtain purified populations of macrophages and B cells that were then returned, in different combinations, to a responding population of bovine peripheral blood lymphocytes that had itself been depleted of all antigen-presenting cells by using the fluorescence-activated cell sorter. The resultant populations were then cultured and tested in comparison with undepleted peripheral blood lymphocytes.

In the sheep erythrocyte-specific cultures, no cell proliferation was observed after the lymphocyte populations were depleted of macrophages; if macrophages were returned to a population depleted of all antigen-presenting cells, cell proliferation returned to expected levels. In contrast, putting purified B cells back into a population did not initiate cell proliferation. In the ovalbumin-specific cultures, macrophages were responsible for antigen presentation and the number of macrophages added to the lymphocyte population correlated with the degree of the proliferative response. B cells also presented antigen in this system, but less efficiently than macrophages.

These results seem to indicate that in cattle the presentation of particulate antigens is mediated by macrophages alone, whereas the presentation of soluble antigens is mediated by both macrophages and B cells. The relative efficiency of the two cell types in presenting antigen, as well as structural immunological aspects of the presentation process, will be the

subjects of future studies.

Immune responses of goats to *T vivax* infection

In 1988 a number of *T. vivax* stocks from different parts of Africa were cloned in goats and defined and/or purified from isolates containing mixed infections. Goats were then infected with these stocks and the course of infection and the humoral response of the host were studied. All infected goats showed a capacity to mount antibody responses against surface and internal antigens of *T. vivax*, but the timing of the appearance of these antibodies varied with the timing of the appearance of the parasites. Antibody responses occurred 5 to 6 days after the appearance of parasites in infections that subsequently became chronic, 7 to 8 days after the appearance of parasites in acute infections, and coincidentally with the appearance of parasites in transient infections.

Parasite-specific responses were tested by immune lysis and ELISA. Purified VSG of the infecting VATs was used as antigen in the ELISA. Recurrent peaks of lytic antibody and IgM, and sometimes IgGI, to the infecting VAT occurred in both cattle and goats, suggesting that the reappearance of VATS, or serologically cross-reactive VATs, is more common in *T. vivax* infections than in *T*. *brucei* or *T congolense* (Figure 23).

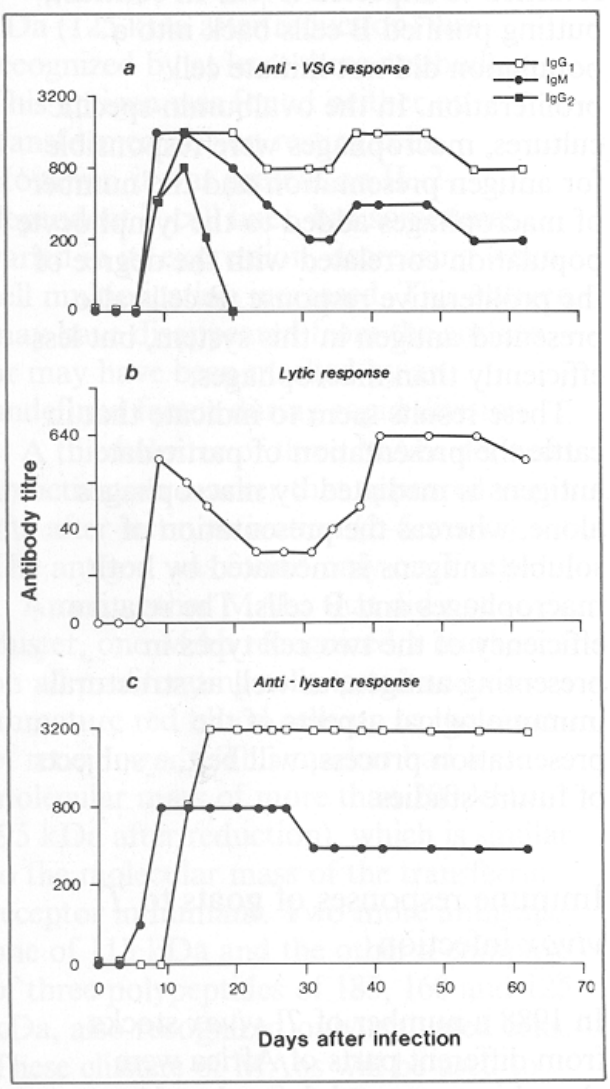


Figure 23. *Evidence for recurring peaks of anti-VSG (variable surface glycoprotein) activity*

appearing in a goat infected with Trypanosoma vivax *ILDat 1.2 shows: (a) that there are second peaks of antibodies to isolated ILDat 1.2 VSG; (b) that the anti-VSG response is mirrored by the lytic response against live ILDat 1.2 trypanosomes; but (c) that there is no secondary response to the common antigens present in a lysate of ILDat 1.2 trypanosomes.*

Antibody secretion in single cells

The studies using ruminant hosts have been supported by investigations into the immunological basis of resistance in mice to *T. b. brucei* infection. Previous work with a resistant, C57B 1 / 6, and a susceptible, C3H/ He, strain of mice infected with a clone of *T. b. brucei* (GUTat 3.1) had shown that the two strains developed similar numbers of cytoplasmic Ig-positive B cells in the spleen with similar kinetics, and that these cells contained similar amounts of parasite-specific antibody. However, the resistant strain had detectable parasite-specific antibody in its serum from day 5 onwards, whereas the susceptible strain had little or none. From this it has been inferred that there might be a block to Ig secretion in the susceptible strain.

Two new techniques were developed at ILRAD during the year: immunoblots to visualize the secretion products of individual cells and a parasite-specific ELISA using fixed trypanosomes. Use of these techniques showed that secretion was normal in the spleen cells from the susceptible mouse strain, both in the proportion of cells secreting antibody and the amount secreted. Spleen-cell cultures from infected susceptible or resistant mice showed that total and parasite-specific antibody production *in vitro* in both strains were similar up to the point at which the parasitaemia decreased in the resistant strain (the parasitaemia staying high in the susceptible strain). However, after the disappearance of detectable numbers of trypanosomes in the resistant mouse strain, the levels of total IgM and parasite-specific IgM increased greatly. Fixing trypanosomes immediately after removing them from infected mice and using immunofluorescence staining showed that mouse antibody was present on the surface of trypanosomes from both strains of mice. Control trypanosomes from irradiated mice had very little antibody. Thus, the infected, hypersusceptible C3H / He mice produce parasite-specific antibody both *in vivo* and *in vitro.*

Trypanosomes were added to spleen-cell cultures from the resistant mice on day 7, when the mice had no detectable parasites. The amount of parasite-specific antibody decreased in correspondence with increasing numbers of trypanosomes added. The antibody was thus being absorbed by the trypanosomes. The greater numbers of parasites in the susceptible mice—2 to 5 times greater than the numbers in resistant mice at peak parasitaemia—may effectively absorb parasite-specific antibodies so that the antibodies are undetectable and the antibody concentrations remain too low to be able to destroy trypanosomes.

Further investigations of clonal virulence have revealed that the different growth rates of clones of *T. b. brucei* stock S427 parasites in C3H/ He, C57B1/6 and Balb/c mice were related to the infective dose of parasites, the clone studied and the host used. Parasites of different virulence produced different parasitaemic profiles in a single infected host. The capacity of a host to control a parasite of low virulence may be dependent on a host cell or cellular process that is sensitive to irradiation.

The long-lasting effect of *bacterium acnes* (formerly called *Corynebacterium parvum)* treatment to reduce the level of parasitaemia in mice was associated with a relative absence of dividing forms of *T. brucei* in the bloodstream. Previously described biological mediators generally did not induce dividing forms of *T. brucei* to become non-dividing forms in cultures free of accessory cells. However, on injection into mice prior to infection with *T. brucei,* one mediator, tumor necrosis factor (also called TNF/ cachectin), delayed the onset of parasitaemia by an unknown mechanism.

In summary, the advances in ruminant immunology have shown that the number of helper T cells in trypanotolerant N'Dama cattle increases during trypanosome infection, whereas in sensitive Zebu cattle their number decreases. N'Dama are also better able to maintain the activity of their complement system than Zebu. Furthermore, as noted last year, the reticulo-endothelial cells of the N'Dama are better able to ingest and destroy antibody-coated trypanosomes than are those of Zebu. Further research in this area may provide the first indications of the mechanisms involved in trypanotolerance and how they may be manipulated by immunological means to enhance resistance to trypanosomiasis.

Improving host resistance to the pathogenic effects of trypanosomiasis

Research into the mechanisms in livestock that enable the animals to resist the pathogenic effects of trypanosomiasis can be divided into two broad areas: studies of trypanosomal anaemia in cattle, including bovine bone-marrow cultivation *in vitro,* and studies to elucidate effector pathways and molecules that may be involved in host responses to disease.

