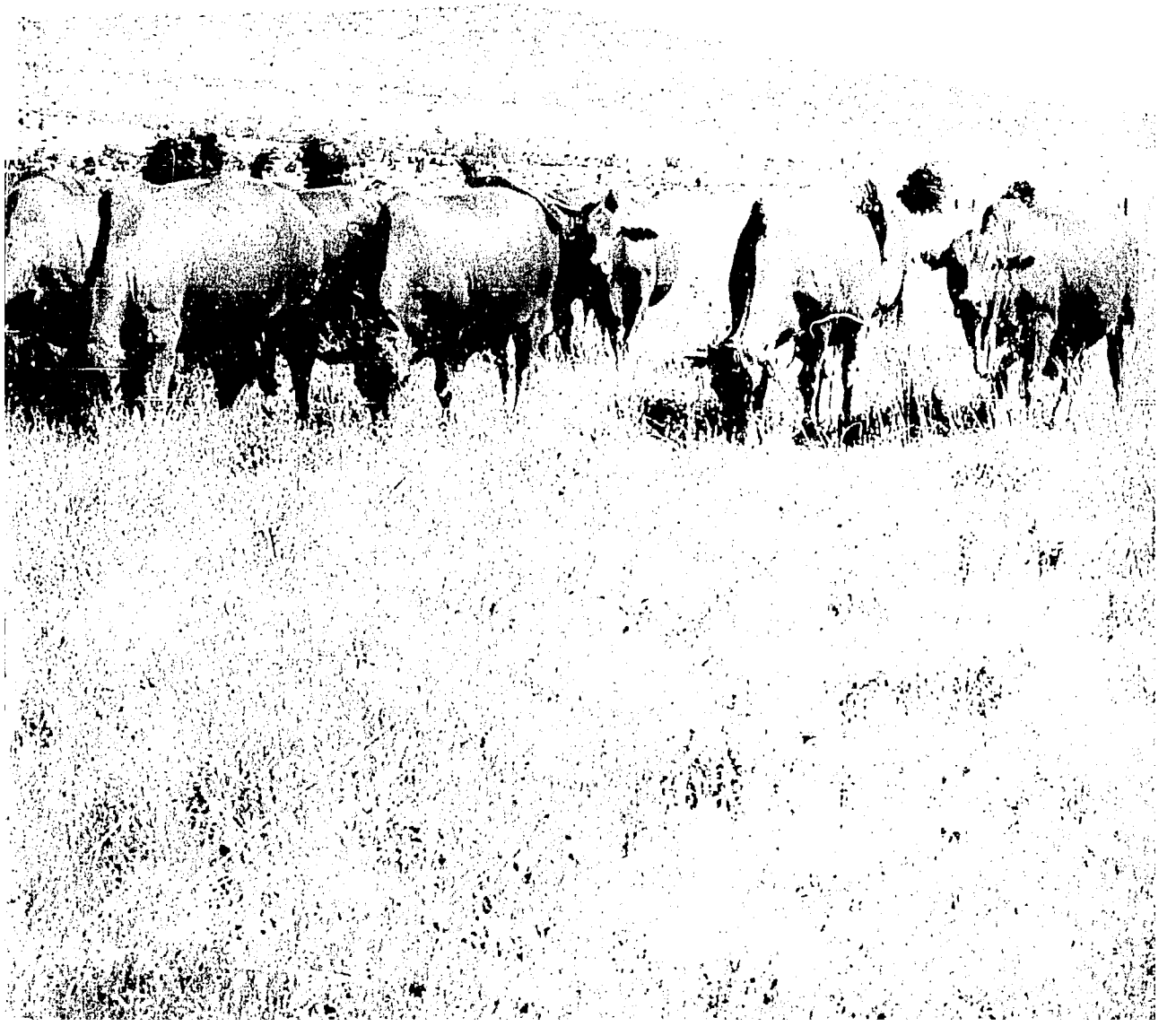
ILRAD 1984



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 1983, core funding was provided for ILRAD's research and training activities by the World Bank (International Bank for Reconstruction and Development—IBRD), the United Nations Development Program (UNDP) and the Governments of Australia, Belgium, Canada, the Federal Republic of Germany, France, Italy, Japan, Norway, Saudi Arabia, Sweden, Switzerland, the Netherlands, the United Kingdom and the United States of America. Special research projects, conferences and training activities were supported by the International Atomic Energy Agency (IAEA), the Australian Centre for International Agricultural Research (ACIAR), the Commonwealth Foundation, the Ford Foundation, the Government of the Netherlands and May and Baker Ltd.

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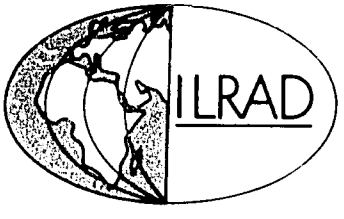
INTERNATIONAL LABORATORY
FOR RESEARCH ON ANIMAL DISEASES



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 programs focus on African animal trypanosomiasis and East Coast fever (ECF), a form of theileriosis. These two disease complexes have adversely affected animal production and human welfare in Africa and other tropical regions for many years, causing direct and indirect economic losses and human suffering over substantial areas.

The development of improved control measures for trypanosomiasis and ECF requires extensive and detailed research on the protozoan parasites which cause these diseases as well as on their arthropod vectors. After several years of preliminary investigation, ILRAD prepared projections for research on both target diseases in 1983, covering the next 10 years: 1984 was the first year of implementation of this long-term strategy.

With the recruitment of additional scientific and supporting staff to provide expertise in previously neglected areas, ILRAD now has in operation substantial research programs employing teams of specialists in different scientific fields. In addition, training and outreach programs have been developed in an effort to ensure that existing knowledge is used to the best advantage and that new research findings are disseminated and applied as quickly as possible. These outreach programs are also aimed at integrating ILRAD's activities with those of other international and national organizations with similar objectives and needs.

ILRAD's present organization and the activities and achievements of 1984 are chronicled in the body of this report. As in previous years, the annual report has been written to impart information about ILRAD's work to a wide audience, many of whom may lack detailed knowledge of the two target disease complexes. Specialists interested in greater detail will find references to technical publications in international journals.

It is not necessary to reiterate here the numerous developments and achievements made at ILRAD during the year, but it might be worth emphasizing a few particularly exciting areas of activity. Turning first to theileriosis, methods have been developed over a period of years for serotyping isolates of *Thieleria parva*, the parasite which causes ECF. These have paved the way for promising vaccination trials, based on infection with *Theileria* sporozoites and simultaneous treatment, in collaboration with the Kenya Governments's Ministry of Agriculture and Livestock Development.

This work is now being followed by research using recombinant DNA techniques which could provide the ultimate diagnostic tools for epidemiological investigation and surveillance. The reagents being developed will be able to tell us the species and strain of *Theileria* parasite affecting livestock in a given area. They may be used with material taken directly from an infected animal, obviating the present necessity for extended culture and transmission before serotyping can be performed.

After a number of years of basic immunological research, scientists at ILRAD have succeeded in establishing and maintaining lines of bovine T lymphocytes in culture. This capability will be invaluable in investigating the problems associated with immunization of cattle against ECF using schizont-infected lymphocyte cultures, including problems related to the inhibition of immunogenesis which appear to arise from involvement of the bovine histocompatibility antigens.

Turning to trypanosomiasis, there has been a shift of emphasis in the research program over the past 3 years away from *Trypanosoma brucei* towards *T congolense* and *T vivax*, the more important pathogens in cattle. This new emphasis is beginning to show results: *T congolense* and *T vivax* can now be grown in culture, and highly specific diagnostic tests are being developed to identify parasite species and serodemes using monoclonal antibodies and recombinant DNA techniques.

Major experiments are also in progress on the immunogenesis and pathogenesis of trypanosomiasis in cattle. During the past year, investigations in this area have provided reagents and techniques for identifying many different types of bovine cells which are involved in the immune response and have probed deeply into the mechanisms of pathogenesis during trypanosome infection. The association between ILRAD and the International Livestock Centre for Africa (ILCA) in the trypanotolerance research network has begun producing information on livestock productivity under tsetse challenge. The network is still expanding, and collaboration with the new International Trypanotolerance Centre in The Gambia will soon increase the flow of information on this important topic.

The research effort on both theileriosis and trypanosomiasis is still predominantly laboratory based. This is because real progress towards the development of improved control methods will depend on the results of basic research on the biology and properties of the parasites as well as the immune responses and other defence mechanisms of domestic livestock.

In making this research possible, the activities of the support units at ILRAD assume major importance. Substantial contributions have been made by the electron microscopy laboratory, the tick and tsetse laboratories and the laboratory animal breeding unit. In 1984, the farm associated with the laboratories at Kabete and the cattle production facility at Kapiti Plains Estate have played particularly valuable roles. In a year when Kenya was affected by severe drought, with an estimated 20% of the national cattle herd lost, ILRAD was able to obtain enough calves from its own breeding facilities to ensure the continuation of vital experimental work. Furthermore, the use of Boran heifers at Kapiti as surrogate mothers for embryos imported from The Gambia has provided trypanotolerant N'Dama calves for important research on genetic resistance to trypanosomiasis.

The section in this report on ILRAD's training and information programs indicates expanding activity in these areas. ILRAD plays a leading role in training scientists and technicians who can contribute to research on and the improved control of theileriosis, trypanosomiasis and other important livestock diseases. The results of ILRAD's research are widely distributed through contributions to scientific meetings, papers appearing in international journals, presentations at regular internal seminars, and through the quarterly news-

letter, *ILRAD Reports*, and the annual report, both of which are produced in English and French. ILRAD's newsletter won an award of excellence in 1984 from Agricultural Communicators in Education, a professional editors' association in the USA.

It is a pleasure to note with gratitude the financial contributions received from donor governments and organizations in 1984 to support ILRAD's core activities. During the year, ILRAD continued to receive funds from the World Bank (International Bank for Reconstruction and Development—IBRD), the United Nations Development Program (UNDP) and the Governments of Australia, Belgium, Canada, France, Germany (Federal Republic), Italy, the Netherlands, Norway, Sweden, Switzerland, Saudi Arabia, the United Kingdom and the United States of America. The government of Japan added its support during the year, and the government of Denmark pledged support for 1985. In addition to ILRAD's core programs, scientists have been provided through special funding from the governments of Belgium, France, Italy, Japan, the Netherlands and the United Kingdom.

During the year, ILRAD was honoured to receive visits by Their Excellencies the Ambassadors of the United States of America, the Federal Republic of Germany, Norway, Switzerland and Japan and the High Commissioners of the United Kingdom, Canada and Australia. 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.

Last year, I indicated the importance of collaborative research activities to ILRAD's program. ILRAD cannot alone carry out all the research required to improve control of theileriosis and trypanosomiasis, and links are maintained with a number of organizations, laboratories and field projects in Kenya, throughout Africa and around the world. These links range from research contracts with other laboratories to the exchange of material and personnel.

Special projects in progress during 1984 included research on trypanosomiasis and theileriosis infections in wildlife, in collaboration with the Kenya Government and partially funded by the Netherlands, the extension of the trypanotolerance research network with funding from the European Economic Community, research on trypanosomiasis in collaboration with the Swiss Tropical Institute involving Swiss financial support, and research on in vitro cultivation of trypanosomes with support from the Japanese International Cooperation Agency.

In 1984, ILRAD staff participated in four international meetings in collaboration with other centres in the network supported by the Consultative Group on International Agricultural Research (CGIAR). These were a meeting on Nutritional Aspects of International Agricultural Research, held at ILCA in Addis Ababa, a meeting on Farming Systems Research in Eastern and Southern Africa, held in Nairobi, a meeting on Biotechnology in International Agricultural Research, held at the International Rice Research Institute (IRRI) in Manila, and a meeting on the Role of Women in Agricultural Development, sponsored by the International Service for National Agricultural Research (ISNAR). Senior government officials from all the countries in Africa affected by ECF came to ILRAD in October to meet with research scientists to discuss current problems of ECF control and explore possibilities for the wider use of immunization by sporozoite infection and treatment. A review team commissioned by UNDP visited ILRAD in December to examine the trypanosomiasis program, and preparations began for ILRAD's second quinquennial external program review which will take place in 1985 and 1986.

We now look ahead towards the second external program review with the knowledge that we have sound research programs in place aimed at developing safe, effective and economic control measures for our two

target diseases. At the same time, work at ILRAD is beginning to have an impact on livestock production in African countries, particularly related to improved protection against theileriosis and the availability of highly specific diagnostic materials of major value in epidemiological surveillance and disease control. We are also being invited to expand our horizons to the large areas in Asia and America where different forms of trypanosomiasis and theileriosis are endemic.

Finally, I would like to repeat our warmest thanks to the Government and people of Kenya for their continuing generosity and support in serving as hosts to ILRAD. I commend this report to you with confidence that it accurately portrays the laboratory's progress during the past year.

Dr A.R. Gray
Director General
ILRAD

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THEILERIOSIS

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

The most important *Theileria* species are *T parva*, the causative agent of East Coast Fever (ECF) that affects cattle in a large part of East and Central Africa, and *T annulata*, the cause of tropical theileriosis or Mediterranean Coast fever in cattle in the Mediterranean littoral and the Middle East, extending eastward to India. 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 Kenya, Uganda, Tanzania, Rwanda, Burundi, Zaire, Malawi, Zambia, Mozambique and southern Sudan. 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 the drug industry have resulted in a number of effective therapeutic compounds for the treatment of ECF, but prevention will always remain a more desirable option 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. This is in contrast to many protozoal infections, where immunity may be short-lived unless constantly boosted by challenge. Different strains of *T parva* exist and immunization of cattle with one strain will not necessarily protect them against challenge with an unrelated strain. However, the number of strains found in cattle appears to be small and the problems associated with extensive antigenic diversity, as seen in other protozoal diseases, appear to be less acute. Thus, there are good prospects for 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 it is difficult to control the quality and quantity of parasites used.

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 constrains 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 undergoes a complex lifecycle in cattle and in ticks. The brown ear ticks, *Rhipicephalus appendiculatus*, which transmit ECF feed on cattle three times during their lives—as larvae, as nymphs and as adults. Transmission of ECF occurs 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 themselves to lymphocytes, white blood cells of the immune system. Some sporozoites enter lymphocytes and develop into forms called schizonts. The infected lymphocytes are transformed by the parasites into 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 in the cell lining of the gut into kinetes. The motile kinetes move through the gut wall into the body cavity and migrate to the salivary gland where they enter one cell type, 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.

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[illegible]

In *Theileria* infection, the sporozoite binds to the surface of the host lymphocyte, invades the host cell, differentiates to a schizont, multiplies and induces the host cell to multiply. Studies carried out in 1984 concentrated on the types of host cells which are targets for sporozoite binding and invasion and the nature of the parasite-specific receptor molecule(s) on host cells which binds with a sporozoite ligand during parasite entry. To ascertain the range of cell types binding *Theileria* sporozoites, cells harvested from the peripheral blood (peripheral blood leucocytes) and cells from different organs of cattle were incubated with sporozoites harvested from the salivary glands of infected ticks. The proportions of cells binding sporozoites in each preparation are shown in Figure 1. These results indicate that sporozoites bind to some B and T lymphocytes and some monocytes/macrophages, but not to other cell types.

Electron microscopic studies in past years have concentrated on the formation of *Theileria* sporozoites in the salivary gland of the tick and on their attachment and entry into bovine lymphocytes. One of these studies showed that *T parva* sporozoites can enter bovine lymphocytes at temperatures as low as 2°C. At this temperature, cell metabolism is greatly reduced, which indicates that the sporozoite entry process requires little or no energy, either from the host cell or parasite. Electron microscopy studies in 1984 showed that sporozoites can also enter monocytes/macrophages in vitro at 2 to 4°C. Successful entry appears to depend upon the site of initial parasite attachment. If the sporozoites first lodge between projections on the macrophage surface, binding of these processes occupies all of their ligands and they progress no further. However, if their initial contact is with a site free of surface processes, the zippering up of ligands to receptors progresses laterally and draws them into a deepening recess in the cell surface. When binding progresses around the entire circumference of the parasite, the host membrane fuses over it to complete the entry process (Figure 2).

Passive entry of a parasite into a host cell seemed so unlikely that further confirmatory evidence was needed. It has now been found that sporozoites can enter lymphocytes in the presence of cytochalasin B, a drug known to inhibit active movement in cells. Similar results were obtained when the cells were exposed to high concentrations of magnesium ions, a treatment known to paralyze cell motility. Although sporozoite entry is more rapid and more efficient in vivo, these experiments demonstrate that entry does not actually require motility by parasite or host cell once the initial contact has been made.

Cell Source or Type	Number of Experiments	Total Cells Counted	% Total Cells Binding Parasites (mean ± SD)
Peripheral blood leucocytes (PBL)	4	400	30 ± 3.5
Thymus	4	400	53 ± 3.0
Lymph node	2	200	30 ± 2.0
Alveolar macrophages	5	500	6 ± 1.5
Peripheral blood monocytes	3	300	5 ± 1.5
Spleen	2	200	29 ± 1.5
Concanavalin A blasts	3	300	51 ± 7.0
BL20 leukaemia cells	2	200	19 ± 1.5
Bone marrow	3	300	4 ± 0.5
BL3 leukaemia cells	3	300	2 ± 0.5
Neutrophils	2	200	0

Figure 1. Binding of *T p parva* Muguga sporozoites by cells from bovine peripheral blood and various organs. For each experiment, 4,000 infected tick acini were incubated with a preparation of 10^6 viable cells for 2 hours at 37°C in continuous rotation.

The fact that infections with low doses of parasites are often self-curing, while infections with high doses are lethal, suggests that resistance to theileriosis might correlate with the ability of the host to limit initial parasite binding. However, comparative studies in vitro indicate that there is no difference between the number of target cells among buffalo *(Syncerus caffer)* or bovine peripheral blood leucocytes which bind *T p parva* (Muguga) sporozoites, though buffalo are much more resistant to *Theileria* infection than cattle. Preliminary studies also suggest that sporozoites of *T p lawrencei*, the *Theileria* subspecies which normally infects buffalo, invade the same cell types in buffalo and in cattle. In addition, no differences were observed in the number of bovine peripheral blood leucocytes binding sporozoites of three different *Theileria* strains, either incubated together or singly, indicating that different *Theileria* strains bind to the same receptors on the same cell types. These findings suggest that host susceptibility is unlikely to be related to the number or type of cells which are targets for parasite binding.

