PN-AAV-768

ANNUAL REPORT OF THE INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES

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

ILRAD is one of 13 centres in a worldwide agricultural research network sponsored by the Consultative Group on International Agricultural Research (CGIAR). In 1985, core funding for ILRAD's research and training activities was provided by the World Bank (International Bank for Reconstruction and Development—IBRD), the United Nations Development Program (UNDP) and the Governments of Australia, Belgium, Canada, Denmark, the Federal Republic of Germany, France, Italy, Japan, Norway, Sweden, Switzerland, the Netherlands, the United Kingdom and the United States of America. Special research projects were supported by the European Economic Community (EEC), the International Atomic Energy Agency (IAEA), and the Government of The Netherlands.

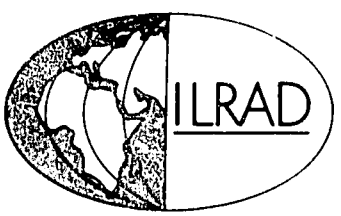
All responsibility for views and information expressed in this report remains with ILRAD. The use of trade names does not imply endorsement of any product.

The correct citation for this report is: ILRAD (International Laboratory for Research on Animal Diseases). 1986. *ILRAD 1985*. Nairobi: ILRAD.

ISBN 92-9055-085-6.
US Library of Congress Catalog Card Number 82-981264.

ILRAD: 1985

ANNUAL REPORT OF THE
INTERNATIONAL LABORATORY
FOR RESEARCH ON ANIMAL DISEASES



P O BOX 30709 NAIROBI, KENYA

FOREWORD

The International Laboratory for Research on Animal Diseases (ILRAD) was established in 1973 with a mandate to develop effective control measures for livestock diseases which seriously limit world food production. ILRAD's research programs focus on African animal trypanosomiasis and East Coast fever (ECF), a form of theileriosis. These two disease complexes are a serious constraint on livestock production and rural economic development in Africa and other tropical regions, causing direct and indirect economic losses and human suffering over substantial areas.

There are unlikely to be any quick or easy solutions for improving the control of animal trypanosomiasis or theileriosis. The complexity of these animal health problems has been recognized for many years. A major research effort is required to improve our understanding of the the protozoan parasites which cause these diseases, of the disease processes and responses of affected livestock and of ways to prevent or reduce further losses.

To meet these needs, ILRAD has steadily increased its capacity to carry out scientific research, starting in 1975 with the construction of basic facilities at Kabete on the outskirts of Nairobi, Kenya, and the arrival of a small nucleus of scientific staff. In 1985, ILRAD occupied a complex of research laboratories and support units, including a cattle breeding ranch at Kapiti about 80 km from Nairobi, and was staffed by a full complement of 50 internationally recruited scientists and associated specialists, with appropriate supporting personnel.

Research programs at Kabete are complemented by collaborative research agreements with laboratories in Africa and other parts of the world. ILRAD has developed important contacts with national research systems in many African countries affected by trypanosomiasis and theileriosis—through collaborative research networks, scientific meetings, training activities and the distribution of research reports. ILRAD also maintains close contact with international organizations and bilateral technical assistance agencies concerned with animal disease control and agricultural development. These include the World Bank (International Bank for Reconstruction and Development—IBRD), the Food and Agriculture Organization of the United Nations (FAO), the United Nations Development Program (UNDP), the World Health Organization (WHO), the Organization of African Unity (OAU) and the International Livestock Centre for Africa (ILCA).

As one of 13 international agricultural research centres supported by the Consultative Group on International Agricultural Research (CGIAR), ILRAD is subject to external

program and management reviews every 5 years. Late in 1985 and extending into January 1986, all aspects of ILRAD's research and training programs were reviewed by panels of scientific and administrative specialists commissioned by the CGIAR. Professor Jos Mortelmans of the Prince Leopold Institute of Tropical Medicine (Belgium) was chairman of the External Program Review panel, and Mr Frank Raymond, former Chief Scientist in the Ministry of Agriculture, Fisheries and Food (UK) headed the panel conducting the External Management Review. The review teams concentrated on progress made since ILRAD was first reviewed in 1980. By any standards, achievements have been substantial. Some of the highlights may be summarized as follows:

- completion of a detailed study on trypanosomiasis epidemiology at a site in Kenya's Coast Province, with special emphasis on serodeme characterization and the possibility of acquired immunity under chemotherapeutic treatment

- establishment of a collaborative research network with ILCA and national livestock production services in 8 African countries to assess the productivity of trypanotolerant livestock under different levels of trypanosomiasis risk

- development of new techniques to diagnose trypanosomiasis and determine the origin of tsetse bloodmeals

- establishment of *in vitro* culture systems to maintain the three major pathogenic trypanosome species for use in a variety of studies, including *in vitro* testing of drug sensitivity

- investigation of the genetic mechanisms underlying antigenic variation in *Trypanosoma brucei* and *T congolense* and studies on the synthesis of variable surface antigens in *T brucei*

- discovery that host responses influence trypanosome differentiation from rapidly-dividing to nondividing bloodstream forms and that this differentiation triggers the humoral immune response

- description of the immunosuppressive effects of *T brucei* infection which involve inhibition of B lymphocyte maturation and

disruption of the structure of the lymph nodes and spleen

- demonstration of resistance to trypanosome infection in N'Dama calves brought to ILRAD from The Gambia by embryo transfer and in different species of wildlife

- use of monoclonal antibody technology to characterize strains of *Theileria parva parva* and *T p lawrencei* from Kenya and other countries in eastern and southern Africa

- ECF epidemiology studies and 'infection and treatment' immunization trials in Kenya's Coast Province and support to national veterinary services and FAO projects conducting related field trials in Burundi, Malawi Rwanda, Zambia, Tanzania (Zanzibar), and Zimbabwe

- identification, isolation and characterization of *Theileria* sporozoite antigens which may provide the basis for an improved vaccine

- immunological and biochemical studies to identify *Theileria* schizont-associated antigens which could also be used in an improved vaccine

- application of recombinant DNA techniques to construct genomic and cDNA libraries aimed at producing *Theileria* antigens for improved vaccines.

Important activities in 1985 included the launching of a special project within the ILCA/ILRAD trypanotolerance network to study N'Dama cattle at several sites in The Gambia and Senegal. This project is being conducted in cooperation with the International Trypanotolerance Centre in The Gambia and is supported by the European Economic Community (EEC). Scientists working in the trypanotolerance network met at ILRAD in December for a major review of achievements and discussion of future activities.

Studies on trypanosomiasis epidemiology have indicated chromosomal differences between isolates of *T congolense* which could be regarded as a basis for description of a new parasite species. Basic research continues on trypanosome biochemistry and antigenicity, including efforts to expand the cultivation of the three major trypanosome species *in vitro*. ILRAD is collaborating with the Swiss Tropical Institute in a study to identify trypanosome growth factors, and the Japanese International Cooperation Agency

is supporting work on *T brucei* cultivation through a special project. Scientists are using cell biology and immunoelectron microscopy techniques to investigation aspects of trypanosome metabolism: the goal is to identify vulnerable receptor mechanisms which could be blocked by chemical or immunological means.

Studies continue on host responses which stimulate or inhibit trypanosome growth and on parasite-induced suppression of immune responses. Material for comparative studies is provided through collaboration with the wildlife section of the Kenya Government's Veterinary Research Laboratory as part of a special project funded by the Government of The Netherlands. This research area provides challenges to scientific innovation and originality at the highest level with the ultimate goal of enhancing protective responses against trypanosome infection.

Scientists working to produce a vaccine against *Theileria* sporozoites processed protein materials for amino acid sequences in 1985 and screened DNA libraries to identify genes coding for sporozoite antigens. In May, an international workshop on parasite antigens brought together specialists in immunology and parasitology from around the world.

Studies on *Theileria* schizont-associated antigens have involved considerable basic research on the bovine immune system, including the role of major histocompatibility antigens and their interactions with parasite antigens at the surface of infected lymphocytes. T lymphocyte clones have been established in culture, and these are being used to investigate several aspects of cellular immunity.

However impressive these achievements may be, it is of continuing importance to ensure that ILRAD's research programs retain their relevance to the real needs of African countries affected by trypanosomiasis and theileriosis. In 1985, ILRAD provided support enabling scientists from several African countries to participate in scientific meetings on trypanotolerance, parasite antigens, control of ECF and other aspects of research on protozoan diseases. The exchange of information and views in gatherings sponsored by ILRAD has been invaluable in maintaining the focus of research efforts on the practical aspects of disease control. The training program continued to expand in 1985 with additional support for post-doctoral scientists from African countries who spend 6 to 9 months at ILRAD expanding their knowledge and skills. Training and information activities will continue to receive emphasis in future.

In concluding this brief foreword, I would like to emphasize the role of ILRAD's Board of Directors, the scientific and supporting staff and the donor organizations and nations who collectively support efforts to identify and introduce improved control measures for theileriosis and animal trypanosomiasis. In 1985, Dr Leo Jenni of the Swiss Tropical Institute joined ILRAD as a member of the Board of Directors and two senior scientists, Dr Max Murray and Dr Tony Irvin, left to take up new positions after many productive years on ILRAD's research staff. Another senior scientist, Dr Onesmo Ole MoiYoi, was appointed to the Council of Kenyatta University by His Excellency Daniel Arap Moi, President of Kenya.

We acknowledge with gratitude the interest and financial support ILRAD receives for its research and training activities from many countries and organizations. In 1985, ILRAD continued to receive funds from the World Bank, UNDP, EEC and the Governments of Australia, Belgium, Canada, France, Germany (FR), Italy, Japan, The Netherlands, Norway, Sweden, Switzerland, the United Kingdom (UK) and the United States of America (USA). The Government of Denmark added its support during the year, and the Rockefeller Foundation pledged support beginning in 1986. In addition to funding for ILRAD's core programs, the Governments of Belgium, France, Germany,

Italy, Japan, The Netherlands and the UK provided scientists through special project funds.

During the year, ILRAD was honoured to receive visits by Mrs M. Moriyama, Deputy Minister of Foreign Affairs of the Government of Japan, Mr S. Husain, Chairman of the CGIAR, and Their Excellencies the Ambassadors of Belgium, Japan, Norway, Switzerland and the United States of America and the High Commissioners of Australia, Canada and the United Kingdom. We value these visits, as well as contacts with scientific and technical advisors and representatives of all the donor nations and organizations which support our work.

Finally, it is a pleasure to acknowledge the support ILRAD has received through the years from the people and Government of Kenya. We look forward to the continuation of this warm relationship with our Kenyan hosts.

A.R. Gray
Director General
ILRAD

CONTENTS

Foreword 2

Theileriosis 9
- Lifecycle of the parasite 10
- The sporozoite 10
  - Invasion of host cells 10
  - Immune responses against sporozoite antigens 12
- The schizont-infected cell 13
  - Proliferation of infected cells 13
  - Antigenic changes on infected cells 14
  - Immune responses against schizont-infected cells 14
    - Definition of cell types 16
    - Definition of MHC antigens 18
    - Cell-mediated immune responses 19
- Epidemiology and experimental immunization 20
  - Strain characterization 21
  - Experimental immunization 23
- Heartwater: some preliminary observations 27
- Immune responses and the tick 29

Trypanosomiasis 33
- Improved control of the parasites 34
  - Antigenic variation: *T brucei* and *T congolense* 35
  - Antigenic variation: *T vivax* 37
  - The trypanosome lifecycle: stage-specific proteins 39
  - Aspects of trypanosome metabolism 40
  - Trypanosome cultivation *in vitro* 42
- Improved host responses 43
- Pathogenesis: effects of trypanosomiasis on bovine reproduction 46
- Epidemiology 47
  - Characterization of trypanosome populations 47
    - Serological typing: a common trypanosome antigen 47
    - Serological typing: species-specific antigens 47

Serological typing: *T congolense* serodemes from Kililfi 48
In vivo studies 48
Use of genomic probes 48
Isoenzyme analysis 52
Tsetse flies as trypanosome vectors 52
Livestock production under trypanosomiasis risk 54
Trypanocidal drugs: detection and testing *in vitro* 54
Trypanocidal drugs: evaluation of protection 56
Studies on resistance to trypanosomiasis 58
Trypanotolerance research network 61

Training and information services 66
Training 66
Information services 68
The ILRAD library 69

Research support 70
Tsetse laboratory 70
Tick laboratory 70
Farm animal production 72
Laboratory animal production 72
Clinical and diagnostic services 73

1985 publications 75

Board of Directors 79

Staff 80

Financial statements 86

THEILERIOSIS

Theileriosis is a complex of tick-transmitted diseases caused by protozoan parasites of the genus *Theileria*. The parasites infect wild and domestic animals—particularly cattle—and in many parts of the world they are a major constraint to expansion and improvement of the livestock industry.

The most important *Theileria* species are *T parva*, the causative agent of ECF affecting cattle in East and Central Africa, and *T annulata*, the cause of tropical theileriosis in cattle in the Mediterranean littoral and the Middle East, extending eastward to India, southern Russia and the far east. Other important *Theileria* parasites include *T sergenti*, *T orientalis* and *T mutans* in cattle and *T hirci* in sheep and goats.

Work at ILRAD concentrates on ECF, which occurs in Burundi, Kenya, Malawi, Mozambique, Rwanda, Sudan, Tanzania, Zaire, Zambia and Zimbabwe. Altogether, some 25 million cattle are at risk. At present, ECF is controlled principally by dipping or spraying cattle with acaricides to kill the vector ticks, but this approach is becoming increasingly difficult and expensive and alternative methods of control are urgently required. Recent advances in chemotherapy have resulted in the development of a number of effective compounds for the treatment of ECF, but prevention will always remain more desirable than treatment.

The prospects of immunizing cattle to prevent infection with ECF are promising, as cattle which recover from ECF show a long-lasting immunity even in the absence of disease challenge. However, different strains of *T parva* exist and immunization of cattle with one strain will not necessarily protect them against challenge with an unrelated strain. The number of strains found in cattle is unknown but certain isolates or combinations of isolates appear to provide a broad protection. Thus, there are good prospects of controlling ECF by vaccination, and ILRAD places particular emphasis on this approach.

The development of a vaccine requires knowledge of both the parasite and the host. Studies of the parasite, initiated at the former East African Veterinary Research Organization, have led to a control strategy of immunizing cattle by infection with parasites and concurrent treatment. Currently, this method offers the most practical and effective form of vaccination when properly controlled, and it is being used in extended field trials in a number of countries. The disadvantages are that infection with live parasites is potentially lethal if misused, full protection may not be achieved in areas where

several strains of the parasite are present, and cattle may become carriers of the parasites used in immunization.

Work is in progress at ILRAD to overcome these disadvantages, but more emphasis is placed on developing purified antigens for immunization which will not be subject to the problems inherent in a live vaccine. The second major component of ILRAD's research effort is directed towards identifying the mechanisms of host immunity to ECF. This work has involved considerable basic research on the immune system of cattle. Details of work along all these lines are given in the report which follows.

LIFECYCLE OF THE PARASITE

The *Theileria* parasite has a complex lifecycle in the bovine host and the vector tick. The brown ear tick, *Rhipicephalus appendiculatus*, which transmits ECF feeds on cattle three times—as a larva, a nymph and an adult. The parasites are transmitted most commonly when ticks feed on infected cattle as nymphs and then on disease-free animals as adults.

Theileria sporozoites develop in the salivary glands of infected ticks and are passed to cattle along with tick saliva when the ticks feed. In cattle, they attach to lymphocytes, white blood cells of the immune system. Some sporozoites enter lymphocytes and develop into forms called schizonts. Infected lymphocytes become enlarged cells called lymphoblasts that multiply along with the parasites, resulting in a rapidly expanding population of parasitized cells. The final stages of infection are characterized by large-scale destruction of cells and often by death of the host.

During the course of infection, some of the *Theileria* schizonts differentiate into merozoites. These are released from the lymphoid cells into the bloodstream and invade red blood cells where they develop into forms called piroplasms. Ticks feeding on the host animal become infected when they ingest red blood cells containing *Theileria* piroplasms. In the tick gut, the parasites differentiate into gametes which fuse to form zygotes; these then develop into kinetes in the cells lining the gut. The motile kinetes move through the gut wall into the body cavity and migrate to the salivary gland where they enter the E cell of the type III acinus. Here they form an elaborate intracellular sporoblast syncytium. Stimulated by tick feeding, the sporoblast undergoes segmental fission and gives rise to 30,000 to 50,000 sporozoites. These are introduced with tick saliva into a new mammalian host, initiating a new cycle of parasite development.

THE SPOROZOITE

INVASION OF HOST CELL

Studies at ILRAD have shown that *Theileria* sporozoites bind *in vitro* to a variety of bovine target cells—including thymocytes, both B and T lymphocytes and a small subset of macrophages—but active infections are only established in lymphocytes. These studies have been conducted with monoclonal antibodies which are used to identify cell

types by recognizing specific cell-surface antigens. Monoclonal antibodies are produced from hybrid cells formed by the fusion of mouse spleen cells, primed to produce specific antibodies, and mouse tumour cells capable of growing and multiplying in culture.

A study initiated in 1984 compared *in vitro* sporozoite binding and infection of peripheral blood mononuclear leucocytes taken from cattle and from African buffalo (*Syncerus caffer*). The goal of this work is to elucidate the mechanisms which enable African buffalo to resist *Theileria* infection. Sporozoites were used from two *T parva* subspecies: *T p parva* (Muguga) which causes classical ECF in cattle and *T p lawrencei* which is carried asymptomatically by wild buffalo but is highly pathogenic to cattle, usually causing fatal infections. There was no significant difference in the number of target cells from cattle or buffalo which bound *T p parva* or *T p lawrencei* sporozoites or became infected: about 30% of cells from both species bound *T p parva* sporozoites, and about 20% developed schizonts after 1 week's exposure *in vitro*. However, many fewer buffalo cells than cattle cells were transformed by *T p parva* infection into a rapidly dividing state resulting in a continuously growing cell line. This limited transformation of buffalo cells may explain the lower susceptibility of buffalo to *T p parva* infection. Among cells infected with *T p lawrencei*, similar numbers of buffalo and cattle cells were transformed into a dividing state. This suggests that buffalo possess other mechanisms of resistance to *T p lawrencei* which enable them to remain carriers of infection without suffering signs of disease: this resistance is not related to differences between buffalo and cattle in the mechanisms of cell binding and the establishment of infection.

Sporozoites from different *Theileria* strains appear to bind to the same receptor molecule(s) on host cells, suggesting that parasites bind host cells by a common mechanism. Experiments were conducted in 1985 to elucidate this mechanism by identifying and isolating the molecule(s) on the surface of bovine lymphocytes which serve as receptors for invading sporozoites. The lymphocyte receptor was destroyed by treatment with proteinase K or endoglycosidases, suggesting that the molecule is a glycoprotein (protein/carbohydrate compound). Monoclonal antibodies raised against bovine peripheral blood leucocytes were screened to identify antibodies which blocked sporozoite binding. One of the monoclonal antibodies identified was shown to recognize three molecules on bovine leucocytes of apparent molecular weights of 200,000, 135,000 and 50,000 daltons. Work is now in progress to characterize these molecules more fully and also to identify the carbohydrates which are linked to the proteins to form the receptor molecules. The goal is to find a way to block the sporozoite entry process and thus protect cattle against infection with different *Theileria* strains.

Monoclonal antibodies produced by bovine cells might recognize different antigens from those recognized by mouse monoclonal antibodies and could extend present studies of the bovine immune system considerably. A study was initiated in 1984 aimed at producing hybrid cells from a fusion of antibody-secreting bovine B cells with mouse tumour cell lines, applying a technique developed at the University of Alabama (USA) for human cells. Continuation of this work in 1985 has failed to produce stable populations of murine x bovine hybrid cells, probably because of loss of bovine chromosomes. As a result, efforts now concentrate on producing hybrid cells from the fusion of bovine cells. Two continuously growing bovine leukaemia cell lines are being investigated as possible partners for fusion with bovine antibody-producing cells.

Sera from cattle in ECF endemic areas have been shown to contain antibodies which neutralize *Theileria* sporozoites thus potentially conferring protection against infection. The sporozoite antigens recognized by antisera from immune cattle have molecular weights of 105,000, 67,000, 43,000 and 18,500 daltons. Several monoclonal antibodies have been raised against *T p parva* sporozoites which prevent sporozoite entry into bovine lymphocytes *in vitro*. One of these monoclonal antibodies recognizes a 68,000-dalton antigen. The antigen can be fragmented into molecules of approximately 55,000 and 25,500 daltons which are also recognized by the monoclonal antibody. These sporozoite antigens might be capable of stimulating protection if used in a vaccine against ECF.

Scientists are producing polyvalent antisera in rabbits to characterize the *Theileria* sporozoite antigens in more detail. An antiserum produced against the 25,500-dalton molecule also reacts with the antigens of 55,000 and 68,000 daltons. It is now being used to isolate the 68,000-dalton antigen. Biochemical investigations have revealed that this antigen is a glycoprotein, though the carbohydrate moiety does not appear to play a role in inducing protective antibody responses. Present efforts also focus on producing a polyvalent antiserum in rabbits against the 105,000-dalton antigen recognized by sera from resistant cattle.

It has not yet been possible to produce sufficient antigenic material from *Theileria* sporozoites for detailed chemical characterization or use in immunization trials. It has been recognized for some time that a protective sporozoite antigen, once it is characterized, will have to be produced by molecular biology techniques. Considerable work was carried out in 1985 to identify *T p parva* gene sequences which code for protective sporozoite antigens and to develop systems for the expression of these genes.

One type of expression library has proved useful in this work—a sheared genomic DNA library using piroplasm DNA. This library has been screened with a variety of antisera and monoclonal antibodies which recognize sporozoite antigens. The most successful experiments have used antisera raised in rabbits. One rabbit antiserum which was raised by inoculating rabbits with the purified 18,500-dalton sporozoite antigen blocks sporozoite infectivity *in vitro*. Nine clones from the genomic library, recognized by this antiserum, are being screened to confirm whether they represent part of the gene for the 18,500-dalton protein.

Genomic DNA will be screened further to obtain complete DNA sequences for the 55,000-, 25,500- and 18,500-dalton proteins. The next step will be to purify these proteins in large quantities for use in experiments to block sporozoite entry *in vitro*, either by direct competition or by using antisera raised against the proteins, and then for use in immunization experiments in cattle. DNA libraries are also being screened with monoclonal antibodies and antisera from resistant cattle in order to identify additional gene sequences coding for proteins which could potentially stimulate immunity.

Because protection against sporozoite infection would be based on a humoral immune response, studies were conducted in 1985 to identify the cells responsible for humoral immune responses in cattle. The first step was to establish an *in vitro* culture system which would demonstrate the antibody responses of bovine cells. Peripheral blood lymphocytes, spleen cells and lymph node cells were taken from cattle and cultured in the presence of pokeweed mitogen. Sheep red blood cells were added to the cultures to stimulate an immune response. Several experiments were conducted with cells taken from many different animals, but results were not encouraging: cell growth was strongly

dependent on the mitogen, and few cells developed to an antibody-producing state. When macrophages were depleted to less than 1% of the cultures, the proportion of cells producing antibody increased 2 or 3 fold. However, cell growth was still dependent on the mitogen. Present work involves culturing cells from cattle primed by subcutaneous injection of sheep red blood cells and boosting the cultures *in vitro* with sheep red blood cells, but without the mitogen. The aim is to measure antibody production and identify the different classes of antibody involved. The study will also focus on the interactions between different subpopulations of bovine cells in the generation of humoral immune responses.

THE SCHIZONT-INFECTED CELL

PROLIFERATION OF INFECTED CELLS

The proliferation of bovine cells infected with *Theileria* schizonts is an important factor in the lifecycle of the parasite and leads to severe pathogenic effects in the infected host. When cells are transformed by certain strains of viruses into tumours, which also multiply rapidly, a class of enzymes called protein kinases has been shown to play an important role. For this reason, scientists at ILRAD have determined the levels of protein kinases in normal bovine cells and in cells infected with *Theileria* schizonts.

Protein kinases change the molecular structure of proteins by attaching phosphates, a process called phosphorylation. Such modifications of proteins play a role in cell growth and regulatory events. There is evidence that phosphorylation may be involved in the proliferation of *Theileria*-infected cells. Studies were initiated in 1985 comparing protein kinase activity in a cloned, uninfected T-cell line and in the same cell line infected with *T parva* (Muguga). The goal is to identify mechanisms specific to infected cells which might be modified to control the disease.

During these experiments, the infected cell lines incorporated 90 times as much phosphate into protein as the uninfected cells. Kinetic analyses showed that the infected cells were larger, divided more rapidly and synthesized protein at least twice as fast as the uninfected cells. Polyacrylamide gel electrophoresis has revealed at least five proteins, with molecular weights ranging from 12,000 to 100,000 daltons, which are specifically modified by the phosphorylation process. In order to establish that this pattern of phosphorylation is not a peculiarity of one cloned cell line, uncloned cells infected with *T parva* (Muguga) and cloned cells infected with *T parva* (Marikebuni) were shown to phosphorylate proteins as rapidly as the original cell line used in the experiment.

Present investigations focus on whether the observed phosphorylation activity is due to the activation of a specific enzyme in the host cell or to the presence of an enzyme of parasite origin. The amino acids within the host cell which are targets for phosphorylation are also being characterized, as well as the genes coding for kinases and other transforming activities.

Theileria-infected cloned bovine T lymphocytes have also been shown to produce a factor, similar to T-cell growth factor, which can support the growth of uninfected bovine T-lymohocyte clones dependent on the growth factor. This suggests that the

autonomous growth of *Theileria*-infected lymphocytes may be due to constitutive production of T-cell growth factor or other hormone-like growth factors.

ANTIGENIC CHANGES ON INFECTED CELLS

ILRAD scientists have used monoclonal antibodies in efforts to distinguish antigenic markers on the cell surface specific to *Theileria*-infected cells. The principal obstacle was thought to be competition from immunodominant normal and activated host-cell antigens which prevent recognition of weaker, *Theileria*-specific antigens. Work in 1985 concentrated on developing techniques to block the production of antibodies to these other antigens and thus improve the specificity of monoclonal antibodies against *Theileria*-infected cells. The most promising approach involves injecting mice simultaneously with *T parva*-infected cells and with a strong antiserum against uninfected blasting cells from the same animal.

Several experiments have revealed antigenic changes on bovine T cells associated with *T parva* infection, though these antigens are also expressed by some noninfected lymphocytes. Two monoclonal antibodies—J5 and J6—detect a T-lymphocyte antigen associated with cell proliferation. T cells stimulated by the mitogen concanavalin A or by cell lines from other animals express this antigen. It is also expressed on all cells infected by *T parva*, including what appear to be infected B cells. This implies that cells transformed by *Theileria* express an antigen associated with proliferating T cells, regardless of their precursor origin.

Two other monoclonal antibodies, BT3/8.12 and J3, recognize a subpopulation of uninfected T lymphocytes as well as all cells infected with *T parva*. Using recently developed techniques to produce T-cell clones, it was possible to raise two distinct populations of uninfected T cells—one with and one without the antigenic determinant recognized by BT3/8.12 and J3. A cloned cell line that did not express the antigen was cultured in the presence of *T parva* sporozoites and the percentage of cells with schizonts and with the antigen was monitored. It was shown that the schizonts appeared first, while the antigen was detected after a delay of 2 days. The antigen is not essential for cell multiplication; its real function is still unknown. Research continues using monoclonal antibodies to define antigenic changes on the surface of *Theileria*-infected lymphocytes. Changes are sought that are directly related to immune responses which could protect the host against infection.

IMMUNE RESPONSES AGAINST SCHIZONT-INFECTED CELLS

Susceptible cattle undergoing acute infection with *T parva* produce cytolytic (cell-killing) lymphocyte cells which are capable of killing their own infected and uninfected cells. These killer cells are probably important in mediating the destruction of the immune system which is a prominent feature of the disease. By contrast, cattle which have developed immunity against ECF produce cytolytic lymphocytes which only kill cells infected with *Theileria* schizonts (Figures 1 and 2). In this case, the killer cells recognize a parasite-induced antigen on the surface of the parasitized cell.

Although a number of antigens have been identified on the surface of sporozoites which stimulate immune responses both *in vitro* and *in vivo*, the antigenic changes which appear on the surface of schizont-infected lymphocytes have not yet been fully defined.