Anaemia

Anaemia, a reduction in the number of red blood cells normally present in the blood, as well as an apparent dysfunction of leucocytes, white blood cells that include lymphocytes and monocytes, occur in animals suffering from trypanosomiasis. The causes of the anaemia and leucocyte dysfunction are unknown. Studies of the formation of blood cells in cattle infected with a haemorrhagic stock of *T. vivax* continued in 1988. As reported last year, the infected animals developed anaemia; leucopenia, a reduction in the number of leucocytes; and thrombocytopenia, a reduction in the number of platelets in the blood. Other than a moderate increase in the proportion of monocytes in the blood, the leucopenia reflected a reduction in the numbers of all the other white blood cell types.

In this experiment extensive haemorrhage involving many organs occurred in the cattle during and after the second week of infection. Extensive phagocytosis—ingestion of red and white blood cell precursors, erythrocytes (Figure 24) and trypanosomes by macrophages—was also observed. Hyperactivation of the phagocytic system may thus partly account for the reduction in the numbers of normal red and white blood cells in the infected cattle. The underlying stimuli that activate macrophages are still unknown but these may include a direct effect of trypanosome products on host cells. The techniques developed in the ILRAD studies in this area will be extended to include comparisons between the relatively trypano-resistant N'Dama cattle, which suffer only mild anaemia, and the more susceptible Boran animals, which suffer severe anaemia.

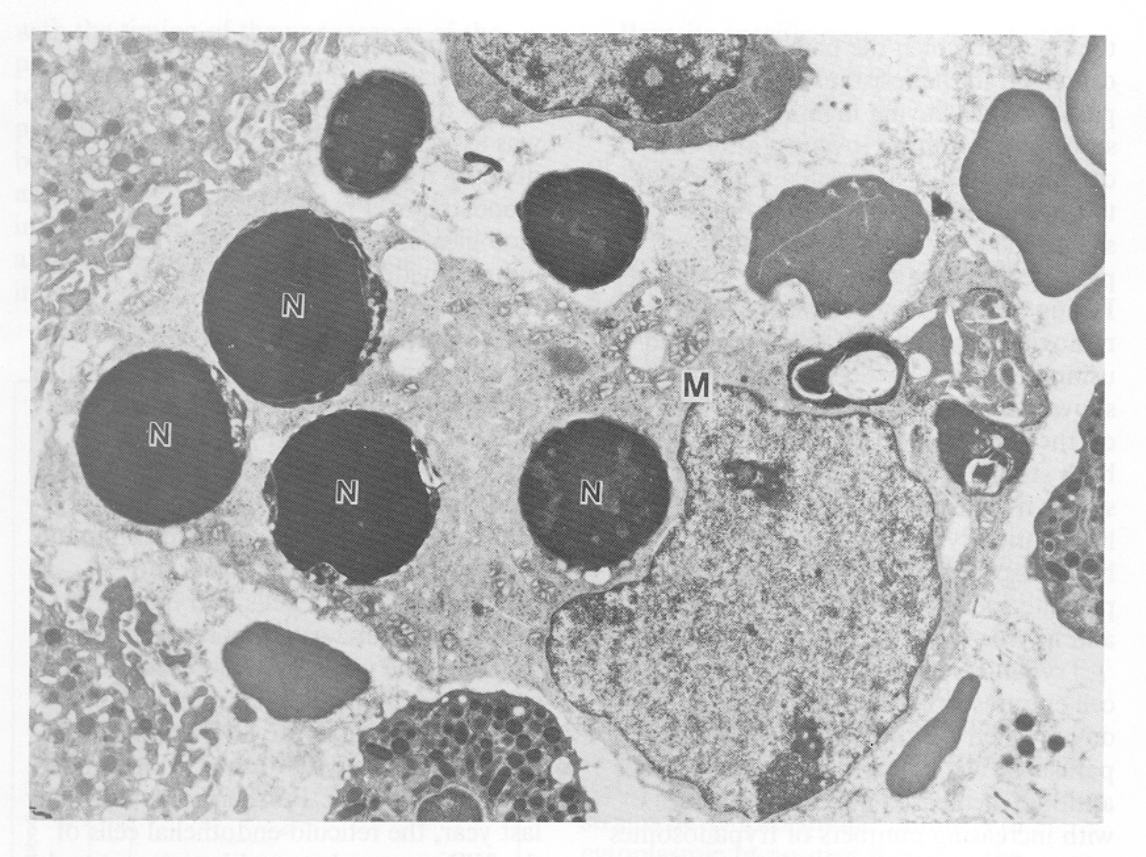


Figure 24. *Electron micrograph of a macrophage (M) in the bone marrow of a* Trypanosoma vivax*-infected calf; the macrophage has phagocytized several normoblasts (immature red blood cells) (N). Magnification = × 5,300.*

Bovine bone marrow cultivation

Both the anaemia and the inefficient immune responses in cattle infected with trypanosomes may arise in part from impaired bone marrow functions. Using the right growth factors, researchers have been able to determine the number of various progenitor cell types—those cells that give rise to the various types of red and white blood cells—in humans and rodents. In cultures of human and rodent cells, soft agar has been used to grow colonies of white blood cells (myeloid), and methylcellulose has been used to grow red blood cells (erythroid) and blood platelets (megakaryocytes). Bone marrow stromal cells were also grown for several months in fluid phase cultures.

The work done in this area in 1988 was focused on finding the most suitable sites and means of obtaining bovine marrow and ways of enriching bone marrow biopsy material for the earliest possible progenitor cells. Use of the many monoclonal antibodies produced at ILRAD that identify bovine cell types enabled workers to eliminate relatively mature cells from bone marrow preparations by incubating the mixed, biopsied cell population with antibodies to mature cell types in the presence of complement. Magnetic beads coated with such antibodies have also been used in similar selection procedures. Using fluorocytometry and cell separations in the fluorescence-activated cell sorter has also made it possible either to eliminate certain cell types or to enrich cell preparations for progenitors.

The long-term goals of this project are to study the effects of trypanosomiasis on bone marrow functions and to gain an understanding of the causes of trypanosome-associated anaemia and immunodepression. In attempts to optimize the growth of cells that will give rise to colonies of cells of a particular lineage, various conditioned media and growth factors,

including human and bovine recombinant IL-2, human recombinant granulocyte-monocyte colony stimulating factor, and murine IL-3, were tested for their ability to stimulate or maintain colony growth. None of these growth factors or interleukins gave good results. However, medium from bovine peripheral blood mononuclear cells, which had been stimulated with the mitogen Con A, contained factors that supported neutrophil, macrophage and eosinophil colonies.

With the use of Iscove's medium supplemented with methylcellulose, 1 % deionized bovine serum albumin, and recombinant erythropoietin, it has been possible to grow short-term erythroid colonies that last between 8 and 15 days, depending on the maturation stage of the progenitors. Attempts to grow long-term cultures, essential for testing the effects of materials from trypanosome-infected animals, have been unsuccessful to date, but use of media containing 2-mercaptoethanol with hydrocortisone has yielded encouraging results.

The difficulty in maintaining pluripotential cells—which are able to give rise to many different types of blood cells—in long-term bone marrow cultures may be due to ignorance of the growth factors required by these cells. Attempts are now being made to improve the survival of such pluripotential cells. As sources of bovine interleukins, produced commercially by recombinant DNA technologies, become available, the role of these substances in controlling the growth and differentiation of blood cells will be studied.

Pathways and molecules involved in host responses to trypanosomiasis

Studies were begun in 1988 to determine the role of complement in trypanosome infections. Complement is a system of blood proteins that may be activated to help destroy microorganisms. In the first of these, a haemolytic assay was developed to quantify the activation of the alternate pathway of complement. Comparison between the haemolytic activity in sera recovered from fully susceptible and relatively resistant animals showed that the latter had higher complement levels. The decrease of complement activity seemed to correlate with parasite load. N'Dama cattle that were immune to a particular serodeme of trypanosomes had higher complement levels than Boran cattle exposed to the same parasite. Polymorphisms of complement components may thus contribute to trypanotolerance and are being studied further.

Previous studies showing the presence of a peptidase in the sera of trypanosome-infected rodents were extended in 1988 to analyse sera of cattle infected with *T*. *congolense.* The parasite peptidase has been shown not only to be present during the course of detectable parasitaemia, but also to persist for several days after the animals have been successfully treated. Elevated levels of circulating hydrolases of parasite origin could well contribute to the pathogenesis of trypanosomiasis, particularly by having subtle effects on regulatory molecules such as hormones.