Several experiments were carried out to determine the nature of the *Theileria*-specific receptor molecule(s) on the surface of bovine cells. A number of monoclonal antibodies were raised which recognize different antigens on the surface of bovine leucocytes. Monoclonal antibodies are products of hybrid cells created by a fusion of mouse spleen cells, which produce antibodies, and tumour cells, which are capable of growing and multiplying in culture. These were tested for their ability to block sporozoite binding, but none had any effect. However, polyspecific antisera raised in rabbits against bovine peripheral blood leucocytes blocked parasite attachment, as did preincubation of target cells with the enzymes proteinase K, α-glucosidase or acetylglucosaminidase. The cell-surface molecules which are altered by these treatments are now being analysed, with the aim of identifying the molecules which serve as receptors for parasite invasion.

Figure 2. *T parva* sporozoite which has entered a bovine monocyte in vitro at 4°C.

Blood samples collected from cattle in ECF endemic areas and screened at ILRAD have shown antibodies against both *T p parva* sporozoites and schizonts, using neutralization of infectivity and the indirect fluorescent antibody (IFA) test. Some of these sera contain antibodies which are capable of neutralizing *Theileria* sporozoites, thus possibly conferring protection against infection.

Evidence has accrued that sporozoites of several *T p parva* isolates possess a common antigen which is capable of stimulating protective antibodies in cattle and rabbits: a vaccine based on this common antigen might protect cattle against ECF over a wide geographic area. Considerable effort has been devoted to isolating and purifying the protein(s) bearing this antigenic determinant as possible starting material for a vaccine. Antibodies from infected cattle and from rabbits immunized with isolated *T p parva* sporozoites were found to recognize three specific sporozoite proteins.

At the same time, monoclonal antibodies were raised against *T p parva* sporozoites which block sporozoite entry into bovine lymphocytes in vitro. Immunoelectron microscopy has shown that the antigen recognized by one of these monoclonal antibodies is located on the surface of the sporozoite. This antigen appears to be one of the three proteins recognized by antibodies from infected cattle and rabbits. An investigation of its synthesis in the salivary gland of infected ticks showed that it is associated with parasite endoplasmic reticulum, micronemes and the surface of sporoblasts. Mature sporozoites are completely covered by this antigen (Figure 3), but as they enter bovine lymphocytes the antigen is shed (Figure 4).

The cattle and rabbit antibodies which recognize three sporozoite proteins appear to be capable of neutralizing the infectivity of sporozoites from a wide range of different *T p parva* stocks. These three proteins have now been isolated in pure form. Total messenger RNA, including the transcripts for the proteins, has been used as a template to synthesize cDNA, and the resulting mRNA/cDNA hybrids have been inserted into an expression vector which has been amplified in the bacterium *Escherichia coli*. Libraries of random fragments of *Theileria* genomic DNA have also been constructed. The resulting clones are being screened with polyspecific rabbit serum and sporozoite-specific monoclonal antibodies to detect clones expressing any of the three sporozoite proteins. Such clones will be isolated and cultivated in sufficient quantities to carry out immunization tests in cattle.

THE SCHIZONT-INFECTED CELL

While considerable research concentrates on the identification and purification of a sporozoite antigen(s) which may provide the basis for an ECF vaccine, it is impossible to know yet for certain whether a sporozoite antigen vaccine will protect cattle effectively against infection. Thus, research also concentrates on the schizont stage of parasite development, with the goal of identifying an antigen(s) or other factor which could lead to a vaccine directed against this form of the parasite. Work in 1984 concentrated on the changes induced in host cells invaded by *Theileria* parasites and on host responses, including lymphocyte proliferation and cell-mediated killing of parasitized cells. These investigations have involved considerable research on the structure and function of the bovine immune system.

HOST CELL POPULATIONS

A great deal of information has been obtained over the past 9 years on different cell types which comprise the bovine immune system and how cells interact during the generation of an immune response. Progress in this area has accelerated rapidly following the development of techniques to produce specific monoclonal antibodies which recognize antigens on the surface of different populations of cells. The development of highly sensitive immunofluorescence and immunohistochemical

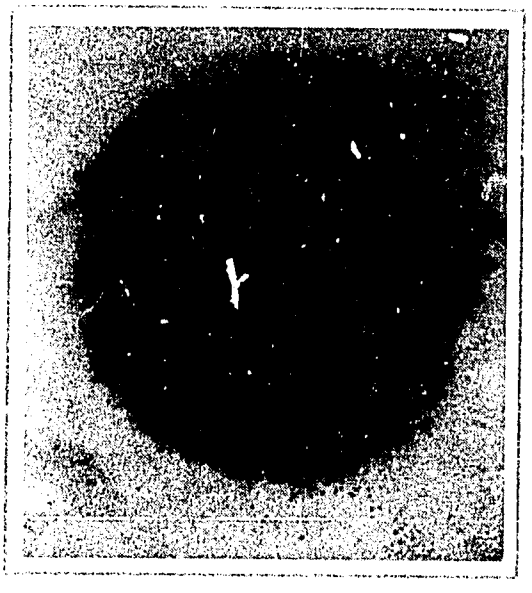

Figure 3. Electron micrograph of an ultrathin cryosection through a *T parva* sporozoite labeled with a monoclonal antibody directed against the sporozoite surface coat plus protein A/colloidal gold (5 nm particle size). The label is uniformly distributed over the entire surface of the parasite.

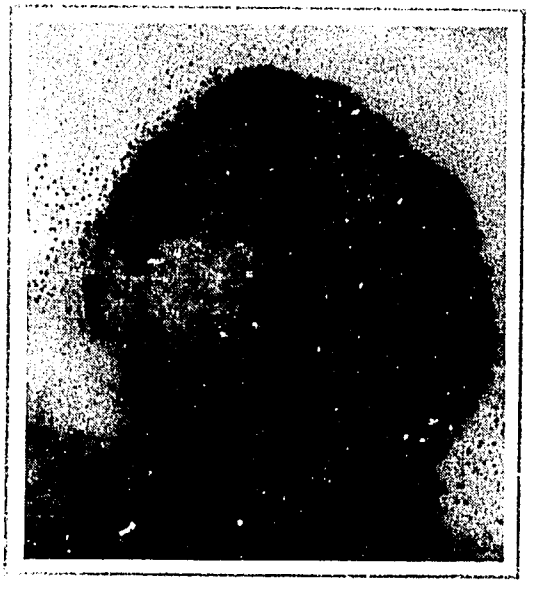

Figure 4. Electron micrograph of a section, obtained by cryo-ultramicrotomy, through a *T parva* sporozoite in the process of entering a bovine lymphocyte. The section was labeled with a monoclonal antibody directed against the sporozoite surface coat plus electron-dense protein A/colloidal gold (5nm particle size). The sporozoite surface is labeled except where it is in close contact with the lymphocyte membrane.

techniques for the detection of cells in tissues has made it possible to use these monoclonal antibodies—together with more traditional histological, ultrastructural and enzyme-histochemical techniques—to identify different types of bovine cells, cells at different stages of maturity and functionally important molecules on cell surfaces.

Monoclonal antibodies have been produced at ILRAD which recognize bovine T lymphocytes, B lymphocytes, and monocytes/macrophages and granulocytes. These have been used to produce purified cell populations containing over 95% of the desired cell type. An early finding was that populations of T and B lymphocytes can be infected with *Theileria* sporozoites in vitro and transformed into proliferating lymphoblasts. Although sporozoites can bind and enter monocytes/macrophages, they cannot establish a viable infection in this cell type. The monoclonal antibodies are now being used to investigate the role of monocytes/macrophages in the generation of cell-mediated immune responses.

Results of studies carried out over the past 2 years indicate that immune responses against *Theileria* schizont infections are directed against the class I major histocompatibility (MHC) antigens of the host cells along with surface antigenic changes induced by the parasite. The MHC antigens are cell surface molecules which show clear differences between individuals of a species. In addition to being the major antigens against which graft rejection responses are directed, they are important in the communication between different cell types during the generation of an immune response. There are two main classes of MHC antigen: class I MHC antigens are expressed on most cell types, whereas class II MHC antigens are expressed predominantly on cells of the immune system. In 1983, as part of a cooperative project supported by the British Government's Overseas Development Administration (ODA) through the Agriculture and Food Research Council's Animal Breeding Research Organization in Edinburgh, ILRAD gained the capacity to type bovine cells in terms of class I MHC antigens using alloantisera which have been evaluated in a series of international workshops. These antisera identify specificities on class I antigens coded by one genetic locus—the A locus.

In 1984, over 350 cattle in the ILRAD breeding herd were typed for class I MHC determinants. The objective was to establish the frequencies of the various international specificities among the Boran (*Bos indicus*) animals in the ILRAD herd, to select suitable animals for experimentation, and, to a limited extent, to select breeding groups with a view to producing offspring by embryo transfer from animals of particular MHC types. Among the animals typed, about 45% of the alleles at the A locus of the class I MHC were represented by the international specificities. Eight additional alloantisera have been produced against other alleles represented in the ILRAD herd.

Four monoclonal antibodies have been derived which appear to recognize determinants on bovine class I MHC antigens. These antibodies react with the majority of peripheral blood and lymph node cells and a minor population (less than 15%) of thymocytes. Some recognize A locus determinants, whereas others probably recognize products of other MHC loci. By sequential precipitation using these and other antibodies, it should be possible in 1985 to identify the products of a second and possibly a third class I locus.

The monoclonal antibodies produced at ILRAD which recognize bovine cell populations are listed in Figure 5, although some of the specificities given are still tentative. This work is now being extended, with emphasis in 1985 on identifying monoclonal antibodies which recognize functionally important T-cell subpopulations.

Efforts are also being devoted to producing monoclonal antibodies using bovine, rather than murine, antibody-producing cells. Success in this area should extend considerably the usefulness of monoclonal anti-

Monoclonal Antibody	Specificity
B5/4, B4/27	B lymphocytes
P5	T lymphocytes & monocytes
K1	most, if not all, T lymphocytes
T1/D8	T-lymphocyte subpopulation
BT3/8	T-lymphocyte subpopulation, monocytes, granulocytes
J3	T-lymphocyte subpopulation, monocytes
J5, J6	T lymphoblasts
P13	some T lymphoblasts, intracellular fibrillar material
P8	monocytes/macrophages & granulocytes
P12, B4/18, P3, M4, M5, M7, T1/C6	class I MHC
R1, P2	class II MHC

Figure 5. Reactivities of monoclonal antibodies produced at ILRAD with bovine cell populations.

body technology for research on bovine immunology. It could also make it possible to obtain an antibody to the antigenic determinants on the surface of *Theileria*-infected cells responsible for inducing protective, cell-mediated immune responses. The approach followed at ILRAD is based on a fusion technique developed for human cells at the University of Alabama (USA). Bovine peripheral blood cells were stimulated with pokeweed mitogen (PWM) and B lymphoblasts were isolated and stimulated to begin making antibodies. Work now concentrates on fusing these bovine lymphoblasts with a mouse tumour cell line. Clones from the fused cell line will be used as fusion partners for further bovine B lymphoblasts in order to create hybrid (murine x bovine) x bovine cells which are likely to retain the ability to produce bovine monoclonal antibodies in culture.

GROWTH AND TRANSFORMATION OF INFECTED CELLS

Studies were initiated in 1984 to compare the establishment and development of in vitro infection with *T p parva* (Muguga) in peripheral blood cells taken from cattle and buffalo. The objective was to analyse how buffalo resist or control *Theileria* infection, in order to identify possible approaches to increasing resistance in cattle. Although sporozoites were shown to bind readily to cells taken from buffalo, the rate of parasite development was much slower in buffalo cells than in cattle cells. Seven days after the parasites were introduced into the cell cultures, 7% of the buffalo cells contained *Theileria* schizonts, compared with 31% of the cattle cells. Fourteen days after the parasites were introduced, nearly 100% of the cattle cells contained schizonts compared with less than 1% of the buffalo cells. The buffalo cells tended to contain more schizont nuclei than the cattle cells and many contained what appeared to be degenerating schizonts.

Further experiments are in progress to determine whether the control of infection in buffalo cells is an intrinsic property of the infected lymphocytes or is related to interaction with other cells in the culture. It is still early to draw conclusions, but it is reasonable to postulate that the control of *T p parva* infection in buffalo is not related to the ability of sporozoites to invade host cells and develop into schizonts, but rather to the inability of the parasite to stimulate the rapid proliferation of the host cell. Studies are being extended to other strains of *T p parva* and to *T p lawrencei*, the subspecies which infects buffalo in the field.

CELL PROLIFERATION

Immune responses to *Theileria* infection involve the proliferation of T lymphocytes and eventually the generation of T cells which kill infected cells. The stimulation of T-lymphocyte proliferation and the generation of cytolytic (cell-killing) T lymphocytes during an immune response are believed to involve several steps in which both major subpopulations of T lymphocytes—T helper cells and T cytolytic cells—participate. When T helper cells are stimulated with a specific antigen, they proliferate and release the soluble mediator interleukin 2 (IL-2). This step may also depend on the release of the mediator IL-1 from monocytes macrophages. IL-2 binds to precursors of cytolytic T cells bearing specific IL-2 receptors and promotes their proliferation.

Studies were conducted in 1984 on T-cell proliferation stimulated by *Theileria*-infected cells, focusing on the role of monocytes/macrophages as accessory cells. It was found that monocytes/macrophages are required to stimulate T-cell proliferation in mixed leucocyte reactions of uninfected cells from the same animal (autologous) or from two different animals of the same species (allogeneic). However, proliferation stimulated by viable *Theileria*-infected cells does not require the presence of monocytes/macrophages. Yet when infected cells are rendered metabolically inactive by fixation with 0.05% glutaraldehyde, they no longer stimulate the proliferation of mixed cell populations unless monocytes/macrophages are present. This suggests that viable parasitized cells may produce a growth factor, not produced by fixed cells, which replaces the requirement for monocytes/macrophages in stimulating cell proliferation.

When bovine peripheral blood leukocytes are stimulated in vitro by the mitogen concanavalin A (con A), they develop into lymphoblasts (Figure 6) and produce T-cell growth factor (TCGF), designated as IL-2. This factor has been purified by gel filtration, isoelectric focusing and high-pressure liquid chromatography (HPLC) to a protein of 20,000 to 23,000 daltons which retains IL-2 activity. By repeated stimulation of con A-induced lymphoblasts with TCGF it has been possible to obtain bovine T cell lines and clones. The clones do not respond to con A and are dependent on the presence of TCGF for continued growth. However, when these TCGF-dependent cells are infected with *T p parva* (Muguga), they no longer require TCGF to maintain proliferation, and supernatants from the cultures contain a low level of TCGF-like activity. This raises the possibility that parasitized T cells may produce and utilize growth factors in an autocrine fashion to maintain proliferation. Research is now in progress to characterize the factor secreted by *Theileria*-infected cells and determine whether it is IL-2 or some other growth factor. In addition, production and assay systems are being developed aimed at purifying IL-1 and investigating its role in the stimulation of cell proliferation.

CYTOLYTIC CELLS

Immune reactions to the schizont stage of *T p parva* appear to involve the generation of cytolytic T lymphocytes which selectively destroy infected cells, but not uninfected cells, from the same animal. Cells from most other animals are not destroyed, whether infected or not. By contrast, in fatal cases of ECF, cytolytic cells appear in the peripheral blood which are capable of killing both infected and uninfected lymphocytes from different animals. Studies over the past 2 years have focused on defining the specificity of cytolytic T cells from immune cattle and in particular on the role of class I MHC antigens.

Cytolytic cells are generated in a mixed culture combining leucocytes from two different animals. When such an allogeneic

mixed leucocyte reaction combines cells from two cattle which are mismatched at the A locus of the MHC, the cytolytic cells are directed principally against A locus determinants. However, cytolytic cells can also be generated between cattle which are matched at the A locus, suggesting that other loci of the MHC can be involved in the recognition events which lead to a specific cytolytic response. Current efforts are directed towards cloning cytolytic cells which recognize MHC antigens in order to

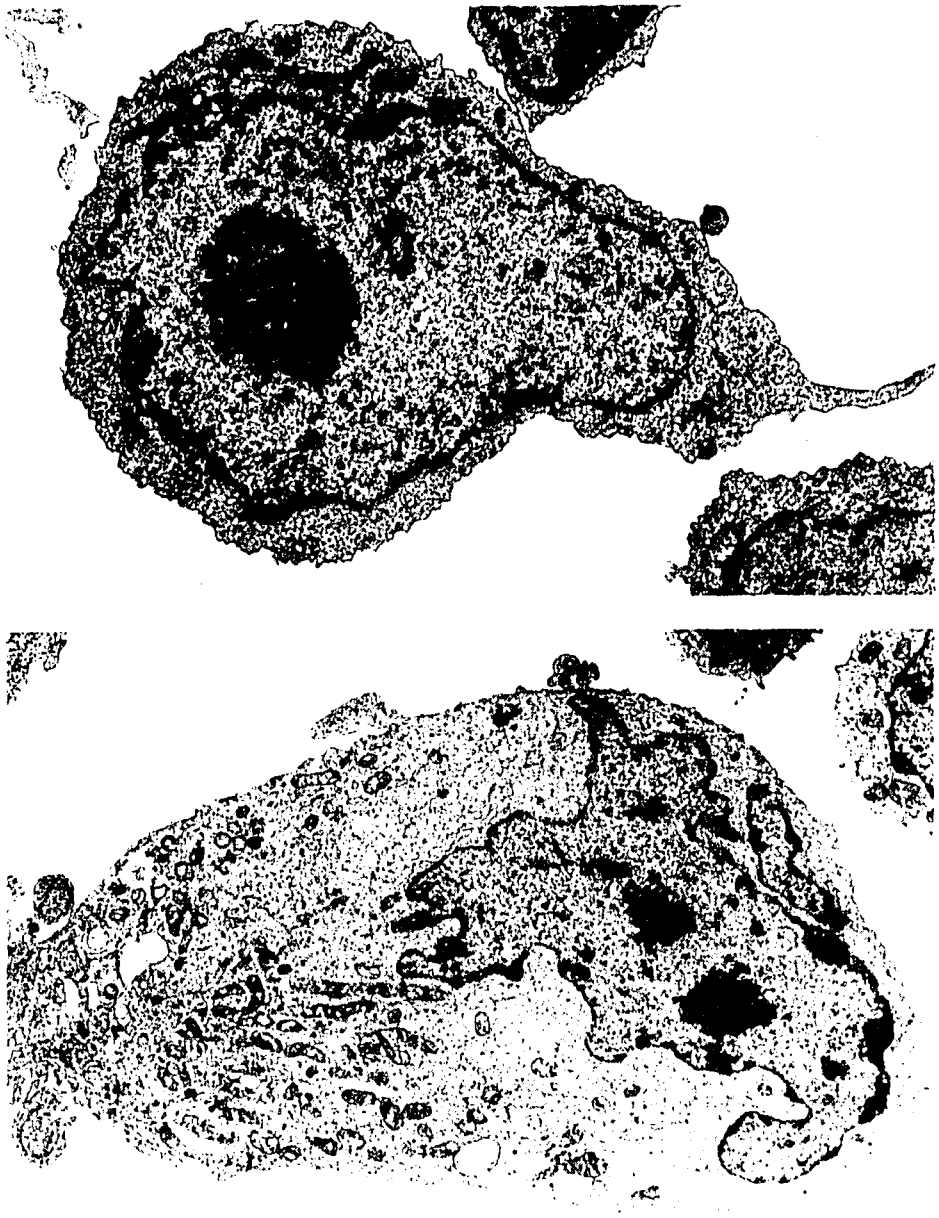

Figure 6 The electron micrograph at the top shows lymphoblast cells produced by stimulating bovine peripheral blood leucocytes in vitro with the mitogen concanavalin A (Con A). Below is an electron micrograph of bovine lymphoblasts stimulated by *Theileria* infection

obtain more precise information on their target specificity. Several cloned cell lines have been derived which either kill or proliferate in response to the specific allogeneic stimulator cell.