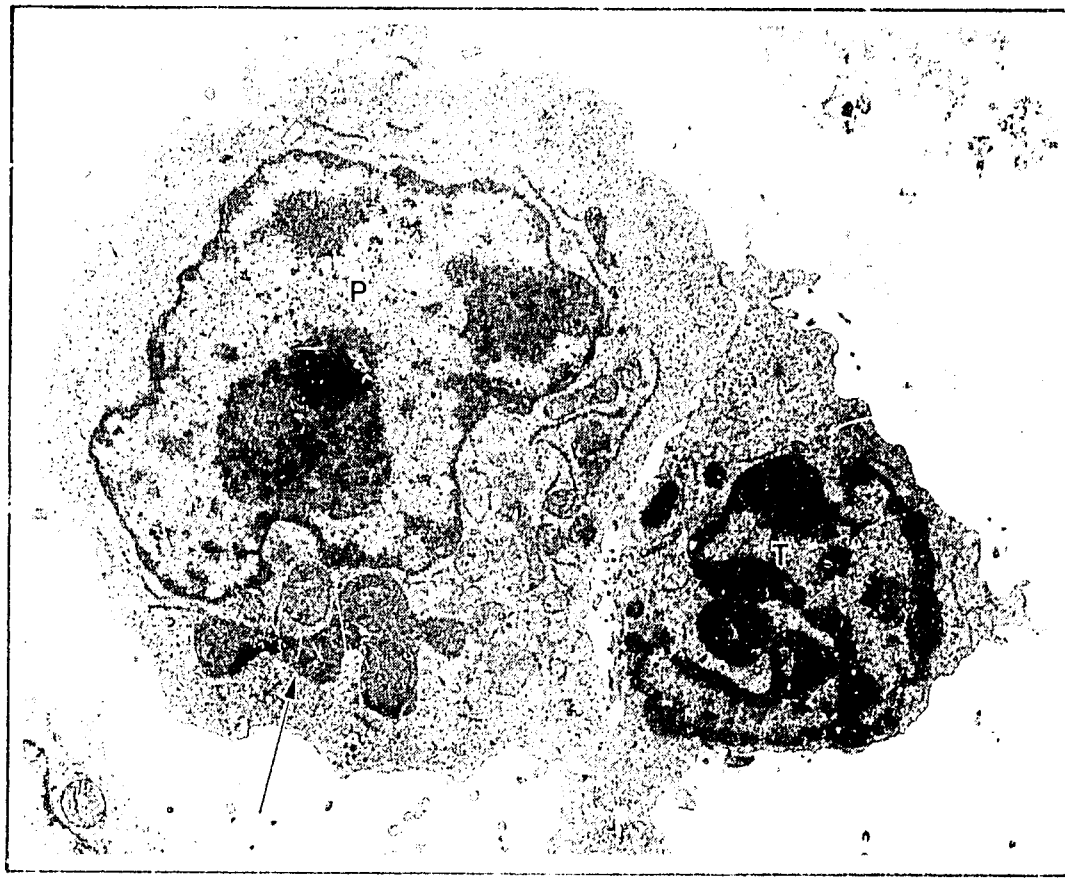


Figure 1. Electron micrograph showing a cytolytic T lymphocyte (T) attached to the surface of a parasitized lymphocyte (P). The arrow indicates the *Theileria* schizont in the parasitized cell.

The mechanisms involved in cell-mediated immunity are also complex, involving several cell types within the immune system. In 1985, ILRAD scientists made considerable progress in the development of *in vitro* systems and reagents which specifically recognize different cell types involved in the immune response. The development of lymphocyte cloning techniques has also led to a better understanding of the mechanisms of the immune response against cells infected with *Theileria* schizonts.

Cytolytic cells belong to a particular subpopulation of T (thymus-derived) lymphocytes. An important feature of T lymphocytes is that they react to foreign antigen presented on the surface of other cells in connection with normal 'self' molecules known as major histocompatibility (MHC) antigens. Because the MHC molecules differ between individuals of a species, T lymphocytes can only recognize foreign antigen on the surface of cells from the same animal or an animal which shares the relevant MHC molecule. This phenomenon is known as MHC restriction and T cells are often described as restricted by particular MHC molecules. Because these MHC-restricted T cells are believed to be important in mediating immunity against the schizont stage of *Theileria* parasites, ILRAD scientists have made a major effort during the past 2 years to develop reagents for identifying T lymphocytes and for defining different MHC molecules.

Figure 2. Electron micrograph showing the destruction of a parasitized lymphocyte (P) by a cytolytic T lymphocyte (T). The arrow indicates a *Theileria* schizont in the parasitized cell.

Definition of cell types

Monoclonal antibodies have been produced at ILRAD which recognize the different types of bovine leucocytes—cells involved in the immune response in cattle. These are T lymphocytes, B lymphocytes, monocytes/macrophages and granulocytes. Studies in 1985 concentrated on defining functionally important T lymphocyte subpopulations and populations of monocytes/macrophages. Once it becomes possible to identify bovine cell populations, their role in responses to *Theileria*-infected cells can be ascertained.

Several monoclonal antibodies have also been derived which recognize bovine MHC antigens. There are two main classes of MHC antigens: class I MHC antigens are expressed on most cell types, whereas class II MHC antigens are expressed predominantly on cells of the immune system. The important monoclonal antibodies identified at ILRAD in 1984 and 1985 are listed in Figure 3, along with their apparent specificities.

Two of the monoclonal antibodies screened at ILRAD—IL-A26 and IL-A27—recognize all mature T lymphocytes. The antibodies IL-A11 and IL-A12 identify a subpopulation of T lymphocytes which recognize target cells that express antigenic changes in conjunction with class II MHC antigens. Another antibody—IL-A17—identifies most T cells which recognize target cells expressing antigenic changes in

Cell Type	Monoclonal Antibody	Specificity	Antigen Designation	Antigen Molecular Weight (Kdaltons)
T cells	IL-A26	all T cells	BoT2	55
	IL-A27,IL-A28	all mature T cells	BoT3	NI
	IL-A11,IL-A12	T-cell subset restricted by class-II MHC	BoT4	52, 55
	IL-A17	T-cell subset restricted by class-I MHC	BoT8	34, 35
Monocytes/ granulocytes	IL-A22,IL-A24	monocytes/ macrophages & granulocytes		NI
	IL-A23	subpopulation of monocytes/ macrophages		NI
	IL-A25	pulmonary macrophages		NI
MHC molecules	IL-A19	monomorphic determinant on class-I molecules		12, 44
	IL-A1,IL-A4 IL-A5,IL-A7 IL-A8,IL-A9 IL-A10,IL-A13	polymorphic determinants on class-I molecules		12, 44
	IL-A21	monomorphic		26, 34
	J11	determinant on class-II molecules		29, 31

[NI]Not yet identified.

Figure 3. Monoclonal antibodies derived at ILRAD which recognize specific classes of bovine leukocytes. Most of these antibodies were produced in 1984 and 1985.

conjunction with class I MHC antigens (Figure 4). Functional studies of the cell populations identified by these antibodies and biochemical analyses of the target molecules they recognize suggest that they distinguish the two major subsets of T cells in cattle, one subset involved predominantly in 'helping' the induction of immune responses (recognized by IL-A11 and IL-A12) and the other the principal mediators of cell killing (recognized by IL-A17).

The T lymphocytes which mediate killing of *Theileria*-infected cells belong to the population identified by the monoclonal antibody IL-A17 and, as expected, are restricted by class I MHC molecules. Indeed the IL-A17 antibody has been shown to inhibit the cytolytic response of some T lymphocytes, suggesting that the molecule recognized by IL-A17 has an important function in the recognition or binding of the cytolytic lymphocyte to the parasitized cell.

The two subpopulations of cells recognized by the IL-A17 and IL-A12 monoclonal antibodies can be infected with *T parva* sporozoites with identical frequency. Most *Theileria*-infected T cell clones are recognized by either IL-A12 or IL-A17, indicating that the majority of lymphocytes that become infected and transformed by the parasite arise from these two populations.

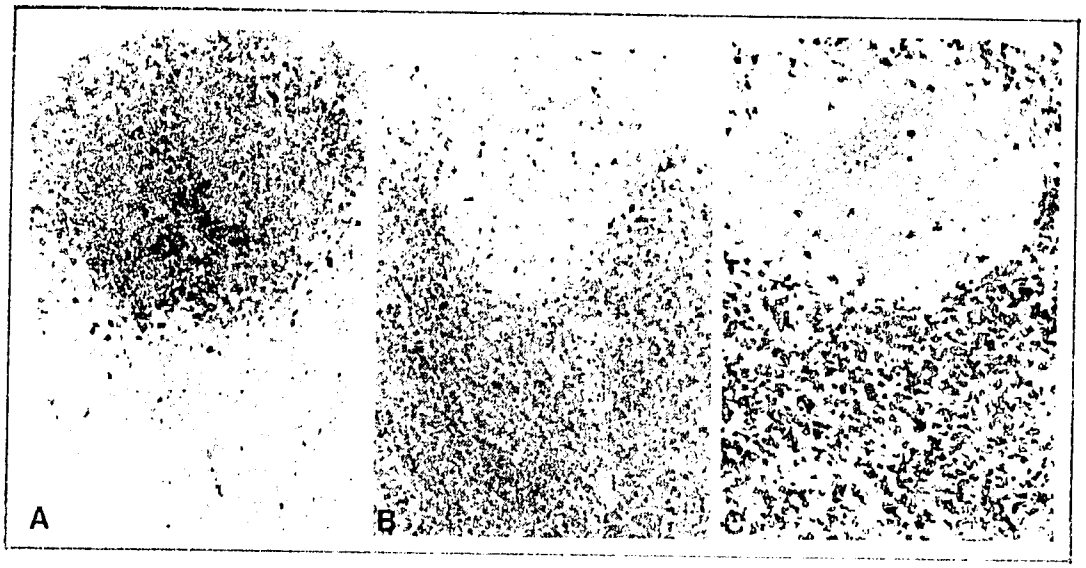


Figure 4. Frozen sections of a normal bovine lymph node stained by the immunoperoxidase technique with monoclonal antibodies that react with (A) all B lymphocytes, (B) all T lymphocytes and (C) a subpopulation of T lymphocytes.

Three monoclonal antibodies were also produced in 1985 which recognize bovine monocytes/macrophages. They appear to recognize cells at different stages of maturation or activation. Further studies are in progress to examine their functional properties.

Definition of MHC antigens

The genes which code for MHC antigens occupy a number of sites, called loci, along a bovine chromosome. By comparison of a series of alloantisera in two international workshops, 17 class I MHC specificities have been identified which are believed to be encoded by genes at one locus, called the BoLA (bovine lymphocyte antigen) A locus. ILRAD joined this international effort in 1983 in a collaborative project initially supported by the British Government's Overseas Development Administration (ODA) through the Agriculture and Food Research Council's Animal Breeding Research Organization in Edinburgh.

Beginning in 1984, over 1000 Boran (*Bos indicus*) cattle in ILRAD's breeding herd have been typed for class I MHC determinants using antisera and monoclonal antibodies. The objectives are to compare the MHC antigens found in the Boran herd with those identified by the international workshops, to select suitable animals for experimentation and to select breeding groups with a view to producing offspring of particular MHC types. Five clearly defined antigens have been detected which were not recognized by the international workshops. These appear to be specific for *B indicus* cattle: they appear frequently among ILRAD's Boran cattle but not at all among a sample of *B taurus* animals tested. As an important component of this work, computer programs have been designed specifically for the analysis of bovine MHC antigens. This aspect of the project is being conducted in collaboration with Strathclyde University (UK), and several other laboratories have expressed interest in using the programs developed at ILRAD.

Particularly interesting class I antigens have been analysed using T-cell clones as well as antisera and monoclonal antibodies. T-cell clones have also been raised which recognize class II MHC antigens: this work represents an important advance because this technique provides the best means now available to define differences between individual cattle.

Bovine MHC antigens have been precipitated from cell lysates with antisera and monoclonal antibodies and then analysed according to molecular weight and isoelectric point. Several distinct class I molecules have been revealed. Studies initiated in 1984 have compared class I MHC antigens from *Theileria*-infected and noninfected bovine cells, but as yet no biochemical changes have been detected which are associated with *Theileria* infection.

In 1985, cytolytic T cells taken from nine cattle undergoing challenge with *T parva* were screened on panels of parasitized cells with which they were matched or mismatched with respect to six MHC antigens coded at the A locus. The cytolytic cells only killed target cells with which they shared A locus antigens. Of the six antigens examined, three consistently played a more important role in the induction of cytolytic responses. These findings suggest that parasite-induced changes on the surface of the host cell are either associated preferentially with certain MHC molecules or may stimulate immunity more effectively when associated with these molecules.

Bovine T lymphocytes infected with *Theileria* schizonts are readily maintained in a state of proliferation *in vitro*. To study cell-mediated immune responses in cattle, *in vitro* techniques have been developed to maintain populations of noninfected bovine T lymphocytes specifically reactive against *Theileria*-infected cells. Cultures are initiated by adding T lymphocytes from an immune animal to irradiated parasitized cells from the same animal in a mixed leucocyte reaction. Irradiation of the parasitized cells (referred to as stimulator cells) prevents them from proliferating, but the T lymphocytes (referred to as responder cells) are able to proliferate in response to the antigens on the parasitized cells. Adding stimulator cells to the culture on several occasions markedly increases the proportion of T lymphocytes reactive against the parasitized stimulators. Individual T lymphocytes can then be cloned from these populations by limiting dilution in the presence of appropriate growth factors. Repeated stimulation and addition of growth factors maintains their proliferative status, thus producing cloned cell populations. Cloned T-lymphocyte populations have also been produced for other purposes by stimulating T lymphocytes repeatedly with the mitogen concanavalin A and maintaining the proliferating cells with T-cell growth factor.

Two main types of T-lymphocyte clone have been identified which respond to *Theileria*-infected cells:

1. Cells restricted by class I MHC antigens which kill parasitized target cells and are specific for particular *Theileria* strains. These clones belong to the T-lymphocyte subpopulation identified by the monoclonal antibody IL-A17.

2. Cells restricted by class II MHC antigens which proliferate in response to *Theileria*-infected target cells but are not cytolytic. These clones belong to the T-lymphocyte subpopulation identified by the monoclonal antibody IL-A11.

The cloned, cytolytic T lymphocytes which are restricted by class I MHC molecules and are specific for particular *Theileria* strains (1) are almost certainly analogous to the cells generated *in vivo* in an immune animal. These clones have been used to examine the role of specific MHC antigens in the generation of an immune response as well as differences among parasite strains. They have provided the first conclusive evidence that the cytolytic T-lymphocyte response may be parasite strain specific; this finding supports the idea that the antigen on the surface of the parasitized cell is a parasite product.

The clones which proliferate in response to *Theileria*-infected cells but are not cytolytic (2) are restricted by the class II MHC and are also parasite strain specific. These clones are being used to study the role of class II MHC antigens in the immune response to *Theileria* infection as well as associated strain specificity. Their functional properties are also being investigated, for instance their possible role as helpers to cytolytic cells or in the production of soluble factors important in immunity.

The *Theileria*-specific, cytolytic T-lymphocyte clones are not only valuable reagents for defining parasite strain heterogeneity, but also may be of use in identifying the parasite-induced antigen(s) on the surface of the parasitized cell. Studies are in progress to screen for the relevant antigens by monitoring the responses of *Theileria*-specific T-lymphocyte clones to uninfected cells which have either been transfected with parasite genes or incubated with antigenic subunits of the parasite.

Another study conducted in 1985 compared the cytolytic responses of cells taken from buffalo with findings obtained from studies on bovine cells. Peripheral blood lymphocytes taken from a buffalo showed a strong specific cytolytic response when cultured *in vitro* with cells from the same animal infected with *Theileria*: up to 100% of infected cells were killed, with cytolytic-to-target-cell ratios as low as 7.5 to 1. However, this response was only observed when lymphocytes were taken from a buffalo which had previously been infected with *Theileria*. Cells taken from an uninfected buffalo did not show a cytolytic response to autologous infected cells. Current work involves analysis of the cell populations and mechanisms involved in resistance to *Theileria* infection in buffalo as well as comparisons with findings obtained in cattle.

EPIDEMIOLOGY AND EXPERIMENTAL IMMUNIZATION

ECF epidemiology studies at ILRAD have concentrated on developing methods to distinguish *Theileria* subspecies and strains. Early studies focused on serological screening using antischizont monoclonal antibodies to characterize *Theileria* populations isolated from cattle and from African buffalo. In 1985 efforts were also directed towards obtaining cloned parasite stocks and DNA probes to characterize *T parva* strains.

ILRAD scientists are conducting ECF immunization trials in cooperation with the Kenya Ministry of Agriculture and Livestock Development and the Agricultural Development Corporation. Experimental immunization techniques are based on infection with live sporozoites and simultaneous treatment with a long-lasting oxytetracycline antibiotic.

STRAIN CHARACTERIZATION

To develop immunization procedures against ECF which will protect cattle effectively in the field, scientists must be able to identify subspecies and strains of *Theileria* and determine which strains of the parasite are crossprotective. Several approaches have been explored at ILRAD for the precise characterization of parasite isolates. Large numbers of isolates have been made from infected cattle and ticks in Kenya and other countries, and these have been cryopreserved in the laboratory as stabilates of sporozoites or schizont-infected lymphoid cells. Stocks have been characterized both *in vitro* and by crossimmunity testing in cattle.

In vitro characterization has been based primarily on the recognition of schizont antigens by specific monoclonal antibodies. It appeared originally that this type of serological screening could be used to classify *Theileria* isolates into a limited number of antigenic groups or strains, but by 1985, as more monoclonal antibodies were produced and more isolates tested, greater antigenic diversity was detected. Although anti-schizont monoclonal antibodies have proven useful for comparing *Theileria* isolates and selecting stocks for further characterization, it now appears that monoclonal antibody responses alone are not sufficient to classify *Theileria* isolates into groups of parasites which are crossprotective *in vivo*.

ILRAD scientists are now investigating the use of DNA probes to characterize *Theileria* subspecies and strains. The first step has been to develop techniques for purifying *Theileria* DNA. A simplified method is now being developed to produce highly purified DNA from *Theileria* piroplasms. DNA has been extracted from blood infected with piroplasms of four *T p parva* strains—Mariakani, Muguga, Kilifi and Marikebuni. Comparison of restriction enzyme patterns shows a number of variations between the Muguga and the Mariakani strains. Interestingly, the Muguga and Kilifi patterns show fewer variations, which is in agreement with monoclonal antibody profiles and clinical studies showing crossprotection between these two strains.

Two genomic expression libraries have been constructed from *T p parva* Muguga DNA and one from DNA of *T p parva* Mariakani. These libraries carry complete gene sequences and also fragments of regions between genes. Hybridization experiments are now being carried out to identify sequences which hybridize differently in the two strains. These will be used to identify both repetitive and single-copy gene sequences in the different genomes.

Several studies have suggested that *Theileria* antigenic diversity may be limited in field situations where *T p parva* is maintained in a simple cattle/tick transmission cycle. However, in places where cattle come into contact with African buffalo, the situation becomes much more complex. *T p lawrencei*, the *Theileria* subspecies almost invariably carried by wild buffalo, is characterized by low levels of schizont and piroplasm parasitaemia in cattle, but high mortality rates. A collaborative study was initiated in 1984 to investigate the antigenic diversity of *T p lawrencei* isolates and to improve available techniques for distinguishing this subspecies from *T p parva*. Schizont-infected

cell lines obtained from the Kenya Government's Veterinary Research Laboratory and the Kenya Agricultural Research Institute were screened with 20 anti-schizont monoclonal antibodies raised against the two subspecies. By the end of 1985, scientists had screened over 50 *Theileria*-infected buffalo cell lines and 50 bovine cell lines which were either infected *in vitro* with sporozoites from ticks which had fed on buffalo or were isolated from cattle exposed to buffalo.

Marked antigenic diversity was seen not only in isolates from different animals in the same area, but also in different isolates taken from the same animal at weekly or monthly intervals. One explanation for this striking antigenic diversity is the occurrence of mixed parasite populations in isolates taken from buffalo. For example, after limiting dilution cloning, an infected lymphoid cell line from one buffalo was shown to contain at least four antigenically distinct *Theileria* populations. Once cloned, these populations retained their specific antigenic profiles throughout an 8-month *in vitro* cultivation period.

Another possible explanation for observed antigenic diversity in *T p lawrencei* infections is that these parasites may change antigenically during the course of infection in buffalo. To investigate this possibility, a 2½-year study was conducted in collaboration with the Kenya Government's Veterinary Research Laboratory, partially supported by the Government of the Netherlands. This work was completed in 1985. A buffalo maintained under experimental conditions was infected with a single stabilate of *T p lawrencei* and a steer was infected with the stabilate at the same time. The steer developed severe clinical signs of *T p lawrencei* infection and was treated with parvaquone (Clexon: Wellcome). The buffalo became a carrier. A schizont-infected cell line was isolated from the steer before treatment and tick and cell culture isolates were made from the buffalo at regular intervals. Screening with a battery of monoclonal antibodies revealed antigenic differences among cells isolated from the buffalo at different times and between cells isolated from the buffalo and from the steer.

Due to the very small number of parasites seen in *T p lawrencei* infections, it is not possible to obtain sufficient quantities of piroplasm DNA for genomic studies of this subspecies. However, DNA has been prepared from schizont-infected buffalo cell lines, and these preparations will be screened with genomic probes derived from *T p parva* piroplasm DNA. The goal is to identify probes which will distinguish between the two *Theileria* subspecies.

The possibility of antigenic variation in *T p lawrencei* infection cannot be determined conclusively until the existence of mixed infections can be ruled out. This could be done by infecting *Theileria*-free cattle and buffalo with cloned parasites and checking for antigenic diversity in sequential isolates taken over a period of time. Considerable effort at ILRAD now concentrates on developing techniques to obtain *Theileria* clones derived unequivocally from single parasites. Three approaches are being investigated. These involve the isolation of sporozoites from a single infected tick acinus, the implantation of small numbers of *Theileria* kinetes (the parasite form which develops in the tick gut and moves to the salivary gland) into an uninfected tick, or the infection of bovine lymphocytes with small numbers of sporozoites obtained by limiting dilution, followed by cloning the infected cell line.

Theileria infection rates are highly variable in ticks, making it difficult to identify ticks with only one infected acinus. In a collaborative project conducted in 1985, scientists examined salivary glands from ticks fed on an infected buffalo: salivary glands which contained only a single infected acinus were crushed to extrude the *T p lawrencei* sporozoites into medium, and this suspension was used to infect three Boran steers. *Theileria* schizonts were detected for a brief period in two of the steers, but no piroplasms

were observed. All three animals survived the challenge and all showed anti-*Theileria* antibodies 4 weeks after infection. When the three recovered steers and two susceptible controls were challenged with a *T p lawrencei* stabilate prepared from ticks fed on the same infected buffalo, both controls and one recovered animal died of typical *T p lawrencei* infection. The two steers which had demonstrated schizonts following the initial challenge both recovered.

This experiment showed that it is possible to infect susceptible cattle with a direct inoculation of sporozoites derived from a single tick acinus, although infections were transient. The fact that one out of three steers was susceptible to homologous challenge could be due to antigenic differences between the inoculated sporozoites derived from a single acinus and the bulk stabilate. Another possibility is that the infective dose was insufficient to elicit a protective immune response. This work was carried out in collaboration with the Kenya Agricultural Research Institute and the Kenya Government's Veterinary Research Laboratory.

Another project carried out in 1985 aimed at establishing *Theileria* clones by transplanting small numbers of kinetes into uninfected ticks. A mixture of haemolymph (equivalent to blood) and gut content was taken from ticks infected with *T p parva* (Muguga), diluted and injected into uninfected ticks. The number of kinetes contained in each inoculum was estimated by analysis of the residual haemolymph/gut mixture, and recipient ticks were grouped according to the estimated number of parasites received. After incubation, the recipient ticks were fed on rabbits for 4 to 5 days, their salivary glands were dissected out and stained, and the number of infected acini in each tick was determined.

The results showed considerable mortality among recipient ticks, no doubt due in part to the crude nature of the inoculation. However, transplantation of 18 to 138 kinetes gave an infection rate of 1 to 5 acini in a high percentage of surviving ticks. This kinete transplantation technique could make it possible to obtain tick salivary gland acini with low *Theileria* infection rates more reliably than infection of ticks by natural feeding.

Efforts to obtain *Theileria* clones by limiting dilution have concentrated on techniques to infect cloned bovine lymphoid cell lines with very small numbers of sporozoites. Sporozoites are serially diluted and incubated with a fixed number of lymphocytes in the presence of T-cell growth factor. After 4 days, the growth factor is removed and all noninfected cells die off. The infected cells are then recloned. The aim is to produce cloned cell lines arising from a single lymphocyte infected with a single sporozoite.

EXPERIMENTAL IMMUNIZATION

ILRAD scientists conducted an extensive field trial in 1985 at Kiswani, near the Kenya coast, in collaboration with the Ministry of Agriculture and Livestock Development and the Agricultural Development Corporation. The objective was to determine the effect of ECF immunization combined with different acaricide treatment regimes on the productivity of beef cattle.

Eighty 2-year-old Boran steers were immunized by infection and treatment against ECF using a local stock, *T p parva* (Marikebuni). Eighty similar nonimmunized cattle were used as controls. All cattle were maintained in an ECF endemic area for 9 months, beginning in May 1985. They were divided into four groups of 20 immunized and 20 nonimmunized animals under different tick-control regimes, as follows:

1. Forty cattle were sprayed with acaricides twice a week

2. Forty cattle were sprayed with acaricides once every 3 weeks

3. Forty cattle wore acaricidal ear tags

4. Forty cattle had no tick-control treatment.

All cattle were examined regularly: weights, tick counts and packed cell volumes (PCVs—an indication of anaemia) were recorded weekly. Sera from all cattle were examined for antibodies to *T parva* every month, and temperatures were taken every 2 days. Blood and lymph node smears from all cattle with fever were examined daily, and most nonimmunized cattle with ECF were treated with an antitheilerial drug. At the end of the 9-month trial period, the treatment groups were compared in terms of the effectiveness of ECF immunization and the different tick control regimes.

Eighteen of the 80 immunized cattle became infected with ECF. Of these, 15 recovered spontaneously and 3 died: none were treated. Among the 80 nonimmunized animals, 58 cases of ECF were recorded: 44 were treated with either parvaquone or halofuginone and 14 were not treated. Fifteen died of ECF. Immunized cattle showed better weight gains than nonimmunized controls under all four tick-control regimes. More detailed results are shown in Figure 5.

One constraint on the widespread implementation of ECF immunization by infection and treatment has been the absence of a reliable *in vitro* assay to quantitate *Theileria* sporozoites. At present, it is impossible to standardize the number of sporozoites inoculated during immunization. It is also impossible to compare the infectivity or virulence of *Theileria* stocks when observed differences may be due to unknown variations in sporozoite numbers.

A new method for counting *Theileria* sporozoites is being explored, using the monoclonal antibody D_1 which recognizes sporozoites and sporoblasts in different types of fixed and unfixed preparations, but does not crossreact with any other developmental stage of the parasite. The monoclonal antibody is labeled with fluorescein isothiocyanate and incubated with the parasites, and the culture is analysed using ILRAD's fluorescence-activated cell sorter (FACS). Three distinct populations appear which have been identified as sporozoite particles, intact sporozoites and sporoblasts. Sporozoites sorted with the FACS maintain infectivity for cattle and for bovine lymphocytes *in vitro*; they are about 0.8 μm in size.

Two *T p parva* stocks, Muguga and Marikebuni, have been analysed by this system. A comparison of suspensions obtained from infected ticks which had fed for 4 days suggested that the two stocks may have different maturation cycles: sporoblasts accounted for about 10% of suspensions from ticks infected with *T p parva* (Muguga), compared with 30 to 40% of suspensions from ticks infected with the Marikebuni stock. Gene sequences which are being identified in different *Theileria* populations may also provide useful tools for determining the number of sporozoites present in parasite stabilates. The eventual aim of this work is to produce *Theileria* stabilates which are well characterized in terms of number of sporozoites, infectivity, monoclonal antibody and crossimmunity responses and sensitivity to oxytetracycline.

Immunization and Acaricide Regime	Number of Cattle	Number Surviving	Mean Weight Gain of Survivors (kg)
Immunized			
Sprayed twice/wk	20	20	67.6
Sprayed once/3 wks	20	19	51.2
Ear tags	20	19	62.4
No acaricide	20	19	55.6
Total or overall	80	77	59.2
Nonimmunized			
Sprayed twice/wk	20	19	54.2
Sprayed once/3 wks	20	15	38.8
Ear tags	20	16	34.2
No acaricide	20	14	41.1
Total or overall	80	64	42.1

Figure 5. Productivity in terms of survival and average weight gain of immunized and nonimmunized Boran steers kept for 9 months in an ECF-endemic area at the Kenya coast under different acaricide treatment regimes.