Earlier studies to assess the endocrine status of *T*. *congolense*-infected cattle showed no significant differences in the basal plasma cortisol levels between control and trypanosome-infected animals. In 1988 workers assessed the capacity of the pituitary to respond to exogenous bovine corticotropin-releasing factor during *T*. *congolense* infection. Sera collected from infected cattle had significantly lower levels of plasma cortisol than sera collected from the same animals before or after their infection (Figure 25). Levels of plasma ACTH (adrenocorticotropic hormone) during patent parasitaemia were not significantly altered by the parasitaemia, which suggests that in cattle infected with *T*. *congolense* there is either an end-organ (adrenal) malfunction or a decreased half-life of cortisol.

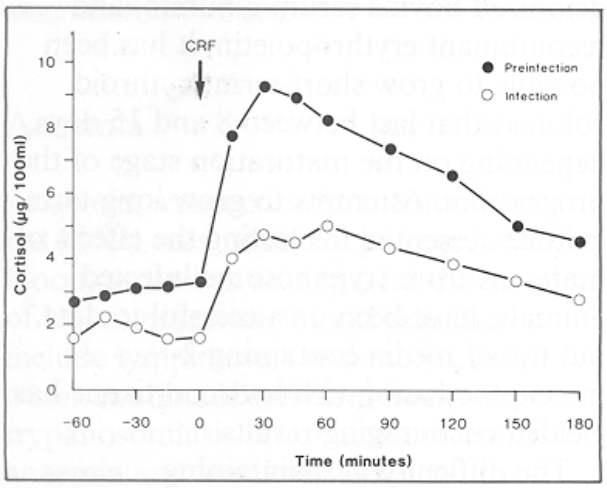


Figure 25. *Graph showing the effect of intravenous administration of bovine corticotropin-releasing factor (bCRF) on plasma cortisol concentrations in cattle before and during their infection with* Trypanosoma congolense. *After injection of bCRF, the mean peak plasma cortisol concentration was 9.3µg/100 ml in animals before they were infected and 4.8 µg/100 ml after they were infected.*

Future trypanosomiasis research work at ILRAD will continue to focus on both the basic biology of the trypanosome and the interactions between the parasite and its animal host, with the aim of identifying targets for immunological or chemotherapeutic attack or ways to enhance livestock resistance to trypanosomiasis.

Epidemiology and Socioeconomics

To feed the growing human population of Africa, land and other natural resources on the continent must be used more efficiently and the health of domestic animals must be improved. ILRAD's newest research unit, Epidemiology and Socioeconomics, is attempting to identify factors that govern the successful application of improved livestock disease control measures—particularly the widespread use of immunization—and to assess the likely impact of improved disease control in epidemiological, economic, social and environmental terms.

Livestock are an important part of African smallholder farms. In addition to providing two of the most important foods in Africa—milk and meat—livestock provide manure, which is commonly used for both fertilizer and fuel, as well as transport and traction for ploughing. The sale of livestock and livestock products also gives small farmers the cash with which to buy essential agricultural inputs such as fertilizers. Many farmers use surplus income to buy more livestock, and such acquisitions in Africa, where livestock often represent a family's total wealth, help households to accumulate much-needed capital.

For these reasons, new methods for controlling the debilitating and often fatal livestock diseases still common in Africa are eagerly sought. In the past, however, the implementation of new control measures that have led to increases in livestock populations have in some instances also led to the erosion of marginally productive lands. Reducing or removing the constraints to livestock production that are now imposed by disease in Africa is therefore not the whole answer to increasing livestock productivity. ILRAD's Epidemiology and Socioeconomics Unit is not only assessing measures to increase productivity, but also examining ways to sustain this increase without degrading the environment.

In 1988, its first complete year of work, the Unit focused on the control of theileriosis and other tick-borne diseases in the countries of eastern, central and southern Africa in which theileriosis occurs. Once the methodologies the Unit is developing to study the impact of tick-borne diseases and their control have been thoroughly tested, they should be applicable to other economically important African livestock diseases.

The economic impact of effective and widespread control of several livestock diseases in Europe and North America has been calculated and these calculations used by policy makers. A few such calculations have also been made for disease control in Africa. The reliability of such calculations depends on the accuracy of the data acquired on both disease incidence

and livestock productivity and on the relationship between the two. Although reliable estimates of the benefits and costs of control programs should help policy makers to evaluate the viability of control measures, insufficient attention has been given, especially in Africa, to the social feasibility and impact of control programs. ILRAD's Epidemiology and Socioeconomics Unit is attempting to redress this inadequacy.

Making a benefit-cost analysis of implementing disease control measures on a single farm can be relatively straightforward if accurate and complete data are available, but such an analysis becomes complex when applied to administrative regions, countries and continents. Furthermore, sociological factors may diminish or negate the apparent benefits of a disease control program. It is difficult to identify and set values on costs that are social as well as financial; identifying and evaluating benefits are even more difficult. For this reason the Epidemiology and Socioeconomics Unit is studying new ways to assess the impact of livestock diseases and their control. This year the Unit has focused on acquiring data, assembling databases and developing research methodologies.

Assembling baseline data

The Unit is assembling an extensive database on factors related to the epidemiology and control of theileriosis and other tick-borne diseases in eastern, central and southern Africa where pathogenic forms of theileriosis occur. These data, which include socioeconomic factors in several countries in this region, were obtained from scientific journals and publications of non-governmental, national and international organizations. In a few study areas information has also been obtained on a farm-by-farm basis on land use, farming systems, livestock production and marketing, access to services such as piped water and roads, agricultural labour and the division of labour within households. All this information is stored in both spreadsheet and database formats to facilitate its retrieval and cross-referencing.

Assessing the impact of disease control

One of the first aims of the Unit was to identify the epidemiological, economic and social factors likely to indicate the impact of immunization in given areas. These factors were then incorporated into models of the impact of disease control being written by the Unit. Once sufficient information relating to these factors has been fed into the model, it should be possible to assess accurately the impact of immunization at the community, country and regional levels. The following kinds of epidemiological data were used as indicators of the impact of immunization in given areas:

- distribution, abundance and movement of livestock
- size, composition, mortality, morbidity and performance of livestock herds
- prevalence of antibodies in defined age groups of cattle to the parasite species that cause disease: *Theileria parva, T. mutans, Babesia bigemina, B. bovis* and *Anaplasma marginal.*
- suitability of the climate and vegetation for the survival and development of ticks, which transmit the parasite that causes East Coast fever
- tick abundance and infection rates
- policy and practice of acaricide use

In many areas the introduction of new, effective measures to control theileriosis will be followed by an introduction of exotic and grade livestock, which formerly could not be raised in these areas because of their susceptibility to tick-borne diseases. The introduction of improved livestock breeds should have a major impact on smallholder dairy farms. The Unit has selected socioeconomic factors with which to identify areas that are suitable for smallholder dairy farming if theileriosis can be controlled. The factors include the following:

- density of the human population
- intensity of land use
- system of land tenure in use
- types of farming and farm management employed
- functions and importance of livestock on the farms
- breeds of livestock kept
- local constraints to livestock production
- farmers perceptions of the risks of livestock production in their areas
- degree to which livestock production is commercialized
- availability of veterinary services
- access to milk-buying centres

Techniques for data analysis

Most research on the epidemiology and control of tick-borne diseases and the socioeconomics of livestock production has been carried out at only a few sites within the study region. Because the epidemiological, ecological and demographic circumstances differ, and often differ greatly, from one area to another among and within the eleven countries affected by theileriosis, these site-specific data cannot be used meaningfully to assess livestock diseases and their effects on production across districts, provinces or countries. On the other hand, it is at the district, provincial and national levels that decisions on disease control policy and implementation are usually made. The Unit is therefore attempting to define general geographical zones and social circumstances according to which particular areas may be classed with respect to ecology, farming systems, disease epidemiology and socioeconomics. These broad categories will then be used to tailor field research and disease control implementation to the circumstances of particular areas.

Geographic information systems

Geographic information systems are computer software that combine sophisticated mapping techniques with powerful database programs so that spatially distributed information may be easily displayed, assimilated and analysed. In a pilot project carried out in collaboration with the Global Environment Monitoring System of the United Nations Environment Programme (UNEP), the Unit has assembled databases comprising the three main determinants of livestock disease epidemiology in Africa: host distribution, vector distribution and disease incidence.

Host distribution

The distribution of cattle across Africa was obtained from the Inter-African Bureau of Animal Resources (IBAR). The distributions of Zebu and Sanga cattle breeds and Zebu-Sanga crosses, as well as the distribution of Cape buffalo, an indigenous wild animal that carries but is not affected by the parasite *T. parva lawrencei,* were obtained from published literature. Accurate distribution data on exotic and grade cattle, the types most susceptible to East Coast fever and other tick-borne diseases unfortunately do not exist in several of the countries under study.