Data obtained from cattle undergoing immunization against *T parva* indicate that the *Theileria*-specific cytolytic T cells are also restricted principally by determinants of the A locus of the class I MHC. Thus, when tested on a panel of *Theileria*-infected target cells, the cytolytic cells were found to kill cells from animals matched or semi-matched with respect to A locus specificities. The possibility that these cytolytic cells merely recognize antigens expressed on normal lymphoblasts maintained in culture for long periods was excluded by the finding that the effectors did not kill uninfected autologous T-cell lines maintained in vitro with TCGF. Furthermore, the absence of cytolytic activity in supernatants of the cytolytic cells or cytolytic-cells-plus-targets excluded the possibility that killing was mediated by a soluble factor.

Further definition of the specificity of anti-*Theileria* cytolytic cells has been hampered by the difficulty of generating these cells in vitro. Current work is focused on this area, the eventual aim being to obtain cloned populations of *Theileria*-specific cytolytic cells. Such cloned cell lines would be of potential value in distinguishing between immunologically differing parasite strains and identifying immunologically important parasite gene products. Cloned T cells have been infected with different strains of *T parva* in vitro, and these are being used as targets for cytolytic T cells to determine the specificity for different parasite strains of the cytolytic response. Other experiments have involved the repeated stimulation of peripheral blood leucocytes from immune cattle with autologous *Theileria*-infected cells. Preliminary results have been promising.

Work has been in progress for some time aimed at producing monoclonal antibodies specific for antigens on the surface of *Theileria*-infected cells which may act as targets for the specific cytolytic response. Several possibilities are being investigated. For instance, the *Theileria* schizont might induce some chemical alteration of host-cell MHC antigens, which allows these antigens to be recognized as targets by cytolytic cells. Class I MHC antigens from *T p parva*-infected and uninfected cells were analyzed biochemically in 1984, but no modifications were detectable in the MHC antigens expressed by the infected cells. It is still possible that parasite-induced molecules on the surface of infected cells cause a conformational change in the MHC antigens by close association which cannot be detected by biochemical analysis. Molecules closely associated with MHC antigens may be detectable after chemical crosslinking, and this is one focus of present research.

One approach to identifying the *Theileria* or *Theileria*-induced antigens recognized by cytolytic T cells involves cloning *T p parva* DNA in the Lambda virus which proliferates in the bacterium *E coli* and expresses the cloned DNA sequences as protein. *T p parva* (Mariakani) piroplasms harvested from the red blood cells of infected cattle have been used as a source of parasite DNA. The *T p parva* DNA is broken into fragments and inserted into a restriction site within the viral genome. Several libraries of cloned piroplasm DNA have been constructed. These are now being screened with monoclonal antibodies to identify clones producing parasite antigens. Eventually, these clones can be used in transfection and cytotoxicity assays to determine which gene products are capable of eliciting a cytolytic T-cell response. Clones produced from different *Theileria* isolates can also be compared to identify strain-specific gene sequences. This may provide a valuable tool for ECF epidemiology studies.

DEVELOPMENT OF [illegible] AND THEIR [illegible]

Investigations continue on the the development of *T parva* merozoites—how they escape from infected lymphoid cells and invade red blood cells and their subsequent development before ingestion by feeding ticks. *Theileria* piroplasms, probably *Theileria velifera*, have been studied by electron microscopy in red blood cells taken from an infected waterbuck. A parasite organelle not previously described was observed in the piroplasms in association with the haemoglobin-containing food vacuole (Figure 7). An organelle of similar form but smaller size was observed in piroplasms of *Theileria parva* in red blood cells of cattle. Many of the *Theileria*-infected red blood cells from waterbuck also contained crystals of haemoglobin, though these were less common in infected cattle cells. Crystallization of haemoglobin is probably induced by the intracellular accumulation of parasite metabolites.

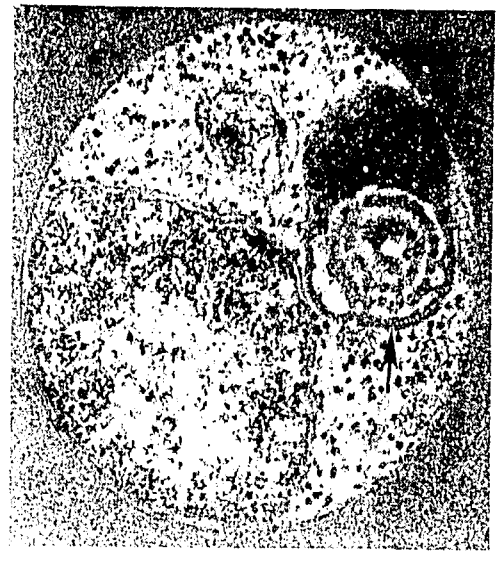

Figure 7. Electron micrograph of a *Theileria* piroplasm in the red blood cell of an infected waterbuck. A highly regular three dimensional network of anastomosing tubules is indicated by an arrow, continuous with the lumen of the food vacuole. This organelle is believed to be involved in degradation of the ingested haemoglobin.

ECF epidemiological studies at ILRAD concentrate on the characterization of parasite strains and the identification of cross-protective patterns between different *T parva* stocks. Emphasis in 1984 was on characterizing *T p lawrencei* stocks isolated from buffalo or from cattle in buffalo-infested areas.

ILRAD scientists are also 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.

Previous studies have suggested that antigenic diversity among *Theileria* stocks may be limited in situations where *T p parva* is maintained in a simple cattle-tick cycle. The results of characterizations made in 1983 and 1984 indicated that *T p parva* stocks isolated from Kenya, Malawi, Rwanda, Uganda, Zambia and Zimbabwe could be placed into four distinct groups—labeled A,

B, C and D—based on patterns of schizont antigen recognition by a battery of 16 monoclonal antibodies using the IFA test.

Up until now it has not been possible to carry out totally satisfactory cross-immunity trials in cattle because no in vitro assay has been available to quantify *T p parva* sporozoites reliably in order to standardize the doses of parasite material. A more accurate technique is being developed to determine the number of sporozoites present in material taken from infected ticks, using a monoclonal antibody (D_1) which recognizes a common antigen on sporozoites of different *Theileria* strains. The parasite material is incubated with antibody which has been labeled with a fluorescent stain (fluorescein isothiocyanate) and then analyzed using ILRAD's fluorescence-activated cell sorter (FACS). This method distinguishes sporozoites from tick material and yields information on the number of sporozoite particles, intact sporozoites and sporoblasts present in a sample.

Past studies of antigenic diversity in *T p lawrencei* parasites carried by buffalo have suggested that this *Theileria* species may change antigenically over time. In collaboration with the Kenya Agricultural Research Institute, a buffalo was infected with a tick stabilate of *T p lawrencei* at the end of 1983, and parasites have been collected at regular intervals and tested against ILRAD's battery of monoclonal antibodies. The results suggest that changes do occur in parasite antigenicity, and cross-immunity trials are being carried out in cattle to test the significance of the changes observed.

Four trials have been been conducted in Kilifi District of Kenya's Coast Province to determine the effectiveness of immunizing cattle against ECF by infection with well characterized local stocks and simultaneous treatment with oxytetracycline. These were at Mtwapa, Kiswani and two at Kibarani. Results can be summarized as follows:

1. Local *Theileria* stocks characterized by screening with monoclonal antibodies can be used in an infection and treatment regime to protect cattle against virulent *T p parva* challenge in areas where there is no contact with buffalo. In areas of heavy challenge, dipping with acaricides even twice a week is not sufficient to prevent ECF in non-immunized cattle.
2. Different breeds of cattle can be protected effectively, including Jersey, Boran and Sahiwal crosses.
3. Immunization 12 months prior to exposure provides solid protection against field challenge.
4. More prolonged treatment with oxytetracycline is occasionally required to reduce severe reactions on immunization.
5. Immunization against ECF must be accompanied by appropriate measures to control other tick-borne diseases. During the challenge periods, cattle were exposed without dipping for up to 3 months. No ECF breakthroughs occurred out of 52 immunized animals, but 3 cattle died of heartwater, a tick-transmitted rickettsial disease caused by *Cowdria ruminantium*. Cases of anaplasmosis and other tick-borne infections were also recorded. This suggests that dipping should not be discontinued altogether, but dipping intervals can safely be extended once animals have been immunized against ECF, resulting in considerable saving in costs.

Cattle immunized in the second Kibarani trial have now been integrated into smallholder farming schemes and their performance is being monitored. Veterinary authorities in several countries affected by ECF have shown interest in immunization by infection and treatment, and a workshop was held at ILRAD in October 1984 to discuss this approach and consider its extension to other affected areas.

Scientists also conducted in vitro experiments in 1984 to investigate how tetracycline works in conferring immunity against ECF. When established cultures of infected lymphocytes were treated with tetracycline, their growth rates were significantly reduced. When tetracycline and *Theileria* sporozoites were added to cultures of uninfected lymphocytes, the effect was even more pronounced, with certain concentrations of tetracycline causing complete inhibition of lymphocyte transformation. Recent work suggests that the effects of the drug may be due to inhibition of protein synthesis, and studies are in progress to investigate this further.

Experiments conducted in 1984 tested whether immunity by infection and treatment can be broken down by massive parasite challenge. In the first experiment, two cattle which had been immunized against *T p parva* (Muguga) were challenged with a dose of 20,000 acini from ticks infected with the Muguga strain, the equivalent of approximately 20 million sporozoites, while a non-immunized control animal was challenged with only 2000 infected acini. Both of the immunized cattle survived the massive challenge, while the control died of acute ECF. In the second experiment, 30 cattle were immunized with *T p parva* (Muguga) or *T p parva* (Muguga/Marikebuni) and then challenged with 140, 1400 or 14,000 acini prepared from ticks infected with *T p parva* (Muguga). The 20 immunized cattle receiving the smaller doses survived. In the group which was challenged with a dose of 14,000 infected acini, 4 out of 10 had to be treated for allergic reactions to tick components in the inoculum, but only 1 became infected and died of ECF. All of the control animals died of acute ECF. These two experiments support the hypothesis that when cattle are immunized by infection and treatment and then challenged with sporozoites from different monoclonal antibody profile groups, the differences in levels of protection demonstrated are due to the presence of different antigenic types, and not merely to variations in doses of infective sporozoites.

Trials have also been conducted to determine how soon immunity develops in cattle after immunization by infection and treatment and whether the protection conferred shortly after immunization is specific for particular disease strains. Four groups of 12 cattle were immunized with *T p parva* (Muguga) and challenged with *T p parva* (Muguga) or *T p parva* (Marikebuni) at different intervals after immunization, along with non-immunized controls. Results are shown in Figure 8. This experiment demonstrates that protective immunity to a specific strain of ECF is fully developed by 20 days after immunization. These findings were later utilized in a field trial at Kibarani, where 12 cattle were exposed to field challenge 3 weeks after immunization and all survived.

In many areas where ECF is endemic, cattle are also exposed to trypanosome infection. In a collaborative project with the International Atomic Energy Agency, experiments were conducted in 1984 to determine whether problems might arise when cattle chronically infected with trypanosomiasis are immunized against ECF, either in terms of increased trypanosome parasitaemia or an enhanced reaction to the *Theileria* parasites used in immunization.

In a preliminary experiment, six cattle were immunized by infection and treatment against ECF: two of these were chronically infected with *Trypanosoma congolense*, two had been infected with *T congolense* and cured by treatment with the trypanocidal drug diminazene aceturate (Berenil), and two were uninfected controls. All six showed mild or inapparent reactions following immunization and similar reactions following potentially lethal ECF challenge. One of the experimental cattle and one of the controls died of trypanosomiasis. In a second experiment, 7 cattle were infected with *T congolense* and later immunized against ECF. They underwent mild or inapparent ECF reactions during immunization and all

Time and Strain of Challenge	Number Treated	Survived	Untreated Controls	Survived
5 days post-immunization				
Muguga	6	4	2	0
Marikebuni	6	2	2	1
10 days post-immunization				
Muguga	6	5	2	0
Marikebuni	6	5	2	0
20 days post-immunization				
Muguga	6	6	2	0
Marikebuni	6	5	2	0
30 days post-immunization				
Muguga	6	6	2	0
Marikebuni	6	5	2	0

Figure 8. Development of homologous and heterologous protective immunity at different intervals after immunization by infection and treatment. Cattle were immunized with *T p parva* (Muguga) and then challenged at different intervals with *T p parva* (Muguga) or *T p parva* (Marikebuni).

resisted potentially lethal ECF challenge which killed all non-immunized controls. Thus, under the conditions of the experiment, it appears that chronic trypanosomiasis is neither exacerbated by ECF infection-and-treatment immunization, nor does it result in enhanced ECF reactions. Similar studies will be conducted using cattle chronically infected with other blood-borne parasites, such as *Babesia bigemina* and *Anaplasma marginale*.

Other tick-transmitted diseases which occur in endemic ECF areas include *Theileria mutans* infections which cause chronic anaemia, and heartwater which is often fatal. Preliminary studies have concentrated on isolating and establishing these parasites in the tick vector, *Amblyomma variegatum*. A study was carried out in 1984 on *Theileria taurotragi* in collaboration with the Kenya Agricultural Research Institute: two eland and two sheep were successfully infected with a *T taurotragi* stabilate, suggesting that this *Theileria* species may have a wide host range in the field.

ASPECTS OF TICK PHYSIOLOGY AND *THEILERIA* TRANSMISSION

The brown ear tick, *Rhipicephalus appendiculatus*, which transmits ECF remains attached to and continues feeding on bovine hosts for periods of several days. It is crucial for the survival of the tick that it be able to prevent coagulation at the site of attachment and promote the flow of blood and tissue fluids during feeding. Although the secretion of anticoagulants by ticks during feeding has been known for some time, little is known of their chemical nature, their mode of action or their possible role in induction of immune responses.

Research at ILRAD in 1984 concentrated on purifying the anticoagulant molecule contained in tick salivary gland extracts. Physicochemical characteristics suggest that this molecule is the same in larvae, nymphs and adult ticks. Analysis of the mechanism of anticoagulant activity in 1984 showed that the anticoagulant exerts its effect by inhibiting the prothrombinase complex which

catalyses the conversion of prothrombin to thrombin. Attempts to raise antibodies in rabbits against the purified anticoagulant molecule have been unsuccessful, suggesting that the molecule is a relatively poor stimulator of immune responses. However, the anticoagulant activity can be detected in bovine blood and fluids from the immediate vicinity of tick attachment sites, and its role in stimulating resistance to tick infestation is being investigated.

Although it has not yet been possible to raise antibodies against the purified tick anticoagulant molecule, both laboratory animals and cattle are known to produce antibodies against other products secreted by feeding ticks. These antibodies mediate acquired resistance to tick infestation, demonstrated by a reduction in the number of ticks attaching to a host and in the weight of ticks after completion of feeding. Resistance to ticks involves delayed-type hypersensitivity reactions in the skin and is acquired naturally in the field after multiple tick infestations. This acquired resistance may help reduce the vector population and disease transmission. Efforts to induce resistance by immunization with tick antigens, most often using crude tick extracts, have not been successful. Work was thus continued in 1984 to identify and purify the specific tick antigens which stimulate resistance. Antisera which recognize tick antigens were raised in rabbits and guinea pigs infested with ticks, and these are being used to identify tick antigens.

Electron microscopy studies at ILRAD have concentrated for several years on the histophysiology and ultrastructure of the *R appendiculatus* salivary gland, in particular on the E cells of the type III acinus where *Theileria* sporozoites develop. Studies of the other acini—types I, II and IV—were completed in 1984, including descriptions of several secretory cell types. This research has established ultrastructural criteria for the identification of cell types, new findings on the sexual dimorphism of the gland, and evidence for participation of the type II acinus in fluid and electrolyte excretion during tick feeding. In addition, examples have been discovered of secretory granule formation without involvement of the Golgi complex, and the utilization of innumerable gap-junctions for bulk transcellular movement of water and electrolytes across transporting epithelium has been observed.

TRYPANOSOMIASIS

In recent history, it is unlikely that any continent has ever been dominated by one disease to the extent that Africa is dominated by trypanosomiasis. Different species of the protozoan parasites which cause trypanosomiasis 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 the flies require as breeding and resting sites. More recently, most tsetse control programs have been based on the appliction 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 management 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 activites are broadly based, but can be considered under three headings: improved control of the parasites, enhanced resistance to infection and trypanosomiasis epidemiology.

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All three major 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 b 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 of another animal along with tsetse saliva.