Two types of drug strategy are commonly used in infection and treatment immunization: oxytetracycline cover, usually at the time of infection, or parvaquone therapy 8 days after infection. In situations where the two approaches are equally effective, oxytetracycline treatment would be preferred because it is considerably cheaper and requires the animals to be handled only once. However, some ECF infections are not contained by oxytetracycline treatment.

Studies are in progress at ILRAD to elucidate the mechanisms of action of oxytetracycline. The objective is to improve the effectiveness of this drug in infection and treatment immunization and also to make it possible to anticipate situations where the drug may not be effective. Oxytetracycline was added to bovine lymphocytes *in vitro* at various stages of infection with *T parva* in concentrations similar to the level which could be expected in treated animals. Results suggest that the drug affects the development of infected lymphocytes after sporozoite attachment and entry. Enzyme studies indicate that oxytetracycline may affect energy processes or the synthesis of proteins required for the establishment of the parasite in the host cell. The drug appears to reduce the rate of establishment of infection rather than inhibiting infection completely. Studies are now in progress to determine whether the observed effects are due to direct action on the parasite, the host cell or both.

Studies were initiated in 1985 to explore the immunogenic potential of a *T parva* stock isolated in Zimbabwe, *T p bovis* (Boleni), which appears to cause mild reactions in cattle. When a stabilate of the Boleni stock was inoculated into Boran cattle at ILRAD, all experimental animals developed mild infections but recovered without treatment. These cattle then resisted challenge with two virulent *T p parva* stocks, Muguga and Marikebuni.

A stabilate prepared from ticks which had fed on two of the experimental animals was inoculated into 28 cattle. Most of these animals underwent mild reactions, though a few reacted severely and one died. The survivors were divided into four groups and challenged with stabilates of *T p parva* (Marikebuni), *T p parva* (Mariakani), *T p lawrencei* (Ngong) or *T p lawrencei* (Buffalo 2306). Pairs of susceptible controls were also

challenged with each of the four stabilates. Most of the experimental cattle showed mild or inapparent reactions but those infected with *T p parva* (Mariakani) reacted more severely. All the controls reacted severely, and several died.

This experiment suggests that *T p bovis* (Boleni) generally causes mild reactions in *B indicus* cattle and confers resistance to lethal challenge with some *T p parva* and *T p lawrencei* stocks. Most attempts to grow the Boleni stock in culture have failed. It may be that this parasite does not readily cause host cell transformation, making it difficult to establish infection both *in vitro* and *in vivo*. This would account for the mild reactions observed in infected cattle. Studies continue on *T p bovis* (Boleni) in light of its potential value as a key immunizing stock.

While work continues on improving techniques for ECF immunization, several countries have initiated field trials aimed at expanding immunization by infection and treatment. Long-term studies are being conducted by national programs in Kenya, Rwanda, Zimbabwe and Tanzania (Zanzibar), and by international programs operating in Burundi, Malawi and Zambia. ILRAD is providing training and technical advice and support.

Two international workshops were held at ILRAD, in 1984 and 1985, to discuss the activities and needs of national programs conducting ECF immunization field trials. In response to requests made at these workshops, ILRAD is setting up a computerized database which can be used by national programs to process and analyse data relating to the performance of cattle immunized against ECF. Specific ILRAD support activities have been as follows:

1. Burundi—characterization of local *T parva* isolates *in vitro* and *in vivo*, inservice training

2. Kenya—characterization of local *T parva* isolates *in vitro* and *in vivo*, assistance in immunization trials (Ngong), inservice training

3. Malawi—characterization of local *T parva* isolates *in vitro*, provision of monoclonal antibodies

4. Rwanda—characterization of local *T parva* isolates *in vitro* and *in vivo*, provision of stabilates

5. Tanzania (Zanzibar)—consultancy and epidemiological survey, isolation and characterization of local *T parva* stocks

6. Zambia—characterization of local *T parva* isolates *in vitro*

7. Zimbabwe—characterization of local *T parva* isolates *in vitro* and *in vivo*, assist-

ance in establishing theileriosis immunization program, inservice training.

HEARTWATER: SOME PRELIMINARY OBSERVATIONS

Heartwater is a tickborne disease which causes serious illness and death in cattle, sheep and goats. ECF immunization trials in Kenya's Coast Province have revealed a steady incidence of this disease, and it is likely that the importance of heartwater will increase as more animals are immunized against ECF. Heartwater is caused by the microorganism *Cowdria ruminantium*, transmitted to livestock by the tick *Amblyomma variegatum*.

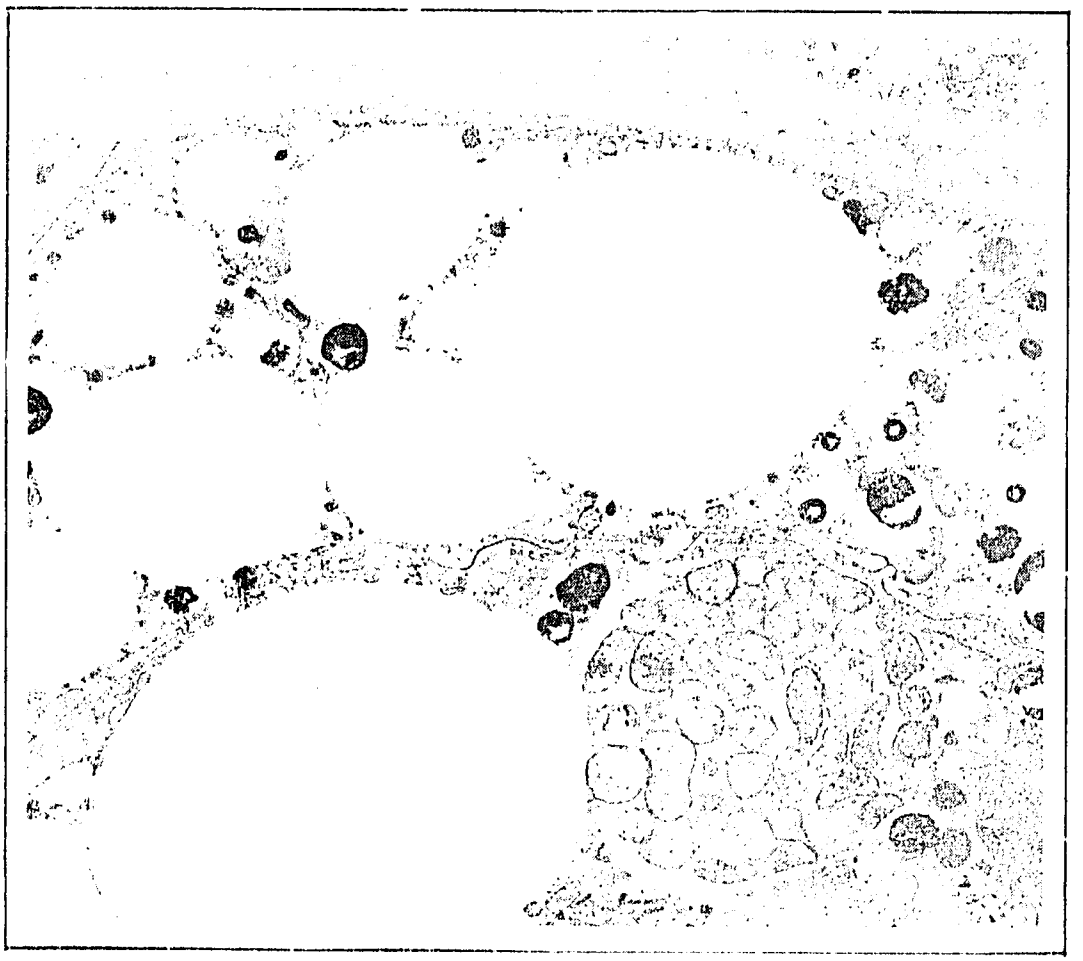


Figure 6. Electron micrograph of a section of a midgut epithelial cell taken from an adult female *Amblyomma variegatum* tick. The tick had fed as a nymph on a cow infected with *Cowdria ruminantium* and after moulting into an adult had attached to an uninfected animal. The cell contains large lipid droplets and one vacuole containing microorganisms believed to be *C ruminantium*. Another packed group of organisms in a round vacuole at the right edge of the micrograph may be a different stage or form of the same parasite. The gut lumen is shown at the top of the micrograph. Original magnification was 12,000.

Because of the potential importance of heartwater in ECF-endemic areas, ILRAD scientists are looking at the development of the *Cowdria* organism in the tick vector and mammalian host. One objective is to identify stages which could be used as a source of antigen for a diagnostic test.

A stabilate of *C ruminantium* was derived from adult ticks originating at Kiswani in Kenya's Coast Province. This stabilate is infective to susceptible cattle and sheep. Infected tick larvae, nymphs and unfed adults have been examined by electron microscopy, as well as blood and tissues from the brain, heart, kidney, liver and spleen of fatally infected cattle.

No *Cowdria* was found in larval ticks, but *Cowdria*-type microorganisms were observed in nymphs and unfed adult ticks in vacuoles within cells of the midgut (Figures 6 and 7). These were observed from as early as 12 days up to 40 to 43 days after completion of feeding on an infected cow. The gram-negative, bacteria-like microorganisms were of a generally similar morphology, although slight variations in form and size were observed. Similar microorganisms have not been observed in other tick organs, though this does not necessarily prove their absence. Detection of the parasites will be greatly facilitated once a specific anti-*Cowdria* antibody is available.

Figure 7. Electron micrograph of parasites believed to be *Cowdria ruminantium* within a large vacuole of a tick midgut cell. The specimen was fixed 35 days after the *Amblyomma variegatum* tick completed feeding on an infected animal. The parasites are bacillary or cocco-bacillary in shape, about 0.5 to 0.75 μm in diameter and up to 2 μm long. Some profiles show evidence of binary fission (arrows). Each parasite is enclosed by an outer and inner limiting membrane typical of gram-negative bacteria. The cytoplasm contains fine DNA filaments and particles resembling ribosomes. No internal membranes are present. Original magnification was 35,000.

IMMUNE RESPONSES AND THE TICK

The brown ear tick, *Rhipicephalus appendiculatus*, which transmits ECF remains attached and continues feeding on its bovine host for several days. During this period, the tick releases a powerful anticoagulant into the host, along with attachment cement, a number of enzymes, enzyme inhibitors and pharmacological agents. This 'cocktail' of biologically active molecules is required to prime the attachment site, to allow tissue penetration, to assimilate the blood meal and to block host defence mechanisms. However, there is evidence that some of these substances are capable of stimulating a delayed-type hypersensitivity response in the host which confers a degree of resistance to tick feeding.

When rabbits are sensitized by inoculation with tick salivary gland extract or by tick feeding, they develop strong immunological responses to feeding ticks. This is shown by a reduction in the number of ticks attaching to sensitized rabbits and by lower engorgement weights in the ticks that do attach and feed. Similar immune responses are acquired naturally by cattle in the field after multiple tick infestations. Researchers have tried to enhance this resistance by immunizing cattle with tick antigens, generally using complex tick extracts. These procedures have not improved upon the limited resistance attained naturally and they are probably also impractical for field application. Research is in progress at ILRAD to identify specific immunogenic substances in tick saliva and to explore the possibility of using high concentrations of these substances in different adjuvant systems as a vaccine against ticks.

Rabbits and guinea pigs which have developed immune responses to feeding ticks generate antibodies that recognize a number of tick antigens. These antibodies have been used to study the development of antigens during the course of tick feeding, indicating that the presence and concentration of many tick salivary antigens vary in the course of the feeding cycle. Larvae and nymphs also appear to lack many of the antigens present in adult ticks.

Because of the importance of anticoagulant activity for the survival of feeding ticks, investigations in collaboration with the University of Nairobi's Department of Biochemistry focused on purifying and analysing the anticoagulant molecule contained in tick salivary gland extracts (Figure 8). This work was completed in 1985. The purified anticoagulant (Figure 9), which is a prothrombinase complex inhibitor, does not stimulate an immune response in rabbits, perhaps due to the extensive manipulation required for purification of the molecule or to its rapid metabolism in the host. Thus, the anticoagulant is not a likely candidate to provide the basis for a vaccine against tick infestation. However, studies on its structure may elucidate underlying molecular mechanisms which could be manipulated to improve its immunogenicity.

Other components of tick saliva are now being investigated with the aim of isolating individual salivary components which might serve as antigens in a vaccine against ticks. Studies are in progress to determine the role of different types of molecules in relation to enhancing tick infestation and sporozoite transmission, circumventing host defences and mediating inflammatory reactions. Antihistamines, enzyme inhibitors and immunosuppressive and anticomplement molecules are being analysed.

In particular, scientists are looking for potentially immunogenic molecules which are present during the initial stage of tick attachment. Analysis of tick salivary gland proteins by SDS polyacrylamide gel electrophoresis (SDS-PAGE) indicates numerous changes during the course of attachment and feeding. One dominant molecule of 67,000 daltons is absent in unfed ticks, evident soon after tick attachment and absent thereafter,

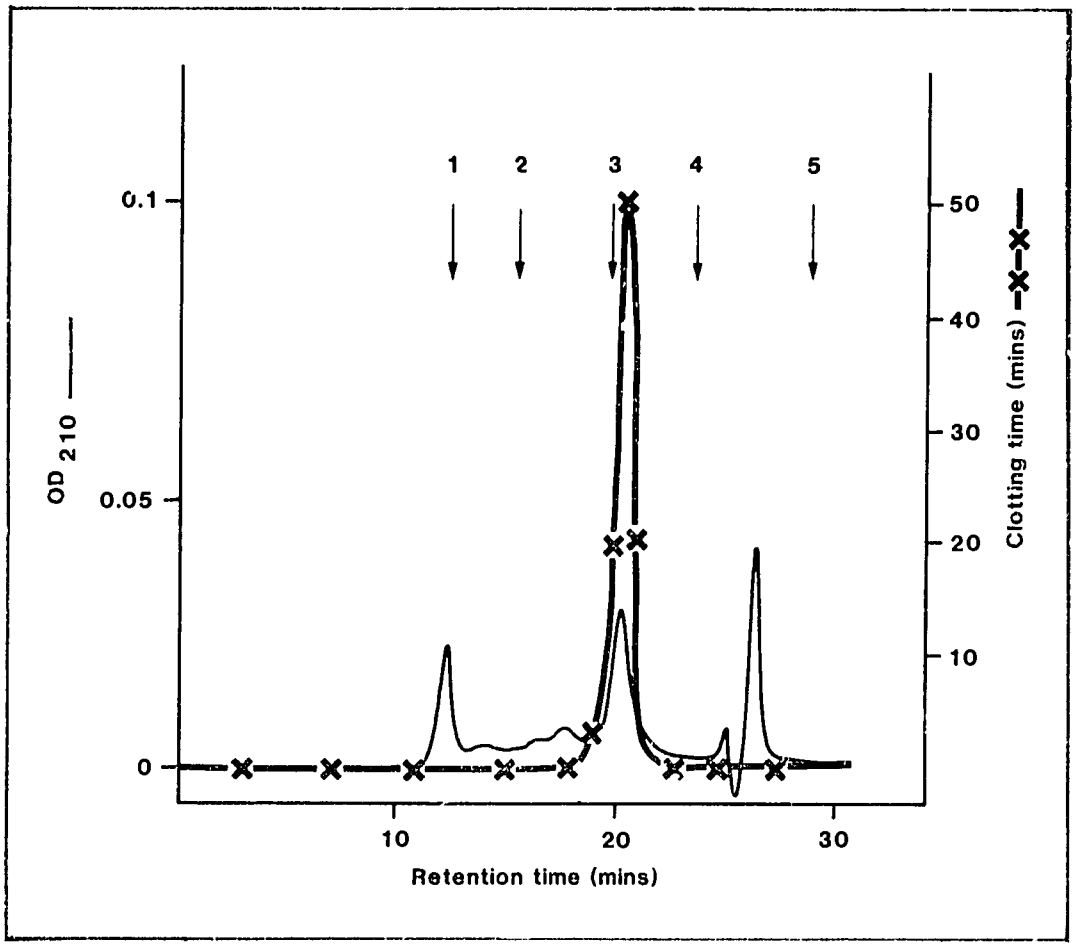


Figure 8. The HPLC elution profile of an anticoagulant molecule purified from *R appendiculatus* salivary gland homogenates. The chromatogram depicts retention times of standard sizing proteins (arrows), protein absorbance at 210 nm. The tick anticoagulant activity coelutes with a symmetrical protein peak with a retention time of about 20 minutes.

suggesting that it may be secreted or converted to other substances required for tick development as feeding progresses. Anti-tick antibodies are now being used to purify this molecule.

Another antigenic protein, with a molecular weight of 90,000 daltons, has been isolated from tick salivary glands. Localization of this protein within the salivary gland suggests that it may contribute to the development of the water secretion mechanism during the process of tick feeding. It might be possible to interfere with this important physiological process in the tick by immunizing livestock with this antigen. This possibility is now being investigated.

Previous work at ILRAD has shown that when *R appendiculatus* ticks feed on an animal immunized against ECF, anti-*Theileria* antibody present in the host is ingested by the ticks and passes unchanged across the gut into the haemolymph. In 1985, scientists investigated whether such antibody could interfere with the development of

Theileria parasites within the tick, thus interrupting the disease transmission cycle. Experiments were based on a monoclonal antibody (D_1) raised against *T parva* sporozoites and on antisera from cattle immunized against ECF.

The monoclonal antibody D_1 neutralizes the infectivity of *T parva* sporozoites *in vitro*. Earlier studies showed that it recognizes an antigenic determinant synthesized during sporozoite development in the tick salivary gland 2 to 3 days after tick feeding commences. In the first experiment conducted in 1985, a rabbit was injected daily for 4 days with 800 μl of D_1 as mouse ascites fluid, while a control rabbit was given injections of phosphate saline buffer.

Immediately after the first injections, 20 male and 20 female *R appendiculatus* ticks were applied to the ears of each rabbit. These adult ticks had been infected as nymphs by feeding on a calf infected with *T p parva* (Muguga). After 4 days' feeding, the partially engorged ticks were removed and their salivary glands were dissected out and examined for parasites. Haemolymph collected from the ticks was also examined.

About 0.006 μl of D_1 could be detected in the haemolymph of the ticks fed on the antibody-treated rabbit. There was a significantly lower percentage of mature sporozoites in both male and female ticks fed on this rabbit, as compared to the percentage of sporozoites reaching maturity in ticks fed on the control. This indicates that anti-*Theileria* antibody passed across the tick gut during feeding, entered the haemolymph and affected the development of parasites within the salivary gland.

In a second experiment, immunoglobulin (IgG_2) was prepared from the sera of cattle which were immunized against *Theileria* sporozoites and showed high anti-sporozoite antibody titres. A rabbit was injected daily for 4 days with 500 μl of bovine IgG_2 from an immunized cow and a control rabbit was given injections of normal bovine IgG_2. After the first injections, 20 male and 20 female *R appendiculatus* ticks infected with *T p parva* (Muguga) were applied to the ears of each rabbit. Examination of their salivary glands after 4 days' feeding revealed fewer mature sporozoites in ticks fed on the rabbit treated with immunized bovine IgG_2 than in ticks fed on the rabbit treated with IgG_2 from nonimmunized cattle.

Evidence has already accumulated that cattle which recover from ECF produce anti-sporozoite antibodies which may play a role in protecting them against subsequent infection. This work suggests that anti-sporozoite antibodies might also suppress parasite development within the tick.

Figure 9. A sodium dodecylsulphate polyacrylamide gel showing crude tick (*R appendiculatus*) salivary gland homogenate (left lane) used as the starting material for the purification of a tick salivary anticoagulant. The anticoagulant is shown in the right lane as a single band of purified protein.

TRYPANOSOMIASIS

Trypanosomiasis is caused by protozoan parasites which infect cattle, sheep, goats, pigs, horses, donkeys, camels, many types of wild animals and man. The most important trypanosome species in economic terms are those which infect domestic ruminants in Africa: *Trypanosoma congolense*, *T vivax* and *T brucei brucei*. Closely related *T brucei* subspecies—*T b rhodesiense* and *T b gambiense*—cause human sleeping sickness.

Trypanosomes are most often transmitted cyclically by about 30 species and subspecies of tsetse fly (*Glossina* spp). These insects infest a wide range of habitats over about 10 million km^2 of Africa, representing 37% of the continent or about half the habitable land. In regions infested with tsetse flies, chronic trypanosomiasis causes a severe reduction in animal productivity, reflected in poor growth, low milk yields, reduced capacity for work and infertility. In severely infested areas, it is very difficult to keep livestock at all, and even human habitation may be threatened.

Trypanosomiasis also occurs in Latin America, the Middle East and Asia. In these areas, the parasites are transmitted mechanically by biting flies. Outbreaks of *T vivax* have been reported in several South American countries, though the epidemiology of the disease has not been fully documented. Another trypanosome species, *T evansi*, occurs in Africa, the Middle East, Asia and South America. This mechanically-transmitted species causes a severe disease in horses and camels and loss of productivity in cattle and domestic buffalo.

Programs to control trypanosomiasis have been in operation for nearly 100 years. Initial attempts involved control of the tsetse vector, either by destroying the wild animal populations on which tsetse feed or clearing the bushy vegetation that tsetse require as breeding and resting sites. More recently, most tsetse control programs have been based on the application of insecticides. Considerable success has been achieved where control measures using insecticide have been properly implemented—notably in Nigeria, Botswana, Zambia and Zimbabwe. However, insecticide spraying is not entirely effective in areas of heavy tsetse infestation, dense vegetation or high rainfall, and spraying programs require a substantial investment and a high level of management. For these reasons, areas infested by tsetse flies are expanding in many parts of Africa. There is also a risk that repeated, large-scale insecticide spraying will have harmful effects on the environment.

Regular treatment with trypanocidal drugs has been an important control method since the early 1920s. This approach has been successful in situations where livestock man-

agement has been competent and consistent over time. Disease control regimes based on chemotherapy must be implemented indefinitely because domestic animals are exposed to an extensive reservoir of infection in the wildlife population. The possibility of drug resistance developing in local parasite populations is also a serious consideration as no new trypanocidal compound has been introduced for general use in the last 25 years.

The goal of ILRAD's research program is to develop improved measures to control trypanosomiasis—measures which are effective, safe and economically sound. Research activities are broadly based, but can be considered under three headings: improved control of the parasites, enhanced host resistance to infection and trypanosomiasis epidemiology.

IMPROVED CONTROL OF THE PARASITES

The three major salivarian trypanosome species proceed through several distinct stages during their lifecycle in the tsetse vector and mammalian host. When a tsetse fly feeds on an infected animal, it ingests trypanosomes along with blood, and these undergo a cycle of development within the fly. *T brucei* develops in the tsetse midgut, proventriculus and salivary gland, while *T congolense* develops in the midgut, proventriculus and mouth parts and *T vivax* develops entirely in the mouth parts. All three species finish this phase of development as metacyclic forms. When the infected fly next feeds, the metacyclic trypanosomes are injected into the skin along with tsetse saliva.

When *T congolense* or *T brucei* parasites are transmitted to an uninfected animal by a tsetse fly, a local skin reaction several centimetres in diameter—called a chancre—develops at the site of the bite. The metacyclic trypanosomes develop further in the chancre, invade the local lymph vessels and move through the regional lymph node into the bloodstream. *T vivax* parasites also move from the skin through the lymphatic system to the bloodstream, but the local skin reaction, if it occurs, is less severe.

Infection in the bloodstream is characterized by successive waves of parasitaemia as trypanosome populations multiply rapidly, then most of the parasites die, but a few survive and begin multiplying again. *T vivax* and *T brucei* parasites may also invade the connective tissues, and, in later stages of infection, all three trypanosome species may be observed in the central nervous system.

Metacyclic trypanosomes and the forms which develop in the mammalian bloodstream are coated with a dense layer of antigen 12 to 15 nm thick. This surface coat is composed of glycoproteins, referred to as variable surface glycoproteins or VSGs. Trypanosomes developing in the tsetse fly are not coated with VSGs until they reach the metacyclic stage, ready for transmission to a mammalian host.

When an animal becomes infected, it produces antibodies against the VSGs displayed by the first wave of invading trypanosomes. However, before all the parasites can be eliminated, new trypanosomes emerge which are coated with different VSGs, and these are capable of evading the animal's initial immune response. These trypanosomes multiply rapidly and the host produces new antibodies, but then parasites appear with yet another VSG, always keeping a step ahead of the host response. In laboratory animals, more than 100 different VSGs have been observed amongst the descendents of

a single trypanosome, and scientists estimate that descendants of a single parasite could display several hundred different types of surface coat.

This remarkable ability to change the antigenic composition of the surface coat—called antigenic variation—is the primary mechanism which prevents most domestic animals from developing effective immunity against trypanosomiasis. The same mechanism would enable the parasite to evade a conventional vaccine, which works by priming an animal's antibody responses against one or a small number of antigens.

Research at ILRAD concentrates on the mechanism of antigenic variation—its genetic basis and the way VSGs are synthesized in the trypanosome and moved to the surface coat. Studies also focus on different stages of the trypanosome lifecycle and the mechanisms involved in the transition from one stage to the next. The goal is to identify factors which could be manipulated to disrupt the development of the parasites or make them vulnerable to host defences.

ANTIGENIC VARIATION: *T BRUCEI* AND *T CONGOLENSE*

Trypanosomes contain a large number of VSG genes—probably several hundred. Each codes for a different protein which can potentially form the protein component of the surface glycoprotein. Generally, only one of these genes is expressed at a time. Molecular biologists at ILRAD and in other laboratories have been working to elucidate the mechanisms involved in VSG gene expression and the genetic switching which takes place when trypanosomes change from one surface coat to another.

To be expressed, a VSG gene must be located in an expression site near one end of a chromosome, called a telomeric site. From this site, the gene is transcribed from DNA into mRNA, and from mRNA it is translated into the protein of the surface coat. There are several expression sites in the trypanosome genome, but a gene is transcribed into mRNA from only one site at a time. Antigenic switching may occur when VSG genes are exchanged in an active expression site or when a different expression site becomes activated. The mechanism involved in the activation of a VSG-gene expression site remains unknown.

Antigenic switching also occurs when one gene replaces another in an active expression site. The most thoroughly studied situation occurs when a VSG gene is replaced in an expression site by a duplicated copy of another VSG gene. Gene replacement most commonly involves recombination between repetitive sequences within a 'barren' region on one side of the VSG gene in its expression site. ILRAD scientists have been analysing this flanking region to see if it might also be involved in the mechanism of gene switching without gene replacement.

These studies have concentrated on the region surrounding two copies of the *T brucei* ILTat 1.3 VSG gene, one expressed and one not expressed. Both gene copies are located in telomeric sites—one on a minichromosome and one in an expression site. The 5′ barren region of the gene copy in the expression site was sequenced in 1985 and was found to be identical whether the site was active or not, suggesting that DNA sequence alteration in the barren region does not have a necessary role in the regulation of activity of this site. Comparison with the 5′ flanking sequence of the minichromosome gene copy suggested that the two gene copies were not derived independently from a nontelomeric gene, but rather derived one from another by serial duplications. This would imply that trypanosome VSG genes may move from one telomeric site to another by a mechanism similar to that involved in movement from a nontelomeric to a telomeric site.

A segment of DNA from an expression site formerly occupied by the ILTat 1.3 gene was also cloned from a trypanosome in which the expressed copy of the gene had been lost. Transcription studies indicated that the loss of the ILTat 1.3 gene was the result of replacement by another VSG gene in the expression site. Experiments using orthogonal field-alternation gel electrophoresis (OFAGE) showed that the new DNA segment came from a minichromosome. Sequence analysis showed that at least 16 barren region repeats were brought into the expression site from the minichromosome along with the new VSG gene. This indicates that minichromosome VSG genes may be flanked by extended repeat arrays like those flanking expressed genes, in contrast to the short arrays next to nontelomeric genes. These observations also suggest that VSG genes associated with relatively long flanking arrays may be more likely than other genes to move into expression sites.

This work showed that the ILTat 1.3 VSG gene remained on the same small chromosome whether it was expressed or not. In conjunction with mapping data, this observation provides evidence that VSG gene expression switches may not always involve chromosome rearrangements. On the other hand, translocations and deletions of large segments of chromosomes can lead to alteration of VSG gene expression. Using OFAGE, scientists observed an expressed *T brucei* ILTat 1.1 VSG gene at the end of a chromosome which was later lost by a large deletion.