The areas in which livestock may be raised in Africa are restricted for two main reasons: the presence of tsetse flies and the presence of game parks and other protected reserves. Databases on these two parameters were obtained from IBAR and UNEP's Global Environment Monitoring System project and have been used to estimate the present cattle distributions and numbers on the continent.

Vector distribution

Distributions of *R. appendiculatus* and related tick species, such as *R. duttoni, R. nitens* and *R. zambeziensis,* which transmit the parasites that cause theileriosis, were derived from data in published literature, government reports and personal communications. For sites at which sample tick collections have never been made, tick specialists estimated the probable distributions of tick species on the basis of their experience.

For sites from which meteorological data were available, a model called CLIMEX, developed by the Commonwealth Scientific and Industrial Research Organization (Australia), was used to calculate a climatic suitability index of the survival and development of the tick *R. appendiculatus* (Figure 26). In addition to a suitable climate, this species needs vegetation cover in the form of grass and tree canopy to survive. An assessment of vegetation cover is given by the satellite-derived 'Normalized Difference Vegetation Index', or NDVI, which provides a measure of the photosynthetically active vegetation, or 'greenness', of every part of the world every ten days at a resolution of 1 km. The Unit obtained NDVI data for Africa from the Nairobi offices of UNEP and the Regional Centre for Services in Surveying, Mapping and Remote Sensing. These data are being evaluated for their usefulness in predicting the distribution of *R. appendiculatus.*

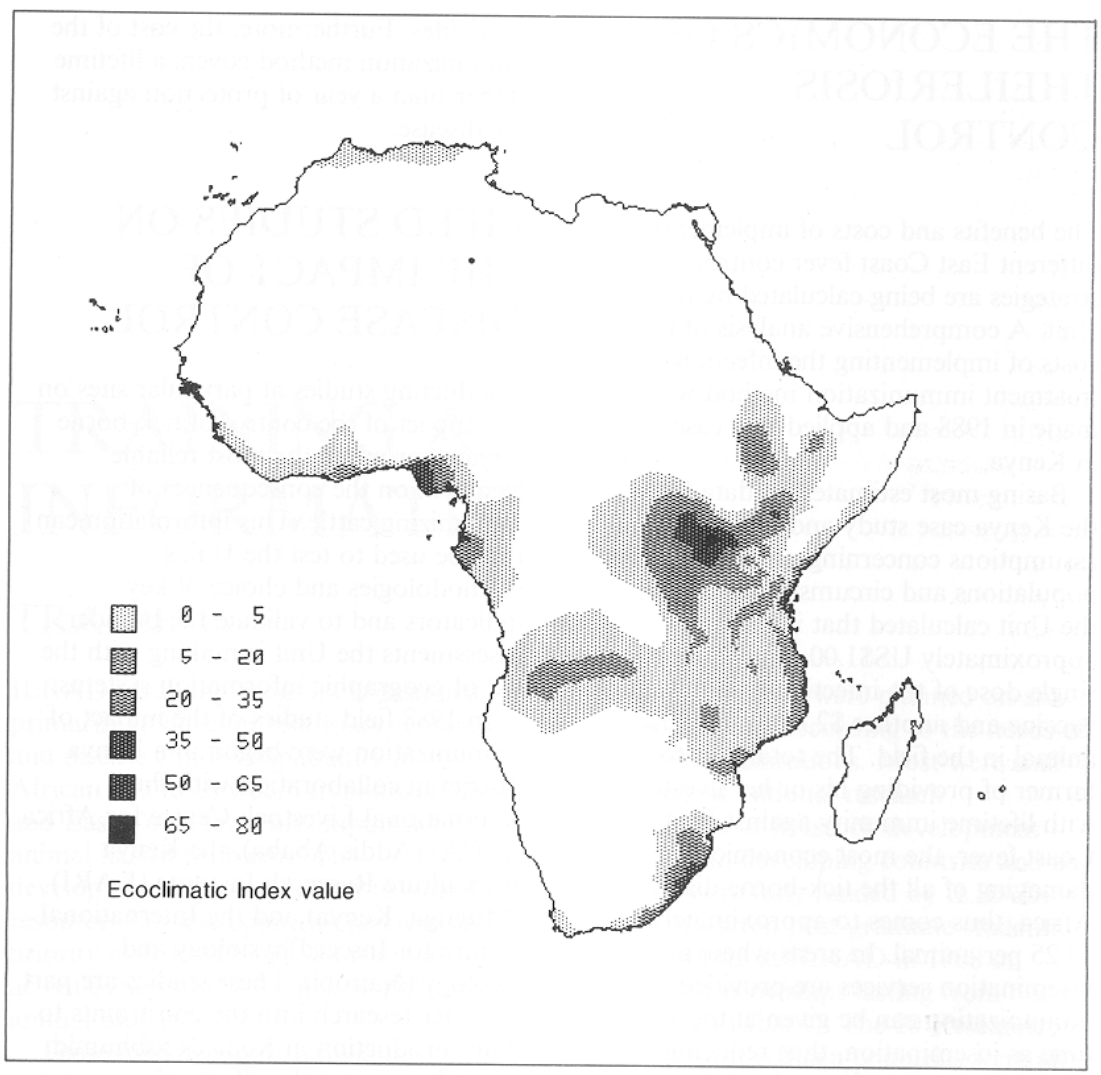


Figure 26. *Map showing the ecoclimatic index values for the survival and development of the tick* Rhipicephalus appendiculatus *in areas of Africa where East Coast fever is endemic. The indices were derived using a CLIMEX computer model and are based on meteorological data*

from approximately 150 meteorological stations on the continent.

Disease incidence

The Unit has assembled from published literature, government reports and personal communications the reported distribution of clinical theileriosis and antibodies to *T*. *parva* in cattle in Africa.

Preliminary epidemiological maps

Using ARC/ INFO, a geographic information systems software package, the Unit produced preliminary maps of the distribution of theileriosis and the animal hosts and tick vectors of the disease in the study region. Relationships among the factors studied were determined by combining and overlaying the maps.

In 1988 the Unit determined the areas in which indigenous livestock are at risk from East Coast fever, the levels of risk and the areas into which the disease may spread. Lack of data on the distribution of exotic and grade livestock makes it difficult to assess accurately the risk of disease to populations of these breeds. The Unit also began to develop a map of areas that are ecologically suitable for the survival and development of the tick *R*. *appendiculatus.* In collaboration with UNEP, the Unit is broadening its epidemiological databases to include factors regarding the socioeconomic status of farmers in the study area and the types of farming systems they employ. In addition, data from field sites in Kenya will be examined to determine relationships between observations made on the ground and remote sensing data obtained by satellite.

The economics of theileriosis control

The benefits and costs of implementing different East Coast fever control strategies are being calculated by the Unit. A comprehensive analysis of the costs of implementing the infection-and-treatment immunization method was made in 1988 and applied to a case study in Kenya.

Basing most estimates on data from the Kenya case study and making some assumptions concerning particular target populations and circumstances of use, the Unit calculated that it will cost approximately US$1.00 to produce a single dose of the infection-and-treatment vaccine and another $2.25 to treat each animal in the field. The total cost to the farmer of providing his or her livestock with lifetime immunity against East Coast fever, the most economically damaging of all the tick-borne diseases in Africa, thus comes to approximately $3.25 per animal. In areas where artificial insemination services are provided, immunization can be given at the same time as insemination, thus reducing the field delivery costs for each of these services.

In contrast, the cost to a Kenya farmer of applying acaricide to one animal for one year ranges from about $0.60 in low-input semi-arid pastoral systems to $6.00 in ranching systems and $8.50 in high-input crop-livestock systems. Although it is true that this relatively high price for acaricide protection covers the cost of controlling all tick-borne diseases for the year rather than controlling East Coast fever alone, it is also true that in much of Kenya East Coast fever is the main tick-borne disease for which farmers use acaricides. Furthermore, the cost of the immunization method covers a lifetime rather than a year of protection against the disease.

Field studies on the impact of disease control

Conducting studies at particular sites on the impact of the control of tick-borne diseases provides the most reliable feedback on the consequences of immunizing cattle. This

information can then be used to test the Unit's methodologies and choice of key indicators and to validate the broader assessments the Unit is making with the aid of geographic information systems.

In 1988 field studies of the impact of immunization were begun in a Kenya district in collaboration with the International Livestock Centre for Africa (ILCA) (Addis Ababa), the Kenya Agriculture Research Institute (KARI) (Muguga, Kenya) and the International Centre for Insect Physiology and Ecology (Nairobi). These studies are part of wider research into the constraints to dairy production in Kenya's subhumid coastal zone and the effects of immunizing livestock against East Coast fever on small and medium-sized farms in the country. Together with KARI and ILCA, the ILRAD team is carrying out farm surveys in Kilifi District, in Kenya's Coast Province, to define basic farming systems, livestock production and disease risk. In longer term studies on selected farms the Unit will identify diseases and other factors that constrain livestock productivity and monitor epidemiological and socioeconomic factors following the immunization of livestock against East Coast fever.