When *T congolense* or *T b brucei* parasites are transmitted to an uninfected animal by a tsetse fly (see Figure 9), 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 into the bloodstream. *T vivax* parasites are also transmitted from the skin through the lymphatic system to the bloodstream, but the local skin reaction, it if 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 sometimes be observed in the cental nervous system. When a tsetse fly feeds on an infected animal, it ingests trypanosomes along with blood, completing the parasite transmission cycle.

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 (protein-carbohydrate compounds), 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. The successive waves of parasites which appear in the mammalian bloodstream during the course of infection display different VSG coats. In laboratory animals, more than 100 different VSGs have been observed on the descendents of a single trypanosome, and scientists estimate that one trypanosome clone could produce cells bearing several hundred different surface coats.

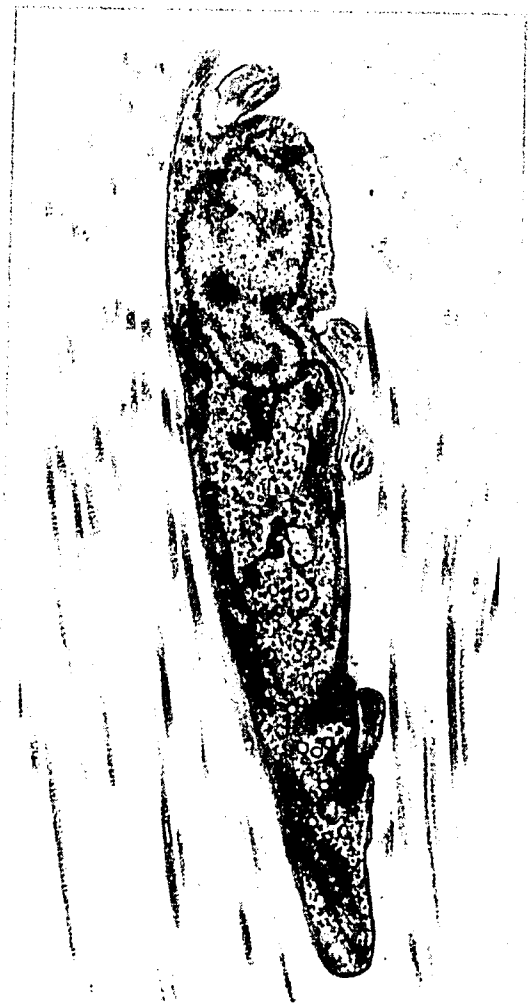

Figure 9 Micrograph of a *T congolense* parasite in the skin of a goat 7 days after transmission by an infected tsetse fly The striation around the trypanosome consists of bundles of collagen fibres Photograph courtesy of Dr W Rudin Swiss Tropical Institute

When an animal becomes infected, it produces antibodies specifically against the VSG displayed by the first wave of invading trypanosomes. However, before all the parasites can be eliminated, new trypanosomes appear coated with a different VSG, and these are capable of evading the animal's 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.

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

Evidence has accumulated that metacyclic trypanosomes produce a smaller number of VSGs than the parasites which develop during infection in the bloodstream, and scientists at ILRAD and elsewhere have immunized animals successfully against metacyclic forms of specific cloned trypanosome populations. However, in most field situations animals are exposed to many antigenically distinct trypanosome populations—belonging to different serodemes—each with its own characteristic repertoire of VSGs. Thus 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 concentrate on different stages of the trypanosome lifecycle and the mechanisms involved in the transition from one stage to the next. This work is supported by the development of techniques to maintain different species and stages of trypanosome in vitro. The goal of all these projects is to identify factors which could be manipulated to disrupt the development of the parasites or make them vulnerable to host defences.

Trypanosomes contain several hundred genes, each of which codes for a different variant of the protein chain of the VSG.

The mechanism which enables a population of trypanosomes to display one variant molecule at a time on their surface and to switch between these molecules has aroused considerable interest among scientists around the world.

For the variant molecule encoded by a particular VSG gene to reach the surface of the trypanosome, the gene must be located in a special 'expression site', which is called a telomeric site because of its location near the end of a chromosome. From this site, the gene can be copied into an RNA molecule which directs the synthesis of the appropriate VSG protein chain. There are many telomeric expression sites in the trypanosome genome that carry VSG genes. Only one of these is actively copied into RNA at a time. The mechanism of this exclusive expression is not yet understood. Neither is the process by which expression is switched to a different telomeric site, leading to the appearance of a different VSG on the trypanosome surface. The telomeric VSG genes provide a pool of VSGs most likely to be expressed early in an infection. Switching of VSG expression may also occur by replacement of the VSG gene in the active telomeric site by a duplicated copy of a non-telomeric VSG gene, or of another telomeric gene.

Research at ILRAD has initially concentrated on four VSG genes cloned from a stock of *T brucei*. The expressed copy of one of these genes was shown to be located at the end of a small chromosome containing about 200,000 base pairs of DNA. Restriction enzyme mapping revealed a 'barren' region of DNA on one side of the gene exceptionally lacking in restriction enzyme sites. This barren region is a characteristic structural feature of all VSG gene expression sites so far described. Studies were initiated to investigate the role of this region in the control of VSG expression and in the gene replacement mechanism.

A segment of DNA containing the barren region was cloned from trypanosomes where the site was active and from trypanosomes in which it was inactive, and the sequences of the two cloned regions were determined. The barren region consisted entirely of an array of imperfect repeats of an 80 base pair sequence motif. No differences were found in the sequences from the active and inactive expression sites. This showed that alterations of the DNA sequence in the barren region were not involved in control of expression of the adjacent VSG gene.

Two VSG gene replacement events affecting this expression site have been studied in some detail. Both involved the replacement of the gene in this site by a duplicated copy of a gene located on a minichromosome. This work showed that a minichromosome gene was also flanked by a long array of barren region repeats, and that gene replacements involved recombination within the repeat arrays. Short repeat arrays are also involved in many replacements by non-telomeric VSG genes. In conclusion, the barren region plays a major role in antigenic switching by gene replacement, but its role, if any, in the mutually exclusive control of the multiple telomeric expression sites is not clear.

Research on the genetic basis of VSG gene expression has been extended to studies of *T congolense* in collaboration with the Vrije Universiteit Brussel. ILRAD scientists have cloned the genes which code for four different *T congolense* VSGs, each from an antigenically distinct parasite population representing a different serodeme. These clones have been used to investigate the organization of VSG genes during antigenic variation (Figure 10). Results suggest that the mechanisms involved in VSG gene expression in *T congolense* are similar to those observed in *T brucei*. The gene which was analyzed in 1984 is duplicated during expression and the copy is transposed to a telomeric site, leading to the presence of an extra copy in clones which express the associated VSG.

The DNA sequences encoding certain *T congolense* VSGs may be specific to particular parasite serodemes. Clones of the

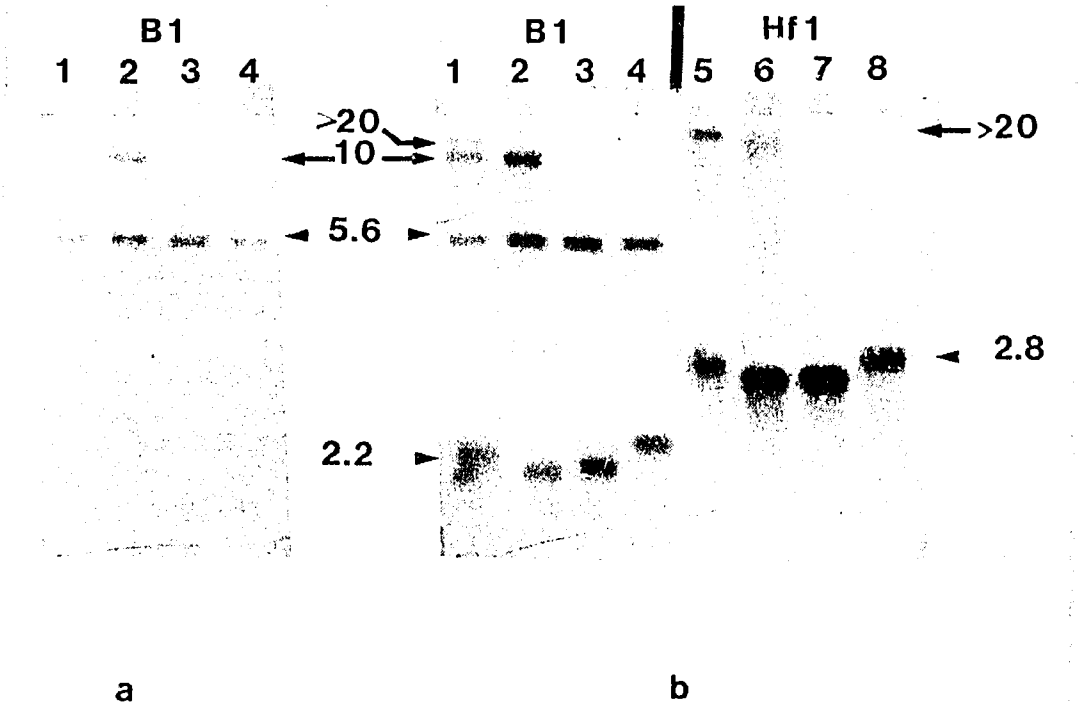

Figure 10. Autoradiograph of a VSG gene expressed after duplication. Expression of the *T congolense* IL Nat 2.1 VSG gene is preceeded by duplicative transposition, leading to the presence of an extra copy of the gene in trypanosome clones expressing this VSG. The DNA was specifically cut with an enzyme, Bgl 1 (lanes 1 to 4) or Hinf 1(lanes 5 to 8), and probed with either the radiolabeled 5' end of the gene (panel a) or the radiolabeled whole gene (panel b). DNA from trypanosomes X3 and X4, which express the VSG, is shown in lanes 1, 2, 5 and 6, while DNA from trypanosome clones IL34 and IL36, which do not express the VSG, is shown in lanes 3, 4, 7 and 8. Arrows point to fragments of DNA present only in those trypanosome clones expressing the VSG, while arrowheads point to fragments of DNA present in all the trypanosome clones shown.

IL Nat 2.1 VSG gene have been used experimentally as hybridization probes to determine whether similar DNA sequences occur in other *T congolense* clones isolated from geographically separate regions of Africa. Out of seven isolates tested from different locations in Kenya, Uganda and Tanzania, two showed insignificant signals of hybridization and five showed no signals at all (see Figure 11), indicating that they do not share the IL Nat 2.1 VSG gene sequences. This suggests that different *T congolense* populations may be identified by characteristic VSG gene sequences. These findings contrast with results obtained from studies of *T brucei* VSG genes which showed common DNA sequences among a number of trypanosome clones isolated originally from different geographic areas. Research is now in progress to determine the number of distinct serodemes represented by the *T congolense* isolates available at ILRAD, in particular among the isolates collected in Kilifi District at the Kenyan coast.

Blot hybridization tests are also being undertaken to determine whether the different *T congolense* isolates in the ILRAD collection may fall into VSG isotypes. Where evidence suggests that isotypes may occur, antisera will be used to find out

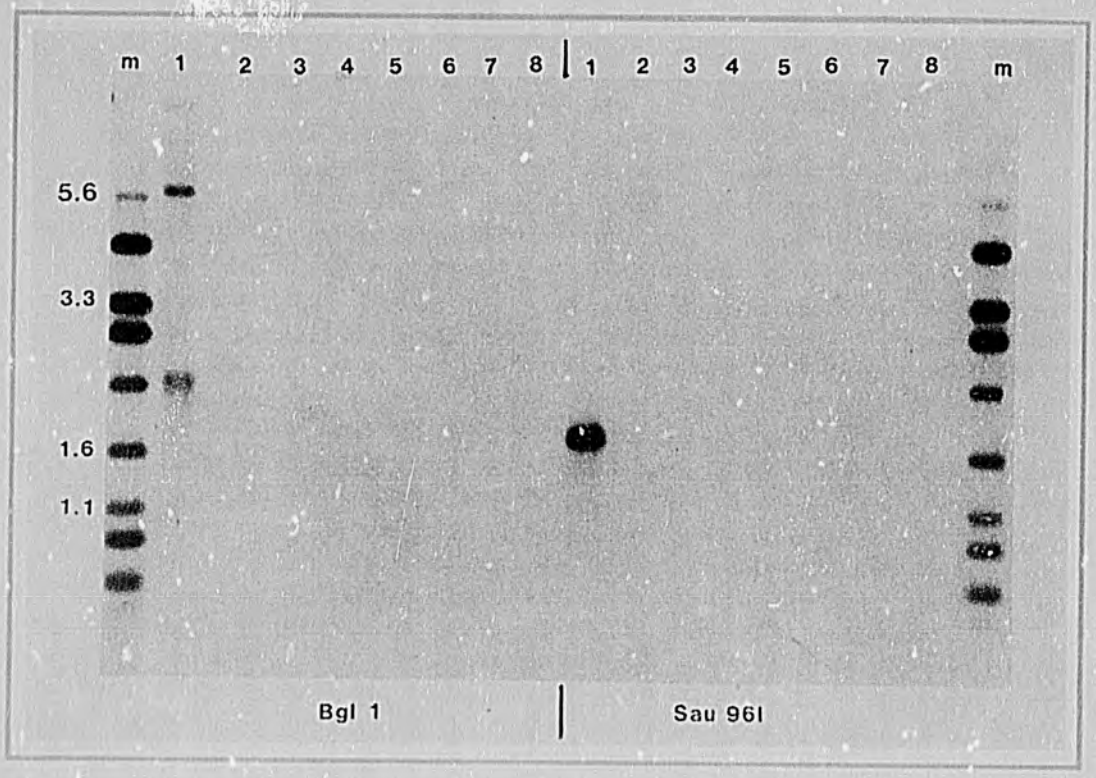

Figure 11. Autoradiograph showing the presence or absence of a VSG gene in the DNA of different trypanosome clones. The *T congolense* ILNat 2.1 VSG gene was used to probe DNA prepared from *T congolense* clones derived from isolates from different locations in East Africa. The DNA was specifically cut with either the enzyme Bgl 1 (left) or Sau 96I (right). Washes after probe hybridization were for 1 hour with two changes of 0.1 x SSC, 0.1% SDS at 65°C. DNA shown in the different lanes was taken from *T congolense* clones as follows: (1) ILNat 2.1 Transmara, Kenya; (2) ILNaR 1 Nairobi, Kenya; (3) ILNaR 3 Serengeti, Tanzania; (4) SC 60 Kilifi, Kenya; (5) SC 31 Ankole, Uganda; (6) clone 1616 Serengeti, Tanzania; (7) SC 45 Kilifi, Kenya; (8) clone 776 Kigoma, Tanzania. DNA from the ILNat 2.1 VSG gene used as the probe only hybridized with DNA from the same trypanosome clone (lane 1).

whether the isolates share common metacyclic antigens. Antigens derived from metacyclic trypanosomes of two distinctive serodemes are now being cloned.

SYNTHESIS OF THE SURFACE COAT

The thick VSG coat protects the trypanosome from destruction by the immune response of the mammalian host. The surface coat also prevents host antibodies from gaining access to other parts of the parasite. Biochemical studies of *T brucei* and *T congolense* have revealed that the surface coat contains at least two classes of carbohydrate side chain bound to the protein by a process called glycosylation. Earlier work showed that the protein component of the VSG is synthesized in the rough endoplasmic reticulum of the trypanosome in 6 to 8 minutes. It takes approximately 8 minutes for the VSG to move from the rough endoplasmic reticulum to the Golgi complex. During this time, the VSG passes through the smooth endoplasmic reticulum, and evidence has accumulated suggesting that the internal carbohydrate side chain may be incorporated into the VSG in this organelle.

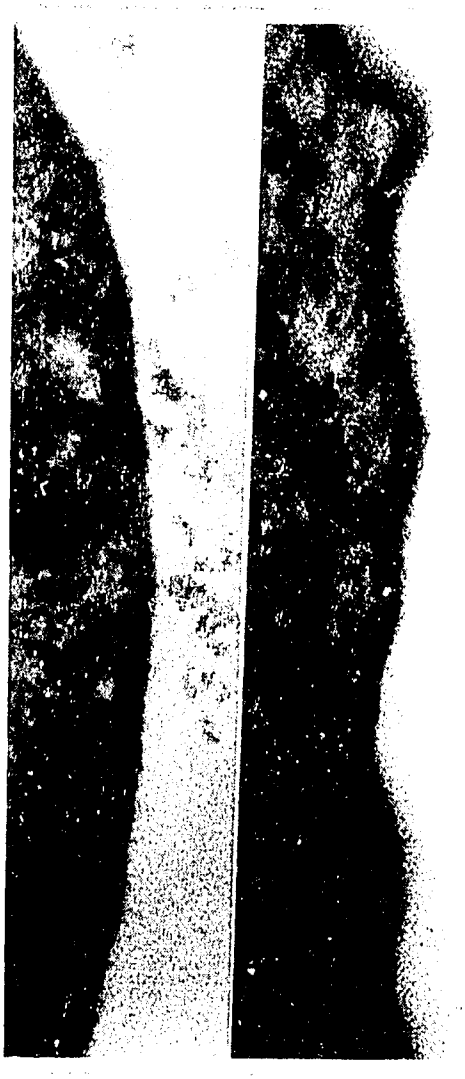

Figure 12. Transmission electron micrographs of sections through East African *T vivax* (IL 2292) parasites. These were extruded by infected tsetse flies (*G morsitans centralis*) which were allowed to probe into a culture medium through a silicone membrane. Only 20 to 50 parasites could be collected in this way from 50 tsetse flies. They were processed using a novel technique developed at ILRAD that allows single cells to be cut into thin sections and examined individually with the electron microscope. The section on the left is an extruded trypomastigote without a surface coat. The section on the right is an extruded metacyclic form from the same sample. The surface coat can be clearly seen.

The second carbohydrate side chain is potentially of greater biological importance because it appears to remain constant on all *T brucei* and *T congolense* VSGs so far studied. This is probably the cross-reacting determinant, responsible for the common antibody recognition of different VSGs. Previous research showed that in *T brucei* this side chain is added to the VSG, or its final maturation occurs, in the trans-Golgi region of the cell facing the flagellar pocket.

Using improved electron microscopy techniques, ILRAD scientists have shown a coat of surface glycoprotein on metacyclic forms of both East and West African *T vivax* stocks extruded by infected tsetse (Figures 12 and 13). Uncoated trypanosomes were found in the same tsetse probes, which suggests that metacyclic forms of *T vivax* may synthesize their surface coat relatively late in the development cycle. The trypanosomes injected by an infected tsetse fly might include individual parasites with a thick surface coat, others with a thin, fuzzy coat as described in earlier reports, and others without coats.