Relatively little work has been published on the molecular biology of antigenic variation in *T congolense*. In collaboration with the Vrije Universiteit Brussel (Belgium), ILRAD scientists are investigating whether the mechanisms underlying antigenic variation in *T congolense* are comparable to those observed in other trypanosomes. Cloned *T*

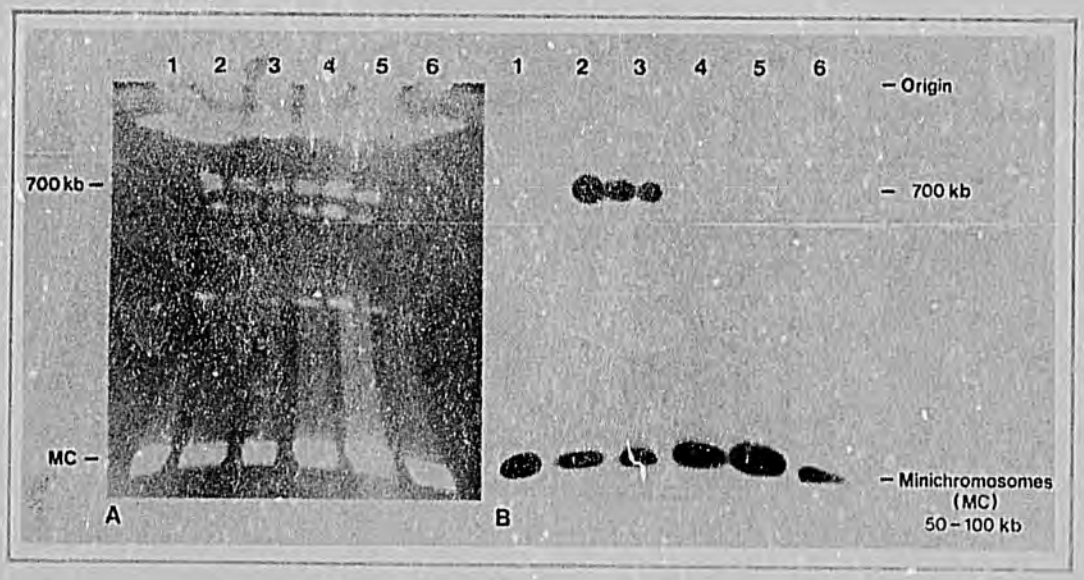


Figure 10. Interchromosomal duplicative translocation of the *T congolense* ILNat 2.1 VSG gene. Chromosome-sized DNA molecules from six ILNaR 2 trypanosome clones, expressing (lanes 1-3) or not expressing (lanes 4-6) ILNat 2.1 VSG, were resolved in a 1.5% agarose gel by OFAGE at 20-second pulse intervals. A. Photograph of Ethidium bromide (EtdBr)-stained gel. The chromosome-sized DNA in the gel was transferred to nitrocellulose filter paper and hybridized with pcN68, a recombinant plasmid containing sequences encoding ILNat 2.1 VSG. B. Autoradiograph of this filter. Approximate sizes of chromosomes, estimated by comparison with those of *T brucei*, are indicated.

congolense VSG genes have also been used as hybridization probes to determine their usefulness in studies of the epidemiology of trypanosomiasis.

Detailed investigations have concentrated on two VSG genes, each from antigenically distinct *T congolense* populations representing different serodemes. Trypanosome clones from the ILNaR 2 repertoire retained a basic copy of the ILNat 2.1 VSG gene on a minichromosome with an average size of 100 kb, whether the gene was expressed or not. Clones which expressed the ILNat 2.1 gene were found to have an extra copy of the gene located on a 700 kb chromosome, but the extra copy was not present in clones which had switched off expression of the gene. No alteration in the molecular karyotypes of the ILNaR 2 clones was observed: the number and size of chromosomes between 200 and 1000 kb appeared constant among all the variants examined (Figure 10). These observations indicate that the ILNat 2.1 VSG gene is expressed by duplicative transposition. They also suggest that rearrangement of the ILNat 2.1 gene is not associated with large-scale chromosome translocations.

By contrast, trypanosome clones in the *T congolense* ILNaR 3 repertoire showed the same number of copies of the ILNat 3.2 VSG gene whether the gene was expressed or not. This VSG gene was not found in the genomes of *T congolense* clones from other serodemes. No signals of hybridization were detected, either in restriction enzyme digests of total DNA or on whole chromosomes separated by OFAGE (Figure 11). This observation extends results from similar investigations using the ILNat 2.1 VSG gene in 1984. The sequences encoding these two *T congolense* VSGs occur only in parasites of the two respective serodemes. If sequences encoding other *T congolense* VSGs are also present only in their respective serodemes, then, as hybridization probes, they might be a useful adjunct for serodeme characterization.

ANTIGENIC VARIATION: *T VIVAX*

Electron microscopic studies in 1984 revealed a surface glycoprotein coat on metacyclic forms of both East and West African stocks of *T vivax*, similar to the surface coat on *T brucei* and *T congolense*. Studies on *T vivax* VSGs in 1985 concentrated on extending the antigenic analysis of the ILDaR 1 serodeme from West Africa. Work was hampered by the very small numbers of *T vivax* parasites available for study and by the apparent instability of VSGs displayed by this species. However, employing a recently developed culture system, new antigenic types were generated not previously recognized in early bloodstream populations in goats and mice. Since the culture system apparently mimics the lifecycle of the parasite in the tsetse vector, it will be of interest to compare the new antigenic variants produced in culture with the antigenic types displayed by metacyclic trypanosomes from tsetse.

Serodeme relationships were established in *T brucei* and *T congolense* infections by examining the resistance of animals to homologous or heterologous challenge following a regimen of cyclical infection and treatment. However, this was not always possible with *T vivax*. A detailed investigation in goats employing stocks and clones from East and West Africa showed that homologous immunity is difficult to elicit, sometimes even after several challenges by infected tsetse. This was principally ascribed to the very low number of metacyclic trypanosomes extruded by *T vivax*-infected tsetse and to the antigenic lability of the bloodstream trypanosomes, as demonstrated in earlier studies. However, in further experiments, it was possible to demonstrate immunity in cattle to homologous tsetse challenge when the animals were allowed to recover for several

months following self-cure of the primary infection. In some instances, protection against heterologous challenge was also elicited, using stocks of *T vivax* from different areas of Kenya. This suggests that immunity to *T vivax* infection may entail responses to the entire antigenic repertoire of a serodeme.

A relatively stable bloodstream variant—ILDat 1.2—was identified, and work is in progress in collaboration with the University of Victoria (Canada) to characterize the

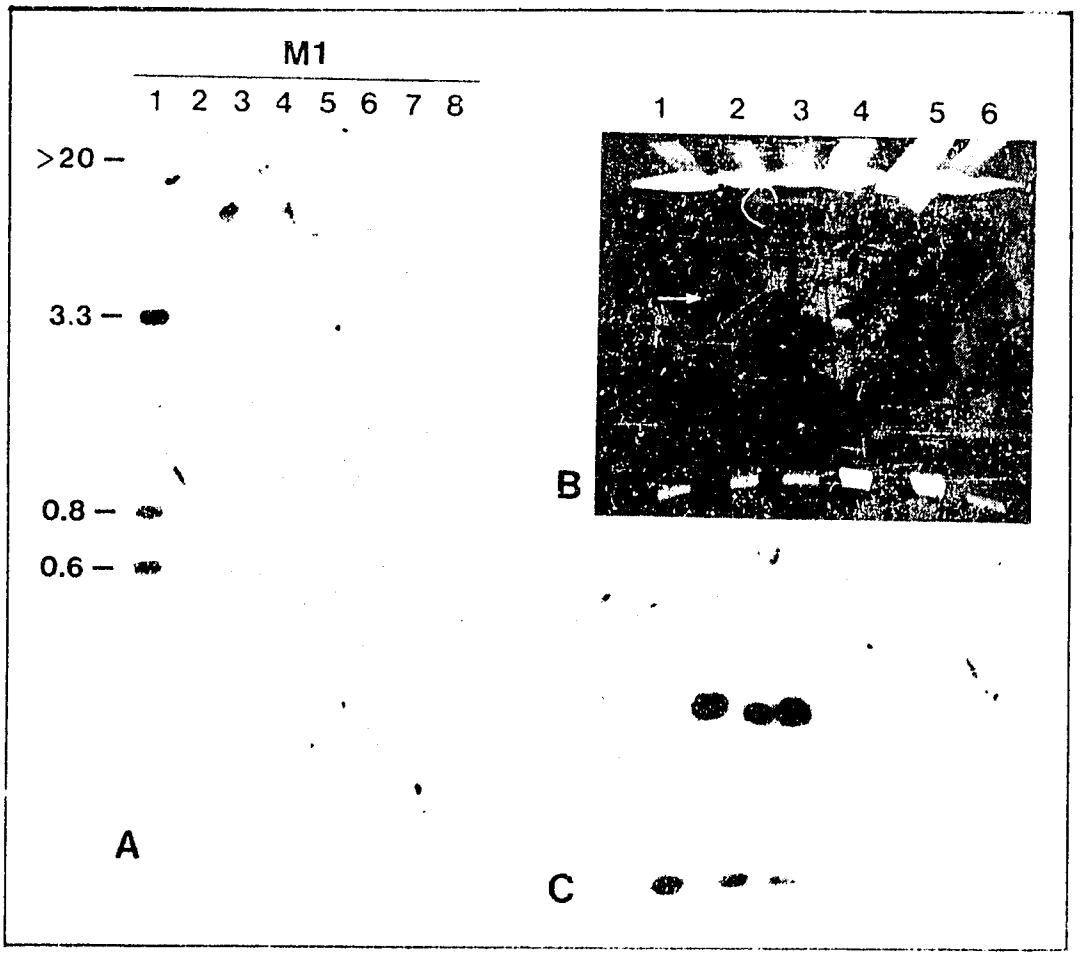


Figure 11. *T congolense* ILNat 3.2 VSG cDNA sequences are absent from heterologous genomes. A. DNA (2 μg) from *T congolense* clones from eight different serodemes was digested with *Mbo*I, size-fractionated in a 0.8% agarose gel, blotted and hybridized with ILNat 3.2 VSG cDNA. Lanes contain DNA from clones as follows: (1) ILNat 3.1, Serengeti, Tanzania; (2) ILNat 2.1, Transmara, Kenya; (3) 776, Kigoma, Tanzania; (4) ILNaR 1, Nairobi, Kenya; (5) C1, Busoga, Uganda; (6) C47, Kigoma, Tanzania; (7) C36, Ikoma, Tanzania; (8) ILNat 5.1, Kilifi, Kenya. DNA fragment sizes are given in kb. B. Chromosome-sized DNA molecules from trypanosome clones belonging to three distinct serodemes were fractionated by OFAGE in a 1.5% agarose gel for 8 hours at 20-second pulse intervals, stained with EtdBr and photographed. C. DNA in the gel shown in panel B was transferred to a sheet of nitrocellulose filter and hybridized with ILNat 3.2 VSG cDNA. An autoradiograph of the hybridization is shown. White arrow in panel B points to the medium-sized chromosomes of the ILNaR 3 clones with which the ILNat 3.2 VSG cDNA hybridizes. Lanes contain chromosome-sized DNA molecules from trypanosome clones as follows: (1) ILNat 3.1, (2) ILNat 3.3, (3) ILNat 3.2, (4) ILNaR 1, (5) 776, (6) ILNat 2.1.

ILDat 1.2 VSG with respect to molecular weight, amino acid composition and the presence of a cross-reactive determinant/fatty acid tail. This VSG will also be compared to VSGs from other trypanosome species.

THE TRYPANOSOME LIFECYCLE: STAGE-SPECIFIC PROTEINS

Trypanosomes develop in the mammalian bloodstream from rapidly dividing to nondividing forms. In *T brucei*, the rapidly dividing bloodstream stage is associated with a long-slender shape, while the nondividing form has a short-stumpy appearance. Parasite transition to nondividing forms helps to limit the severity of infection and is also thought to prepare the trypanosomes for transmission to tsetse. Once the parasites enter the fly, they lose their VSG coat and develop into procyclic forms in the tsetse midgut. A full understanding of the mechanisms involved in these transitions may suggest specific ways to disrupt parasite development. Interventions which hasten the change to nondividing or uncoated forms would limit the growth of trypanosome populations and make the parasite vulnerable to the host's immune system.

Unique proteins synthesized at different stages of the trypanosome lifecycle present possible targets for chemotherapeutic or immune attack. Protein profiles of rapidly dividing and nondividing bloodstream forms and uncoated procyclic forms of *T brucei* are being compared using two-dimensional gels. Proteins are also being identified which may be specific to particular species or subspecies and could be used for epidemiological or diagnostic purposes.

Several stage-specific proteins have been observed. The most obvious are the VSGs which are present on both bloodstream forms but absent on procyclics. Among other proteins noted, several were present in only one of the three stages, some were present only in the two bloodstream forms and others were present only in nondividing bloodstream and procyclic forms.

A collaborative study with the University of Massachusetts/Amherst (USA) has focused on some nonvariant antigens specific to bloodstream forms of *T brucei* and *T congolense*. These antigens were shown to be proteins, and antibodies generated against them in rabbits were of the IgG class. Electron microscopic studies suggested that some of these nonvariant antigens may be located on the surface of the parasites, making them possible candidates for immunization studies. These antigens will be analyzed in more detail and then tested for their potential protective capacity.

Analysis of microsomal and putative Golgi fractions and high-speed supernatants of ruptured trypanosomes using SDS-PAGE showed that rapidly dividing bloodstream parasites lack a number of proteins which are prominent in nondividing bloodstream forms. Present investigation of developmental changes in *T brucei* includes the preparation of antisera against these stage-specific proteins. Histones, which are nuclear proteins involved in the expression of different genes, were taken from highly purified trypanosome nuclei and analysed in 1985. Differences were noted among histones from rapidly dividing and nondividing bloodstream forms and procyclic forms.

Research was initiated in 1985, in collaboration with the Vrije Universiteit Brussel (Belgium), to detect differences in trypanosome gene expression associated with specific stages of the parasite lifecycle. This work has concentrated on dividing and nondividing bloodstream forms of *T brucei* and on bloodstream and metacyclic forms of *T congolense*. The method consists of preparing cDNA from mRNA of one stage, hybridizing it to excess mRNA from another stage and selecting nonhybridized material which should

be enriched for stage-specific transcripts. Preliminary studies have shown that this technique should be useful in identifying transcripts specific for *T brucei* nondividing forms.

ASPECTS OF TRYPANOSOME METABOLISM

Studies on trypanosome metabolism have as their ultimate goal the identification of avenues for blocking parasite growth. These studies may also lead to a better understanding of the pathogenic effects of trypanosomes on their hosts, thereby suggesting approaches for preventing or alleviating the most serious results of infection.

Trypanosomes ingest biologically important molecules from the host internal environment, such as nutrients and differentiation signals, through a process of endocytosis by coated vesicles (Figure 12). To study the host bloodstream molecules required for parasite development, coated vesicles from bloodstream forms of *T brucei* were purified and characterized. Electron microscopic examination revealed a difference between *T brucei* coated vesicles and similar endocytotic vesicles from other nucleated cells: in addition to an external coat of clathrin, the trypanosome vesicles have an internal coat of electron-dense material, presumably enclosed amounts of surface VSG. Antisera tested so far have detected host IgG and IgM immunoglobulins and serum albumin in

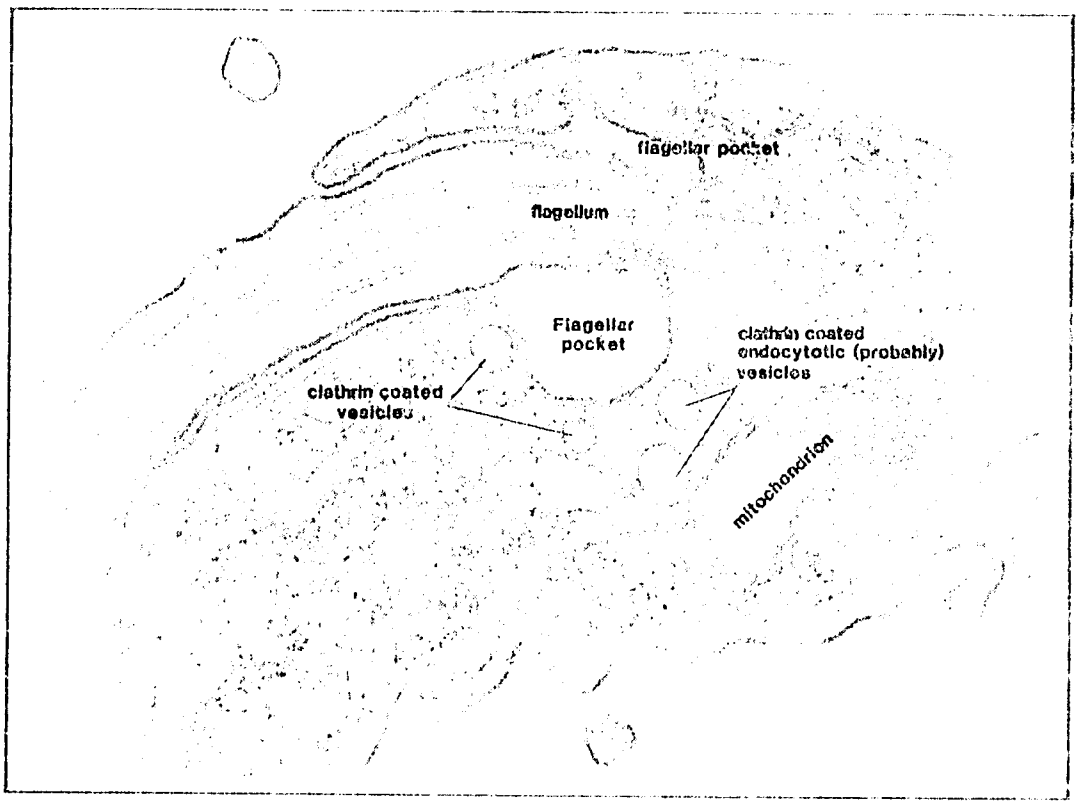


Figure 12. Coated vesicle endocytosis in *T brucei*. The arrows indicate coated vesicles near the flagellar pocket of a trypanosome. Original magnification was 100 000 (S Ito).

trypanosome coated vesicles. Immunological analysis conducted in collaboration with Yale University (USA) indicated that clathrin derived from trypanosomes differs antigenically from mammalian clathrin.

In the tsetse vector, *T vivax* parasites develop entirely within the proboscis. An interesting phenomenon associated with *T vivax* cultivation *in vitro* is the attachment of last-stage bloodstream forms to Matrix Gel Green A beads. Work in 1984 indicated that attachment is based on a receptor-ligand mechanism between the parasites and the Green A dye on the surface of the beads. Ultrastructural studies in 1985 confirmed that *T vivax* attachment to beads *in vitro* closely resembles parasite attachment to the proboscis of infected tsetse.

Biochemical studies have involved modifying the Green A dye beads or exposing the parasites to a variety of different but structurally related dyes bound to the beads. These studies have shown that the receptor-ligand interaction responsible for binding to the dye is specific for a certain molecular structure. The binding process is energy dependent and membrane mediated: binding is inhibited at 4°C, but resumes when cultures are returned to 27°C. So far attempts to generate an antibody to the Green A dye have been inconclusive. Isolation of either the *T vivax* or tsetse molecules involved in the binding process might provide a novel method of intervention leading to improved disease control.

An awareness of the important protective role played by the trypanosome VSG has led to interest in the mechanisms of attachment and release of the parasite's surface coat. The VSG appears to be anchored in the parasite membrane by a hydrophobic lipid moiety at its C-terminus. When trypanosomes are disrupted *in vitro*, VSG molecules are released from fragments of plasma membrane by enzymatic cleavage of the lipid residue. If trypanosomes in the mammalian bloodstream shed their surface coat by a similar mechanism, then pharmacological stimulation of the lipase responsible for cleavage might be developed into a new drug therapy. Preliminary studies at ILRAD suggest that *T brucei* parasites do shed their surface coat by enzymatic cleavage *in vivo*, both as bloodstream forms and during transition to the uncoated procyclic stage.

In order to identify the sites of important metabolic functions in trypanosomes, biochemical and electron microscopy techniques have been developed for isolating and identifying subcellular fractions of the parasites. Earlier studies on the process of VSG synthesis included the purification of rough and smooth endoplasmic reticulum and Golgi fractions. In 1985, highly purified fractions of trypanosome flagella, lysosomes and glycosomes were obtained (Figure 13). In addition to research on VSG formation, these fractions have been used to study trypanosome proteolytic (protein-splitting) enzymes.

Studies focusing on bloodstream forms of *T brucei* and *T congolense* have demonstrated the presence in parasite lysosomes of a family of thiol-dependent proteolytic enzymes sharing similar biochemical properties. Biochemical studies have shown that they are similar to mammalian lysosomal thiol proteases such as cathepsins B or L. Other non-thiol-dependent proteases have also been observed, indicating that trypanosomes contain at least two classes of proteolytic activity. Antibodies are now being prepared against these enzymes to confirm their location within the parasite and to ascertain whether they are released into the host bloodstream. Research is also in progress to identify parasite and host factors which may control their activity.

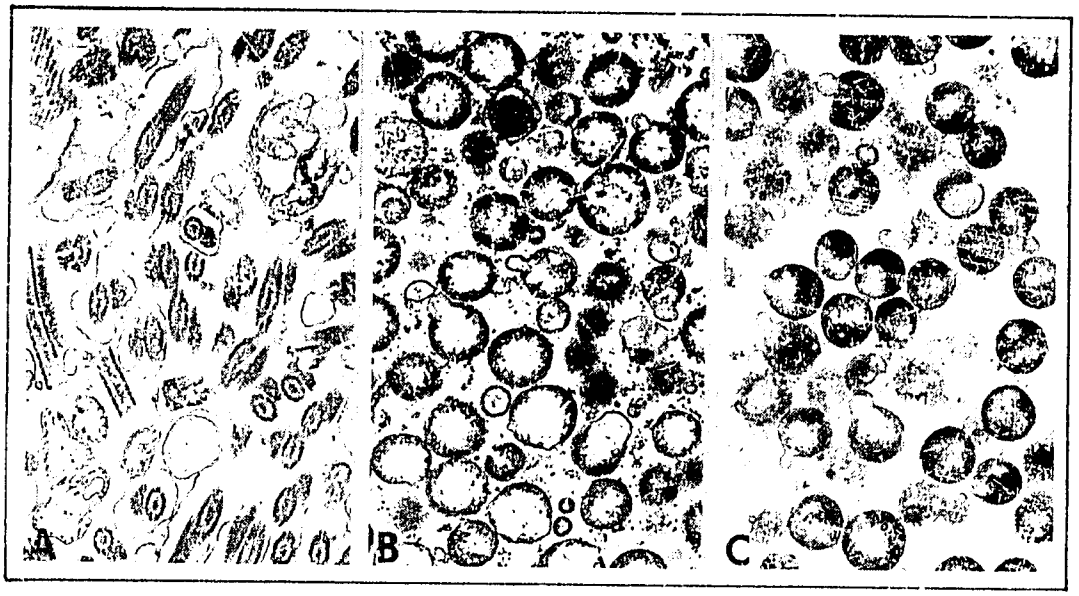


Figure 13. Flagella (A), primary lysosomes (B) and glycosomes (C) isolated from *T brucei* MITat 1.2 by density gradient centrifugation in Percoll.

TRYPANOSOME CULTIVATION *IN VITRO*

The *in vitro* cultivation of trypanosomes was one of ILRAD's first major achievements. By the end of 1983, culture systems were established which support the development of all three major African trypanosome species throughout their lifecycles. Work in this area in 1985 concentrated on simplifying and standardizing culture systems to produce large numbers of parasites at the metacyclic stage of development.

Two culture systems were developed in 1984 to propagate metacyclic forms of *T vivax*. Large numbers of metacyclic forms of five *T vivax* stocks can now be harvested on a regular basis. Observations on the *in vitro* development of metacyclic forms of this species have suggested that they may pass through two morphologically distinct stages of development. Present work includes further investigation of this possibility as well as *in vitro* cultivation of additional *T vivax* stocks, particularly stocks newly isolated in the field.

Metacyclic forms of three *T congolense* stocks were cultivated initially in 1983. In 1985, a fourth stock of *T congolense*—Trans Mara—was cultivated successfully; large numbers of metacyclic forms of this stock can now be produced.

Although *T brucei* was the first trypanosome species to be maintained in culture throughout its lifecycle, efforts to produce metacyclic forms of this species in large numbers have not yet been successful. Work continued in this area in 1985. Bloodstream forms of *T b brucei* (TC221) taken from infected mice were maintained *in vitro* and the culture system was manipulated to produce forms which resembled metacyclic parasites and were infective to mice. However, it is not yet possible to produce large numbers of these metacyclic-like parasites on a continuous basis.

IMPROVED HOST RESPONSES

Early studies on *T brucei* infections in cattle, eland and African buffalo showed that two responses are involved in host control of parasitaemia in the bloodstream—control of parasite growth by nonimmunological processes and destruction of parasites by host antibodies. Subsequent work in resistant and susceptible strains of mice has indicated that *T brucei* parasites require host-derived growth factors in order to multiply: these factors are responsible for maintaining the parasites in the rapidly dividing (long-slender) stage and inhibiting or preventing their transition to the nondividing (short-stumpy) stage. Host antibody responses capable of destroying trypanosomes are induced by degenerating stumpy-form parasites: these responses are specific for exposed or external epitopes on parasite VSG molecules and are depressed in some infected animals.

Studies have been undertaken to identify the host-derived growth factors required for the maintenance of trypanosome populations. Bovine serum components which stimulate trypanosome multiplication in a feeder layer-dependent culture system are of a molecular mass greater than 100,000 daltons. The molecules are not lipids, lipoproteins or immunoglobulins and appear to be of a similar size range in sera from mice, rats, goats and cattle. This area of investigation did not progress substantially during 1985. However, new culture systems have been developed at the University of Bordeaux (France) which permit the cultivation of bloodstream forms of *T brucei* without feeder layer cells. The introduction of these techniques at ILRAD provides hope that *T brucei* growth-factor requirements can be investigated more thoroughly in coming years.

The growth rate of *T brucei* parasites in mice can be modified by treatment of the host with dead *Corynebacterium parvum*, a bacterium which acts as a nonspecific immunostimulator. In treated mice, parasitaemia is reduced by more than 200 fold, though the effect is short lived. This effect appears to result from an acceleration in the rate of transition from dividing to nondividing parasite forms. The effect is greatest in normal mice, but is also detected in mice irradiated either before or after *C parvum* treatment. Radiolabeling of *C parvum* organisms indicates that they are located predominantly in phagocytic cells of the liver and lungs of treated mice, but they can also be detected for a few days in the spleen. Serum or spleen cells from treated mice have no detectable effect on parasites during short-term (3 h) cultivation *in vitro* and do not increase resistance when transferred to untreated animals. These results suggest that *C parvum* treatment might inhibit the production of host-derived factors which promote the growth of *T brucei*, although blocking factors cannot be excluded.

Clarification of how *C parvum* treatment works to accelerate parasite differentiation could shed light on the regulation of parasite populations in resistant breeds of cattle and wild animals. However, full elucidation of the effects of *C parvum* treatment awaits the identification of trypanosome growth factors. Present studies also aim to determine which host cells and which components of the *C parvum* organisms are involved in control of trypanosomes.

Some strains of mice, notably C3H/He, are highly susceptible to trypanosomiasis. They do not produce detectable antibodies against the parasites although their antibody-producing B lymphocytes are extensively activated during infection. ILRAD scientists measured B-lymphocyte activation in susceptible mice infected with *T brucei* by assessing the incorporation of ^{125}I-iododeoxyuridine as an indication of DNA synthesis. Maturation of the B-lymphocyte response was measured by assessing the presence of immunoglobulin in the cytoplasm of the cells. By the time of peak parasitaemia,

approximately 10 to 18% of spleen cells in infected susceptible mice contained immunoglobulin, a proportion of which could bind to the VSG of the infecting trypanosomes, yet no parasite-specific antibodies were detected in their sera. These findings suggest that parasite-specific B lymphocytes are suppressed at a terminal stage of maturation.

This possibility was examined by *in vivo* and *in vitro* biosynthetic labeling with L-^{35}-S-methionine, a radioactive amino acid which is incorporated into newly synthesized proteins, including immunoglobulins. It was found that immunoglobulin secretion by immunoglobulin-containing cells was severely impaired in susceptible mice infected with *T brucei*. A similar phenomenon was observed in susceptible mice infected with *T vivax*.

This block in lymphocyte function in susceptible mice infected with *T brucei* or *T vivax* can be relieved by elimination of the trypanosomes. Antibody was detected in the bloodstream within 20 hours of parasite removal by treatment with diminazene aceturate (Berenil: Hoechst), which suggests that the block in antibody secretion was maintained by the presence of living trypanosomes or short-lived components of dying parasites.