Training and information

Training

ILRAD's training program is designed primarily to develop veterinary research and disease control capabilities in African countries where trypanosomiasis and East Coast fever are important animal health problems. The development of scientific and technical resources in these countries receives high priority at ILRAD: in 1988 training activities accounted for nearly 10% of the annual budget.

These funds support individual training programs for scientists and technicians from laboratories and field programs in Africa and other developing areas, post-graduate training for students working towards master's and doctoral degrees and post-doctoral positions to enhance the professional knowledge and experience of young scientists from many parts of the world. In addition to individual training, workshops, courses and conferences are held throughout the year on topics directly related to ILRAD's research program.

In 1988, 22 scientists and technicians came to ILRAD for specialized technical training for periods lasting from two weeks to four months (Table 1). Their training programs were planned on an individual basis according to the needs of their home institutions. Most were staff members of national research laboratories or livestock development programs in developing countries and all but two were fully funded by ILRAD.

Twenty-seven post-graduate students were working at ILRAD in 1988 on Research Fellowships lasting from one to four years (Table 1). The 20 Research Fellows from African countries were all supported by ILRAD; the 7 Research Fellows from developed countries were supported by their home governments or by funding agencies. These students worked with ILRAD scientists on projects closely related to ILRAD's research program. During 1988 one Research Fellow received a Ph.D. degree, one submitted a thesis for a Ph.D. degree and two submitted work for M.Sc. degrees based on research carried out at ILRAD.

Two Senior Research Fellows worked at ILRAD during 1988, one from Kenya and one from Uganda. This program is for young post-doctoral scientists working at universities or research institutes, principally in Africa, who are invited to ILRAD for several months to enhance their research experience.

Table 1. I*ndividual training at ILRAD in 1987 and 1988: number of participants in different types of training by country of origin. Post-graduate training includes both Research Fellows and Visiting Research Fellows. Post-doctoral training includes both Senior Research Fellows and Post-Doctoral Fellows.*

Country	1987	1988	Country	1987	1988
Specialized technical training					
Benin	–	1	Nigeria	1	1
Burundi	1	–	Sudan	–	2

Canada	–	1	Tanzania	1	-
Côte d'Ivoire	–	1	Uganda	2	2
Ethiopia	–	3	UK	–-	1
India	1	–	Zimbabwe	–	1
Kenya	7	9			
Post-graduate training					
Canada	1	3	Sudan	1	1
Ethiopia	1	1	Tanzania	–	1
Ghana	1	1	Uganda	1	1
Italy	2	3	UK	2	–
Kenya	9	13	USA	1	–
Netherlands	1	1	Zaire	–	1
Nigeria	1	2	Zambia	–	1
Post-doctoral training					
Australia	1	1	Tanzania	1	–
France	1	–	UK	–	5
Ireland	2	2	West Germany	2	2
Kenya	3	2			

Post-Doctoral Fellows are selected on an international basis to meet specific requirements of ILRAD's research program. They normally work at ILRAD for two years. Twelve Post-Doctoral Fellows worked at ILRAD in 1988 (Table 1).

In addition to training activities organized on an individual basis, three training courses were held in 1988, attended by a total of 25 participants from veterinary and research laboratories, universities and ministries in 14 African countries. In July the International Atomic Energy Agency and ILRAD jointly conducted a six-day course on improving the diagnosis and control of trypanosomiasis and other vector-borne diseases of African livestock using immunoassay methods. In September and October ILRAD conducted a seven-week course on the preparation and use of reagents for the diagnosis of haemoprotozoan cattle diseases. In October and November a three-week course was conducted on the control of tick-borne diseases with emphasis on East Coast fever immunization.

ILRAD held a major international workshop in August/ September on protein traffic in parasites and mammalian cells. Participants at the four-day meeting included eleven specialists from seven countries, nine participants from research institutes in Kenya and the University of Nairobi and several ILRAD staff members. In September ILRAD, the Food and Agriculture Organization of the United Nations, the Organization of African Unity and the Malawi Government jointly conducted an international workshop on East Coast fever immunization. The workshop was held in Lilongwe, Malawi, and was attended by 50 scientists working on East Coast fever immunization in eastern, central and southern African countries. ILRAD sponsored eight participants from Burundi, Mozambique, Rwanda, Sudan and Tanzania, one representative from the Southern Africa Development Coordination Conference and six ILRAD staff members working on East Coast fever immunization. The proceedings of both workshops are being published by ILRAD.

Information

The primary responsibility of ILRAD's Information Unit is the publication in English and French of the *ILRAD Annual Report,* an annual *ILRAD Highlights* and a quarterly newsletter, *ILRAD*

Reports, as well as the publication in English and Kiswahili of a weekly *Internal Newsletter,* which is distributed to all staff members and the Board of Directors.

The annual report, annual highlights and quarterly newsletter are intended for a wide range of readers, with emphasis on laboratory and field workers in Africa who are responsible for animal health. In 1988 major articles in *ILRAD Reports* reviewed the development of simple and accurate methods to detect trypanosomes in infected tsetse flies and mammals; recent progress in developing *in vitro* culture systems for the three most important species of African trypanosome; the importance of the major histocompatibility complex (MHC) in bovine immune responses; a report of an international workshop held at ILRAD on MHC class genes and their products; ILRAD's training program; the development of simple, rapid and reliable techniques to diagnose African trypanosomiasis in livestock; and ILRAD's use of embryo transfer techniques to produce groups of Boran calves of particular major histocompatibility types. The newsletter also listed scientific articles and other publications written during the quarter by ILRAD staff.

An *Annual Scientific Report,* comprising short statements on each research project conducted in the institute, was produced for the first time in 1988. The abstracts are written by the scientific and technical teams conducting the projects and outline experimental results they obtained during the year. This publication is intended for scientists and technicians who work outside ILRAD in areas related to the institute's research program, as well as for new ILRAD staff.

Detailed results of ILRAD's research work are published for the most part in international journals and scholarly books that serve a specialized scientific audience. In 1988 ILRAD staff members produced a total of 98 publications, comprising 51 papers in proceedings of international scientific meetings, 41 papers refereed in scientific journals, 4 book chapters, 1 edited proceedings and 1 article in a general magazine. These are listed in the chapter '1988 Publications', appearing below in this annual report.

Papers presented at a week-long international conference held at ILRAD in September 1987 were published in 1988 by Blackwell Scientific Publications for the International Society for Animal Blood Group Research in a special supplement of *Animal Genetics* (Volume 19, Supplement 1). This 83-page volume, *MHC Class II Genes and Products and Their Significance for Disease Research in Livestock Species,* was edited by A.J. Teale, an ILRAD scientist. The volume reviews what is known about the major histocompatibility complex class II regions in livestock species, what still needs to be researched in this area, the most efficient ways of conducting this research in light of similar research being conducted in human and laboratory animals and what the rewards of this research are likely to be regarding ILRAD's search for genes associated with livestock resistance or susceptibility to diseases.

ILRAD and the International Livestock Centre for Africa jointly published a second proceedings volume: *Livestock Production in Tsetse-Affected Areas of Africa.* This 473-page volume contains papers presented at a five-day international workshop held at ILRAD in November 1987. The papers review livestock production in tsetse-affected areas of Africa, collaborative research networks in developing countries, the role of tsetse in African animal trypanosomiasis, the epidemiology of trypanosomiasis in livestock in Africa, genetic and environmental influences on trypanotolerance, the production potential of trypanotolerant livestock and the use of chemotherapy against animal trypanosomiasis.

A publication outlining ILRAD's long-term plan for the years 1988 to 1997, *Meeting the Challenge of Livestock Diseases: ILRAD in the 1990s,* was also published in 1988. This document defines strategic issues and their resource implications that will determine the direction and success of the Laboratory's work over the next decade. The publication describes research progress to date, ILRAD's second external program review, conducted in

1986, and ideas formulated by ILRAD's directors since that review.

Considerable effort is devoted to maintaining and verifying ILRAD's distribution list and responding to requests for publications sent from around the world. The mailing list continued to expand in 1988 as scientists, government officials, librarians, journalists and others wrote asking to receive ILRAD publications on a regular basis (Table 2).

Table 2. Comparison of *ILRAD Reports* distribution at the end of 1987 and the end of 1988.