African trypanosomes replicate primarily by longitudinal binary fission, but evidence has accumulated that a sexual phase of development may also occur at some stage in the parasite lifecycle. Sexual reproduction would be associated with significantly increased possibilities for genetic diversity, which would make immunological control of trypanosomiasis much more difficult. When scientists from the Swiss Tropical Institute transmitted a mixture of *T b gambiense* and *T b brucei* clones through tsetse flies, they found that clones subsequently derived from mice infected with these tsetse showed isoenzyme patterns of both parental clones. In a collaborative project, ILRAD scientists have investigated the antigenic relationship

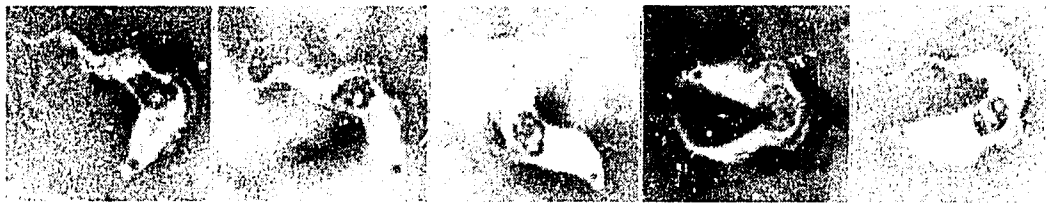

Figure 13 Light micrographs of five typical metacyclic forms of East African *T vivax* (IL 1480) extruded by infected tsetse flies (*G morsitans centralis*). The tsetse were allowed to probe onto warmed (37 C) teflon-coated slides and the salivary probes were dried following mild acid hydrolysis and Giemsa staining.

between metacyclic trypanosomes of these hybrid clones and the two parental clones.

When metacyclic parasites of the different clones were analyzed by indirect immunofluorescence, there was no crossreaction between the VSGs of the two parental clones. Antisera against the metacyclic forms of each clone were prepared by exposing rabbits repeatedly to the bite of infected tsetse, and the antisera against each of the parental clones recognized a high proportion of the hybrids. Double immunofluorescence showed that 76% of the hybrids were recognized by antisera against both parental clones, 10% of the hybrids were recognized by the *T b gambiense* antiserum only, and 14% were recognized by the *T b brucei* antiserum only. Antisera against the hybrid metacyclics recognized 100% of the parental *T b gambiense* clones, but only 50 to 80% of the *T b brucei* parental clones. This indicates that the entire metacyclic genome of one parental clone was represented in the hybrid, while the other parental clone was only partially represented. Cross-neutralization assays and immunization tests gave similar results. When mice were immunized with hybrid metacyclics by infection and treatment, they were fully protected against challenge with the tsetse-transmitted *T b gambiense* parental clone, but not against challenge with the *T b brucei* clone.

These results indicate that a recombination of metacyclic antigen genes occurred between clones of *T b gambiense* and *T b brucei*, providing evidence for a sexual phase in the developmental cycle of the *brucei* group of African trypanosomes. Scientists from ILRAD and the Swiss Tropical Institute are now examining this phenomenon using *T congolense*, *T vivax* and other serodemes of *T b brucei* to ascertain the frequency and stability of genetic recombination, as well as its significance with regard to the diversity of antigens displayed by metacyclic trypanosomes.

Studies on the proteins specific to different stages of the trypanosome lifecycle have been in progress for some time, focusing on rapidly-dividing and nondividing bloodstream forms of *T b brucei* as well as the procyclic form which develops in the midgut of the tsetse fly. Attention is concentrated on subsets of trypanosome proteins which are likely to be involved in the transformation from one stage to the next. These include nuclear proteins, which are potential regulators of gene expression, and proteins from pinocytotic vesicles, organelles which act as receptors for differentiation signals and growth factors. The organelles containing these proteins have been purified, and polyspecific antisera have been produced which recognize specific antigens. Monospecific antisera are now being derived which will be used to investigate the synthesis, modification and intracellular localization of these antigens during parasite differentiation.

In a collaborative project with the European Molecular Biology Laboratory and Yale

University, an IgG monoclonal antibody has been tested at ILRAD and found to recognize membrane-bound intracellular structures in trypanosomes which resemble lysosomes, organelles containing digestive enzymes. This antibody is now being used in studies of the intracellular digestion process (endocytosis) in trypanosomes.

CULTIVATION OF TRYPANOSOMES IN VITRO

Culture systems have been developed at ILRAD which support the development of all three major African trypanosome species throughout their lifecycles. Parasites in most stages of development can now be supplied routinely for a variety of research activities.

In 1984, ILRAD's cell biologists concentrated on developing culture systems which support East African stocks of *T vivax*, and particularly on cultivating and characterizing animal-infective metacyclic forms. This work followed earlier research which resulted in the successful cultivation of one West African *T vivax* stock through all stages of the lifecycle. The cultivation of *T vivax* in vitro is particularly important because most isolates of this species do not infect laboratory rodents. The only method available for maintaining the parasites has been by continuous passage through infected tsetse flies and ruminant hosts. However, infected tsetse extrude very few metacyclic forms of *T vivax*, so only small numbers of these parasites have been available for study.

Parasites resembling metacyclic forms have been propagated successfully from four *T vivax* isolates obtained from different locations in Kenya. None of these isolates is infective to rodents, but they are all highly infective to cattle. Two *T vivax* culture systems have been developed, both requiring bovine serum and feeder layer cells. The first was initiated with infected tsetse proboscides. During the first 4 weeks of culture, parasites in an uncoated insect stage of development (epimastigotes) increased in number, and by the end of the fourth week metacyclic-like forms appeared. During the next 5 months, 124 subcultures were made of these mixed populations.

The second culture system was initiated with bloodstream forms of the parasite separated from the blood of infected cattle. The parasites transformed into two uncoated insect stages (trypomastigotes and epimastigotes) within 10 days, and subsequently metacyclic-like forms appeared. More than 20 subcultures were made over a period of 3 months. All the trypanosome populations containing metacyclic-like forms were infective to cattle and goats by intravenous inoculation. Current work involves further characterization of the in vitro-propagated metacyclic forms and simplification and standardization of the culture systems. The goal is to produce sufficient numbers of *T vivax* metacyclic-like parasites for use in a number of research projects.

An interesting phenomenon associated with *T vivax* cultivation is the attachment of elongated parasite forms harvested from the last stage of bloodstream infection to Matrix Gel Green A beads (Amicon). These trypanosomes may attach themselves to the mouth parts of tsetse flies in a similar way. Using the West African *T vivax* strain originally cultivated at ILRAD, studies were carried out in 1984 to analyze the attachment process. A variety of sugars, salts and other agents were added to the parasites in the presence of the beads, but none inhibited attachment, suggesting that a receptor-ligand mechanism was operating. When the dye molecule was removed from the beads the trypanosomes failed to attach, and parasites preincubated with partially purified dye showed reduced attachment to intact beads. Current work involves analysing molecules in the dye and on the surface of the trypanosomes in order to identify the specific ligands and receptors involved in the binding process.

Similar studies concentrate on bloodstream forms of *T congolense*. These tryp-

anosomes attach themselves to endothelial cells in the blood vessels of their mammalian hosts, and they require endothelial cells in the culture medium to survive in vitro. Research focuses on the receptor-ligand interaction between parasite and host-cell membranes and also on proteins secreted by the host cell which may be bound and metabolized by the parasite. This work may lead to improved in vitro cultivation systems for *T congolense* and a better understanding of the requirements of this parasite species in the host bloodstream.

Two in vitro assay systems have been developed for screening the activity of trypanocidal drugs against *T b brucei*. These have proven useful for testing the susceptibility of different strains to standard trypanocidal drugs used in the field and for screening new compounds for their trypanocidal potential. In the first test, the trypanocidal activity of a compound is measured in terms of the minimum drug concentration required to inhibit trypanosome growth by more than 50% during 24 hours' exposure, expressed as the MIC_{50}. By the end of 1984, over 25 compounds had been tested using this system. Chlorpromazine (Sigma) and two other phenothiazine compounds demonstrated trypanocidal activity in vitro, with MIC_{50}s ranging from 3 to 10 μg/ml. These compounds may be effective against infections in the central nervous system, as they are known to cross the blood-brain barrier. However, *T brucei* bloodstream forms had to be exposed to chlorpromazine for at least 90 minutes, even at a concentration of 30 μg/ml, to render them 100% non-infective for mice.

Efforts in 1984 were directed towards developing and standardizing assay systems to test the activity of trypanocidal drugs against *T congolense* and *T vivax*. The second assay developed originally to test activity against *T b brucei* detects trypanocidal activity by measuring the inhibition of trypanosome protein synthesis. Trypanosomes are first incubated in a methionine-free medium, then radioactively labeled methionine is added and its incorporation into trypanosomal protein is measured in the presence and absence of the drug. This assay has been used to test the activity of the four cattle trypanocides in current use—diminazene aceturate (Berenil: Hoechst), isometamidium chloride (Samorin: May and Baker), ethidium chloride (Novidium: May and Baker) and quinapyramine sulphate (Trypacide: May and Baker)—against three *T congolense* isolates from Kenya and three from Bourkina Faso. The drug sensitivity patterns revealed by this in vitro assay are being compared with patterns detected by experimental infections in mice. If the in vitro results can be shown to reflect accurately the situation in vivo, then it should be possible to use this assay system to detect drug resistance in isolates collected from the field. The test, which can be carried out in 24 hours, could be an important diagnostic tool in situations where drug-resistant *T congolense* strains are assuming increasing importance.

TOWARDS IMPROVED HOST RESPONSES

EARLY EVENTS IN THE SKIN AND LYMPHATIC SYSTEM

The development of a local skin reaction, or chancre, at the site of an infected tsetse bite is often the first host response to trypanosome infection (see Figure 14). Resistant livestock and wild animals develop smaller chancres than susceptible animals and the

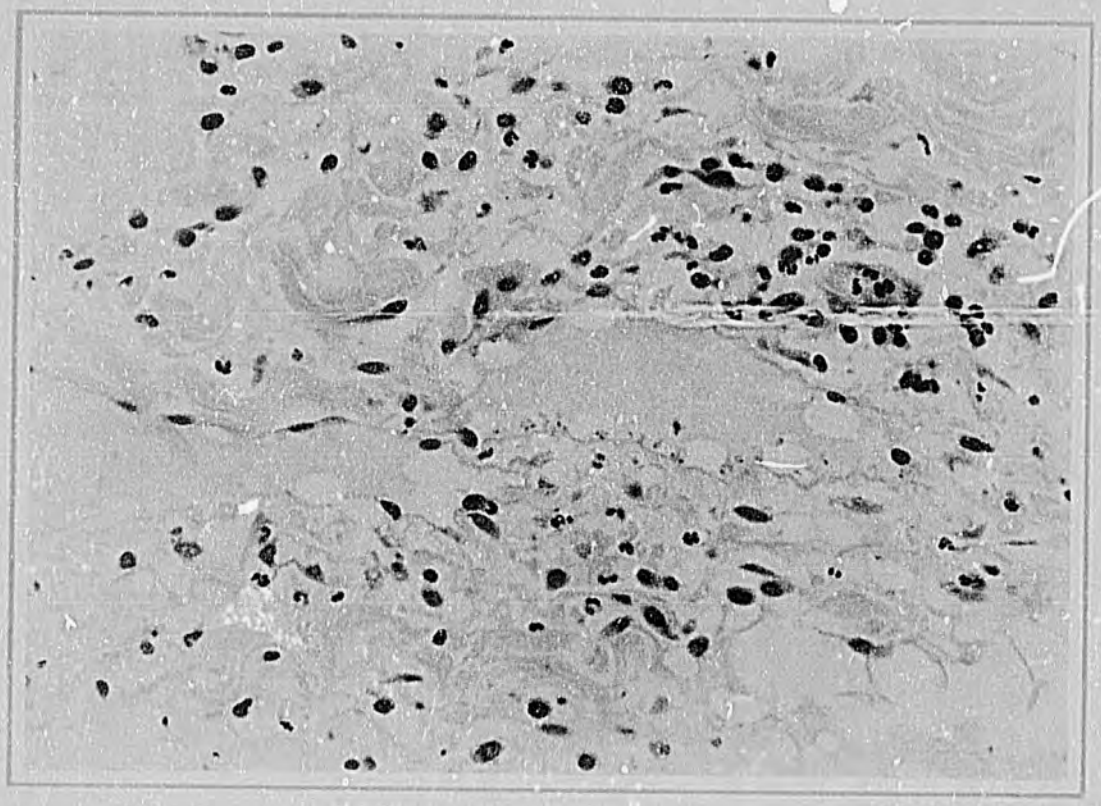

Figure 14. The local skin reaction in a goat 7 days after *T congolense* transmission by an infected tsetse fly. The enlarged lymphatic vessel in the centre and the lymphocytes and neutrophils gathered around are signs of a local inflammatory reaction. Parasites can be detected inside the lymphatic vessel around the edges.

period before trypanosomes appear in the bloodstream is usually longer in resistant animals, suggesting that parasite development has been slowed down in the skin during the initial stages of infection.

When domestic ruminants infected with *T congolense* are superinfected with a different serodeme of the same species, an interference phenomenon occurs which delays or prevents the second infection. Resistance to the superinfection appears to occur largely in the skin. In a cooperative study with the University of Edinburgh's Centre for Tropical Veterinary Medicine, an experiment was carried out to investigate the influence of the time interval between the two infections. In a series of experiments initiated in 1983, goats were infected with one *T congolense* serodeme and then challenged at different intervals with a second serodeme. All parasites were transmitted by infected tsetse flies. Goats challenged with both serodemes at the same time developed chancres at all sites of infection, and skin removed from these chancres 7 days after infection showed a marked cellular reaction and the presence of many trypanosomes. By contrast, when goats were challenged with the second serodeme 7 to 35 days after infection with the first, either no skin reaction occurred or the reaction was delayed and significantly reduced (Figure 15). The cellular response was less pronounced in skin removed 7 days after superinfection, and trypanosomes were not seen, but 16 days after superinfection both cellular infiltration and trypanosomes were observed, indicating a delayed reaction at a time when the local skin reaction in control goats had already disappeared.

All the goats were treated with the trypanocidal drug diminazene aceturate 21 days after superinfection, and 3 weeks later they were challenged again with the second *T congolense* serodeme. The animals that had been infected with the two serodemes simultaneously were immune to rechallenge, but most of those challenged with the second serodeme 7 to 35 days after the first were not, showing that infection had been established in the first group during the initial experiment, but had been delayed or prevented in the others.

More recently, it has been found that interference also occurs between different species of trypanosome. Chancre and antibody development were delayed when goats were infected with *T congolense* and later superinfected with *T brucei*, or vice versa. However, there was no delay in the establishment of a tsetse-transmitted superinfection with *T vivax*, either in goats previously infected with *T congolense* or with another *T vivax* serodeme.

The interference phenomenon could not be overcome by increasing the level of challenge. Thus, irrespective of whether 1000 metacyclic forms of *T congolense* were used for superinfection (equivalent to the number transmitted by 10 infected tsetse

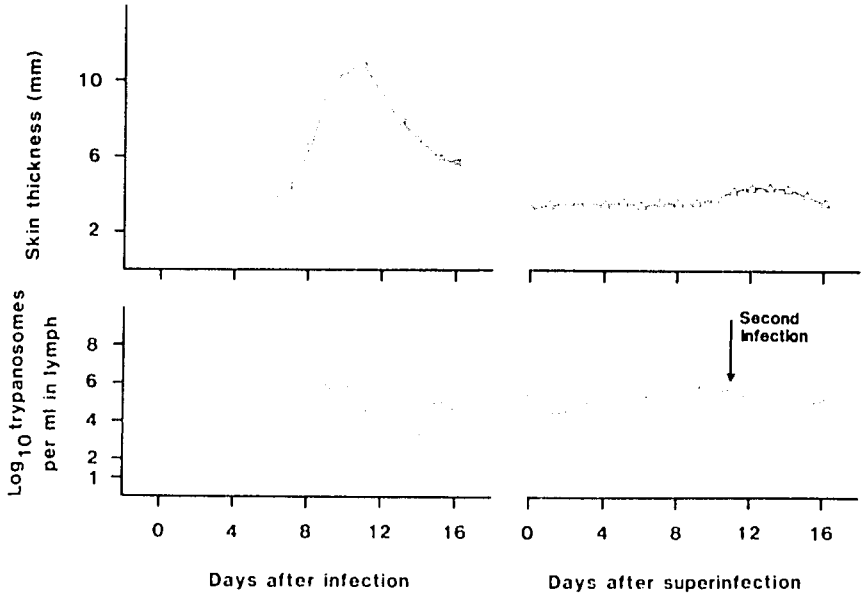

Figure 15 Local skin reaction and parasitaemia in the draining lymphatic vessel of a goat infected with *T congolense* and subsequently superinfected with a second *T congolense* serodeme. The efferent vessel of a prefemoral lymph node was cannulated and the goat was exposed to an infected tsetse fly around the cannulation site. The local skin reaction at the site of the tsetse bite, as measured by skin thickness, began to increase dramatically 6 days after the initial infection and reached a peak on day 12. Trypanosomes were detected in the lymph 4 days after infection and reached a level of 500,000 parasites per ml on day 8. In an infected goat superinfected after 12 days with a different *T congolense* serodeme, no local skin reaction could be detected. Only trypanosomes of the first infecting serodeme were present in the lymph until 11 days after superinfection, indicating a delay of 7 days before the appearance of the parasites used for superinfection. Trypanosomes were detected in the lymph by subinoculation in mice. The two serodemes were distinguished by specific monoclonal antibodies and DNA probes

bites) or 100,000 (equivalent to the number transmitted by 1000 infected tsetse bites), goats already infected with a different serodeme failed to develop local skin reactions, showed no increase in parasitaemia and exhibited no detectable antibody response to superinfection.

In summary, factors present in the skin of goats infected with *T congolense* or *T brucei* delay the development of a second tsetse-transmitted trypanosome infection. This interference phenomenon operates between trypanosome species and between serodemes of the same species. It is time dependent and operates both in the skin and in the circulatory system, and it cannot be overcome by high levels of parasite challenge.