To investigate the link between trypanosome infection and the suppression of B-lymphocyte development, scientists went on in 1985 to investigate the influence of *T brucei* infection on the capacity of mice to produce antibody against other types of antigens which do not crossreact with trypanosomes. Rabbit red blood cells triggered strong immune responses in noninfected mice, but infected mice rapidly lost the capacity to mount immune responses against this antigen. The immunosuppressive effect was relieved within 24 hours of parasite elimination by treatment with diminazene aceturate (Figure 14). Thus parasite-induced suppression of the immune response against rabbit red blood cells appeared to be similar to regulation of the response against the parasites themselves.

Experiments were conducted to examine the influence of the introduction of trypanosomes into mice already producing antibody against rabbit red blood cells. Groups of mice were injected with rabbit red blood cells and also with trypanosomes on the same day or 1, 2 or 3 days later. The parasites had no influence on antibody response to the rabbit cells, as measured on the fourth day. Similarly, when mouse cells secreting

Number of Mice	Day 0 *T brucei* Infection	Day 4 Rabbit Red Blood Cells	Day 7 Diminazene Aceturate	Day 8 Ab-forming Cells/ 500.000 Spleen Cells (± 1 SE)
10	−	+	−	186 ± 18
10	−	+	+	164 ± 24
10	+	+	−	21 ± 6
10	+	+	+	149 ± 16
10	+	−	−	2 ± 1
10	+	−	+	2 ± 2

Figure 14. Influence of *T brucei* (GUTat 3.1) infection on the stimulation of antibody-forming cells against rabbit red blood cells in a susceptible strain (C3H/He) of mice. Six groups of 10 mice each were subjected to different treatment regimes. Parasites were detected in the blood for 24 hours after treatment with diminazene aceturate, and were cleared between 24 and 48 hours after treatment.

antibody against rabbit red blood cells were incubated with serum from infected mice for up to 3 hours *in vitro*, antibody secretion was not inactivated. These results indicate that the presence of parasites *per se* does not inactivate antibody-secreting cells. The parasite-induced response which prevents the final maturation of antibody-secreting cells requires some days to be activated and is dependent on the continuous presence of the parasites.

Trypanosome infection is associated with disruption of the spleen, the organ in which antibody-secreting cells are produced. Studies conducted in 1985 showed that *T vivax* infection causes severe pathology in the spleen of susceptible, but not resistant mice. Within 9 days after infection of susceptible mice, the structure of the spleen was severely disrupted: the boundary between red and white pulp regions became indistinct and within the white pulp it was no longer possible to distinguish T-cell from B-cell regions. There were high levels of cell multiplication and cell death, increased deposits of fibrin and marked production of red blood cells and cells of the monocyte and polymorphonuclear leukocyte lineages (Figure 15).

When susceptible mice were infected with *T brucei*, damage to the spleen was less severe, though the white pulp area was disrupted. The disruption of organ structure did not prevent the development of antibody-producing cells, though these did not secrete antibody.

When susceptible mice infected with either *T vivax* or *T brucei* were treated with diminazene aceturate, their spleen structure rapidly returned to normal and serum antibody responses became detectable. 'Healthy' cells were produced in the spleen 16 hours after trypanocidal treatment. This observation of rapid recovery after trypanocidal treatment indicates that the disruption of spleen structure in infected mice is directly or indirectly related to the presence of living trypanosomes or more likely to short-lived factors from degenerating parasites. Little or no spleen disruption was observed in mice infected with monomorphic lines of *T brucei*, which remain as dividing forms and grow exponentially until death of the host.

To explore the mechanism(s) involved in spleen disruption, the numbers of cells of different types were compared in the spleens of resistant and susceptible mice infected with *T brucei* or *T vivax*. The major difference was that monocytes/macrophages were more numerous, larger and contained more vacuoles in the spleens of susceptible mice than in resistant mice infected with either parasite species. Because monocytes/macrophages can be a source of proteases and free oxygen radicals—which can cause tissue damage—the function of these cells is being investigated.

It remains possible that spleen disruption does not result directly from products of activated host cells, but rather from the release of enzymes or other molecules by trypanosomes. If this is the case, differences in host susceptibility to parasite components could be due to differences in substrate polymorphisms or in the production of plasma inhibitors. Future studies aim to clarify the link between lymphoid organ disruption and defective terminal maturation of B cells in infected susceptible mice, and also to elucidate how these processes are induced and how they contribute to the regulation of antibody responses in resistant mice.

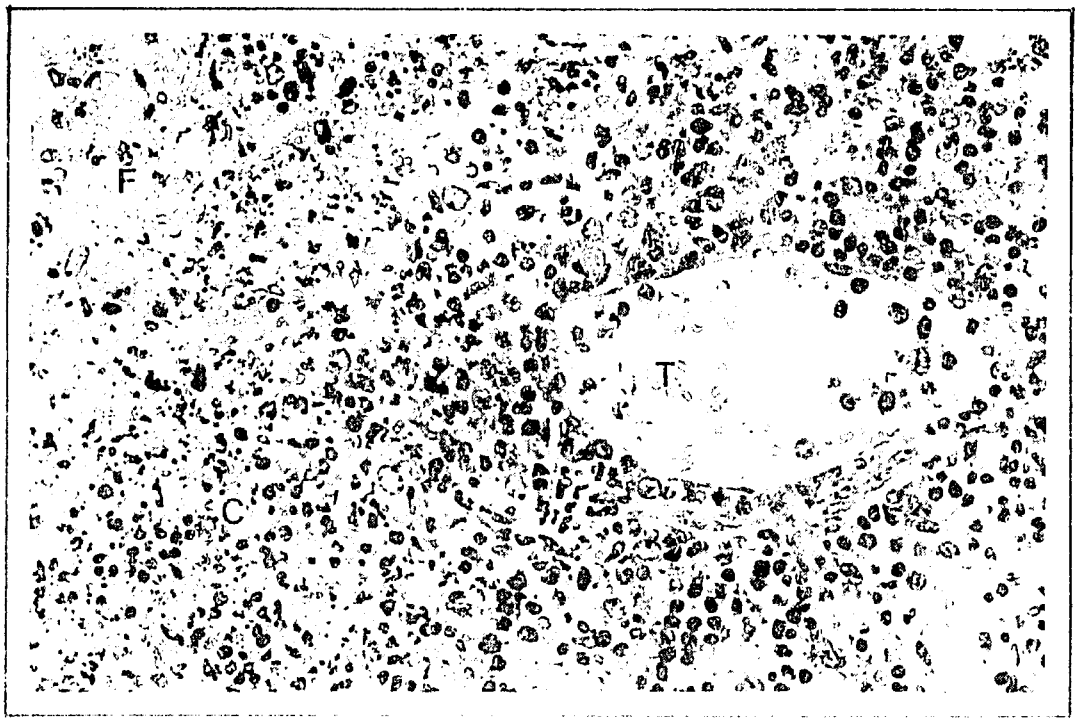


Figure 15 Section through the red pulp of the spleen of a susceptible C3H He mouse 7 days after infection with *T vivax*. The micrograph shows cytolysis (C) fibrinous deposition (F) and trypanosomes within the blood vessel (T)

PATHOGENESIS: EFFECTS OF TRYPANOSOMIASIS ON BOVINE REPRODUCTION

A study was carried out in 1985 in collaboration with the Centre for Tropical Veterinary Medicine of the University of Edinburgh (UK) to investigate the effects of *T congolense* infection on the reproductive cycle of female Boran cattle. Two groups of six Boran cows were infected with *T congolense* and treated with diminazene aceturate, 60 and 90 days after infection respectively. Two groups of five control cows were not infected, but were treated with diminazene aceturate at the same time as the infected animals. All the cows used in the experiment had already completed at least one oestrous cycle.

Following infection, 11 out of 12 cows became acyclical and most showed impaired luteal function, though no pathological structures were observed by rectal palpation. The one cow which continued to cycle showed reduced levels of progesterone. All the infected cows resumed cycling within 2 months after drug treatment. The control cows continued to cycle normally throughout the experiment and no abnormal ovarian structures were observed, indicating that treatment with diminazene aceturate had no effect on the oestrous cycle.

This experiment provided the first clear demonstration of the relation between *T congolense* infection and reduced fertility in cattle. However, it was not possible to distinguish between the direct effect of the parasites on ovarian activity and the effect of weight loss associated with infection.

EPIDEMIOLOGY

CHARACTERIZATION OF TRYPANOSOME POPULATIONS

Research has been in progress for several years aimed at developing simple, accurate and sensitive assays to detect trypanosomes in infected animals and tsetse and to identify parasite species and serodemes. This work is important for diagnosis and the evaluation of treatment as well as for epidemiological studies, for instance to predict the level of trypanosomiasis risk in an area before introducing susceptible livestock or to determine the number of trypanosome species or serodemes present in an area in order to assess the feasibility of controlling trypanosomiasis by a 'cocktail' vaccine.

Serological typing: a common trypanosome antigen

ILRAD scientists have demonstrated a protein with an apparent molecular weight of 83,000 daltons present in *T b brucei*, *T congolense*, *T vivax*, *T b rhodesiense* and *T evansi*. Studies were carried out in 1985 to describe this protein more precisely. It accounted for 0.4 to 1.9 μg per mg of total protein in all six trypanosome species and was shown by electron microscopy to be associated with specific regions of the cell cytoplasm and with dispersed chromatin of the nucleus . None of the protein was found on the plasma membrane or any other cellular membrane. IgG antibody from antisera raised against this protein was found to be monospecific: this monospecific polyclonal antibody will be used to screen trypanosome expression libraries to identify clones producing the protein. If large amounts of the protein can be produced, antibodies can be generated which will provide a useful tool for trypanosomiasis diagnosis.

Serological typing: species-specific antigens

By the end of 1984, ILRAD scientists had derived monoclonal antibodies which recognized antigens specific to the three major African trypanosome species. Used in a sandwich enzyme-linked immunosorbent assay (ELISA), the monoclonal antibodies against *T brucei* and *T vivax* detect circulating antigens in sera from infected cattle. The antibodies against *T brucei* also detect antigens in sera from human sleeping sickness patients. In 1985, the *T brucei* antibody was used successfully to detect trypanosomes in infected tsetse. The monoclonal antibody derived against *T congolense* appears to recognize *T simiae* as well as *T congolense*, but it is not yet sensitive enough to detect antigens consistently in the sera of infected animals.

Present work involves isolating and purifying the species-specific antigens recognized by these monoclonal antibodies. As with the common trypanosomal antigen, the goal is to screen parasite DNA and identify clones which will produce these antigens for use in trypanosomiasis diagnosis and epidemiological studies.

Serological typing: *T congolense* serodemes from Kilifi

Early work on *T congolense* showed that metacyclic forms of this species displayed a limited number of VSGs which were serodeme specific. Subsequent efforts have concentrated on developing serological methods for typing *T congolense* stocks on the basis of their repertoire of metacyclic antigens. These studies started with 81 *T congolense* stocks isolated in 1982 from resident and tracer cattle on a large dairy farm at Kilifi in Kenya's Coast Province.

A total of 61 isolates were prepared from the tracer cattle, 40 of these were passaged and cloned in mice, and 8 clones and 12 stocks were then transmitted through tsetse (*G m centralis*). Metacyclic forms of the clones were screened by indirect immunofluorescence and neutralization assays using anti-metacyclic hyperimmune sera. This work indicated that the eight clones belonged to four distinct serodemes. Cross-challenge experiments in goats and molecular karyotyping confirmed these results. Metacyclic forms of the 12 stocks were then screened by immunofluorescence for reactivity with antisera against metacyclic forms of two of the serodemes, revealing that each stock contained a mixture of serodemes.

This study indicates that serological typing of metacyclic trypanosomes can be used to identify serodemes. It also suggests that the number of *T congolense* serodemes present on the Kilifi farm may be limited. Analysis of sera from the tracer cattle showed antibodies against two of the serodemes as early as 3 months after the animals were introduced on the site. This suggests that exposure of susceptible cattle to tsetse challenge under certain conditions may lead to the development of acquired immunity against local trypanosome serodemes.

In vivo studies

In studies carried out in 1985, stocks and clones of four *T congolense* isolates from Kilifi were transmitted to goats by infected tsetse (*G m morsitans*). A *T congolense* stock (IL 1180) originating from Tanzania was transmitted to control goats for comparison. The trypanosomes originating from Kilifi did not produce significant chancres in the skin, compared with *T congolense* (IL 1180), and infection with the Kilifi trypanosomes did not stimulate protective immune responses against challenge with metacyclic forms of the same stocks and clones. These observations may be relevant to epidemiological studies of trypanosomiasis at Kilifi.

Use of genomic probes

Developments in molecular biology have opened up new possibilities for characterizing parasite species and population groups. Scientists at ILRAD are applying new techniques to identify DNA hybridization probes for the classification of African trypanosomes.

The development of hybridization probes is particularly important for the identification of *T congolense* as this species has demonstrated considerable genomic heterogeneity.

To produce a hybridization probe for trypanosome species identification, total genomic DNA from one clone is hybridized at limited concentration for a short time with DNA from other clones of the same species taken from different geographic areas. This results in the identification of repetitive sequences common to all the clones used. The repetitive sequences are then cloned in a phage or plasmid vector and used as hybridization probes in parasite identification assays. Using this technique, specific DNA probes have been prepared at ILRAD from *T congolense* ILNat 2.1, *T brucei* ILTat 1.4 and the West African *T vivax* IL 1392. These probes have been used for the detection and identification of trypanosome infections in tsetse flies.

The nucleotide sequence contained in the DNA fragment used as the *T congolense* probe was examined in 1985. This fragment belongs to a class of DNA termed satellite DNA which has no known function. Comparisons with satellite DNA from other trypanosome species showed that this fragment is specific for *T congolense* and thus provides a useful marker for the species. When tested against 5 samples of *T b brucei*, 5 of *T b gambiense*, 2 of *T b rhodesiense*, 17 of *T congolense*, 2 of *T evansi*, 2 of *T vivax* and 2 of *T simiae*, the probe hybridized with 15 of the 17 *T congolense* clones or stocks but with none of the other species. The only *T congolense* samples not detected by this probe were two clones prepared from trypanosomes obtained at Kilifi. A hybridization probe from the *T congolense* parasites isolated at Kilifi was cloned and partially sequenced. This new probe should provide further evidence of the relationship between the Kilifi trypanosomes and *T congolense* parasites from other locations in Africa. A probe was also cloned in 1985 which hybridizes only with DNA from *T simiae*, an important trypanosome species in pigs which is difficult to distinguish from *T congolense* by other methods. The *T brucei* probe was sequenced in 1985, revealing that it is not a fragment of satellite DNA but has the potential to encode a trypanosomal protein.

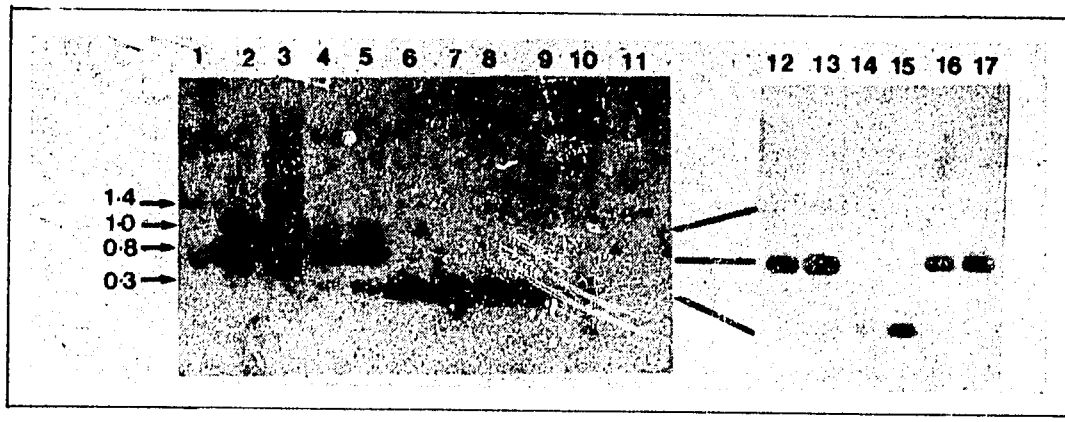


Figure 16. Hybridization of the recombinant plasmid pMLS with Rsa I digests of DNA from different trypanosome species and subspecies. Lanes show DNA as follows: (1, 11) *T vivax* ILDat 1.2; (2, 3, 4, 5) *T congolense* ILNat 2.1, ILNat 3.1, ILNaR 1, clone 776; (6) *T b brucei* ILTat 1.1; (7) *T b gambiense* LiTat 1.6; (8) *T b rhodesiense* ETat 1.10; (9) *T b brucei* ILTat 1.4; (10) *T evansi* KETRI 2443 c1; (12, 13, 16, 17) *T congolense* AnNat 3.1, ILNat 3.1, ILRAD 2463 (from CRTA, Burkina Faso), ILNat 2.1; (14, 15) *T congolense* (Kilifi) ILNat 4.1, ILNat 5.1. DNA fragment sizes are given in kb. This pMLS reveals fragments of DNA that are identical in size in the trypanosomes that belong to one subgenus.

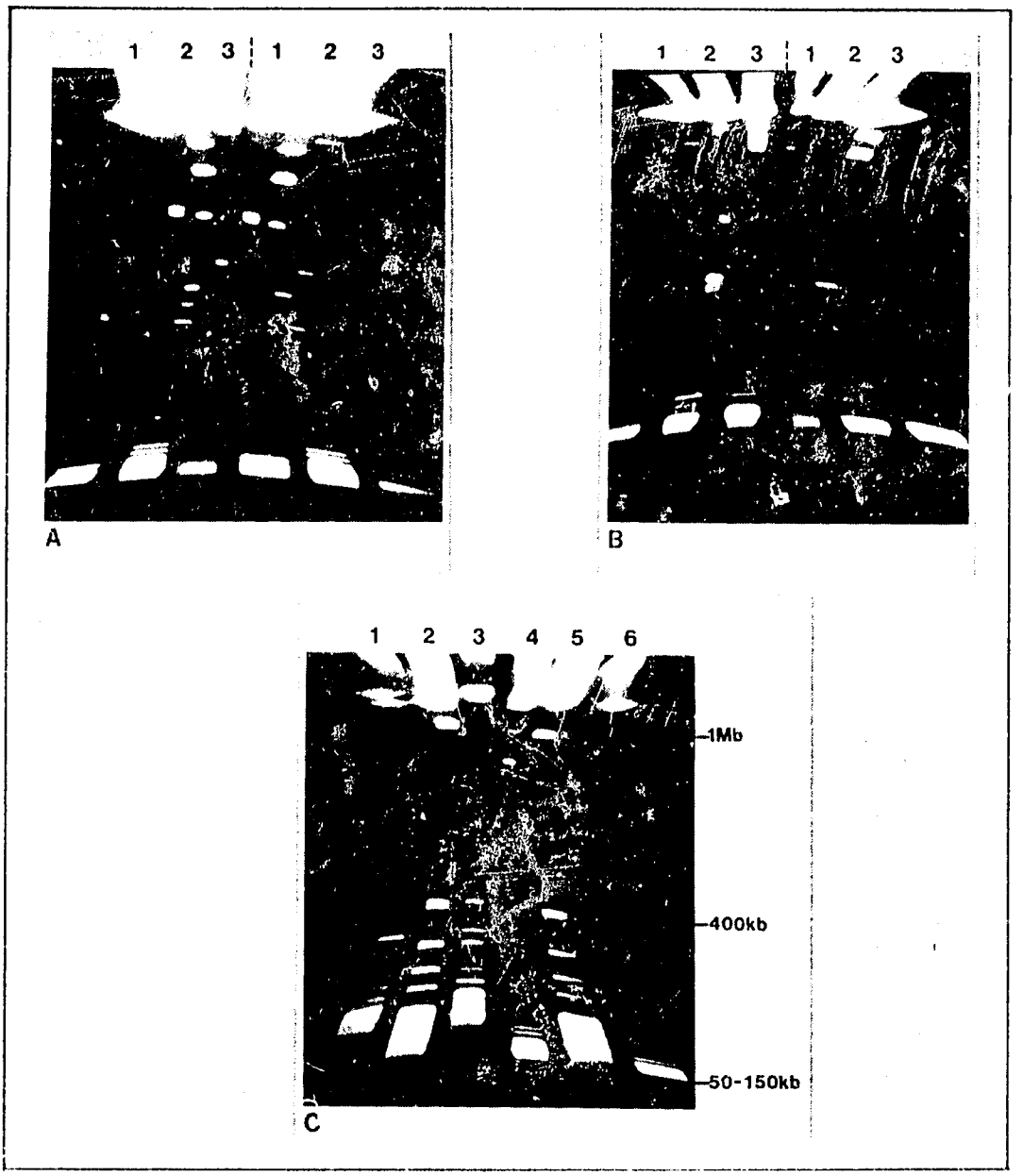


Figure 17. Variable molecular karyotypes in *T congolense* clones from different serodemes. Chromosome-sized DNA molecules prepared from *T congolense* clones, each belonging to a distinct serodeme, were resolved in a 1.5% agarose gel by OFAGE at 20-second pulse intervals. After 15 h, the gels were stained and photographed. A: lanes 1, 2 and 3 contain DNA from clones ILNat 2.1, ILNat 3.1 and C36. B: lanes 1, 2 and 3 contain DNA from clones ILNat 3.1, ILNaR 1 (C57) and 776. C: lanes contain DNA as follows: (1) ILNat 4.1; (2) *T (T) brucei* ILTat 1.1; (3) ILNat 5.1; (4) ILNat 3.1; (5) *T (T) brucei* ILTat 1.1; (6) ILNat 2.1. ILNat 4.1 and ILNat 5.1 are derived from the Kilifi isolates. Clones of *T congolense* from different serodemes have different molecular karyotypes. These, in turn, are distinctly different from the molecular karyotypes of trypanosomes from different species, e.g. *T brucei* (lanes 2 and 5) and the *Nannomonas* from Kilifi (lanes 1 and 3).

The sensitivity of the first DNA probes developed at ILRAD was tested in 1984 using trypanosomes purified from diluted samples of blood. Work is still in progress to improve the sensitivity of these tests. In 1985, the hybridization probes were tested against samples from infected tsetse. The *T congolense* probe detected trypanosomes in the midgut of infected tsetse following overnight exposure and in the proboscis after exposure of 5 hours. No hybridization signal was observed in noninfected samples used as controls. The *T brucei* probe detected infection in tsetse midgut and salivary gland samples after overnight exposure, but did not reliably detect parasites in the proboscides of infected tsetse even after exposure for 1 week. The *T vivax* probe detected infection in over 70% of material taken from the proboscides of infected flies.

A preliminary experiment suggested that the *T brucei* probe may detect as few as 200 trypanosomes/ml in infected cerebrospinal fluid. Results using the hybridization probe to detect parasites in cerebrospinal fluid correlated with those obtained using other tests. One advantage of using a hybridization probe for measuring parasitaemia in cerebrospinal fluid is that this method detects both live and dead or fragmented trypanosomes.

The hybridization probes have been used to characterize a number of parasite samples available at ILRAD. The *T vivax* probe derived from a West African stock (IL 1392) was shown to detect East African isolates of this species less readily than isolates from West Africa. For this reason, a hybridization probe has been developed which is specific for East African *T vivax*, using DNA from a haemorrhagic *T vivax* isolate (IL 2337) from Kenya. A comparison of DNA fragments from this isolate with DNA from the West African *T vivax* revealed clear differences, and one fragment was identified from the East African parasites which may provide a marker to discriminate between the two types.

During the screening of *T congolense* cDNA libraries to identify species-specific hybridization probes, several other nucleotide sequences were identified. Preliminary results suggest that two of these sequences, although their function is unknown, may prove to be useful reagents to distinguish the three trypanosome subgenuses—*Trypanozoon*, *Nannomonas* and *Duttonella* (Figure 16).

Once a trypanosome clone or isolate has been identified by hybridization with one of the DNA probes available at ILRAD, it can be characterized further by hybridization with other, independently cloned DNA sequences. Parasite clones may also be typed by comparing the location of specific sequences on DNA molecules of different sizes, separated by enzymes that digest DNA.

Separation by OFAGE of chromosome-sized DNA molecules from *T congolense* parasites isolated at Kilifi revealed similar patterns among clones shown by serological analysis to belong to the same serodeme (Figure 17). It may be possible to use these patterns, referred to as molecular karyotypes, to predict whether *T congolense* clones are likely to belong to the same serodeme.

For *T congolense* ILNat 3.1, the results of molecular karyotyping have been shown to agree with crossresistance patterns *in vivo*. When goats were infected with *T congolense* of a specific karyotype, they resisted challenge with *T congolense* of the same karyotype but were susceptible to challenge with *T congolense* of other karyotypes, as shown by the development or absence of chancres. These experiments also provided evidence that the molecular karyotype may be a stable property of a specific trypanosome clone: no changes in karyotypes were observed in trypanosomes cyclically transmitted to goats or cattle, even after infection had been maintained in the animals' bloodstream for as long as 90 days.

Isoenzyme analysis

In recent years, considerable genetic diversity has been shown to occur in some of the currently recognized trypanosome species. Such diversity is revealed by detecting differences in the electrophoretic mobility of certain enzymes. For instance, *T b brucei* parasites from East and West Africa can be distinguished in this way, and isolates of *T congolense* from Liberia have been shown to be distinct from *T congolense* parasites from other parts of West Africa.

In 1985, ILRAD scientists compared several stocks of *Nannomonas* trypanosomes from Kilifi with groups of *T congolense* parasites originating elsewhere in Africa. Several enzymes from the Kilifi isolates were shown to differ from enzymes of other *T congolense* parasites (Figure 18). All the Kilifi isolates which have been studied belong to a genetically distinct group of parasites. Enzymes from *Nannomonas* trypanosomes isolated at Muhaka, approximately 100 km south of Kilifi, were also analysed: out of 25 isolates, 23 resembled groups of *T congolense* which had previously been described, while 2 had enzymes characteristic of the Kilifi parasites.

TSETSE FLIES AS TRYPANOSOME VECTORS

In a research project partially funded by the International Atomic Energy Agency (IAEA), ILRAD scientists have shown that male tsetse sterilized by gamma-irradiation can transmit the three major pathogenic trypanosome species as effectively as normal tsetse. Experiments initiated in 1980 and completed in 1985 tested the vectorial capacity of sterilized males of seven tsetse species and subspecies for East and West African stocks of *T vivax*, *T congolense* and *T brucei*. Results, summarized in Figure 19, show very small and inconsistent differences in the infection rates of irradiated and normal tsetse. These findings suggest that the release of large numbers of sterilized male tsetse as part of a tsetse control program could increase the immediate risk of trypanosomiasis. This approach should thus be accompanied by other, short-term measures to control infection in local livestock or human populations at risk from trypanosomiasis.

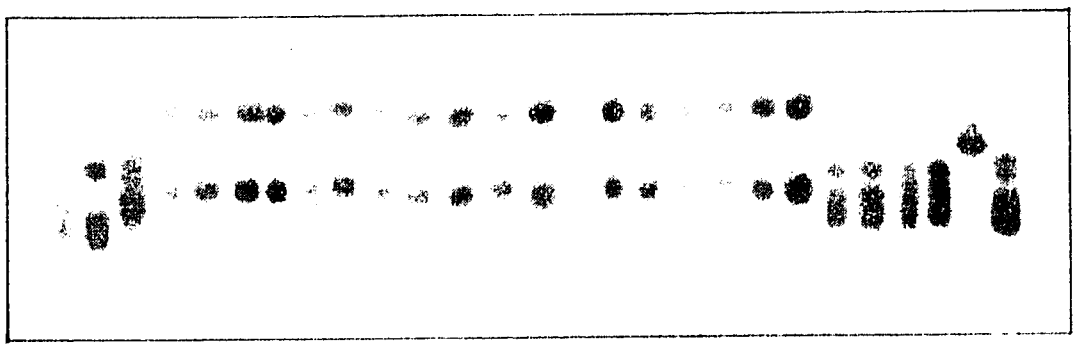


Figure 18. The electrophoretic mobilities of a peptidase enzyme from several isolates of *Nannomonas* trypanosomes. Details of some of the isolates are (from left to right): lane 1, IL 1180 isolated from a lion in Serengeti, Tanzania; lane 2, IL 2411 isolated in West Africa; lane 3, isolated from Muhaka, Kenya; lanes 4 to 19, isolated from Kilifi, Kenya; lanes 20 and 21, isolated from Muhaka, Kenya, possessing Kilifi-like enzymes; lanes 22 to 25, isolated from Muhaka, Kenya; lane 26, mouse red blood cell; lane 27, IL 1180.