	Countries		Institutions/ individuals	
Region	1987	1988	1987	1988
Anglophone Africa	24	24	883	945
Francophone Africa	23	23	316	322
Anglophone and other Europe	19	19	254	257
Francophone Europe	2	2	47	50
North and South America	26	25	286	290
Near and Far East	31	31	274	283
Total	125	124	2060	2147

In addition to keeping journalists informed about scientific progress at ILRAD, the Information Unit distributes press releases and photographs to local newspaper reporters when distinguished or well-known visitors come to ILRAD or when ILRAD hosts meetings of interest to the general public. The daily newspapers and other media in Nairobi published seven short articles and one substantial article in 1988 on the research work conducted at ILRAD. The Information Unit also occasionally produces articles for local and foreign popular publications and provides background material and editorial support to journalists writing articles about ILRAD.

Library

The ILRAD Library serves staff members, visiting scientists and participants in the training program. 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 other research institutes and from government departments and universities in Kenya. The collection concentrates on topics related to ILRAD's research program, with emphasis on scientific journals. In 1988 the 294 subscriptions to international journals and 36 monograph series covered such fields as parasitology, immunology, biochemistry, entomology, cell biology, biotechnology, epidemiology, genetics and molecular biology. The Library acquired 430 books, pamphlets and reports in 1988, bringing the total book collection to 2930 volumes.

The ILRAD Library participates in an interlibrary loan network that includes Kenya's university libraries and national and international research institutes. ILRAD borrowed 916 items from other libraries in the network and loaned 301 items to other libraries during the year. The ILRAD Library also donated 472 books and 121 journals to other libraries, notably the Kenya Agricultural Research Institute, at Muguga, and the University of Nairobi's School of Agriculture and Veterinary Medicine Library, at Kabete.

During the year the ILRAD Library hosted a student from the Kenya Polytechnic for two months. The student did practical training in specialized library and information work. Several staff members from other institutions were given demonstrations and information about ILRAD's computerized information services.

The ILRAD Library offers several current awareness services. Announcements of new accessions are published in the weekly ILRAD *Internal Newsletter* and the Library subscribes to several guides to the scientific literature in ILRAD's research areas, such as *Current Contents, Index Veterinarius* and *Tropical Diseases Bulletin.* The Library also subscribes to selective dissemination of information services provided as printouts by Biosciences Information Services (BIOSIS), the International Information System for Agricultural Sciences and Technology (AGRIS) of the Food and Agriculture Organization of the United Nations, and the International Livestock Centre for Africa. In addition, the Library carries out computer-based literature searches for ILRAD staff. In 1988 ILRAD made 38 online literature searches: 34 were made by the International Service for National Agricultural Research and 4 by the Commonwealth Agricultural Bureaux International.

Reprints of scientific articles by ILRAD staff members are supplied on request and free of charge to scientists, technicians and field workers. In 1988, most of these reprints, 495, were sent to workers in Africa; 99 were sent to Asia, 60 to North America and 35 to Europe.

Research Support

Tsetse laboratory

A great deal of the trypanosomiasis research conducted at ILRAD requires trypanosomes that have developed in tsetse flies. Researchers studying trypanosomiasis transmission and parasite development in the vector work in close collaboration with staff of the Tsetse Laboratory.

In 1988 ILRAD's Tsetse Laboratory maintained stable breeding colonies of eight tsetse species. These were *Glossina morsitans centralis*, originating from mainland Tanzania; *G. austeni*, from Zanzibar; *G. palpalis palpalis*, from Nigeria; *G. p. gambiensis,* from Burkina Faso; *G. fuscipes fuscipes*, from the Central African Republic; *G. tachinoides*, from Tchad; and *G. brevipalpis* and *G. longipennis,* from Kenya. These species represent all three taxonomic groups of tsetse—*morsitans, palpalis* and *fusca*. Their breeding performance in 1988 is summarized in Table 3.

Table 3. *Performance of the eight tsetse breeding colonies maintained at II.RAD in 1988.*

Tsetse species	Mean number of breeding females	Mean daily female mortality %	Mean number of pupae per female per week	Mean puparial weight (mg)	Annual puparial production
G. m. centralis	10,299	0.52	0.57	33.73	305,850
G. austeni	562	0.25	0.59	27.24	17,324
G. p. palpalis	696	0.60	0.54	32.09	19,403
G. p. gambiensis	780	0.48	0.69	28.95	27,931
G. f fuscipes	829	0.20	0.47	36.84	20.165
G. tachinoides	518	0.58	0.65	19.74	17,449
G. brevipalpis	1,204	0.69	0.51	75.04	31,897
G. longipennis	1,068	0.84	0.44	77.32	24,230

In addition, the Tsetse Laboratory established in 1987 a colony of *G. pallidipes* (*morsitans* group), which is still expanding. By the end of 1988 this colony, which originated from the Shimba Hills, Kenya, consisted of 2647 breeding females and had produced 24,180 pupae with a mean puparial weight of 40.4 mg.

These breeding colonies provided all the tsetse required for trypanosomiasis research in 1988. Tsetse puparia and adult flies were also supplied to colleagues at the University of Nairobi, the Biological Control Project (Nigeria), the Swiss Tropical Institute and the University of Bristol (UK).

Tick laboratory

The main work of the Tick Laboratory is the production of a steady supply of ticks and tick salivary glands infected with the sporozoite form of *Theileria parva* for staff in ILRAD's theileriosis research program.

Theileria sporozoites can be obtained only by development of the parasites in the tick vectors. The Tick Laboratory maintains a large colony of *Rhipicephalus appendiculatus* ticks. 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.

Staff from the Tick Laboratory are collaborating with ILRAD scientists on several research projects, including the isolation of new stocks from the field, the rate of transmission of parasite clones from ticks to cattle, pick ups of parasites from *Theileria*- carrier cattle, tick stabilate preparation and determination of *Theileria* infection rates in field ticks.

Rhipicephalus appendiculatus is the most important tick species in East Africa, where it is the principal vector of *T. p. parva* and *T. p. lawrencei.* Colonies of other tick species maintained at ILRAD include another *Rhipicephalus* species, *R. zambeziensis,* as well as *Amblyomma variegatum* and *A. gemma,* vectors of *Cowdria ruminantium,* which causes heartwater, and two relatively benign *Theileria* species, T. *mutans* and *T. velifera.*

Changes continued to be made in the Tick Laboratory to improve disease security. The Laboratory is being extended so that rabbits that have never encountered *Theileria* parasites and rabbits infested with ticks infected by different *Theileria* species, strains and isolates can be kept isolated from each other.

An incubator was purchased to increase the Laboratory's tick-handling capacity and another was modified to provide the long daylight regimes needed to activate the species and strains of ticks from southern Africa. Two data loggers were purchased to monitor the temperature and relative humidity of the incubators. A computer was purchased in August and a software program is being designed to store and retrieve tick data.

Low *Theileria* infection rates in ticks continued to be a problem in 1988. No clear relationship is manifested between parasitaemia in cattle and infection rates in tick batches fed on the cattle. Differences do exist, however, in the susceptibility to *Theileria* infection shown by *R. appendiculatus* and *R. zambeziensis* collected from different areas. The Tick Laboratory is identifying the tick species and isolates most susceptible to infection. It is also studying the effect on infection rates of treating cattle with dexamethasone, a corticosteroid drug.

Large animal production

The large animal facilities on the ILRAD Farm, located next to ILRAD's laboratories, at Kabete, housed 459 cattle and 211 goats and sheep during 1988 that were allocated for research projects at ILRAD. Of the cattle, 61% were used for East Coast fever research, 34% for trypanosomiasis research and 5% for other projects, such as antigen production. Most of the small ruminants were used for trypanosomiasis research. Twenty-five per cent fewer animals were used experimentally in 1988 than in 1987 (670 were used in 1988, 893 in 1987).

ILRAD's ranch, Kapiti Plains Estate, located about 50 km from Nairobi, supplied 279 Boran calves, 54 *Bos taurus* calves, 20 N'Dama calves and 41 Boran steers and heifers to the ILRAD Farm in 1988 and sold 688 cattle to other users. The supplies of animal feeds were stable throughout 1988, the rainfall excellent and the animal health on the ILRAD Farm generally good. Minor improvements were made on the farm to make cleaning the

experimental animal facilities easier. No disease outbreaks or quarantine restrictions occurred during the year and the embryo transfer work continued on schedule in 1988. Altogether, 967 calves were born on the ranch during the year from a breeding herd of 1110 cows, making a calving rate of 87%.

Both Boran and N'Dama calves are regularly produced at ILRAD using embryo transfer techniques. In the last five years ILRAD's Large Animal Unit has tested and standardized methods for synchronizing the ovulation of donor and recipient cows, for stimulating superovulation in donors, for assessing the suitability of recipients and for handling and splitting embryos before implanting them in recipient cows. In both breeds an average of just under four embryos is now obtained from each embryo-producing donor during each superovulation.