Histochemical surveys are now in progress to monitor the activity of different cell types during chancre formation in relatively resistant and susceptible hosts. Anti-bovine IgM and IgG antibodies have been used to demonstrate surface and cytoplasmic immunoglobulin in chancres, and mast cells have been observed by staining with anti-bovine aprotinin. Bovine Langerhans cells, which are major antigen-presenting cells, have also been observed in the skin and shown to display class II MHC antigens. However, it has not been possible to identify subsets of lymphocytes or macrophages/monocytes in tissues of bovine skin using the monoclonal antibodies available at ILRAD. Present work concentrates on developing additional reagents which will recognize important subsets of cells in the chancre.

Immediate hypersensitivity reactions—possibly to tsetse saliva—have been demonstrated in goats bitten repeatedly by tsetse flies, and antibodies (presumably IgG) to purified tsetse saliva have been shown in the sera of hypersensitive goats. However, there is as yet no evidence that these reactions affect the transmission of trypanosomes.

Studies continued in 1984 on the role of the lymphatic system in the development of primary trypanosome infections and superinfections. The vessel draining the prefemoral lymph node was cannulated in goats, and the animals were exposed to tsetse flies infected with *T congolense* near the site of cannulation. In primary infections, trypanosomes were detected in the efferent lymph 4 days after challenge, which was 2 days before a local skin reaction was observed or trypanosomes were detected in the bloodstream. However, in superinfected goats, trypanosomes from the superinfection were not found in the efferent lymph until 11 days after superinfection, as shown in Figure 15.

PARASITE-HOST INTERACTION IN THE BLOODSTREAM

Control of trypanosome growth

Research at ILRAD has shown that the growth of trypanosome populations in the bloodstream is linked to a transition from rapidly-dividing to nondividing forms. Considerable evidence has accumulated that the host influences the growth of the parasite population by controlling, through non-immune processes, the rate of parasite transition to the nondividing form. Past work in mice suggested that the transition of *T b brucei* to nondividing forms is controlled by the availability of host molecules which stimulate parasite multiplication, but inhibit this transition.

A serum-free culture system was developed in 1983 which permits parasite survival and transition, but not multiplication. *T b brucei* parasites were stimulated to multiply when the culture medium was supplemented with bovine serum and bovine fibroblast cells. Work in 1984 concentrated on analyzing the serum components and fibroblast factors which permit parasite multiplication. In the absence of fibroblasts, most batches of foetal bovine serum screened were toxic for trypanosomes. A

few sera were identified which, at low concentrations, promoted trypanosome multiplication in the absence of fibroblasts, but at higher concentrations they too were toxic. These studies suggest that one role of fibroblasts may be to remove a toxin from serum.

Bovine sera were fractionated by a variety of methods, and the serum components which induced trypanosome multiplication (population doubling in 7 to 8 hours) were found to be of a molecular mass greater than 100,000 daltons. They are not lipoproteins or immunoglobulins. Current investigations involve treating bovine sera with different enzymes in order to identify the types of molecules required for parasite multiplication and eventually to purify them.

To determine whether the molecules which control parasite multiplication in this culture system play the same role in vivo, experiments have been conducted analyzing the development of mixed *T b brucei* infections in mice. Some clones of *T b brucei* do not change to nondividing forms in mice. These clones give rise to extremely acute infections, characterized by exponential expansion of the parasite population until death of the host. Other clones change to nondividing forms which die out, resulting in chronic infection with limited parasite population growth. When mice are infected with mixed populations of these two clones, both types of parasite retain their respective traits. This strongly suggests that the clones which cause acute infections are able to utilize murine growth-promoting molecules more efficiently than the clones which cause chronic infections. Efforts now concentrate on reproducing these results in vitro, using mouse fibroblast cell lines and serum.

Further studies to identify the host factors responsible for regulating trypanosome growth have investigated the effects of treatment with the non-specific immunostimulator *Corynebacterium parvum*. When mice are treated with *C parvum* a few days before infection with *T b brucei*, *T congolense* or *T vivax*, trypanosome growth rates are dramatically reduced, and this appears to be associated with earlier parasite transition to nondividing forms. Research conducted in 1983 showed that *C parvum* also inhibits parasite growth in irradiated mice, which indicates that the adjuvant is unlikely to exert its effect through the immune response.

In 1984, the effects of *C parvum* were evaluated in resistant and susceptible mouse strains infected with *T b brucei*, and in mice possessing different immunological abnormalities. Tests were carried out using both tsetse infection and syringe inoculation of parasites. The administration of *C parvum* 2 days before infection increased the capacity of both resistant and susceptible mice to control parasitaemia, with the effect being more marked in the resistant strain. The superior ability to control and remit parasitaemia observed in resistant mice treated with *C parvum* was reflected in the earlier appearance and higher levels of antibody; in all groups the IgM response was much greater than the IgG response. When mouse strains with different types of immunodeficiencies were treated with *C parvum* prior to *T b brucei* infection, the level of parasitaemia was reduced compared to levels reached in untreated controls. Of particular interest, *C parvum* treatment of mutant mice characterized by marked impairment of cell-mediated and antibody-dependent immune responses rendered the majority completely resistant to *T b brucei* infection. This confirms the earlier finding that the mechanism of host resistance enhanced by *C parvum* treatment is not immunological.

An in vitro study was carried out in 1984 to test whether the resistance of buffalo to trypanosome infection might be related to non-specific factors in buffalo serum which affect the growth of the parasites. Sera from two trypanosome-free buffalo and two control cattle were incubated with *T congolense* (iL 1180) for 30 minutes at 4°C and then inoculated intraperitoneally into mice. All the mice became infected, indicating—at least for this *T congolense* serodeme—that there were no non-specific factors in

the buffalo serum which irreversibly halted growth of the parasites.

In general, for an infected animal to mount a protective immune response, antibody-producing cells must be stimulated to proliferate, they must produce antibodies, and these antibodies must be secreted into the bloodstream. Earlier work on the responses of mice to *T. b. brucei* infection showed that protective antibody responses are induced by fragments of parasites derived from senescent, nondividing forms, while actively dividing parasites do not stimulate antibody production. Research in 1984 focused on all stages of the immune response.

Studies on the proliferation of antibody-producing cells suggest that B-lymphocyte proliferative responses are stimulated by senescent trypanosomes. When mice were infected with a *T. b. brucei* clone which does not transform to nondividing forms until very high levels of parasitaemia are reached, they died before their B lymphocytes were stimulated to proliferate. This suggests that host B-cell proliferation, like antibody production, is induced by nondividing trypanosome forms, because in these experiments nondividing forms of the parasite did not appear until just before the death of the host.

Cell proliferation was stimulated when mice were infected with *T. b. brucei* clones which give rise to senescent forms rapidly or when they were inoculated with non-infective forms of *T. b. brucei*, including parasites killed by irradiation or freeze-thawing, formalin-fixed parasites or uncoated procyclic forms. In all cases, T lymphocytes were activated to proliferate earlier than B lymphocytes. Detailed investigations are being conducted on the kinetics of lymphocyte subpopulation proliferation in response to trypanosome infection, including the possible role of factors secreted by monocytes macrophages.

Studies of antibody production and secretion were conducted in 1984, using two mouse strains, one of which is relatively resistant to trypanosome infection and one relatively susceptible. Mice were infected with a *T. b. brucei* clone (GUTat 3.1a) which stimulates strong antibody responses in the resistant strain, but no antibody response in the susceptible strain. Following infection, T and B lymphocytes of both mouse strains were stimulated to multiply, as shown by increased synthesis of DNA. In both strains, these responses led to a high proportion of B lymphocytes (20% in the spleen) which contained cytoplasmic immunoglobulin (predominantly IgM). Some of the cells in both mouse strains contained antibody specific for the VSGs of the infecting parasites. However, only the resistant mice actually secreted serologically detectable, parasite-specific antibodies, which suggests that antibody secretion is blocked in the immunoglobulin-containing cells of susceptible mice.

Many of the immunoglobulin-containing cells produced by the two mouse strains had an unusual cytoplasmic morphology, characterized by highly distended cysternae of rough surfaced endoplasmic reticulum (Figure 16). Normal plasma cells which contain immunoglobulin have extensive rough endoplasmic reticulum organized as a series of flattened sacs. It is not possible without detailed statistical analysis of electron micrographs to ascertain whether or not the proportion of abnormal plasma cells is greater in susceptible than in resistant mice. However, this morphological feature is consistent with a block in antibody secretion by plasma cells in trypanosome-infected animals. In susceptible mice, the block in antibody secretion was rapidly reversed following removal of the parasites by the trypanocidal drug diminazene aceturate, which suggests that abnormal plasma cell function is associated with exposure of the host to living trypanosomes or short-lived factors from degenerating parasites.

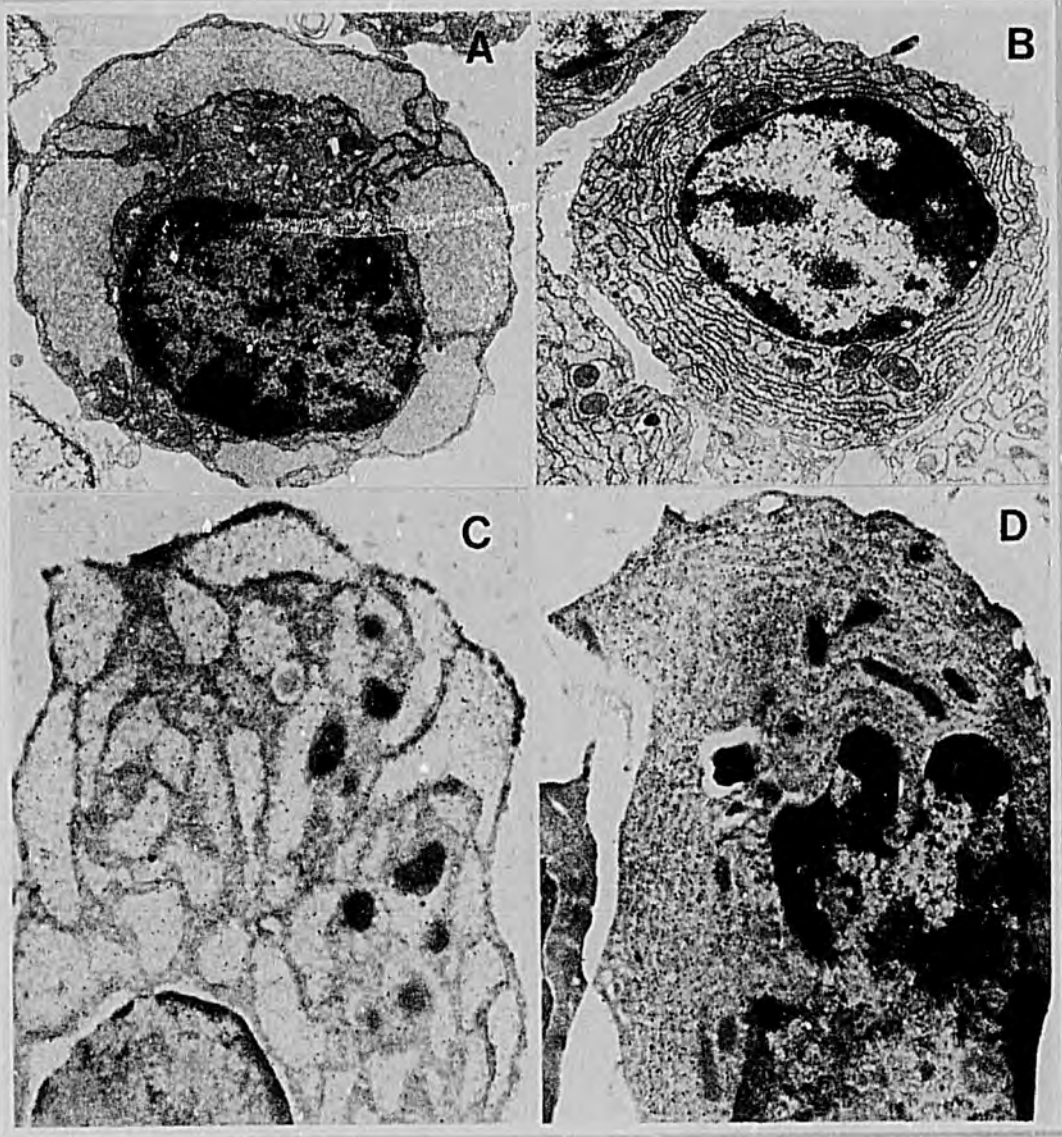

Figure 16. Electron micrograph of epoxy sections (A and B) and ultrathin frozen sections (C and D) of splenic plasma cells from mice infected with *T brucei*. A shows a plasma cell with highly distended endoplasmic reticulum which, when reacted with gold-labeled anti-immunoglobulin reagents (C), is found to contain antibody (black dots). B shows a plasma cell in which the endoplasmic reticulum is arranged as flattened sacs. These sacs also contain antibody (D). It is suspected that the cells shown in A and C have a block in their capacity to secrete antibody, which results in the antibody accumulating in the endoplasmic reticulum. This trypanosome-induced defect in plasma cell function may contribute to the immunodepressed status of trypanosome-infected animals.

It has previously been shown that immune mice produce protective antibodies which coat trypanosomes by binding with the parasite VSG. These coated trypanosomes are then destroyed by phagocytic cells (which digest foreign cells and particles), primarily in the liver. Parasites incubated in immune serum in vitro also become coated with specific antibody, and thus susceptible to phagocytosis when inoculated into normal

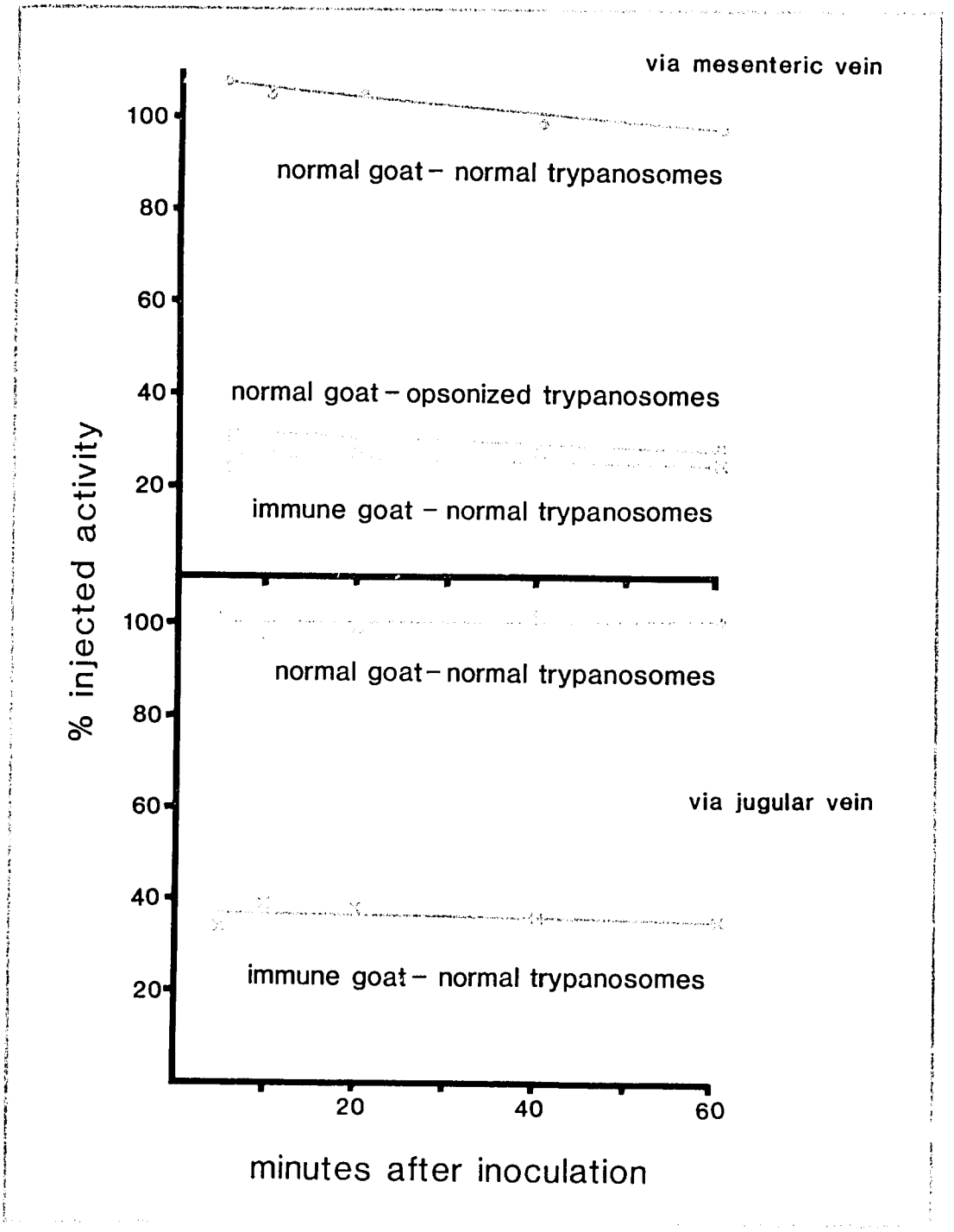

Figure 17. Trypanosomes radiolabeled with ^{75}selenomethionine were inoculated intravenously into goats. The parasites were either preincubated in normal or immune (opsonized) goat serum and inoculated into normal goats, or preincubated in normal goat serum and inoculated into normal or immune goats. The distribution of injected activity was assessed 60 minutes after inoculation. A high degree of clearance of trypanosomes from the bloodstream was achieved only in the presence of specific anti-trypanosomal antibody.

mice. A project carried out at ILRAD in 1984 in collaboration with Glasgow University (UK) showed that similar mechanisms operate in ruminants.

Radioactively-labeled trypanosomes were inoculated into normal goats and into goats immunized against the homologous VSG. Blood samples were collected at intervals over the following 60 minutes, at which point the goats were sacrificed and the lungs, liver and spleen removed. These organs were analysed in a gamma counter to determine the distribution of radioactivity as an indication of sites where phagocytosis had occurred. Results are shown in Figure 17. Trypanosomes were rapidly removed from the bloodstream of immunized goats and also from normal goats that were inoculated with trypanosomes coated with specific antibody. By contrast, trypanosomes remained in the circulation of goats in which antibody was absent. High radioactivity was found in the lungs and liver, the relative contribution of each being determined to some extent by the route of inoculation: inoculation in the jugular vein resulted in higher levels of phagocytosis in the lungs, while inoculation in the mesenteric vein favoured phagocytosis in the liver.