Many field workers have suggested that only immature teneral tsetse could become infected with trypanosomes and transmit infection to mammalian hosts. Based on this assumption, it has been suggested that the release of mature sterilized tsetse might not increase the risk of trypanosomiasis. To test this possibility, 30-day-old male and female *G m centralis* were fed on a goat infected with *T brucei*, then maintained on a noninfected rabbit and dissected 30 days later to determine infection rates. Infection rates in mature tsetse were lower than in teneral flies fed on the same goat, but were still high enough to imply a significant level of disease transmission.

Experiments were also conducted in 1985 to investigate the effects of trypanosome infection on the probing and feeding behaviour of tsetse. *G m morsitans* and *G m centralis* flies were infected with *T vivax*, *T congolense* or *T brucei* and then allowed to feed on noninfected rabbits or mice for 20 consecutive days. There were no significant differences in the frequency of probing or feeding between any group of infected tsetse and noninfected controls. This indicates that the presence of trypanosomes in the tsetse midgut, proboscis or salivary gland does not impair the feeding behaviour of the flies.

Further studies were conducted to investigate the effects of trypanosome infection on tsetse survival and reproductive performance. Groups of teneral female *G m morsitans*

	E African *vivax* (IL 2241)	W African *vivax* (IL 1392)	E African *congolense* (IL 2047)	W African *congolense* (IL 2281)	E African *brucei* (IL 923)	W African *brucei* (IL 2380)
G m centralis						
sterile	55.9	49.5	44.0	55.8	50.9	12.3
fertile	61.1	46.3	35.3	49.2	40.4	6.8
G austeni						
sterile	4.2	50.0	0.0	2.1	1.0	0.0
fertile	1.8	54.8	2.0	3.0	0.2	0.0
G p palpalis						
sterile	0.0	45.3	0.5	1.0	0.4	0.4
fertile	0.0	39.9	0.5	0.7	0.4	0.0
G p gambiensis						
sterile	1.8	58.1	0.0	0.3	0.0	0.2
fertile	1.3	62.8	0.3	0.3	0.4	0.0
G f fuscipes						
sterile	0.3	42.2	1.6	1.5	1.6	0.0
fertile	0.5	41.2	6.0	0.9	2.1	0.0
G tachinoides						
sterile	0.0	41.7	0.3	0.8	1.5	1.3
fertile	0.3	41.5	0.3	2.0	1.0	0.7
G brevipalpis						
sterile	69.7	75.4	18.3	8.8	0.0	0.0
fertile	75.3	81.6	15.7	6.3	0.2	0.0

Figure 19. Infection rates (%) in seven species of mature male tsetse sterilized by gamma-irradiation and fed on animals infected with *T vivax*, *T congolense* or *T brucei* stocks originating from East or West Africa. All groups were compared with fertile controls.

were mated and fed for 63 days on calves or goats infected with *T vivax*, *T congolense* or *T brucei*. Their survival rates and the number and weight of puparia produced were compared with those of female tsetse fed on noninfected calves or goats. Differences between tsetse maintained on infected and noninfected hosts were small and inconsistent, suggesting that a high incidence of trypanosome infection in mammalian populations probably does not have harmful effects on tsetse.

It has been postulated that trypanosome infection rates in tsetse might be increased by slowing the rate of movement of the parasites from the tsetse crop to the midgut. To test this possibility, groups of *G m morsitans* tenerals were fed on a goat infected with *T brucei* and some were transferred to a cooler environment (20°C rather than the normal environment of 25°C) for 1 hour. This had the effect of slowing the movement of trypanosomes from the crop to the gut. However, when surviving tsetse were dissected 35 days later, there was no significant difference in infection rates between the different groups.

Work continued in 1985 to improve serological methods for identifying the source of tsetse bloodmeals. First, antisera were derived which recognized many species of mammals or birds from minute quantities of blood obtained from captured flies and preserved on filter papers. Because it is difficult to standardize antisera, work now concentrates on deriving monoclonal antibodies against mammalian family- or species-specific proteins. Monoclonal antibodies were derived in 1984 and 1985 which recognize bloodmeal samples from cattle; goats and sheep; domestic pigs, bushpigs and warthogs; eland; bushbuck; waterbuck; oryx; and the Alcelaphinae family (wildebeest, hartebeest and topi). ILRAD scientists have made this technology available to colleagues at the International Centre of Insect Physiology and Ecology (ICIPE) and are analysing tsetse bloodmeal samples for the International Trypanotolerance Centre in The Gambia and for ILCA/ILRAD trypanotolerance research network sites in Central and West Africa, Ethiopia and Kenya.

In 1983, scientists at ILRAD purified a thrombin inhibitor from the saliva and salivary glands of *G m centralis* and demonstrated its anticoagulant effect on tsetse bloodmeals. Fibrinolytic enzymes were also identified in the tsetse midgut which probably function as a back-up mechanism to protect feeding tsetse from the potentially harmful effects of blood clotting. Scientists employed several purification and assay systems in 1985 to characterize these enzymes. Two enzymes have been identified and shown to be serine proteases. They consist of single polypeptide chains with molecular masses between 25,000 and 30,000 daltons. The responses of immune sera raised against the two enzymes show that they are closely related, perhaps sharing some common antigenic determinants. Antibody responses to the enzymes are being investigated in more detail; the goal is to determine whether antibodies against the enzymes might be used to kill tsetse by blocking the breakdown of blood clots in the crop.

LIVESTOCK PRODUCTION UNDER TRYPANOSOMIASIS RISK

Trypanocidal drugs: detection and testing *in vitro*

Although trypanocidal drugs have been widely used in Africa for more than 50 years, very few are available commercially and no new compounds have been introduced on the market for over 25 years. A major constraint on the development of new trypanocidal drugs for human and veterinary use is the expense of drug testing on experimental

animals. The testing of existing trypanocidal compounds is also assuming increasing importance with the appearance of parasite populations showing a degree of drug resistance.

Trypanosome culture systems developed at ILRAD are being used to screen the effects of trypanocidal drugs on different parasite species and stocks *in vitro*. This work is being carried out in collaboration with the Swiss Tropical Institute. Although the activity of a trypanocidal compound in the laboratory does not always correspond precisely with its effectiveness in the field, the results of drug screening assays provide important insights into possibilities for improved trypanosomiasis control. Drugs which are highly active *in vitro* but perform poorly in livestock might become useful after modification of their structure or formulation. At the same time, a drug which shows trypanocidal activity *in vivo* but not *in vitro* is likely to be metabolized, and identification of the active metabolite involved could result in a more powerful trypanocidal effect.

An *in vitro* assay system was developed originally using *T brucei* parasites cultured with mammalian feeder layer cells. This assay measures the minimum drug concentration required to inhibit trypanosome growth by more than 50% during 24 hours' exposure (IC_{50}). Work in 1984 and 1985 has concentrated on simplifying and standardizing the assay system and extending it to measure trypanocidal activity against *T congolense* and *T vivax*.

T brucei cultures can easily be initiated from tsetse-derived metacyclic forms or parasitaemic blood. By contrast, bloodstream forms of *T vivax* and *T congolense* are maintained more reliably after passing through the parasite lifecycle *in vitro*, so 'second generation' parasites are used for trypanocide screening against these two species. Efforts to simplify *T congolense* and *T vivax* cultivation by eliminating the requirement for feeder layer cells have not yet been successful.

Seven trypanocidal compounds were tested against *T vivax* (IL 1392) in 1985 and the results compared with their effects against *T brucei* (TC 221). The phenanthridines, isometamidium chloride (Samorin: May and Baker) and ethidium chloride (Novidium: May and Baker), showed higher activity against *T vivax* than against *T brucei*, while the effects of the diamidines, diminazene aceturate and pentamidine isethionate (Pentamidine: May and Baker), were similar for both species. The naphthalene, suramin (Naganol: Bayer), and the quinoline, quinapyramine sulphate (Trypacide: May and Baker), showed much higher activity against *T brucei* than against *T vivax*. These results correspond with studies *in vivo*. Because of the large number of parallel tests carried out on each drug, a computer program has been developed at ILRAD for evaluating and analysing drug screening data.

Parasites exposed to drugs may be tested for infectivity as well as effects on their viability and growth. When *T congolense* (IL 1180) parasites were exposed to isometamidium chloride for 24 hours at low drug concentrations, their growth was not inhibited but they lost their infectivity for mice. The combination of these two parameters—inhibition of growth and loss of infectivity—results in an assay system sensitive enough to detect trypanocidal activity in samples from infected animals. When plasma samples taken from Boran cattle which had been infected with *T congolense* and treated with isometamidium chloride were added to *T congolense* cultures, the plasma killed the parasites or rendered them noninfective to mice. Plasma taken after the cattle became susceptible to reinfection supported trypanosome growth.

This *in vitro* assay is now being used to evaluate the effects on trypanosome viability and infectivity of plasma and cerebrospinal fluid taken from goats which have been treated with different trypanocidal compounds. High-performance liquid chromatogra-

phy (HPLC) analysis is being used in conjunction with the assay system in an attempt to quantify levels of trypanocides in samples taken from treated animals.

Trypanocidal compounds must be extracted from plasma before they can be analysed by HPLC. Diminazene aceturate is recovered from plasma relatively easily and amounts in the low µg range can be detected by HPLC. By contrast, extraction of suramin is more complex because this compound binds with plasma proteins, so only larger amounts (approximately 200 µg/ml) can now be detected accurately by HPLC. The detection of isometamidium chloride in plasma samples is also complicated, due to the breakdown of this drug within the host into active, as yet unidentified, metabolites. Current studies are directed towards identifying active components in the plasma of treated animals which differ from the parent compound and towards increasing the sensitivity of detection of trypanocides in biological fluids.

Drug levels in cerebrospinal fluid from treated animals can be measured directly by HPLC without prior extraction. Analyses of cerebrospinal fluid concentrate particularly on the detection of melarsoprol (Arsobal: Specia), the only commonly used drug known to cross the blood-brain barrier in trypanocidal amounts.

Trypanocidal drugs: evaluation of protection

Despite the wide use of trypanocidal drugs, very little is known about their mode of action *in vivo* or the factors which may have an influence on the effectiveness of a therapeutic regime. Such factors include drug dosage, level of trypanosome challenge, the effect of pre-existing infection at the time of drug treatment and the possible development of immunity while under chemoprophylactic cover.

An experiment conducted in 1984 showed that treatment with isometamidium chloride (1 mg/kg body weight) protected cattle against repeated challenge with *T congolense* (IL 1180) for at least 5 months. Protection appeared to be afforded by the drug alone, as no anti-trypanosome antibodies were detected in any of the animals which resisted challenge.

Group	Previous Challenge	Drug Dosage (mg/kg)	Serodeme	Challenge Method	First Infection (month) Animal: 1	2	3	4
A	none	1.0	IL 1180	5 tsetse	5	6	-	-
B	none	1.0	IL 2642	5 tsetse	6	6	-	-
C	none	0.5	IL 1180	5 tsetse	4	5	5	-
D	IL 2642[a]	0.5	IL 1180	5 tsetse	5	5	6	6
E	none	0.5	IL 1180	5000 metacyclics	4	4	6	6
F	none	0.5	IL 1180	500,000 metacyclics	4	5	5	7

[a]Five infected tsetse 4 weeks before treatment.

Figure 20. Effectiveness of isometamidium chloride treatment against *T congolense* challenge in cattle. Twenty-four 1-year-old Boran steers were divided into six groups of four. All groups were treated with isometamidium chloride (1.0 or 0.5 mg/kg) and challenged with *T congolense* (IL 1180 or IL 2326) monthly from 2 to 7 months after drug treatment. Challenge was either by five infected tsetse flies (*G m centralis*) or by inoculation with 5000 or 500,000 *in vitro*-derived metacyclic trypanosomes. Untreated controls were also challenged every month to confirm the infectivity of the parasites.

In a second experiment conducted in 1985, 24 yearling Boran steers were treated with isometamidium chloride and then challenged monthly with *T congolense* (IL 1180 or IL 2642) from 2 to 7 months after drug treatment (Figure 20). Routine clinical examinations were conducted throughout the period of the experiment. Parasitaemia was monitored after every challenge by direct observation and subinoculation in mice. Breakthrough trypanosome populations were tested for drug sensitivity *in vitro* and all cattle were checked for the development of anti-trypanosomal immune responses. Figure 20 shows the onset of infection in all the treated animals.

Approximately 75% of the treated cattle, on becoming infected, exhibited less pronounced skin reactions and less severe enlargement of the draining prefemoral lymph node than untreated controls. Many treated animals also exhibited a delayed onset of parasitaemia and concurrent anaemia. No trypanosomes from breakthrough infections showed any increased resistance to the drug.

All four cattle challenged by inoculation with 500,000 *in vitro*-derived metacyclic trypanosomes developed low but persistent levels of antibody against the parasites, yet they were still susceptible to infection between 4 and 7 months after treatment. None of the other cattle showed antibody responses during the period in which they were protected by the drug: antibody titres were only observed at the time of onset of parasitaemia. The five animals which remained refractory to infection at the end of the experiment produced no antibodies against the parasites. Conclusions may be summarized as follows:

1. While the experimental results were similar in cattle infected with two unrelated serodemes of *T congolense*, it is not possible to generalize about the situation in the field where other populations of *T congolense* or populations of *T vivax* may differ in terms of virulence or drug sensitivity.

2. Although the number of animals in the experiment was small, statistical analysis of results indicated a direct relationship between drug dosage and the duration of protection.

3. Prior infection (Group D) appeared to have no influence on the duration of protection or the development of immunity.

4. Under the conditions of the experiment, no relationship was detected between the duration of protection and the level of trypanosome challenge (Groups C, E and F).

5. During the period of chemoprophylactic cover, immune responses were only observed in animals subjected to very high levels of repeated parasite challenge (Group F).

6. No evidence of drug resistance was observed. Breakthrough trypanosome populations remained sensitive to isometamidium chloride at doses of 0.5 mg/kg as determined by *in vivo* assay.

Present work concentrates on conditions which may govern the development of protective immune responses in animals treated with trypanocidal drugs. An *in vitro* assay system developed at ILRAD is being used to measure drug levels in plasma taken from treated animals and to evaluate the drug concentration required for effective protection. This work is being conducted in collaboration with the University of Glasgow (UK) and the British Government's Overseas Development Administration (ODA).

A study conducted in collaboration with the Kenya Government's Veterinary Research Laboratory and the Free University of Berlin (FR Germany) tested immunological methods for evaluating the effectiveness of curative trypanocidal drug treatment in camels. Ten camels, negative for antitrypanosomal antibodies at the beginning of the study, were infected with clones of *T evansi* (KETRI 2443). They were treated 8 weeks later, five with suramin and five with melarsoprol. Three camels, which served as positive controls, were infected but not treated and two camels were negative controls.

Samples of blood and cerebrospinal fluid were taken from all animals on a regular basis up to 7 months after the initial infection and several types of analysis were carried out. Parasitaemia was monitored and IgM and IgG antibody levels were determined by micro-ELISA. The camels were weighed periodically and examined for clinical signs of disease.

Infected camels showed the characteristic clinical signs of *T evansi* infection: emaciation, anaemia and oedema. Although subinoculation in mice revealed parasites in the cerebrospinal fluid of all infected camels before treatment, no clinical signs of central nervous system involvement were observed. Treatment with melarsoprol appeared to clear the parasites and no relapse infections occurred. Suramin, which has no trypanocidal activity in the central nervous system, also appeared to provide an effective cure. One relapse infection in the suramin-treated group was detected by mouse inoculation, but parasites were never observed in the camel's bloodstream, nor were there any clinical signs of disease. Both IgM and IgG antitrypanosomal antibodies increased after infection but IgG levels declined after treatment, as shown by ELISA. This suggests that analysis by ELISA could provide a useful tool for field diagnosis of trypanosomiasis in camels.

Studies on resistance to trypanosomiasis

Field studies have established that the N'Dama (*Bos taurus*) cattle of West Africa are more capable of controlling trypanosome infection and resisting clinical signs of disease than Zebu (*B indicus*) cattle in Africa or the European taurine breeds. In 1985, ILRAD scientists began intensive research on the mechanism(s) of N'Dama resistance to trypanosomiasis, using 10 N'Dama cattle brought from The Gambia by embryo transfer and born at ILRAD in 1984 (Figure 21).

Groups of 10 N'Dama and 10 Boran cattle were matched at 12 months of age and an average weight of 140 kg. Two control cattle were included in each group. The other 16

animals were each exposed to eight bites of tsetse (*G m centralis*) infected with *T congolense* (IL 1180)

Skin thickness at the bite sites was measured daily for 21 days and skin biopsies were collected from four cattle in each group 5, 8, 12, 15 and 21 days after infection. PCV and parasitaemia were determined daily for the first 4 weeks and thereafter twice a week up to 5 months after infection. Animals showing PCVs of 15% or lower were treated with diminazene aceturate. All animals were examined clinically twice a week during the first 3 months and thereafter at weekly intervals. White and red blood cells, platelets and reticulocytes were counted and haemoglobin and white cell differentiation were measured. Body weights were recorded weekly.

Chancres developed at all bite sites in both the N'Dama and Boran cattle. The average time to maximum skin thickness was similar in both groups (Figure 22).

Parasites were detected in the blood of the N'Dama on average 1 day sooner than in the Boran, and the first peak of parasitaemia was reached on average 1 day sooner in the N'Dama. The level of first-peak parasitaemia was slightly lower in the N'Dama and from 40 days to 5 months after infection they showed consistently lower parasitaemia levels than the Boran (Figure 23).

The PCVs of six of the eight Boran dropped to 15% between 25 and 50 days after infection and these animals were treated with diminazene aceturate (Figure 24). The N'Dama and the two untreated Boran showed the most severe anaemia 50 days after infection. At that time, the average PCV for the N'Dama was 22% and for the Boran 18%. Afterwards the N'Dama recovered gradually, reaching normal PCV levels by 120 days after infection though parasites were still detectable in their blood.

Figure 21. N'Dama bulls at 18 months of age. These are five of the ten N'Dama cattle brought to ILRAD by embryo transfer and born in 1984.

Skin Reactions	N'Dama	Boran
Time to peak skin thickness (days)	10.00 ± 0.2	10.20 ± 0.6
Skin thickness on day 0 (cm)	0.79 ± 0.2	0.66 ± 0.2
Skin thickness at peak (cm)	1.60 ± 0.2	1.18 ± 0.3
Skin thickness increase (%)	102	79

Figure 22. Skin reactions in 1-year-old N'Dama and Boran cattle after challenge with *G m centralis* infected with *T congolense* (IL 1181). Eight cattle in each group were exposed to eight infected tsetse bites each. None of the cattle had any previous exposure to trypanosome infection.

To summarize the results of this study:

1. Reactions during the first 20 days after challenge with tsetse-transmitted *T congolense* are similar in N'Dama and Boran cattle with no previous exposure to trypanosomiasis.

2. Boran cattle are likely to die from 20 to 50 days after *T congolense* infection unless treated, while N'Dama start to control parasitaemia levels during this period.

3. N'Dama cattle begin to control anaemia about 25 days after infection with *T congolense*; their PCVs start to rise by day 50 and reach normal levels by day 120 after infection without treatment.

4. The growth of 1-year-old N'Dama cattle is retarded by 10 to 15% during 5 months of infection with *T congolense*.

The group of eight infected N'Dama cattle will next be challenged with an unrelated serodeme of *T congolense*. Their responses to this second challenge will be compared with reactions to the first infection.

In 1985, ILRAD scientists studying trypanosome infection in wildlife compared skin responses and antibody production in four buffalo and four susceptible cattle infected by tsetse with *T congolense* (IL 1180). This work was conducted in collaboration with the Kenya Government's Veterinary Research Laboratory partially supported by the Government of The Netherlands. Skin reactions at the sites of infected tsetse bites were much less severe in the buffalo than in the cattle, as indicated by changes in skin thickness. Trypanosomes were never observed in histological sections of buffalo skin, though all four animals became infected. Severe collagen destruction was observed in the skin of the cattle, but not in the buffalo. The skin reaction in the buffalo was clearly diminishing 18 days after infection, but there were no signs of regression in the cattle at at time.

Parasites were detected in the bloodstream sooner in the cattle (10 to 12 days after infection) than in the buffalo (18 to 27 days after infection). Parasitaemia was considerably lower in the buffalo and no parasites were detected after about 30 days. Serological analysis of blood taken from the buffalo revealed antibodies capable of neutralizing *T congolense* metacyclics as early as 15 days after infection. In three out of four of the buffalo, these neutralizing antibodies were observed several days before parasites were detected in the bloodstream. By contrast, a high level of parasitaemia appeared to be a prerequisite for antibody responses in the cattle: neutralizing antibodies were first observed 25 days after infection, more than 10 days after parasites were detected in the blood. The pattern of resistance demonstrated by buffalo in this study differed considerably from the resistance observed in N'D[illegible]a cattle in the experiment reported above.

Trypanotolerance re[illegible]h network

ILRAD scientists are participating in a trypanotolerance research network coordinated by ILCA. In 1985, the network included research sites in six countries of West and

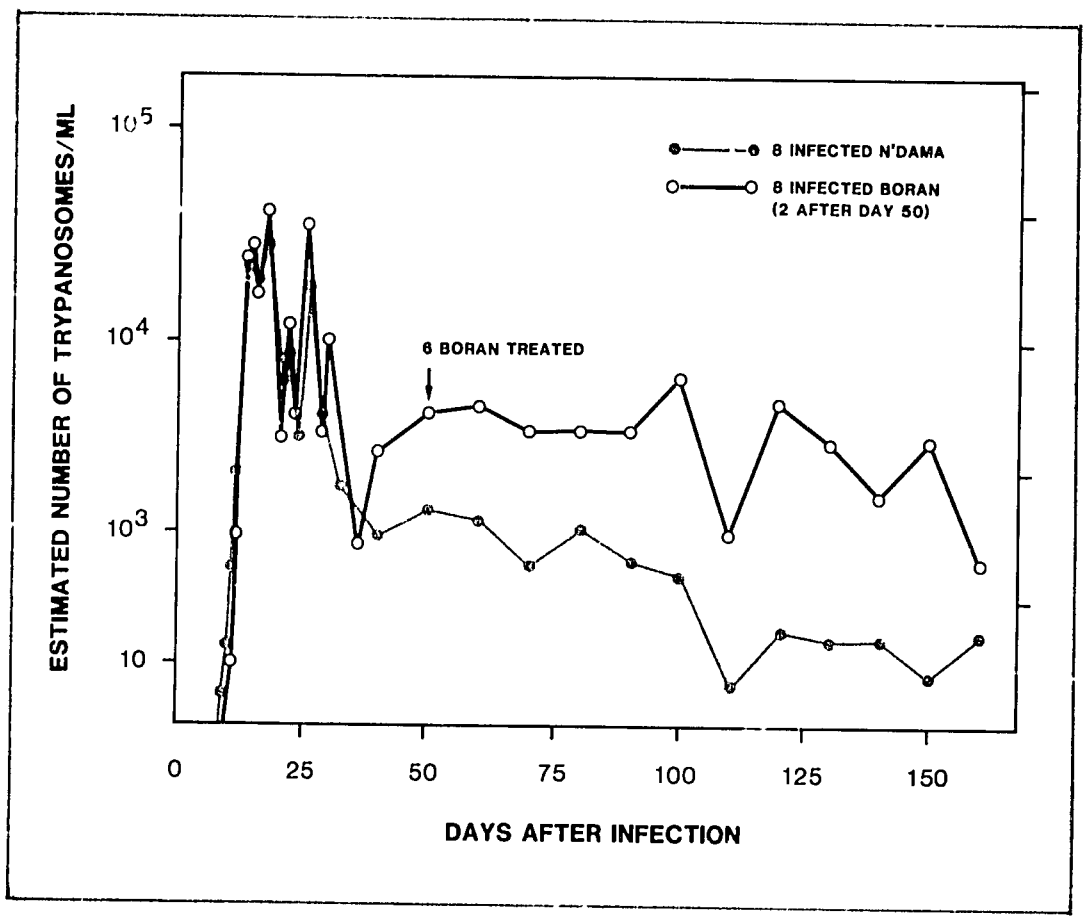


Figure 23. Estimated average number of trypanosomes per ml of jugular blood from eight N'Dama and eight Boran cattle infected with tsetse-transmitted *T congolense* (IL 1180). Six of the Boran were treated with diminazene aceturate around day 50; none of the N'Dama required treatment.

Central Africa—Gabon, Zaire, Nigeria, Ivory Coast, Togo and The Gambia—as well as associated research projects in Kenya and Ethiopia. The goal is to obtain accurate, comparable information on the health and productivity of different breeds of livestock in different ecological zones, under different management systems and exposed to different levels of trypanosomiasis risk. Such information is essential for the rational planning and evaluation of livestock development in the region, including existing or new measures to control trypanosomiasis.

Attention is directed particularly towards the indigenous taurine cattle breeds of West Africa which have shown resistance to trypanosome infection—the N'Dama and West African Shorthorn. At most of the study sites, the health and productivity of these breeds can be compared with that of susceptible cattle kept under the same conditions. The local breeds of sheep and goats also demonstrate a degree of resistance to trypanosomiasis.

ILRAD's role has been to establish and support procedures to monitor tsetse populations, trypanosome infection rates in tsetse and livestock and the health status of animals

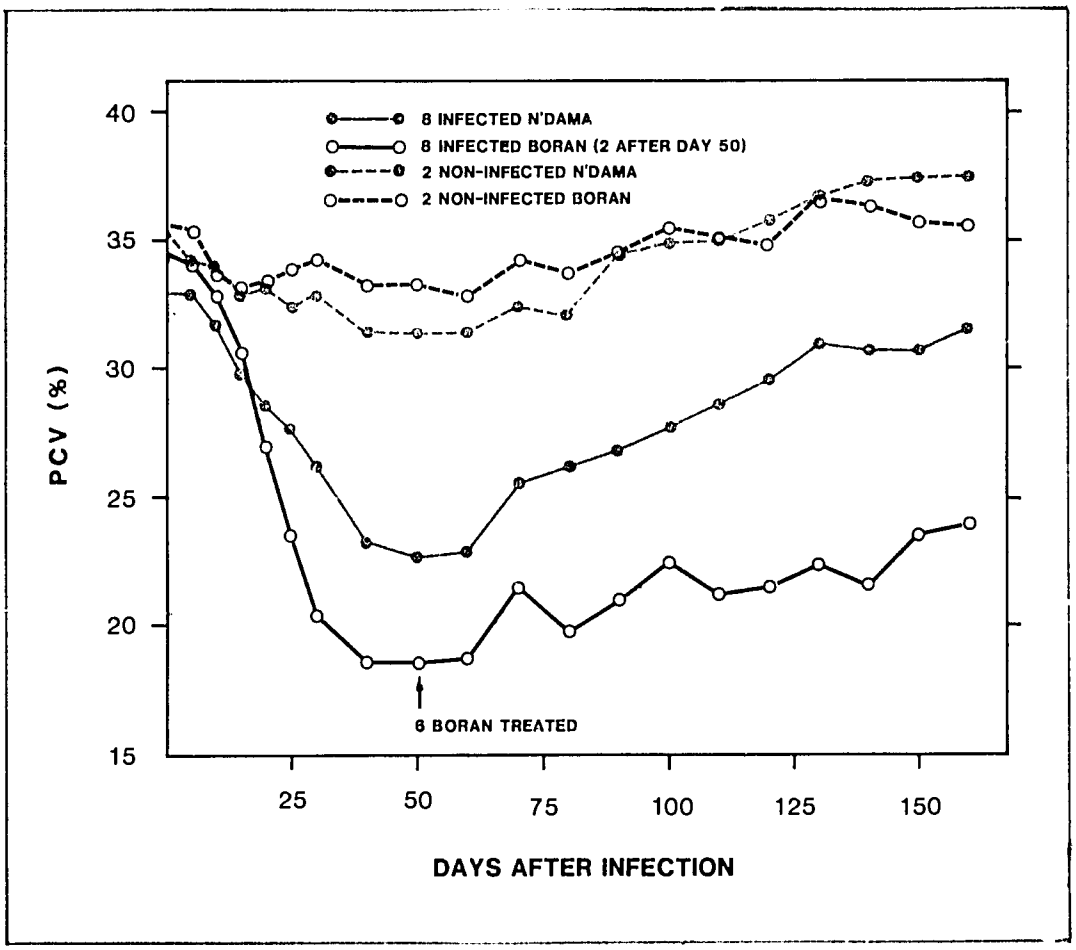


Figure 24. Average PCVs of eight N'Dama and eight Boran cattle infected with tsetse-transmitted *T congolense* (IL 1180). Six of the Boran were treated with diminazene aceturate around day 50 when th ·· PCVs fell to 15%; none of the N'Dama required treatment.

included in the study. ILRAD also has primary responsibility for the introduction and evaluation of trypanosomiasis control measures in the study areas. ILCA is responsible for animal production and nutrition studies and for data processing.