The objective of transferring embryos in Boran cattle is to produce animals with the same major histocompatibility complex (MHC), as well as to produce twins. In 1988, 32 Boran calves were produced by embryo transfer from selected MHC-typed cows and bulls. Boran calves of specified MHC types were also produced during the year from natural matings.

The objective of embryo transfer work in N'Dama cattle is to increase the number of N'Damas available for ILRAD's trypanosomiasis research program as rapidly as possible. ILRAD's five N'Dama heifers were each induced to superovulate twice in 1988. During the second superovulation, three of the N'Damas were infected with trypanosomiasis. Unlike susceptible cattle, these trypanotolerant N'Dama were capable of retaining reproductive activity while being infected with trypanosomes. A total of 13 N'Dama embryos were recovered and surgically implanted in Boran foster mothers, 6 pregnancies were established and 20 N'Dama calves were born in 1988, making ILRAD's total N'Dama calf production 24.

Laboratory animal production

The Laboratory Animal Unit provides ILRAD's research and training programs with a regular supply of mice, rats and rabbits. The Unit also maintains small colonies of meadow voles, cotton rats and guinea pigs, which are occasionally required for experimental work. All laboratory animals are housed and maintained according to international animal welfare conventions.

ILRAD is self-sufficient in mouse and rat production. Colonies of three inbred mouse strains--BALB/c, C3H/He and C57/B16--are maintained. The C3H/He and C57/ B 16 mice are used primarily for studies on host resistance to trypanosomiasis. The BALB/c mice and a colony of (BALB/c × Swiss) FI 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.

ILRAD scientists use rats primarily for the production of trypanosomes. All rats are random-bred and originate from the Sprague Dawley strain. Rabbits are used mainly to produce antisera and to support the tsetse and tick colonies. Expansion of ILRAD's rabbit production continued to be impeded by outbreaks of *Staphylococcus aureus*. Because of this, although 25% more rabbits were weaned in 1988 than in 1987, ILRAD still relied on outside suppliers for about one-third of the rabbits required during the year.

Table 4 shows the total number of mice, rats and rabbits produced and used at ILRAD in 1988. The demand for mice increased by 8% from the 1987 level, while the demand for rats decreased by 39%. In addition to supplying nearly all the laboratory animals required by ILRAD scientists, the breeding unit provided a total of 1548 mice and 588 rats to other research organizations in Kenya. These were the Institute for Primate Research; the International Centre of Insect Physiology and Ecology; the Kenya Agricultural Research

Institute; the Kenya Medical Research Institute; the Kenya Science Teachers' College; the Kenya Trypanosomiasis Research Institute; the Kenya Veterinary Laboratories; the National Veterinary Research Centre (Kabete and Muguga); the Small Ruminant Collaborative Research Support Program; the University of Nairobi; and several secondary schools.

Table 4. *Rats, mice and rabbits produced and supplied to ILRAD scientists in 1988.*

Species and strain	Number weaned	Number supplied to scientists
Mice		
Random-bred Swiss	24,207	21,707
Inbred BALB/c	11,454	9,734
Inbred C3H/He	5,276	3,388
Inbred C57B1/6	2,397	1,456
BALB/c × Swiss F_1	4,407	3,682
Rats	14,785	11,055
Rabbits	1,347	1,260

Biostatistics and computing services

ILRAD's Biostatistics and Computing Services Unit, in conjunction with the University of Strathclyde (UK), offers support to ILRAD's scientific and administrative staff ranging from installing computer equipment; giving advice on hardware and software; storing, presenting and analysing data from laboratory and field studies; and writing computer programs.

The Unit maintains a library of commercial software that includes dBase III for database work; Symphony for spreadsheet work; Multimate for word processing; Smart, an integrated package, for doing all three of these kinds of work; and Harvard Graphics for producing graphs. The Unit also uses Statgraphics, SPSS (Statistical Package for Social Scientists) and other specialized software for analysing scientific data; compilers for high-level languages, such as C; utility packages such as Norton Utilities and PC Tools; and conversion packages such as Word for Word, used to convert documents from one word processing format to another.

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).

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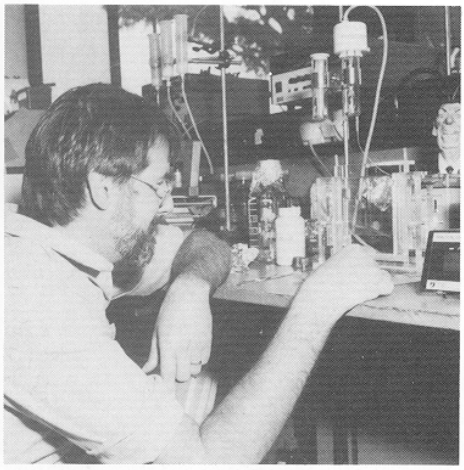

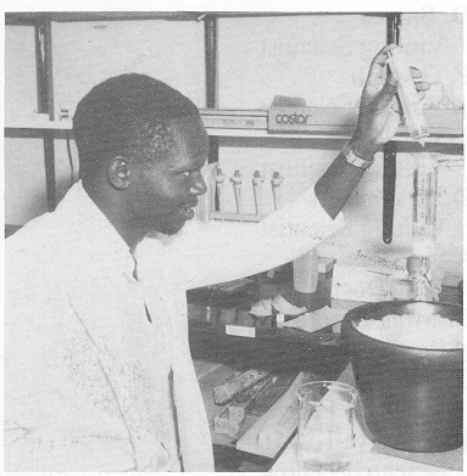

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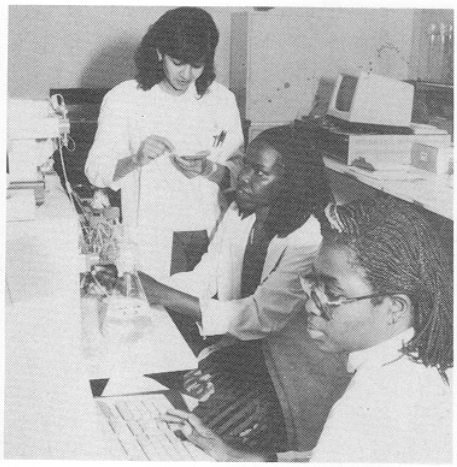

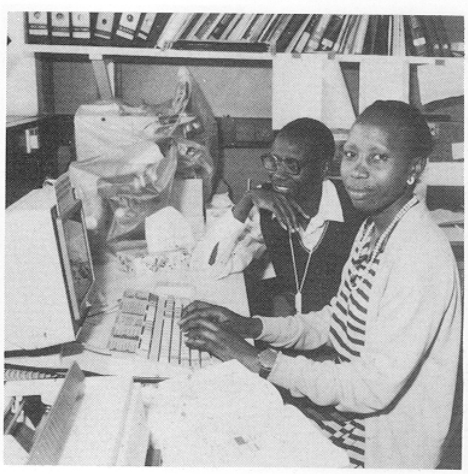

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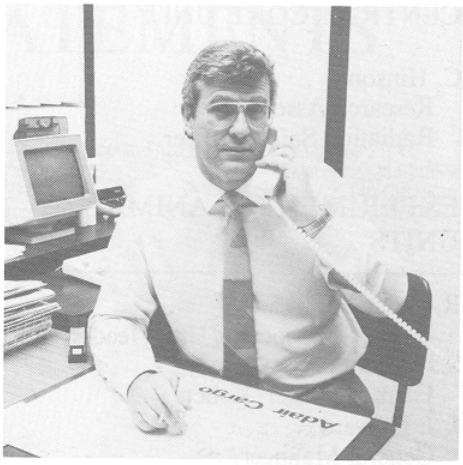

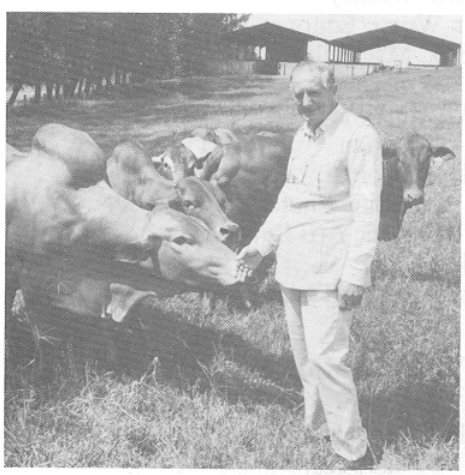

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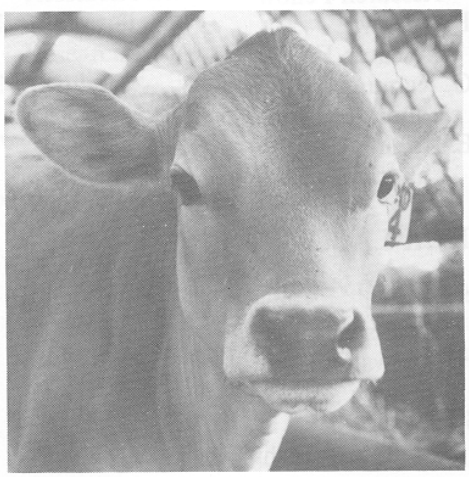

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Cables PRICEWATER

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 Al to A4, which contain information extracted from the accounting records of ILRAD for the years ended 31 December 1987 and 1988.