Another experiment was conducted to monitor bloodstream responses to trypanosome infection in four species of wildlife. An eland, an oryx and a waterbuck were infected with *T congolense* and a wildebeest was infected with *T b brucei*. When sera and peripheral blood leucocytes were taken from these animals and incubated with trypanosomes, high levels of neutralizing and phagocytosis-promoting IgM antibodies were produced compared with levels detected in cattle. This capacity to produce anti-trypanosomal antibodies may be partly responsible for the efficient parasite clearance and reduced pathogenic effects of trypanosomiasis observed in these species.

INVOLVEMENT OF THE CENTRAL NERVOUS SYSTEM

T b brucei, *T congolense* and both West and East African stocks of *T vivax* have been shown to infect the central nervous system of goats and cattle, and *T vivax* parasites have been observed in the aqueous humour of the eye. Trypanosomes in these areas evade the action of the trypanocides commonly used to treat livestock and thus may produce relapse infections following drug treatment. Primary and relapse infections are accompanied by lesions in the brain involving the choroid plexus and meninges. The development of a cannulation technique at ILRAD in 1983 has made it possible to monitor changes in the cerebrospinal fluid (CSF) of infected goats. This work has revealed that the composition of CSF from uninfected and chronically infected goats is markedly different, as shown by high-pressure liquid chromatography. CSF taken from the chronic stage of infection supports the survival and replication of trypanosomes in vitro, whereas normal CSF does not. Studies are now in progress to determine at what point after infection the CSF develops the capacity to support trypanosome growth and to analyze the factors responsible.

[illegible] OF [illegible] INFECTION

One of the most important pathogenic features of trypanosomiasis is anaemia, which is apparently caused by the destruction of red blood cells in the spleen, liver and lymph nodes. For the red blood cells to be recognized as 'foreign' and attacked by the host's own reticulo-endothelial system, their surface membrane must be altered in some way by the presence of the parasites. Trypanosome enzymes have been identified at ILRAD which catalyse the breakdown of phospholipids and perhaps proteins in the membranes of host red blood cells and lym-

phocytes. Research concentrates on purifying these enzymes, determining how they work and identifying chemical compounds which inhibit them.

Phospholipases (fat-splitting enzymes) A_2 and B were first identified by ILRAD scientists in *T brucei* and *T evansi* and in 1984 they were observed in extracts of *T vivax* and *T congolense*. The activities of these enzymes on specific substrates are shown in Figure 18. The phospholipase activities of the four pathogenic species are substantially greater than the activities associated with the non-pathogenic trypanosome, *T musculi*. Phospholipase A_2 is required to initiate the activity of phospholipase B, which in turn appears to be responsible for the destruction of red blood cells in vitro. Several experiments have suggested that the phospholipases act on trypanosome membranes, probably as the parasites are dying, and this action releases free fatty acids which may be responsible for the destruction of red blood cells. Progress has been made in purifying phospholipases from *T brucei*, though it is not yet possible to separate A_2 from B. Research is also in progress to determine whether trypanosomes contain phospholipases C and D.

In a preliminary experiment carried out in 1984, pairs of trypanosome-free waterbuck, oryx, buffalo and cattle were infected with *T b brucei* (MITat 1.2). Red blood cells were collected from these animals over a 38-day period and examined for signs of destruction by trypanosome enzymes. Cells from the two waterbuck were completely resistant to enzyme destruction throughout the study period, cells from the two cattle exhibited very different susceptibilities and cells from the two oryx showed intermediate susceptibility. During the early stages of infection, cells from the buffalo showed intermediate susceptibility, but they became completely resistant to trypanosomal enzyme activity 3 weeks after infection. The anticipated availability of purified trypanosome phospholipases will make it possible to carry out more detailed studies on the effects of these enzymes on the red blood cells of various hosts.

A procedure was developed in 1983 to identify specific trypanosomal proteinases (protein-splitting enzymes). This is done by separating the enzymes according to their molecular weight and analyzing the digestive action of the separated enzymes on specific protein substrates. Proteolytic enzymes are generally classified on the basis of substrate specificity as well as by the type of chemical compounds which inhibit their enzymic activity. Tests with more than a dozen compounds indicate that the proteolytic activity detected in trypanosomes falls into the category of SH- or cystein-dependent proteinases, which we have called trypanopains. Among the compounds tested, both diminazene aceturate, a widely used bovine trypanocide, and pentamidine, a drug used to treat human sleeping sickness, have a strong inhibitory effect on trypanopain activity.

Bloodstream forms of trypanosomes have abundant trypanopain activity, while uncoated procyclic forms show only trace amounts. In *T b brucei*, *T b gambiense* and *T evansi*, the predominant trypanopain activity has a relative molecular weight of approximately 29,000, while the relative molecular weight of trypanopain activity in *T congolense* is approximately 31,000. In addition, these parasites sometimes exhibit proteinase activities at higher relative molecular weights, which may be precursor forms of the 30,000 activity.

Trypanopains extracted from these trypanosome species are now being purified. Using partly purified trypanopains from *T b brucei* and fluorescently labeled synthetic substrates, studies have indicated that these enzymes may be some of the most powerful of the known proteinases, showing, for instance, about 100 times stronger binding between enzyme and substrate than several trypsin-like enzymes. Several observations suggest that the kinetics of protein binding and lysis by trypanopains are complex, and work continues to purify these enzymes with

Trypanosome Species	Phospholipase Activity (pmoles/10^9 trypanosomes/min) B	A_2
T vivax (2337)	773	73
T b brucei (MITat 1.2)	2106	381
T congolense (ILNat 2.1)	221	86
T evansi	288	20
T musculi	3	13

Figure 18. Comparison of phospholipase activities in five trypanosome species.

the aim of carrying out precise kinetic studies. Efforts are also being made to raise antibodies against the trypanopains. The availability of enzyme-specific antibodies should make it possible to identify the location of trypanopains within the parasite and to determine whether the enzymes are released by the trypanosome into the host bloodstream or chancre.

Some East African strains of *T vivax* are capable of producing an acute haemorrhagic syndrome in cattle, characterized by a severe drop in the number of red blood cells and platelets, a dramatic enlargement of the spleen and extensive haemorrhaging, particularly in the gut. This syndrome is of interest to researchers because it demonstrates in extreme form the mechanisms of anaemia which occur in other forms of African animal trypanosomiasis, and perhaps parallels the platelet destruction, haemorrhaging and disseminated intravascular coagulation (DIC) syndrome noted in some cases of human sleeping sickness.

In 1983, ILRAD scientists produced experimental *T vivax* (ILRAD 2337) infections in cattle which demonstrated most of the signs of the haemorrhagic syndrome. These were repeated and extended in 1984, using a second *T vivax* isolate. Intradermal inoculation with bloodstream forms of the parasite produced the syndrome as effectively as tsetse-transmitted infection with metacyclic trypanosomes. Quick-frozen plasma samples taken from infected cattle have provided material for immunological and biochemical analysis to identify trypanosome enzymes. Free sialic acid, suggestive of elevated neuraminidase levels, was found in the plasma, but not at high levels. *T vivax* parasites possess phospholipase A_2 and B activities, but these were not detected in measurable amounts in plasma from infected animals. However, a change in the red blood cells of infected animals is readily apparent, and it seems likely that one of these enzymes, or perhaps trypanosomal free fatty acids, damage bovine red blood cells early in infection.

All the infected cattle produced increased levels of antibodies to normal red blood cells and normal platelets about 30 days after infection. In some animals, these 'autoantibodies' disappeared with increasing control of parasitaemia, whilst in others they persisted at substantial levels until death. These observations suggest that trypanosomal enzymes damage red blood cells and platelets and these damaged cells then become targets for the animal's own immune system. Scientists are now analysing surface changes in the blood cells of infected cattle and investigating to what extent the bone marrow is capable of compensating for the loss of red cells and platelets. Experiments are also in progress to determine whether neuraminidase is the trypanosomal enzyme, or one of the enzymes, responsible for the initial damage to blood cells.

A project was initiated at the end of 1984 to investigate the effects of *T congolense* infection on the reproductive cycle of cattle. This work is being carried out in col-

laboration with the Centre for Tropical Veterinary Medicine and the Veterinary School's Department of Reproduction at the University of Edinburgh.

EPIDEMIOLOGY

CHARACTERIZATION OF TRYPANOSOME POPULATIONS

Considerable research has been conducted at ILRAD aimed at developing simple, accurate and sensitive assays to detect trypanosomes in infected animals and tsetse flies and to determine parasite species and serodemes. This work is important for diagnosis and treatment and 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.

Studies aimed at testing and applying methods for typing trypanosome stocks on the basis of their antigenic repertoire concentrate on *T congolense* populations isolated in 1982 from a large dairy farm near the Kenya coast. Further objectives of this work are to determine the number of serodemes of different trypanosome species affecting animals in a limited geographic area and to determine what degree of immunity, if any, develops in cattle in an area where the the number of trypanosome antigenic types is limited. A total of 81 *T congolense* stocks were isolated from 20 indigenous and 20 tracer cattle exposed to infection in the study area. Analysis by indirect immunofluorescence and neutralization using anti-metacyclic antisera has led to the identification of three different *T congolense* serodemes. The three serodemes are present in all the 12 stocks analyzed so far, which suggests that the 81 isolates may represent a small number of serodemes.

Sera obtained from tracer cattle 3, 6 and 8 months after exposure to infected tsetse on the ranch have been screened for antibody activity against two of the *T congolense* serodemes identified. Neutralizing antibodies were detected in sera of the tracer cattle as early as 3 months after exposure, indicating that they might have acquired resistance against metacyclic forms of these serodemes. However, results show that immunity is lost in the absence of continuing tsetse challenge. This has important implications for the acquisition of immunity in field situations where tsetse activity is highly seasonal.

Studies have continued in the field of *T vivax* epidemiology by focusing initially on a well characterized, rodent-infective serodeme (ILDar 1) originating in Nigeria. Goats were infected by tsetse flies that had ingested single clones of the ILDar series, and the antigens arising in parasite populations from the first peak of parasitaemia were analysed using mouse antisera to over 30 clones of the serodeme. These parasite populations displayed a complex mixture of surface antigens; more than 10 antigenic types were sometimes present although trypanosomes bearing the VSG of the ingested clone were absent. When the parasite populations were expanded in irradiated mice, they continued to show antigenic mixtures, though occasionally there was a tendency towards the development of predominant antigenic types. Few attempts to raise antigenically homogeneous parasite populations have been successful, even in irradiated mice, demonstrating the dramatic antigenic instability of some of these clones.

Particular interest focuses on the antigens displayed by metacyclic trypanosomes because these are the forms which initiate infection in the mammalian host. However, it is difficult to obtain enough metacyclic forms of *T vivax* for antigenic studies because infected tsetse extrude very few metacyclic forms of this species. For this reason, metacyclic forms and 'second-generation' bloodstream forms of West African and East African *T vivax* serodemes are being produced in vitro which will be used to produce monoclonal antibodies. It is hoped that these can be tested against metacyclic forms in fly probes and first-peak parasitaemias in cattle and goats to detect the specific metacyclic antigenic types. This work is being expanded considerably in 1985.

To diagnose trypanosomiasis, it is necessary to demonstrate the parasites themselves or to measure host antibody responses to parasite antigens. However, it is often difficult to detect trypanosomes in an infected host because only small numbers of parasites are present in the bloodstream and these populations wax and wane rapidly during the course of infection. Tests devised to diagnose infection by measuring antibody response require parasites or specific antigens derived from parasites, but no specific antigens are yet available for the diagnosis of African trypanosomiasis.

At present, serological diagnosis of trypanosomiasis is frequently carried out by incubating serum from infected animals with trypanosomes of known species and measuring serum antibody levels by the IFA test. This test is not completely reliable because non-specific immunofluorescence is produced which sometimes leads to false diagnosis. Work was carried out in 1984 to improve the fixation of the trypanosomes used for diagnosis, and the accuracy of the test was also increased by absorbing blood samples with lymphocyte lysates before exposing them to parasites.

Use of monoclonal antibodies

Monoclonal antibodies have been produced at ILRAD which distinguish the three major species of African trypanosomes. The monoclonal antibody derived against *T b brucei* has reacted with all the *T b brucei*, *T b rhodesiense* and *T b gambiense* isolates tested, but with no isolates of *T congolense*, *T vivax* or *T evansi*. Similarly, the monoclonal antibody against *T vivax* reacted with all the *T vivax* isolates tested, but with no isolates of the other trypanosome species. *T congolense* monoclonal antibodies reacted with *T congolense* and *T simiae* only.

The *T brucei* monoclonal antibody was used to detect antigens circulating in the blood of *T b brucei*-infected cattle in a sandwich enzyme-linked immunosorbent assay (ELISA). Circulating antigens were detected 2 weeks after the animals were bitten by infected tsetse flies. Thereafter, the antigens could be detected in blood taken every third day throughout the course of infection, including several days when parasites could not be seen by light microscopy.

This monoclonal antibody was used in the same assay to detect circulating antigens in blood from patients known to be infected with Rhodesian or Gambian sleeping sickness. When 20 samples were tested from Rhodesian sleeping sickness patients, provided through collaboration with the Uganda Trypanosomiasis Research Organization, the monoclonal antibody detected antigens in 18, but did not react to normal human serum controls. The two samples which were not recognized were from patients who had been treated with trypanocidal drugs. The monoclonal antibody also detected antigens in blood samples from 20 out of 21 Gambian sleeping sickness patients provided by the Université de Kinshasa in Zaire.

The *T vivax* monoclonal antibody detected antigens in the blood of cattle 3 weeks after they were bitten by *T vivax*-infected tsetse.

Antigens were detected throughout the course of infection, even at times when trypanosomes could not be detected in blood samples by microscopic examination. Following treatment with diminazene aceturate, some cattle cleared the trypanosome antigens from their circulatory system within 1 week while others took 2 weeks. Present work involves testing monoclonal antibodies which recognize *T congolense* infections and developing assays to distinguish trypanosome species from infected tsetse flies. Efforts are also being made to simplify the assay system for use in the field.

A study completed at ILRAD in 1984 showed that DNA hybridization techniques could be used to distinguish between different trypanosome species, and also between subspecies and serodemes. However, the method involves the isolation of pure trypanosome DNA and Southern blotting, which are too cumbersome for general field use. In addition, the probes are of low sensitivity and large numbers of parasites are required.

Scientists elsewhere have demonstrated the use of repetitive DNA sequences to detect very small numbers of *T cruzi* and *Leishmania* parasites and, in the case of *Leishmania*, to discriminate between different subspecies. ILRAD scientists are thus investigating the use of repetitive sequences from the genomes of *T brucei*, *T congolense* and *T vivax* as probes to detect these trypanosome species.

Most of the repetitive sequences isolated from the three trypanosome species appear to be strongly species specific. The most sensitive probe is one from the *T congolense* genome which can detect amounts of purified DNA equivalent to 24 trypanosomes or 80 to 100 trypanosomes in blood diluted in PSG buffer. Probes for *T vivax* and *T brucei* can detect the DNA equivalents of about 240 parasites. All three *T brucei* probes tested so far recognize five *T b brucei*, two *T b rhodesiense* and five *T b gambiense* strains equally well, and to a lesser extent they also recognize strains of *T evansi*. These probes have been used to identify trypanosome species being propagated in vitro, and for this purpose they have been extremely useful. Scientists are now searching for ways to prepare material from infected tsetse so that the probes may be applied to epidemiological studies in the field.

To use these probes, trypanosome DNA must first be isolated from infected blood and immobilized on nitrocellulose filters. The most promising of the several procedures tested so far involves blood lysis and the extraction of DNA with organic solvents, though some of the potential sensitivity of the probes is lost by this technique. Intensive work now focuses on making this procedure simpler and more efficient. Studies are also in progress to prepare and test stable, nonradioactively-labeled probes—perhaps based on biotin—which would increase the applicability of DNA hybridization for trypanosomiasis diagnosis and epidemiology in moderately equipped laboratories. The methodology may equally well be applied to identifying specific trypanosome subspecies or serodemes.

Tsetse control programs implemented in many African countries have relied primarily on insecticide spraying, but considerable effort has been expended to evaluate the effectiveness of the sterile insect release method against *Glossina morsitans morsitans* in Zimbabwe and Tanzania and *G palpalis gambiensis* in Bourkina Faso. This control method involves the release of large numbers of sterilized male flies. Since female tsetse normally mate only once, if most or all females in an area mate with sterile males

the next generation of flies will be substantially reduced.

In a research project partially funded by the International Atomic Energy Agency, ILRAD scientists have shown that male tsetse sterilized by γ-irradiation can transmit the three major pathogenic trypanosome species as effectively as normal tsetse. Earlier studies on the vectorial capacity of sterilized male *G m centralis*, *G austeni* and *G tachinoides* were expanded in 1984 to test the capacity of sterilized males of these three species plus *G palpalis palpalis*, *G p gambiensis* and *G fuscipes fuscipes* to transmit East and West African stocks of *T vivax* and *T congolense*. Results (Figure 19) show very small differences in the infection rates of irradiated and normal male tsetse flies. As in previous studies, *G m centralis* was a much more efficient vector than the other species and subspecies examined.

In previous years a serological method was developed to identify the source of tsetse bloodmeals by analysing minute quantities of residual blood obtained from captured flies. Antisera have been raised against 42 species of mammals and 4 species of birds. In collaboration with the International Centre of Insect Physiology and Ecology (ICIPE), ILRAD scientists used a panel of 20 antisera in 1984 to analyse 697 bloodmeal samples from tsetse flies captured in western Kenya. Because it is difficult to standardize antisera, work has also been in progress to derive species-specific monoclonal antibodies for the analysis of tsetse bloodmeals. Monoclonal antibodies were produced in 1984 which recognize bloodmeal samples from tsetse flies which have fed on cattle, pigs, eland, bushbuck or the Alcelaphinae family (wildebeest, hartebeest and topi).