As an important contribution to the network, ILRAD scientists collaborate with ILCA staff in conducting intensive training courses for field personnel. Normally two 7-week training courses are held at ILRAD each year—one in French and one in English. By the end of 1985, 41 staff members working in the trypanotolerance network had received training.

Data have been collected in Gabon since 1981, covering trypanotolerant N'Dama and susceptible Nguni cattle and their crosses. The study site is a government-operated ranch in the humid forest zone. The ranch is infested with three tsetse species; trypanosome infection rates are substantial in flies and in susceptible cattle (Figure 25). Cattle are maintained under one of three treatment regimes: total prophylactic treatment, partial prophylaxis or curative treatment when disease becomes apparent. A preliminary analysis of animal health data collected over 2½ years revealed weight losses in infected cattle during periods when noninfected animals were gaining weight. Further results, as shown in Figure 25, indicate that the N'Dama were less likely to become infected, suffered less anaemia (with higher average PCVs) and required trypanocidal treatment less frequently than susceptible cattle or crossbreds.

Studies began in 1983 on two large, privately owned cattle ranches in Zaire (Figure 26). At Kolo Ranch, 25,000 N'Dama cattle are maintained in an area which is free of trypanosomiasis. Research at this site will be completed in 1986 and should provide baseline productivity data for comparison with N'Dama production under different management systems and levels of trypanosomiasis risk. Mushi Ranch supports 16,000 N'Dama cattle in an area of substantial trypanosomiasis risk. Some animals are treated with diminazene aceturate when trypanosomiasis is observed. A third site in Zaire, a village production situation at Idiofa, was added to the research network in 1985; N'Dama cattle were introduced to farmers in this area in 1957. Animal health and productivity data from this site will provide a useful guide to N'Dama production potential under a village management system.

Research began in 1983 in two areas of Ivory Coast—Boundiali and Tengrela—in collaboration with the Société d'Etat pour le Développement de la Production Animale (SODEPRA: Ivory Coast) and the Gesellschaft für technische Zusammenarbeit (GTZ: FR Germany). Local livestock producers maintain cattle and sheep under a seminomadic production system (Figures 26 and 27). Relative tsetse density is similar in both areas,

Breed	Infection Rate (%)	PCV Level (%)	Annual Drug Treatments
N'Dama	5	35	0.6
Nguni	27	28	4.4
crosses	13	30	2.4

Figure 25. Indices of animal health in N'Dama (*Bos taurus*), Nguni (*Bos indicus*) and crossbred cattle maintained under medium trypanosomiasis risk at Okouma Ranch in Gabon. Infected cattle were treated with diminazene aceturate. Data are averages from observations over a period of 2½ years.

but trypanosomiasis risk, as measured by tsetse infection levels, is much higher in Boundiali than in Tengrela. Research is difficult for several reasons: the disease situation is complex, including trypanosomiasis, tick-borne diseases, helminthiasis and the threat of rinderpest and peste des petits ruminants (PPR); the animals do not stay in one place; and it is impossible to record all trypanocidal drug treatment. However, research results from these areas should be representative of production systems practiced over the broad semiarid region of West Africa.

In collaboration with the Centre de Recherche et d'Elevage (CREAT) and GTZ, network field staff are monitoring cattle, sheep and goat production in two village areas of Togo—Avetenou and Sokodé (Figures 26 and 27). Staff of the ILCA Humid Zone Small Ruminant Program also collect data on the nutrition and health status of goats at four village locations in Nigeria. Preliminary results are given in Figure 27.

ILRAD is cooperating with a project initiated in 1982 by the Kenya Ministry of Agriculture and Livestock Development at Muhaka village near the Kenya Coast. Staff

	Tsetse			Cattle		
Country and Site	Species	Mean Relative Density	Mean Infection Rate (%)	Breed	Number in Study	Mean Monthly Infection Rate (%)
Gabon						
Moanda Ranch	*palpalis*	0.30	20.2	N'Dama	900	5.0
	tabaniformis	0.36	23.0	Nguni	100	27.0
	nashi	0.06	8.8	crosses	200	12.5
Zaire						
Kolo Ranch	*palpalis*	0.54	0.3	N'Dama	650	0.0
Mushi Ranch	*fuscipes*	0.93	2.6	N'Dama	650	7.3
	tabaniformis	5.28	11.2			
Ivory Coast						
Boundiali Village	*palpalis*	0.61	20.5	N'Dama	50	14.7
	tachinoides	0.26	20.4	WAS	300	13.9
	morsitans	0.01	NA	Zebu	20	32.8
				crosses	200	19.5
Tengrela Village	*palpalis*	0.94	4.2	N'Dama	40	3.0
	tachinoides	0.07	10.4	WAS	30	2.7
				Zebu	10	3.9
				crosses	300	3.5
Togo						
Avetonou Station & Village	*palpalis*	0.10	14.3(?)	N'Dama	200	3.9
	tachinoides	0.10	9.1(?)	WAS	240	NA
				crosses	60	NA

Figure 26. Tsetse infestation, cattle numbers and trypanosome infection rates in tsetse and cattle at six trypanotolerance study sites in West and Central Africa. Tsetse density is indicated by the average number of flies caught daily in each trap. WAS stands for West African shorthorn.

of the project are measuring relative tsetse density in the area and monitoring the health and productivity of about 500 East African Zebu cattle. Beginning in April 1984, two-thirds of the animals have received prophylactic drug treatments 4 times a year; the others only receive curative treatment as required. PCVs of cattle in the prophylactic treatment group averaged 27.2% during the first year of treatment, compared to 26.4% for the others. On average, 3.1% of cattle under prophylactic treatment required curative treatment each month, compared to 8.1% of the cattle without prophylaxis, and over the 12-month period average weight gains were 33% higher for cattle receiving prophylactic treatment.

A large-scale livestock development project was initiated in 1984 in The Gambia under the auspices of the International Trypanotolerance Centre (ITC). ILRAD is collaborating with ILCA and the ITC in the research component of the project, which began in 1985. Entomological studies are supported by ODA, while the ILCA/ILRAD work on the productivity of N'Dama cattle is funded by the EEC. The health and productivity of 2500 cattle are being monitored at two village sites in The Gambia—Gunjur and Keneba—and two additional sites will be included in the network, one at Bansang in The Gambia and one in Senegal. Scientists also plan to study the mechanisms and genetic basis of trypanotolerance in N'Dama cattle and to test new methods of trypanosomiasis control.

	Tsetse			Livestock		
Country and Site	Species	Mean Relative Density	Mean Infection Rate (%)	Species	Number in Study	Mean Monthly Infection Rate (%)
Nigeria						
Fashola	*palpalis*	0.10	NA	goat	175	3.5
	tachinoides					
Ivory Coast						
Boundiali	*palpalis*	0.61	20.5	sheep	240	7.4
	tachinoides	0.26	20.4			
	morsitans	0.01	NA			
Tengrela	*palpalis*	0.94	4.2	sheep	300	1.9
	tachinoides	0.07	10.4			
Togo						
Avetonou	*palpalis*	0.10	NA	sheep	180	1.9
	tachinoides	0.10	NA			
Sokodé	*palpalis*	0.04	NA	goat	80	7.0
	tachinoides	0.59	4.0	sheep	200	4.1

Figure 27. Tsetse infestation, livestock numbers and trypanosome infection rates in tsetse and small ruminants at five village sites in West Africa. Tsetse density is indicated by the average number of flies caught daily in each trap. All goats are of the West African Dwarf breed; sheep are Djallonké. Both breeds are considered to possess some resistance to trypanosomiasis.

TRAINING AND INFORMATION SERVICES

TRAINING

ILRAD's training activities include individualized training programs for scientists and technicians, post-graduate training for students working towards master's and doctoral degrees, and post-doctoral positions for young scientists. In addition, training courses and conferences are held each year. The emphasis is on developing veterinary research and disease control capabilities in African countries where trypanosomiasis and ECF are important animal health problems. Training activities account for nearly 10% of ILRAD's total budget.

In 1985, 17 scientists and technicians came to ILRAD on study attachments for periods of 2 to 16 weeks. Their training programs were planned on an individual basis according to the needs of their home institutions. They came from 10 African countries—Burundi, Burkina Faso, Ethiopia, Gabon, Kenya, Nigeria, Senegal, Uganda, Zambia and Zimbabwe. Two were associated with national universities in their home countries, five were staff members working with the ILCA/ILRAD trypanotolerance research network and the other ten were laboratory technicians in national veterinary research organizations.

Post-graduate students work as Research Fellows at ILRAD from 1 to 4 years to fulfill requirements for a post-graduate degree. Eighteen Research Fellows worked with ILRAD scientists in 1985 on projects closely linked with the research program. Four Visiting Research Fellows worked at ILRAD for shorter periods during the year. Four Research Fellows were working towards master's degrees, one towards a diploma in veterinary science and the rest towards their doctorates. Two of these were sent overseas to complete their degree training. During the year, three Research Fellows were awarded their degrees. Research Fellows in 1985 came from Ghana, Kenya, Nigeria, Tchad, Uganda, Zaire, Belgium, Italy, Germany (FR), The Netherlands, the UK and the USA. The students from Africa were fully or partially supported by ILRAD, while the students from elsewhere were supported by funds from their home countries.

Post-Doctoral Fellows are recruited on an international basis. They work at ILRAD for 2 years on projects contributing directly to the research program. In 1985, 13 Post-Doctoral Fellows were working at ILRAD—comprising 5 from the USA, 2 from Kenya and 1 each from Belgium, France, Germany (FR), Japan, Rwanda and the UK.

A new training program component was added in 1985 designed specifically for young scientists working in developing countries, principally in Africa. Under this program staff members of universities or research institutes come to ILRAD for periods of 6 to 12 months to work as Senior Research Fellows. The first Senior Research Fellow came to ILRAD in 1985 from the Biochemistry Department of the University of Nairobi.

In addition to training activities for individuals, ILRAD staff conducted three training courses in 1985. A 3-month course was held from September through November on the preparation and use of reagents for the diagnosis of bovine haemoprotozoan diseases. Six participants attended this course from Burundi, Kenya, Senegal and Zambia.

Two courses were conducted in connection with the ILCA/ILRAD trypanotolerance research network. A 7-week course was held in April and May with six participants from Ethiopia, Mozambique, The Gambia and Zaire. This was the seventh course conducted for network staff. As in previous courses, the subject was the diagnosis and control of trypanosomiasis, helminthiasis and tick-borne diseases. The major emphasis was on practical aspects of clinical diagnosis, collection and analysis of diagnostic materials and post-mortem examination of cattle and goats. Training in animal production research focused on data recording and analysis. The course also covered tsetse fly identification, ageing and dissection, determination of trypanosome infection rates and collection and preparation of tsetse bloodmeal samples. Participants practiced tsetse trapping techniques in the Kibwezi Forest. A 2-week course was held in June designed to upgrade the standard of tsetse research at network sites. Four participants took part from Benin, The Gambia and Togo.

An international workshop on parasite antigens was held at ILRAD in May 1985, bringing together 14 distinguished scientists from around the world to discuss their work with scientists from ILRAD, ICIPE, the Universities of Nairobi and Yaoundé (Cameroon), the Kenya Veterinary Research Laboratory and Trypanosomiasis Research Institute and the Uganda Trypanosomiasis Research Organization. The objective of the workshop was to review the current status of research aimed at characterizing, producing and utilizing parasite antigens for improved disease control. Constraints on research were also discussed, as well as new approaches towards the improved control of parasitic diseases. ILRAD's Information Services published a summary of the discussions.

A second international workshop was held in September to discuss the implementation of ECF immunization programs in East and Central Africa. This meeting followed up a joint ILRAD/FAO workshop on ECF immunization held in October 1984. The 1985 workshop covered methods of ECF immunization and the collection and analysis of data on the productivity of immunized cattle. Sessions on data management and economic evaluation techniques included practical demonstrations using microcomputers. Twenty-four invited participants attended, representing research organizations, field projects and national veterinary services in Burundi, Kenya, Malawi, Zambia and Zimbabwe. A report on the workshop was published in 1986.

Seminars are given by ILRAD scientists and visiting researchers throughout the year, presenting the results of ongoing projects. Altogether, 30 seminars were given in 1985, including a special symposium on the characterization of trypanosome isolates from Kilifi, Kenya. These gatherings are well attended by ILRAD staff and scientists from other research institutions in the Nairobi area.

ILRAD provided financial support for two scientists, from Ethiopia and Zaire, to attend the Fifth Annual Seminar on Tsetse and Trypanosomiasis Control, held in February and March 1985 in Harare, Zimbabwe. The seminar was sponsored by the OAU, FAO and WHO. ILRAD also supported two participants, from India and Nigeria,

who attended the Seventh International Congress of Protozoology, held in June 1985 in Nairobi. In addition, ILRAD provided registration fees to cover participation in the Congress for 18 Kenyan scientists and students.

ILRAD makes its conference facilities available for a variety of workshops and seminars sponsored by other organizations. The most important of these meetings held at ILRAD in 1985 were a Research Coordination Meeting on Sheep and Goat Production sponsored by the International Atomic Energy Agency (IAEA), the annual general meeting of the Kenya Veterinary Association, a regional Germplasm Training Course conducted by the International Potato Centre (CIP), a Workshop on Animal Nutrition sponsored by the University of Nairobi, and a major internal review of the ILCA/ILRAD trypanotolerance research network.

INFORMATION SERVICES

The primary activities of ILRAD's Information Services are the publication and distribution of the annual report and the quarterly newsletter, *ILRAD Reports*, in English and French. A weekly *Internal Newsletter* is also published in English and Kiswahili for distribution to staff members and the Board of Director

Two updated brochures were published in 1985 describing ILRAD's training and research activities. These are intended for wide distribution. In addition, a proceedings volume was published—*Immunization against theileriosis in Africa*—which includes papers and discussions presented at the joint workshop sponsored by ILRAD and FAO in October 1984. The summary proceedings of an international workshop on parasite antigens was also produced for the workshop participants who came to ILRAD in May 1985.

The four issues of *ILRAD Reports* published in 1985 included major articles on the structure and function of the bovine immune system; collaborative studies on trypanotolerant livestock; international research on parasite antigens aimed at developing improved vaccines and diagnostic tests; and detailed studies at ILRAD on the immune and nonimmune mechanisms of host resistance to trypanosomiasis. Shorter articles described the workshop on ECF immunization held in September 1985 and the establishment and institutional setting of ILRAD within the CGIAR.

Members of ILRAD's scientific staff published 32 articles in international journals in 1985 and 16 chapters or scientific reports. These scholarly publications, plus numerous

	Countries		Institutions/Individuals	
Region	1984	1985	1984	1985
Anglophone Africa	26	24	749	851
Francophone Africa	24	24	334	378
Anglophone & other Europe	17	20	270	286
Francophone Europe	2	2	39	42
North & South America	27	27	304	317
Near & Far East	34	34	334	248
Total	130	131	2030	2122

Figure 28. Comparison of *ILRAD Reports* distribution at the end of 1984 and the end of 1985.

papers presented at international conferences comprise the bulk of ILRAD's information output for specialized scientific audiences. The information section continues to direct considerable effort towards ensuring the distribution of these publications to scientists in developing countries who may not have access to a wide range of international journals.

The annual report and quarterly newsletter are intended for less specialized readers, with emphasis on animal health workers in laboratories and field situations in Africa. In addition, *ILRAD Reports* and occasional short press releases are distributed to journalists in many parts of the world. A major effort was initiated in the second half of 1985 to verify and update the distribution list for these publications (Figure 28). The goal is not simply to expand ILRAD's audience, but rather to increase the accuracy and appropriateness of information distribution. A major consideration, under continuous review, is the cost-effectiveness of production and distribution of ILRAD publications.

THE ILRAD LIBRARY

The ILRAD library serves staff members, visiting scientists, participants in the training program and staff of other research institutes, government departments and universities in Kenya. A brochure, *The ILRAD library: user's guide*, describes the services offered.

The specialized collection concentrates on topics related to ILRAD's research program. The library subscribes to 210 scientific journals covering fields such as parasitology, immunology, biochemistry, entomology, cell biology and veterinary medicine and including several indexing and abstracting journals. Journal subscriptions are reviewed every year according to the recommendations of the scientific staff. The collection of scientific reference books is steadily expanding: 401 new books were acquired in 1985, bringing the total to just over 3000.

The ILRAD library participates in an interlibrary loan network along with the University of Nairobi, the Kenya Government's Veterinary Research Laboratory, the Veterinary Research Department of the Kenya Agricultural Research Institute, the Ministry of Health and ICIPE. In 1985, ILRAD staff members borrowed 400 items from these other libraries, and the ILRAD library loaned out 215 items through the network. In addition, the ILRAD library donated over 200 books and 15 journal volumes to other libraries in the Nairobi area.

The ILRAD library offers current awareness services through weekly announcements of accessions in the *Internal Newsletter* and through subscriptions to three monthly selective dissemination of information services. These are provided by Biosciences Information Services (BIOSIS), the International Information System for Agricultural Sciences and Technology (AGRIS) of FAO and the abstracts service of the Commonwealth Agricultural Bureaux (CAB). In addition, the library carries out computer-based literature searches for ILRAD staff using agents in Europe and North America.

The library orders copies of scientific articles not available in Kenya as requested by ILRAD staff members. About 400 articles were acquired in 1985, mainly from the British Library Lending Division. Reprints of scientific articles by ILRAD staff are supplied on request to researchers and field workers in developing countries.

RESEARCH SUPPORT

TSETSE LABORATORY

A great deal of the trypanosomiasis research conducted at ILRAD requires parasites which have developed in tsetse flies. Although it is possible to maintain trypanosomes *in vitro* and to infect laboratory animals and livestock by injection, the parasites normally pass through several stages of their lifecycle in tsetse flies and trypanosomes maintained and transmitted artificially are different in some respects from parasites transmitted through the bite of an infected fly.

The three major taxonomic groups of tsetse flies—*morsitans*, *palpalis* and *fusca*—are represented by seven breeding colonies maintained at ILRAD. These are *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* from Kenya. The overall breeding performance of the seven colonies in 1985 is shown in Figure 29.

All breeding colonies are maintained at 25°C; tsetse in the *morsitans* group are kept at 70% relative humidity and the others are kept at 85%. All groups feed 5 days a week on rabbits. No major production problems were encountered in 1985.

Tsetse flies are used in a wide range of trypanosomiasis research projects at ILRAD. In 1985, tsetse puparia and adult flies were also supplied to scientists at the University of Nairobi, the Kenya Agricultural Research Institute, the National Council for Scientific Research and an FAO project in Zambia, the Universities of Bristol and Salford (UK), the Institute of Tropical Medicine (Belgium) and the Swiss Tropical Institute.

TICK LABORATORY

Infective sporozoite forms of *T parva* can only be obtained by continuous passage of parasites between tick vectors and mammalian hosts. The tick laboratory at ILRAD maintains colonies of infected and uninfected ticks by feeding on rabbits and cattle. The laboratory provides ticks, tick organs and tick-derived *Theileria* sporozoites for a wide range of ECF research projects. About 8.5 million ticks were raised in 1985 to provide

	Breeding Females (mean)	Daily Female Mortality (mean %)	Weekly Pupae per Female (mean)	Puparial Weight (mean mg)	Annual Number Puparia
G m centralis	9566	0.44	0.54	33.68	269,584
G austeni	2240	0.15	0.43	25.81	50,374
G p palpalis	1991	0.54	0.41	30.01	41,934
G p gambiensis	2473	0.46	0.47	28.93	60,739
G f fuscipes	1787	0.31	0.45	34.58	41,492
G tachinoides	1857	0 76	0.49	19.29	47,009
G brevipalpis	5238	1.06	0.46	73.06	125,456

Figure 29. Performance of seven tsetse breeding colonies maintained at ILRAD in 1985.

a continuous supply of material for research on ticks and tick-borne diseases. Whole ticks were provided to ILRAD scientists and about 111,000 ticks were dissected to provide parasite material.

The main tick species maintained at ILRAD is *Rhipicephalus appendiculatus*, the principal vector of *T p parva* and *T p lawrencei*. Colonies are maintained of several *R appendiculatus* laboratory strains as well as field isolates from different locations in East Africa. A number of other tick species are maintained in smaller colonies. These include *Amblyomma variegatum* and *A gemma*, the vectors of *Cowdria ruminantium* (heart-warter) and two relatively benign *Theileria* species, *T mutans* and *T velifera*; *Boophilus decoloratus* and *B microplus* which transmit *Babesia* spp and *Anaplasma marginale*; and *Rhipicephalus evertsi evertsi* and *R pulchellus*.

Factors affecting tick survival and growth are investigated on a continuous basis, including the development of host resistance to ticks, tick toxins causing paralysis or death of host animals, residual effects on ticks of host acaricide treatment, toxic effects on ticks of substances in the host diet, climatic and seasonal effects and bacterial and fungal diseases in ticks. Factors are also studied which affect the development of *Theileria* parasites in ticks, including characteristics of specific parasite and tick strains, individual and breed characteristics of host cattle, timing of tick attachment, seasonal factors and the environment during tick feeding and incubation. The goal is to provide the best dietary and environmental conditions for producing large, healthy ticks which in turn are infected with the highest possible number of *Theileria* sporozoites.

Experiments were conducted in 1985 to test whether *Theileria* infection rates could be significantly increased in ticks fed on cattle whose immune responses are suppressed, either by treatment with glucocorticoids or by splenectomy. Very high infection rates were achieved, but results were strongly influenced by the reactions of individual cattle and by the *Theileria* strain used.

Other studies concentrate on developing staining techniques to identify different *Theileria* species in the salivary glands of infected ticks. Methods are also being investigated to assess parasite maturation in the tick salivary gland.

The tick laboratory identifies ticks sent in by field workers from Kenya and other African countries and supplies ticks and tick-derived parasite material to a variety of collaborative research projects. In 1985, ILRAD's tick laboratory provided assistance to national veterinary laboratories in Kenya, Rwanda and Zaire, two development projects in Kenya, the National Tsetse and Trypanosomiasis Control Project in Somalia, the Serengeti Research Station in Tanzania, Kenyatta University (Kenya) and the University of Oklahoma (USA). During the year, field and laboratory workers came to

ILRAD from Burundi, Ethiopia, Mozambique, The Gambia and Zaire for training courses which included tick handling and identification and the diagnosis and treatment of tick-borne diseases.

FARM ANIMAL PRODUCTION

Livestock production and management returned to normal in 1985 after a severe drought in 1984, both on the ILRAD farm at Kabete and on the ILRAD ranch, Kapiti Plains Estate. During the year, 427 cattle, 564 goats and 13 sheep were allocated for experimental purposes. Sixty percent of the cattle were used for ECF research, 35% for trypanosomiasis research and 5% for the production of antigens used by the diagnostic laboratory. Nearly all the small ruminants were used for trypanosomiasis research.

The Kapiti ranch continued to supply nearly all the cattle required for ILRAD's research program. From a breeding herd of 1,252 cows and heifers, 1,029 calves were born during the year, resulting in a calving rate of 82%. The ranch supplied 331 Boran calves, 38 Guernsey calves and 34 Boran steers to ILRAD and sold 328 cattle to other users. Apart from a few sheep and goats born on the ILRAD farm at Kabete, all small ruminants used for experimental work are purchased from local farmers.

Development of the Kapiti ranch in 1985 included the construction of fencing, water troughs and an effluent storage tank. The addition of nearly 50 km of fencing has improved the control of grazing and stock movement. A foundation herd of registered Boran cows is being developed from the total herd of about 2500 animals, with selection based on conformation and weight at 2 years old: sires for future breeding programs will be selected from the progeny.

Embryo transfer work was initiated at the Kapiti ranch in 1984 to produce Boran calves of specific BoLA types using selected BoLA-typed cows and bulls. The first Boran calves produced by embryo transfer were born in 1985. The goal is to produce 50 to 60 calves by embryo transfer each year, including single births, twins and chimaeric twins.

Brief outbreaks of salmonellosis and contagious caprine pleuropneumonia (CCPP) occurred on the Kabete farm during the year, but were quickly controlled. An outbreak of foot-and-mouth disease near the Kapiti ranch interrupted the supply of calves to ILRAD for a short period.

LABORATORY ANIMAL PRODUCTION

The laboratory animal unit provides a regular supply of rats, mice and rabbits for experimental work at ILRAD and maintains small colonies of meadow voles (*Microtus montanus*), guinea pigs and gerbils. All laboratory animals are housed and maintained according to international animal welfare conventions.

Figure 30 shows the total number of rats, mice and rabbits produced and used in 1985. The demand for mice increased by 50% over the 1984 level, the demand for rats dropped by 25% and the demand for rabbits remained constant.

Random-bred rats raised at ILRAD originate from the Sprague Dawley strain. They are used primarily for the production of trypanosomes. In addition, batches of two inbred rat strains were imported in 1985 for specific experiments.

Mice are used primarily to study the mechanisms of resistance to trypanosomiasis, with different strains varying in their susceptibility to trypanosome infection. Random-bred Swiss mice are used for a great deal of experimental work, but colonies of three inbred strains—BALB/c, C3H/He and C57Bl/6—are required for some studies. Small colonies of C3H/HeJ, AJ and DBA/2 mice were also maintained in 1985, and F_1 and F_1-back crosses were produced of BALB/c x Swiss and C3H/He x C57Bl/6. Batches of C57Bl/6 beige and NZB mice were imported for experimental use, as well as batches of two athymic nude strains, D3H/Tif nude and C57Bl/6 nude.

An isolation system is being established for laboratory animals, with three isolation units already functioning. Imported animals can now be maintained in isolation with little or no risk to the main breeding colonies. Virus-free batches of ILRAD's primary inbred strains were imported in 1985; these are maintained in isolation units under sterile conditions.

Rabbits are used at ILRAD primarily to produce antisera and to support the tsetse fly and tick colonies. The rabbit breeding colony slowly increased during the year, though rabbits must still be purchased from outside breeders to meet the demands of the research program. Preweaning losses in the rabbit colony may be attributable to poor natural ventilation. More powerful ventilator fans have been installed in the breeding rooms and some rabbits are being kept under sheltered conditions outdoors.

In addition to supplying nearly all the laboratory animals used at ILRAD, the breeding unit provides mice, rats and other rodents to several organizations in Kenya. In 1985, these included ICIPE, the Kenya Trypanosomiasis Research Institute, the Kenya Medical Research Institute, the Institute for Primate Research, the Biochemistry Department of the University of Nairobi, the National Museums of Kenya, the Kenya Society for the Protection and Care of Animals and several Kenyan secondary schools.

CLINICAL AND DIAGNOSTIC SERVICES

The diagnostic laboratory carries out several types of routine analysis required by ILRAD's research programs and animal production facilities. In 1985, a total of 37,804 samples were analyzed. These comprised 26,341 serological samples, 4728 bacteriological samples, 3108 haematological samples and 3627 helminthological samples—altogether an increase of 26% over the number of samples analysed in 1984.

Species and Strain	Number Weaned	Number Supplied to Scientists
Rats	20,400	13,075
Mice		
random-bred Swiss	25,573	16,538
inbred BALB/c	17,090	16,709
inbred C3H/He	6,589	5,430
inbred C57Bl/6	5,419	4,107
others (purchased)		5,125
Rabbits	420	1,288

Figure 30. Rats, mice and rabbits produced in 1985 and supplied to ILRAD scientists.

Nearly all this expansion was due to the increased number of serological analyses required for ILRAD's collaborative ECF immunization field trials. The diagnostic laboratory analyses *Theileria* isolates collected from the field and checks cattle for previous exposure to ECF and other diseases before they are immunized by infection and treatment. After cattle are immunized, they are monitored for immune responses to *Theileria* parasites on a monthly basis for 1 year.

Serum samples from farm and laboratory animals are screened for antibodies to six bloodstream parasites—*Theileria parva*, *T mutans*, *Trypanosoma brucei*, *T congolense*, *T vivax* and *Anaplasma marginale*. Most routine screening for *Theileria* and *Trypanosoma* species is by the indirect fluorescent antibody (IFA) test. The ELISA test is also used to screen bovine sera for antibodies to *Trypanosoma* species.

A new method for purifying antibodies against *A marginale* was developed in 1984. Work is still in progress to produce defined antigen(s) for the diagnosis of *A marginale* in collaboration with Washington State University (USA).