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 1987 and 1988, on which we expressed an unqualified opinion.

Price Waterhouse
Certified Public Accountants

7 April 1989

Table A1. *Summary costs by program and activity (US$'000).*

	1988	1987
OPERATIONS		
Research		
Parasitology-Trypanosomiasis	645	502
Biochemistry	596	675
Cell Biology	609	656
Immunobiology	630	653
Parasitology-Theileriosis	632	626
Pathology	639	649
Immunoparasitology	704	342
Tsetse Laboratory	332	345
Tick Laboratory	162	121
Electron Microscopy	159	142
Epidemiology and Socioeconomics	288	176
International Trypanotolerance Centre, Gambia	306	204
Wildlife Project	119	220

Total Research	5821	5311
Research Support		
Office of Director of Research	627	470
Farm Animal Production	624	641
Laboratory Animal Production	156	170
Radioisotope and Central Core Services	807	516
Total Research Support	2214	1797
Training and Conferences	980	1022
Library and Information Services	434	448
Administration		
Board of Directors	123	108
Office of the Director General	447	444
Finance	370	334
Personnel	98	88
Purchasing	437	426
Total Administration	1475	1400
General Operations		
Engineering	752	738
Transport	203	208
Services	279	285
Food and Housing	71	76
Stores	58	55
Total General Operations	1363	1362
Total Operations	**12287**	**11340**

Table A2. *Summary of core operating funds from donors (US$'000).*

	1988	1987
Unrestricted And Restricted Funds From Donors		
Unrestricted Funds from Donors		
United States Agency for International Development	2150	2150
World Bank	2020	1250
United Kingdom	1239	930
Canadian International Development Agency	907	760
Switzerland	819	644
Germany (Federal Republic)	781	630
Japan	470	346

Netherlands	444	350
Sweden	417	340
Norway	320	285
Italy	216	232
Belgium	200	223
African Development Bank	200	190
France	155	133
Denmark	109	80
India	26	24
Total Unrestricted Funds from Donors	10473	8567
Restricted Funds from Donors		
United Nations Development Program	695	828
Italy	500	570
Rockefeller Foundation	391	209
Belgium	200	195
Japan	150	215
Australia	85	-
Total Restricted Funds from Donors	2021	2017
Total Unrestricted/Restricted Funds From Donors	**12494**	**10584**

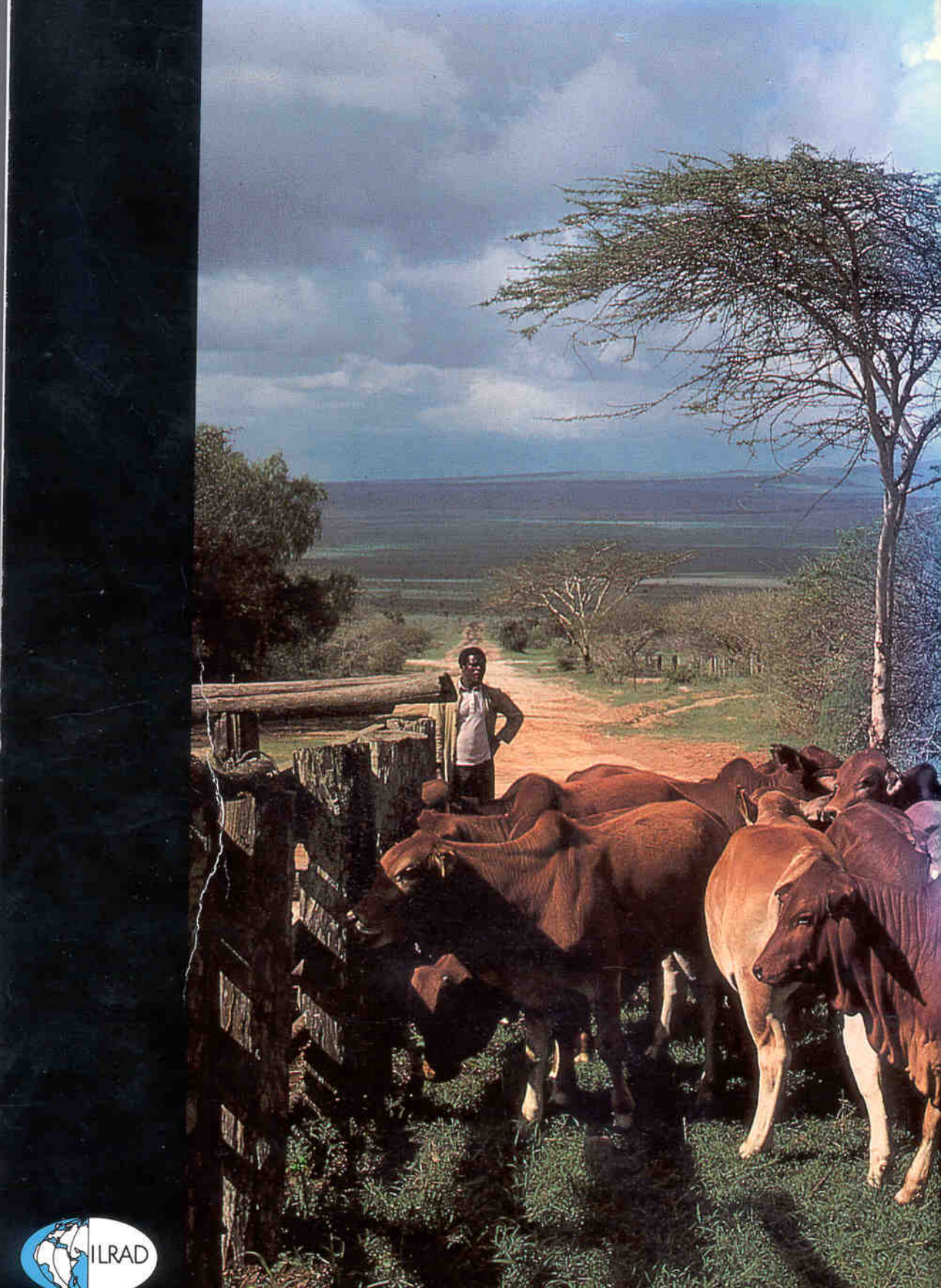
Table A3. *Summary of sources and application of funds (US$'000).*

	1988	1987
SOURCES		
Core Operating Funds		
Unrestricted Funds from Donors	10473	8567
Earned Income Applied in Year	364	727
Restricted Operating Funds	2021	2017
Total Unrestricted/Restricted Operating Funds	12858	11311
Transfer to Capital Funds	(700)	(962)
Net UnrestrictedlRestricted Operating Funds	12158	10349
Capital Funds		
Transferred from Core Operating Funds	700	962
Unexpended Balance from Previous Year	55	714
Balance of Working Funds	1223	1123
Balance of Revolving Fund from Previous Year	100	100
Total Capital Funds	2078	2899
Special Projects		
Wildlife	306	220
Trypanotolerance	119	204

Total Special Projects	425	424
Total Sources	**14661**	**13672**
Applications		
Core Operations	12287	11340
Capital	700	962
Unexpended Balance		
Unrestricted Core	351	55
Working Funds	1223	1123
Revolving Fund	100	100
Capital Development Fund	-	92
Total Funds	**14661**	**13672**

Table A4. *Balance sheet as at 31 December 1988 (US$'000)*.

	1988	1987
Assets		
Fixed Assets		
Land and Buildings	11624	11279
Research Equipment	6798	5807
Other Assets	2112	1967
Subsidiary Company		
Investment	1786	1786
Long-Term Loan	30	20
Total Fixed Assets	22350	20859
Revolving Fund	100	100
Capital Development Fund	92	92
Net Current Assets	1574	1178
Total Assets Employed	**24116**	**22229**
Fund Balances		
Capital Fund	22350	20859
Working Capital	1223	1123
Unrestricted Core Surplus	351	55
Revolving Fund	100	100
Capital Development Fund	92	92
Total Funds	**24116**	**22229**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