A major achievement in 1985 was the adaptation of the ELISA test for screening antibodies against *Babesia bigemina*. Monoclonal antibodies have been generated against this parasite which will be used to purify antigens for use in diagnosis.

In addition to services to ILRAD scientists, the diagnostic laboratory provided antigens, conjugates and control sera to the Veterinary Research Department of the Kenya Agricultural Research Institute, the University of Ibadan (Nigeria), FAO projects in Burundi, Zambia and Zimbabwe and a British Government (ODA) project in Tanzania. The diagnostic laboratory also analysed sera collected from cattle in ECF areas for the Kenya Ministry of Agriculture and Livestock Development and FAO projects in Zimbabwe and Tanzania.

The diagnostic laboratory offered a 3-month training course in 1985 on the preparation of reagents for use in the serodiagnosis of bovine haemoparasites. Six specialists participated in the course from Burundi, Kenya, Senegal and Zambia. In addition, eight laboratory technicians and field veterinarians from developing countries received training in ILRAD's diagnostic laboratory in 1985 on an individual basis.

1985 PUBLICATIONS

Akol, G.W.O. and Murray, Max (1985). Induction of protective immunity in cattle by tsetse-transmitted cloned isolates of *Trypanosoma congolense*. *Annals of Tropical Medicine and Parasitology*. 79: 617-27 (266).

Black, S.J., Sendashonga, C.N., O'Brien, C., Borowy, N.K., Naessens, J., Webster, P. and Murray, Max (1985). Regulation of parasitaemia in mice infected with *Trypanosoma brucei*. *Current Topics in Microbiology and Immunology*. 117: 93-118 (263).

Borowy, N.K., Fink, E. and Hirumi, H. (1985). *In vitro* activity of the trypanocidal diamidine DAPI on animal-infective *Trypanosoma brucei brucei*. *Acta Tropica*. 42: 287-98 (321).

Borowy, N.K., Fink, E. and Hirumi, H. (1985). *Trypanosoma brucei*: five commonly used trypanocides assayed *in vitro* with a mammalian feeder layer system for cultivation of bloodstream forms. *Experimental Parasitology*. 60: 323-30 (324).

Borowy, N.K., Hirumi, H., Waithaka, H.K. and Mkoji, G. (1985). An assay for screening drugs against animal-infective bloodstream forms of *Trypanosoma brucei brucei in vitro*. *Drugs and Experimental Clinical Research*. 11:155-61 (312).

Brown, W.C. and Grab, D.C. (1985). Biological and biochemical characterization of bovine interleukin-2. Studies with cloned bovine T cells. *Journal of Immunology*. 135: 3184-90 (322).

Chumo, R.S.C., Taracha, E., Morzaria, S.P., Irvin, A.D., Voigt, W.P. and Purnell, R.E. (1985). East Coast fever field trial at Ngong, Kenya: isolation of *Theileria parva* stocks and immunization. In A.D. Irvin, ed. *Immunization against Theileriosis in Africa*. Nairobi: ILRAD, pp. 79-81 (390).

Chuley, J.D., Fawcett, D.W. and Chunge, C.N. (1985). Electron microscopy of *Leishmania donovani* in splenic aspirates from patients with visceral leishmaniasis during treatment with sodium stibogluconate. *Annals of Tropical Medicine and Parasitology*. 79: 417-29 (264).

Dobbelaere, D.A.F and Spooner, P.R. (1985). Production in ascites fluid of biosynthetically labelled monoclonal antibody to *Theileria parva* sporozoites. *Journal of Immunological Methods*. 82: 209-14 (268).

Dobbelaere, D.A.E., Shapiro, S.Z. and Webster, P. (1985). Identification of a surface antigen on *Theileria parva* sporozoites by monoclonal antibody. *Proceedings of the National Academy of Sciences of the USA*. 82: 1771-75 (309).

Dobbelaere, D.A.E., Webster, P., Leitch, B.L., Voigt, W.P. and Irvin, A.D. (1985). *Theileria parva*: expression of a sporozoite surface coat antigen. *Experimental Parasitology*. 60: 90-100 (315).

Doyle, J.J., Grandin, B.E. and Paling, R.W. (1985). Women and agricultural technology: understanding the needs of both the direct users and the ultimate beneficiaries of ILRAD's research. In *The Users' Perspective in International and National Agricultural Research*. The Hague: International Service for National Agricultural Research, pp. 111-20 (345).

Fawcett, D.W., Young, A.S. and Leitch, B.L. (1985). Sporogony in *Theileria* (Apicomplexa: Piroplasmida). A comparative ultrastructural study. *Journal of Submicroscopic Cytology*. 17: 299-314 (235).

Hirumi, H. (1985). Battle against African sleeping sickness [in Japanese]. *Newton*. 9: 36-43 (388).

Irvin, A.D. (1985). Immunity in Theileriosis. *Parasitology Today*. 1: 124-28 (409).

Irvin, A.D. (1985). Immunization against *Theileria parva*. In *Ticks and tick-borne disease control: a practical field manual*. Vol. 2. Rome: FAO, 457-507 (402).

Irvin, A.D., ed. (1985). *Immunization against theileriosis in Africa: proceedings of a joint workshop*. Nairobi: ILRAD, 167 pp. (376).

Irvin, A.D., Minami, T., Spooner, P.R., Fujinaga, T., Fujisaki, K., Morzaria, S.P. and Conrad, P.A. (1985). Characterization of strains of *Theileria parva*. In A.D. Irvin, ed. *Immunization against Theileriosis in Africa*. Nairobi: ILRAD, pp. 114-17 (393).

Lenaban J.K. (1985). Training needs in an East Coast fever immunization program. In A.D. Irvin, ed. *Immunization against Theileriosis in Africa*. Nairobi: ILRAD, p. 140 (395).

Lonsdale-Eccles, J.D. (1985). Sulphydryl-dependent enzymes from African trypanosomes. In E.A. Khairallah, J.S. Bond and J.W.C. Bird, eds. *Intracellular Protein Catabolism*. New York: Alan Liss, pp. 229-31 (375).

Majiwa, P.A.O., Matthyssens, G., Masake, R., Nantulya, V.M. and Hamers, R. (1985). *Trypanosoma (Nannomonas) congolense*: identification of two distinct karyotypic groups. *EMBO Journal*. 4: 3307-13 (354).

Majiwa, P.A.O., Matthyssens, G., Williams, R.O. and Hamers, R. (1985). Cloning and analysis of *Trypanosoma (Nannomonas) congolense* ILNat 2.1 VSG gene. *Molecular and Biochemical Parasitology*. 16: 97-108 (387).

Moloo, S.K. (1985). Distribution of *Glossina* species in Africa. *Acta Tropica*. 42: 275-81 (359).

Moloo, S.K. and Dar, F. (1985). Probing by *Glossina morsitans centralis* infected with pathogenic *Trypanosoma* species. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 79: 119 (279).

Moloo, S.K. and Kutuza, S.B. (1985). Survival and reproductive performance of female *Glossina morsitans morsitans* when maintained on livestock infected with salivarian trypanosomes. *Annals of Tropical Medicine and Parasitology*. 79: 223-24 (316).

Morrison, W.I. and Murray, Max (1985). The role of humoral immune responses in determining susceptibility of A/J and C57BL/6 mice to infection with *Trypanosoma congolense*. *Parasite Immunology*. 7: 63-79 (267).

Morrison, W.I. and Musoke, A.J. (1985). Alternative approaches to immunization against East Coast fever. In A.D. Irvin, ed. *Immunization against Theileriosis in Africa*. Nairobi: ILRAD, pp. 97-99 (391).

Morrison, W.I., Murray, Max and Akol, G.W.O. (1985). The immune responses of cattle to trypanosomes. In I.R. Tizard, ed. *Immunology and pathogenesis of trypanosomiasis*. Boca Ratan, Florida: CRC Press, pp. 103-31 (241).

Morzaria, S.P. and Irvin, A.D. (1985). The epidemiology of tick-borne diseases: serological diagnostic tests. In *Ticks and tick-borne disease control: a practical field manual*. Vol. 2. Rome: FAO, 333-72 (401).

Morzaria, S.P., Irvin, A.D., Taracha, E. and Spooner, P.R. (1985). East Coast fever immunization trials in the Coast Province of Kenya. In A.D. Irvin, ed. *Immunization against Theileriosis in Africa*. Nairobi: ILRAD, pp. 76-78 (389).

Murray, Max and Black, S.J. (1985). African trypanosomiasis in cattle: working with nature's solution. *Veterinary Parasitology*. 18: 167-82 (306).

Murray, Max, Hirumi, H. and Moloo, S.K. (1985). Suppression of *Trypanosoma congolense*, *T vivax* and *T brucei* infection rates in tsetse flies maintained on goats immunized with uncoated forms of trypanosomes grown *in vitro*. *Parasitology*. 91: 53-66 (317).

Murray, Max, Trail, J.C.M., D'Ieteren, G., Paling, R.W., Leak, S.G.A., Maehl, J.H.H. and Moloo, S.K. (1985). The trypanotolerance network: a model for an East Coast fever network. In A.D. Irvin, ed. *Immunization against Theileriosis in Africa*. Nairobi: ILRAD, pp. 129-32 (394).

Naessens, J., Newson, J., Bensaid, A., Teale, A.J., Magondu, J. and Black, S.J. (1985). *De novo* expression of T-cell markers on *Theileria parva*-transformed lymphoblasts in cattle. *Journal of Immunology*. 135: 4183-88 (325).

Nantulya, V.M. (1985). Biotechnology at ILRAD. In *Biotechnology in International Agricultural Research*. Manila: International Rice Research Institute, pp. 297-304 (352).

Nwagwu, M., Grab, D.J., ole-MoiYoi, O., Hirumi, H. and Watanabe, M. (1985). Phosphofunctokinases of *T brucei*: purification and properties. *Biochemical Society Transactions*. 13: 890-91 (441).

Parish, N.M., Morrison, W.I. and Pearson, T.W. (1985). Identification of an antigen specific to *Trypanosoma congolense* by using monoclonal antibodies. *Journal of Immunology*. 134: 593-97 (400).

Teale, A.J., Morrison, W.I., Goddeeris, B.M., Groocock, C.M., Stagg, D.A. and Spooner, R.L. (1985). Bovine alloreactive cytotoxic cells generated *in vitro*: target specificity in relation to BoLA phenotype. *Immunology*. 55: 355-62 (311).

Trail, J.C.M., Murray, Max, Sones, K., Jibbo, J.M.C., Durkin, J. and Light, D.E. (1985). Boran cattle maintained by chemoprophylaxis under trypanosomiasis risk. *Journal of Agricultural Sciences*. 105: 147-66 (405).

Trail, J.C.M., Sones, K., Jibbo, J.M.C., Durkin, J., Light, D.E. and Murray, Max (1985). *Productivity of Boran Cattle Maintained by Chemoprophylaxis under Trypanosomiasis Risk*. Addis Ababa: International Livestock Centre for Africa, 76pp. (346).

Webster, P., Dobbelaere, D.A.E. and Fawcett, D.W. (1985). The entry of sporozoites of *Theileria parva* into bovine lymphocytes *in vitro*: immunoelectron microscopic observations. *European Journal of Cell Biology*. 36: 157-62 (318).

Westley, S.B. and Howe, V., eds. (1985) *Parasite Antigens: Summary Proceedings of an International Workshop*. Nairobi: ILRAD, 52 pp. (404).

Westley, S.B. (1985). The fight against East Coast fever. *Kenya Farmer*. 50: 63-65 (403).

Whitelaw, D.D. and Jordt, T. (1985). Colostral transfer of antibodies to *Trypanosoma brucei* in goats. *Annales de la Société Belge de Médecine Tropicale*. 65: 119-205 (327).

Whitelaw, D.D., Kaaya, G.P., Moulton, J.E., Moloo, S.K. and Murray, Max (1985). Susceptibility of different breeds of goats in Kenya to experimental infection with *Trypanosoma congolense*. *Tropical Animal Health and Production*. 17: 155-65 (310).

Whitelaw, D.D., Moulton, J.E., Morrison, W.I. and Murray, Max (1985). Central nervous system involvement in goats undergoing primary infections with *Trypanosoma brucei* and relapse infections after chemotherapy. *Parasitology*. 90: 255-68 (307).

Whitelaw, D.D. and Urquhart, G.M. (1985). Maternally derived immunity in young mice to infection with *Trypanosoma brucei* and its potentiation by Berenil chemotherapy. *Parasite Immunology*. 7: 289-300 (347).

Young, A.S. and Grootenhuis, J.G. (1985). Influence of wildlife on immunization of cattle against theileriosis in East Africa. In A.D. Irvin, ed. *Immunization against Theileriosis in Africa*. Nairobi: ILRAD, pp. 104-9 (392).

BOARD OF DIRECTORS

Dr P. Atang
Chief, Regional Operations
Agricultural Operations Division
Food and Agriculture Organization
of the United Nations
Rome, Italy

Dr L.L. Callow
Director of Pathology
Animal Research Institute
Brisbane, Australia

Dr D.M. Chavunduka
Highlands Veterinary Surgery
Highlands, Zimbabwe

Prof Dr K. Eichmann
Director, Max-Planck Institut
für Immunbiologie
Freiburg, Germany (Federal Republic)

Dr A.R. Gray
Director General, ILRAD
Nairobi, Kenya

Dr G.L. Kazyumba
Chef de Travaux
Laboratoire de Parasitologie
Université de Kinshasa
Kinshasa, Zaire

Mr J.S. Mburu
Managing Director, National Agricultural
Chemicals and Fertilizers Ltd
Nairobi, Kenya

Dr W.K. Ngulo
Deputy Director
Ministry of Livestock Development
Nairobi, Kenya

Prof E.N.W. Oppong
Animal Health/Husbandry Officer
Food and Agriculture Organization
of the United Nations
Jos, Nigeria

Prof W.R. Pritchard (Chairman)
School of Veterinary Medicine
University of California at Davis
Davis, California, USA

Dr K.S. Warren
Director for Health Sciences
The Rockefeller Foundation
New York, NY, USA

Dr K.F. Wells
Agricultural Consultant
Ottawa, Canada

STAFF

(holding positions during all or part of 1985)

ADMINISTRATION

A.R. Gray
Director General
P.R. Rowe
Director of Administration
J.J. Doyle
Director of Research
D. M. Sickelmore
Financial Controller
J. Ouya
Senior Accountant
P.K. Churie
Chief Personnel Officer
M.N. Kanyi
Principal Administrative Officer
M.A. Craig
Food and Housing Superintendent
M.W. Holt
Chief Engineer
M.A. Lobo
Electronics Engineer
S. Kasera
Purchasing Officer
G. Mzera
Stores Superintendent
A. Mathenge
Security Officer
L. Hartley
Transport Officer

PARASITOLOGY: TRYPANOSOMIASIS

V.M. Nantulya
Scientist
P.R. Gardiner
Scientist
G. Knowles
Scientist
T. Asonganyi
Visiting Scientist
R.K.G. Assoku
Visiting Scientist
M. Clarke
Visiting Scientist
S. Ferenc
Visiting Scientist
T.W. Pearson
Visiting Scientist
D. Whitelaw
Visiting Scientist
R.A. Masake
Post-Doctoral Fellow
K. Nguli
Research Associate
A. Adema
Laboratory Technician
H. Gathuo
Laboratory Technician

N. Saigar
Laboratory Technician
V. Nyambati
Laboratory Technician
R. Thatthi
Laboratory Technician

BIOCHEMISTRY/ MOLECULAR BIOLOGY

O. ole MoiYoi
Senior Scientist
D.J. Grab
Scientist
J. Lonsdale-Eccles
Scientist
J.R. Young
Scientist
J. Dame
Visiting Scientist
L. de Almeida
Visiting Scientist
J. Ward
Visiting Scientist
G. Matthyssens
Visiting Scientist
T. Tsukamoto
Visiting Scientist
K. Iams
Post-Doctoral Fellow
B. Kukla
Post-Doctoral Fellow
B. Kimmel
Research Associate
P. Majiwa
Research Associate
J. Mpimbaza
Research Associate
A. Nayar
Laboratory Technician
J. Verjee
Laboratory Technician

CELL BIOLOGY

H. Hirumi
Senior Scientist

W.R. Fish
Scientist
R. Brun
Visiting Scientist
B. Honigberg
Visiting Scientist
R.W. Hampton
Visiting Research Fellow
N.K. Borowy
Post-Doctoral Fellow
K. Hirumi
Research Associate
R.T. Nelson
Research Associate
E. Omolo
Laboratory Technician

IMMUNOBIOLOGY

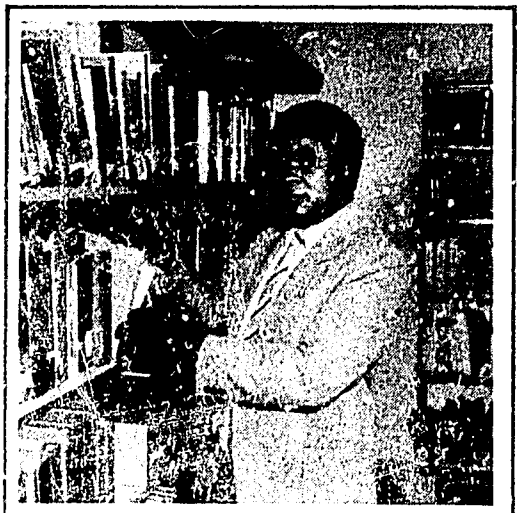
S.J. Black
Senior Scientist
W.C. Barry
Scientist
S.Z. Shapiro
Scientist
A. Bensaid
Visiting Scientist
L. Greenbaum
Visiting Scientist
L. Hendershot
Visiting Scientist
S. Mahan
Post-Doctoral Fellow
J. Naessens
Post-Doctoral Fellow
C. Sendashonga
Post-Doctoral Fellow
K.S. Logan
Research Associate
J. Magondu
Research Associate
J. Newson
Research Associate

PARASITOLOGY/ THEILERIOSIS

A.J. Musoke
Senior Scientist
A.D. Irvin
Senior Scientist
S.P. Morzaria
Scientist
D. Dobbelaere
Visiting Scientist
K. Fujisaki
Visiting Scientist
T. Fukuma
Visiting Scientist
K. Kocan
Visiting Scientist
C. Sugimoto
Visiting Scientist
P. Conrad
Post-Doctoral Fellow
R. Hall
Post-Doctoral Fellow
S.H. Minja
Research Associate
C.G. Nkonge
Research Associate
P.R. Spooner
Research Associate
L.N. Mburu
Laboratory Technician

PATHOLOGY

M. Murray
Senior Scientist
W.I. Morrison
Senior Scientist
R.W. Paling
Scientist
W.C. Davis
Visiting Scientist
D. Emery
Visiting Scientist
B. Goddeeris
Visiting Scientist
C. Llewellyn
Visiting Scientist



C. Munro
Visiting Scientist
A.J. Teale
Visiting Scientist
C. Baldwin
Post-Doctoral Fellow
J. Ellis
Post-Doctoral Fellow
G.M. Lamb
Research Associate
D.N. MacHugh
Research Associate
S. Kemp
Visiting Research Associate

ELECTRON MICROSCOPY

D.W. Fawcett
Senior Scientist
S. Ito
Senior Scientist
C. McCrae
Visiting Scientist
J. Venable
Visiting Scientist
P. Webster
Research Associate

TSETSE LABORATORY

S.K. Moloo
Scientist
S.B. Kutuza
Research Associate
S.G.A. Leak
Research Associate
I. Bakakimpa
Laboratory Technician

TICK LABORATORY

W.P. Voigt
Scientist
F. Mwakima
Research Associate

CENTRAL CORE UNIT

C.A. Hinson
Radiation Safety Officer

EXPERIMENTAL ANIMAL UNITS/ DIAGNOSTIC LABORATORY

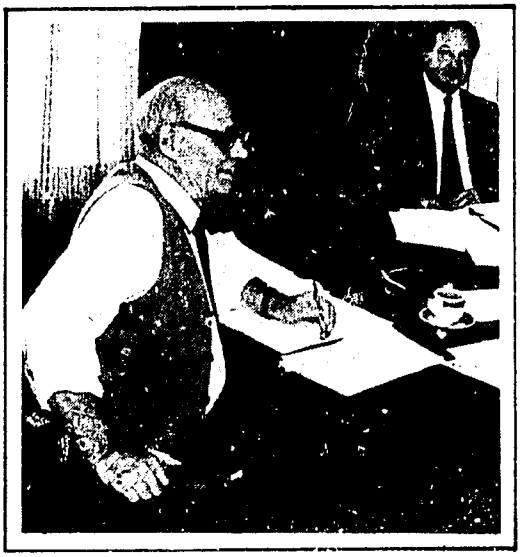
T. Jordt
Scientist
J. Katende
Research Associate
R.C. King
Research Associate
S.J. Kimani
Farm Manager/Kabete
L.J. Howard
Ranch Manager/Kapiti Plains

TRAINING AND INFORMATION SERVICES

J.K. Lenahan
Training and Outreach Officer
W. Umbima
Librarian
D. Elsworth
Graphic Arts Supervisor
S.B. Westley
Writer/Editor
V. Howe
Visiting Editor
J.R. Scott
Research Associate
G. Gettinby
Consultant

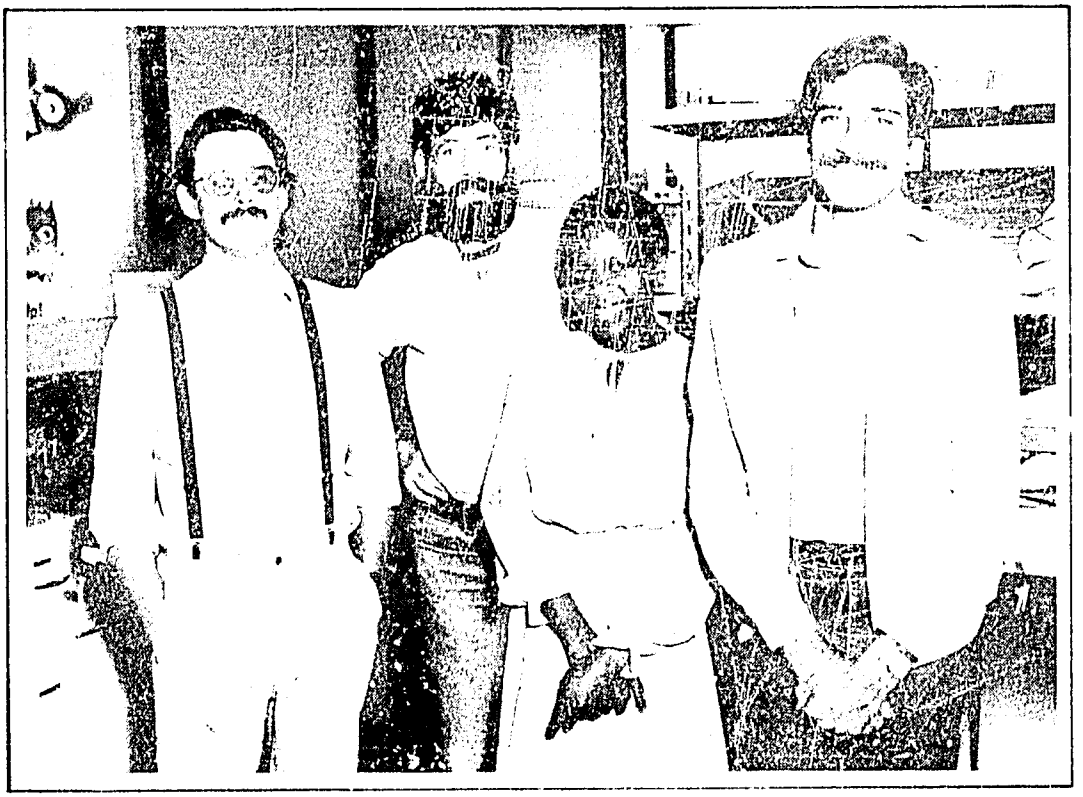
WILDLIFE DISEASE PROJECT

J.G. Grootenhuis
Scientist



N'DAMA TRYPANOTOLERANCE PROJECT

R.H. Dwinger
Scientist
P. Jeannin
Scientist
A S. Grieve
Research Associate



FINANCIAL STATEMENTS

Rattansi Educational Trust Building
Koinange Street
P.O. Box 41968
Nairobi, Kenya

Telephones: 20891, 20418, 21244
Telex: 22140 CHUNGA
Cables: PRICEWATER

Price Waterhouse

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

We have reviewed the abridged financial statements set out in Figures 31 to 34 which contain information extracted from the accounting records of ILRAD for the years ended 31 December 1985 and 1984.

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 1985 and 1984, on which we expressed an unqualified opinion.

Price Waterhouse

Certified Public Accountants

14 March 1986

Figure 31. Summary costs by program and activity (US$'000).

	1985	1984
Research		
Parasitology—Trypanosomiasis	432	315
Biochemistry	656	727
Cell Biology	466	541
Immunobiology	357	356
Parasitology—Theileriosis	516	527
Pathology	605	598
Tsetse Laboratory	297	279
Tick Laboratory	124	106
Electron Microscopy	148	150
Total Research	3601	3599
Research Support		
Office of Director of Research	416	562
Farm Animal Production	614	623
Laboratory Animal Production	148	133
Clinical and Diagnostic Services	72	86
Radioisotope and Central Core Services	341	303
Total Research Support	1591	1707
Training and Conferences	752	685
Library and Information Services	369	313
Administration		
Board of Directors	83	91
Office of the Director General	394	348
Finance	332	342
Personnel	74	85
Purchasing	302	275
Total Administration	1185	1141
General Operations		
Engineering	660	572
Transport	214	252
Services	228	146
Food and Housing	5	14
Stores	48	52
Total General Operations	1155	1036
External Program Review	125	—
Contingency	—	10
Total Core Costs	8778	8491

Figure 32. Summary of core operating funds from donors (US$'000).

	1985	1984
Unrestricted Funds		
United States Agency for International Development (USAID)	2490	2500
World Bank (IBRD)	1200	1570
Canadian International Development Agency (CIDA)	686	699
United Kingdom	546	468
Germany (Federal Republic)	528	556
Switzerland	415	437
Australia	281	208
Netherlands	249	264
Sweden	237	197
Norway	234	255
Japan	201	150
Italy	164	112
Belgium	135	229
France	61	59
Denmark	47	—
Saudi Arabia	—	300
Total Unrestricted Funds from Donors	7474	8004
Restricted Funds		
United Nations Development Program (UNDP)	690	639
Italy	500	500
Belgium	110	105
Total Restricted Funds from Donors	1300	1244
Total Unrestricted/Restricted Funds from Donors	8774	9248

Figure 33. Summary of sources and application of funds (US$'000).

	1985	1984
SOURCES		
Core Operating Funds		
Unrestricted		
Unrestricted Funds from Donors	7474	8004
Balance From Previous Year	—	—
Earned Income Applied in Year	289	144
Total Unrestricted Operating Funds	7763	8148
Total Restricted Operating Funds	1300	1244
Total Unrestricted/Restricted Operating Funds	9063	9392
Transfer to Capital Funds	(285)	(482)
Net Unrestricted/Restricted Operating Funds	8778	8910
Capital Funds		
Transferred from Core Operating Funds	285	482
Unexpended Balance from Previous Year	419	–
Balance of Working Funds from Previous Year	778	687
Balance of Revolving Fund from Previous Year	100	100
Total Capital Funds	1582	1269
Special Projects		
Wildlife	167	236
Trypanotolerance	137	—
Total Special Projects	304	236
TOTAL SOURCES	10664	10415
APPLICATIONS		
Core Operations	8778	8491
Capital	426	391
Special Projects	304	236
Unexpended Balance		
Unrestricted Core	278	419
Working Funds	778	778
Revolving Fund	100	100
TOTAL FUNDS	10664	10415

Figure 34. Balance sheet as at 31 December 1985.

	1985	1984
ASSETS		
Fixed Assets		
Land and Buildings	10125	9882
Research Equipment	5040	4875
Other Assets	1943	1871
Subsidiary Company		
Investment	1786	1728
Longterm Loan	—	58
Total Fixed Assets	18894	18414
Revolving Fund		
Loans	93	86
Cash	7	14
Total Revolving Fund	100	100
Net Current Assets	1056	1197
TOTAL ASSETS EMPLOYED	20050	19711
FUND BALANCES		
Capital Fund	18894	18414
Working Capital		
Unrestricted Core Surplus	778	778
Revolving Fund	278	419
TOTAL FUNDS	100	100
	20050	19711

ILRAD

Published in 1986
Compiled and designed by S.B. Westley
Cover photo by D. Elsworth
Photos by D. Elsworth, S. Ito, F. Shikhubari, P. Webster
Typeset by Waldman Graphics
Printed by English Press
Nairobi, Kenya