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 prob-

	E African *T vivax* (IL 2241)	W African *T vivax* (IL 1392)	E African *T congolense* (IL 2047)	W African *T congolense* (IL 2281)
G m centralis				
sterile	56.0	50.0	26.0	47.0
fertile	61.0	46.0	26.0	42.0
G austeni				
sterile	4.0	50.0	0.0	2.0
fertile	2.0	55.0	0.0	3.0
G p palpalis				
sterile	0.0	45.0	1.0	3.0
fertile	0.0	40.0	0.0	0.0
G p gambiense				
sterile	2.0	58.0	0.0	0.0
fertile	1.0	63.0	0.0	0.0
G f fuscipes				
sterile	0.3	42.0	2.0	1.0
fertile	0.5	41.0	7.0	0.0
G tachinoides				
sterile	0.0	42.0	1.0	1.0
fertile	0.3	42.0	1.0	2.0

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

ably function as a back-up defence mechanism to protect feeding tsetse from the potentially harmful effects of a clotted blood meal. An assay for detecting these fibrinolytic enzymes has been established, and one has been purified to apparent homogeneity and the molecular weight determined. Present activities involve purifying all these enzymes to homogeneity and investigating whether antibodies against the purified enzymes might be used to interfere with tsetse feeding and trypanosome transmission.

Polyspecific antibodies were isolated from sera of rabbits bitten by tsetse flies or injected with crude tsetse salivary gland extract. Electron microscopy studies of tsetse salivary gland sections have shown that these antibodies recognize proteins in the lumen of the salivary gland and the golgi apparatus of the cells surrounding the lumen. They also appear to recognize constituents of tsetse saliva.

LIVESTOCK PRODUCTION UNDER TRYPANOSOMIASIS RISK

While work continues on the development of vaccines or other measures for improved trypanosomiasis control, considerable research also aims at analysing and ameliorating the constraints on livestock production in tsetse-infested areas which must rely on the control measures currently available. Studies at ILRAD concentrate on two approaches to livestock production under trypanosomiasis risk: the use of indigenous African livestock breeds which display a degree of genetic resistance to trypanosomiasis, and the regular use of trypanocidal drugs for prophylaxis or treatment. Studies were also carried out in 1984 to evaluate the effects of age on resistance to trypanosome infection.

A network of research sites is being coordinated by ILCA in West and Central Africa to study the productivity and health of different breeds of domestic livestock in different ecological zones, under different management regimes and exposed to different levels of quantified tsetse challenge. Particular attention focuses on the indigenous taurine cattle breeds of West and Central Africa—the N'Dama and West African Shorthorn—which are significantly more resistant to trypanosomiasis than *Bos indicus* or European *Bos taurus* breeds. ILRAD's role is to advise and assist in recording the health status of the animals under observation, monitoring tsetse populations in the study areas and applying existing and newly developed measures to control trypanosomiasis in field situations.

Network sites are in operation in Gabon, Zaire, Nigeria, Ivory Coast and Togo. Further sites will be added to the network in The Gambia, Benin, Nigeria, Zaire and Guinea, and data are being collected along the same principles at sites in East Africa.

To make valid comparisons between different livestock breeds, tsetse challenge levels and management systems, it is of crucial importance to collect accurate information and to use standardized observations and measurements. To achieve this goal, field staff working at the network sites attend a 6-week training course at ILRAD and are closely supervised by ILCA and ILRAD scientists. A preliminary analysis is carried out after 12 to 18 months of data collection at each site, and final analysis is completed after 3 to 4 years.

Data collection in Gabon began in September 1981, covering trypanotolerant N'Dama and susceptible Nguni cattle and crossbreds on the government-operated Okouma Ranch. ILRAD surveys have detected three tsetse species on the ranch, *G palpalis*, *G tabaniformis* and *G nashi*, and *T vivax* and *T congolense* infections

have been identified in captured flies. Some cattle on the ranch are treated regularly with isometamidium chloride and diminazene aceturate, while others are only treated with diminazene aceturate on an individual basis when disease is apparent or when trypanosomes are diagnosed together with anaemia, indicated by a drop in packed cell volume (PCV).

Trypanosome infection rates varied from 5 to 20% among the N'Dama and from 15 to 55% among the Nguni and crossbred cattle. Mortality in the Nguni groups averaged 53%, while the mortality rates in the N'Dama and crossbred groups were 10%. Calving rates over the 1982/83 breeding season were highest among cows treated regularly with trypanocidal drugs. Among breed groups, the N'Dama achieved the highest calving rates followed by the Nguni and the crossbred groups.

Data have been collected since 1983 from two privately owned cattle ranches in Zaire raising N'Dama cattle. An ILRAD tsetse survey revealed *G palpalis* at Kolo ranch, but none of the dissected flies were infected with trypanosomes. Two tsetse surveys conducted at Mushi ranch detected *G palpalis* and *G tabaniformis*. *T vivax* and *T congolense* infections were identified in captured flies, with infection rates varying according to season. Monthly monitoring on this ranch has revealed a 6.5% trypanosome infection rate in the N'Dama cattle.

Cattle and sheep production under village conditions is also being monitored at two sites in Ivory Coast in collaboration with the German (Federal Republic) Gesellschaft für technische Zusammenarbeit. During the first 4 months of 1984, trypanosome infection rates averaged 8.3% in cattle and 4.2% in sheep, with *T congolense* seen more often in cattle and *T vivax* more often in sheep.

Genetic resistance in East African cattle

Previous work at ILRAD has demonstrated that two East African *Bos indicus* cattle breeds—Boran and East African Zebu—are less susceptible to *T congolense* infection than European taurine breeds. The Kenya Trypanosomiasis Research Institute (KETRI) carried out a 3-year study at Galana Ranch near the Kenya coast which also suggested that certain types of Boran cattle possess a degree of resistance to trypanosomiasis. In 1984, a collaborative study was conducted to assess the basis of these observations.

Thirty 2-year-old Boran steers were brought from Galana to KETRI headquarters at Muguga near ILRAD. These cattle comprised 15 Orma Boran originating from southeastern Kenya and 15 Galana Boran originally from northern Kenya. On arrival at KETRI all were treated with diminazene aceturate and blood tests 50 days later showed no antibody activity against trypanosomes. Twelve cattle in each group were then inoculated intravenously with 1 million parasites of a *T congolense* clone (IL 1180) known to be virulent to cattle. The remaining three cattle in each group were kept as controls. All cattle were then monitored for 14 weeks according to clinical, haematological, parasitological and immunological parameters.

Following inoculation, all cattle became infected and in the course of 14 weeks showed clinical evidence of disease, as judged by lymph node enlargement, weight loss and episodes of fever. Although variation in response was apparent in each group, the Galana cattle were significantly more susceptible to trypanosomiasis than the Orma, as shown by the level and duration of parasitaemia, the onset and significance of anaemia, weight gains and losses, and the number of animals in each group requiring treatment with trypanocidal drugs. The MHC type of resistant and susceptible animals is now being determined. Although it must be assumed that all the animals in this

study had previously been exposed to trypanosomiasis, there was no immunological evidence that they had experienced the *T congolense* serodeme used in the experiment. Thus the results probably reflect a degree of innate resistance among the Orma Boran, rather than any acquired status.

Cattle production using trypanocidal drugs

Chemotherapy and chemoprophylaxis, employing the limited number of trypanocidal drugs available, remain the most widely used methods for controlling bovine trypanosomiasis in the field. However, despite extensive use of trypanocides for more than 30 years, the factors which influence the effectiveness of a therapeutic strategy are poorly understood. These include the effects of drug dosage, level of trypanosome challenge and previous infection on the duration of drug protection, as well as the possibility of immunity developing under different conditions of drug treatment and trypanosome challenge.

In an experiment conducted in 1984 in collaboration with the University of Glasgow, UK, 24 Boran steers from 14 to 18 months old were given 1 mg/kg of the trypanocidal drug isometamidium chloride as a single deep intramuscular injection. They were divided into six groups of four animals each, and challenged at different intervals with five tsetse flies (*G m centralis*) infected with a virulent serodeme of *T congolense* (IL 1180). One group was challenged monthly from 1 month to 5 months after treatment and the other groups were each challenged on one occasion at different periods up to 5 months after treatment.

During the 5-month period, no treated cattle developed detectable local skin reactions or anti-trypanosome antibody responses, and none became infected, whereas all controls developed chancres, became infected and mounted immune responses against the infecting serodeme. Infected tsetse flies which fed on treated cattle showed no effects in terms of infection rates or the ability to transmit infections to mice compared with infected flies which fed on untreated controls.

Six months after treatment, all the cattle were subjected to a final challenge, either by five infected tsetse flies on one occasion as before, or by five infected tsetse on 10 consecutive days, or by intradermal inoculation of in vitro-cultivated metacyclic trypanosomes at doses ranging from 500 to 500,000 parasites. Eight of the 24 treated cattle became infected between 12 and 29 days after challenge, but there was no correlation between breakdown of drug protection and dose or method of challenge or the previous challenge history of individual animals. Antibody responses were detected in all cattle which became infected, but in none of the animals which resisted infection. All breakthrough trypanosome populations were completely susceptible to isometamidium chloride when tested in mice.

Thus, isometamidium chloride, administered at a dosage of 1 mg/kg bodyweight, protected cattle against *T congolense* challenge for at least 5 months. Two-thirds of the cattle tested resisted infection at 6 months, even when subjected to multiple fly challenge or intradermal inoculation of as many as 500,000 cultured metacyclic trypanosomes (equivalent to 5000 infected tsetse bites). Protection appeared to be mediated by the drug alone, since no antibody responses to the trypanosomes were detected. This indicates that trypanosome challenge does not prime immune responses in animals under chemoprophylactic cover, at least under the conditions of the experiment. Experiments are now in progress to confirm the extent of drug protection against other serodemes of *T congolense* and to determine in more detail the effects of drug dosage, previous infection and level of challenge.

ILCA has also coordinated a detailed study of animal health, productivity and trypanocidal drug treatment at Mkwaja ranch in Tanzania in collaboration with ILRAD, May and Baker Ltd and the ranch owners. The ranch is heavily infested with three species of tsetse fly, *G m morsitans*, *G pallidipes* and *G brevipalpis*. Boran cattle have been maintained for over 10 years under prophylactic treatment with isometamidium chloride and less frequently with diminazene aceturate. This situation offered a unique opportunity to evaluate the longterm viability of beef cattle production relying on trypanocidal treatment in an area of severe trypanosomiasis risk.

The most important parameters for beef cattle production are calving rates, cow and calf viability, calf growth rates and cow weight. These characteristics can be combined to build up an index of total weight of weaner calf produced per 100 kg metabolic weight of cow maintained per year. A calculation of this index allows comparison with other livestock production situations in Africa. The most interesting comparison (Figure 20) is with the Boran cattle ranches in tsetse-free areas of Kenya. Although Boran cattle at Mkwaja were inferior in nearly all performance traits, herd productivity, expressed as weight of 8-month-old weaner calf produced per 100 kg metabolic weight of cow maintained per year, was within 20% of productivity achieved by the Kenya ranches, which are considered among the best in Africa. Despite the extensive use of trypanocidal drugs, there was no indication of drug resistance or evidence that the severe inoculation lesions induced by isometamidium chloride had affected productivity.

A project was initiated in 1982 in collaboration with the Kenya Ministry of Agriculture and Livestock Development to monitor the health and productivity of East African Zebu cattle maintained under village conditions at Muhaka on the Kenya Coast. The area is infested with *G pallidipes*, *G brevipalpis* and *G austeni*, and trypanosome infection rates in cattle tested monthly since October 1982 average from 1 to 8%. *T congolense* is detected more

	Mkwaja Boran	Kenya Boran
Reproductive performance		
age at first calving (months)	47	40
calving rate (%)	75	87
Viability		
preweaning viability (%)	92	95
annual cow viability (%)	94	98
Body weights		
weaned calf weight at 8 months (kg)	134	174
estimated mature cow weight (kg)	293	414
Herd productivity index (kg per annum)		
weight of 8-month-old weaner calf produced per cow	87	140
weight of 8-month-old weaner calf produced per 100 kg bodyweight of cow	30	34
weight of 8-month-old weaner calf produced per 100 kg metabolic weight of cow	138	173

Figure 20 Production performance of Boran cattle under heavy trypanosomiasis challenge on Mkwaja Ranch, Tanzania, compared with Boran on 11 ranches in trypanosome-free areas of Kenya. Averages are derived from 12,000 recorded data entries from Mkwaja and 16,000 from the Kenya ranches.

frequently than *T vivax*, though cases of acute haemorrhagic *T vivax* infection are occasionally encountered. Two-thirds of the cattle are treated on a regular basis with isometamidium chloride, while the others, acting as controls, receive only occasional curative treatment. The productivity of these cattle will be compared, with results expected in 1985 or 1986.

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Several studies have suggested that young animals resist trypanosomiasis better than adults. While maternal antibodies acquired in colostrum may contribute to resistance in newborn animals, they cannot account for resistance observed after the first few days of life. Experiments were conducted at ILRAD in 1984 to test the susceptibility to *T congolense* of Boran cattle of various ages, infected both by syringe inoculation and by tsetse flies. Six 9- to 12-month-old Boran cattle and 10 8-week-old calves were each inoculated intravenously with 5 million *T congolense* (IL 552) parasites. All became infected, but clear differences in susceptibility emerged between the two age groups during the 30-week study period. The onset of anaemia and parasitaemia was similar in both groups, but the older animals showed consistently more severe anaemia beginning 6 weeks after infection. The level of parasitaemia in the calves was consistently lower than in the older cattle, though the differences were not as marked as differences in anaemia. This might indicate that the younger animals are more capable of regenerating red blood cells. Two of the older cattle died of trypanosomiasis, while all the calves survived. During the final weeks of the study, all surviving animals in both groups made a complete clinical recovery.

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-doctorate positions for young scientists. In addition, a number of training courses and conferences are held each year. The emphasis is primarily on developing veterinary research and implementation capabilities in African countries where trypanosomiasis and theileriosis are important animal health problems. Altogether, training activities account for nearly 10% of ILRAD's total budget.

Participants in ILRAD's training program work closely with the scientific staff. In 1984, 25 scientists and technicians came to ILRAD for specialized training on an individual basis for periods from 1 week to 9 months. Two were associate experts with FAO (Food and Agriculture Organization of the United Nations) projects and the others were on the staff of veterinary laboratories, universities or research centres; 23 of these scientists and technicians came from African countries, as shown in Figure 21, and two, who were self-sponsored, came from the USA.

Post-graduate students work as Research Fellows at ILRAD from 1 to 4 years to fulfill requirements for a post-graduate degree. In 1984, 17 Research Fellows from 10 different countries worked with the scientific staff on projects closely linked to ILRAD's research programs. In addition, 6 Visiting Research Fellows worked at ILRAD for periods of 2 to 6 months. Out of 23 post-graduate students, 16 came from African countries and 7 from Europe (see Figure 21). The students from Africa were supported fully or partially by ILRAD, while the European students were supported by funds from their home countries.

Post-Doctoral Fellows are selected on an international basis, with emphasis on recruiting well-qualified young scientists from African countries. They normally spend 2 years at ILRAD, with their work contributing directly to the research program. In 1984, 13 Post-Doctoral Fellows were working at ILRAD, including 2 from Kenya, 2 from the UK, 5 from the USA, 1 from Belgium, 1 from France, 1 from Germany (Federal Republic) and 1 from Rwanda.

In addition to training activities for individuals, ILRAD conducted five training courses in 1984. A 4-week course was held in May and June on the diagnosis of haemoprotozoan diseases, with emphasis on trypanosomiasis. The course included a review of basic biochemical and immunological concepts and practical instruction on how to produce antibodies, isolate and purify

Country	1983	1984	Country	1983	1984
Specialized technical training					
Burundi	1	—	The Gambia	1	1
Ethiopia	—	1	Uganda	1	4
Kenya	5	9	Zambia	2	1
Malawi	1	—	Zimbabwe	1	—
Mali	1	—	India	3	—
Rwanda	1	1	Sri Lanka	1	—
Somalia	1	2	Australia	1	—
Sudan	—	1	USA	—	2
Tanzania	1	3	Total	21	25
Post-graduate training					
Kenya	9	10	Belgium	2	1
Nigeria	—	1	France	—	1
Rwanda	1	—	Germany (FR)	—	1
Sudan	1	1	Italy	—	2
Tanzania	—	1	Netherlands	—	1
Tchad	—	1	UK	—	1
Uganda	2	1	Total	15	23
Zaire	—	1			
Post-doctoral training					
Kenya	2	2	Germany (FR)	2	1
Rwanda	—	1	Japan	1	—
Uganda	1	—	UK	1	2
Zaire	1	—	USA	2	5
Belgium	1	1	Total	11	13
France	—	1			

Figure 21. Individual training activities at ILRAD in 1983 and 1984: number of participants in different types of training by country of origin.

immunoglobulins, assess specificities and prepare antigens. Participants also learned how to perform a range of serodiagnostic tests for the diagnosis of haemoprotozoan diseases. Seventeen participants took part in the course from 12 African countries.

A second 4-week course was held in June in Bobo-Dioulasso, Bourkina Faso, covering the same material in French. This course was cosponsored by ILRAD, the Centre de Recherches sur les Trypanosomoses Animales and the Ecole de Lutte anti-Tsétsé. Eleven French-speaking participants attended from 10 African countries. All were staff members of veterinary diagnostic laboratories.

Two 6-week courses were held at ILRAD in April/May and October/November 1984 to train field staff working with the ILCA/ILRAD trypanotolerance research network in Kenya, Nigeria, Somalia, Gabon, Togo and Zaire. Six participants took part in the first course, which was conducted in English, and five participated in the second course, conducted in French. Both courses concentrated on the diagnosis and control of trypanosomiasis, helminthiasis and tick-borne diseases. The major emphasis was on practical aspects of clinical diagnosis, bleeding techniques, serodiagnosis and post-mortem examination of cattle and goats. Course work also included tsetse fly identification, ageing and dissection, determination of trypanosome infection rates and tsetse bloodmeal preparation and preservation, and participants practised tsetse trapping techniques in the Kibwezi Forest.

Six participants attended a 3-month course conducted at ILRAD in 1984 on the preparation and use of reagents in the diagnosis of haemoprotozoan diseases in cattle. All were from African countries. In addition, a senior lecturer from the Department of Mathematics at the University of Strathclyde, UK, visited ILRAD for 2 months in October and November to give lectures and help scientists with experimental design and data analysis techniques. He prepared six training modules which are now available at ILRAD on data presentation and choice of sample size, exploratory data analysis, minitab, two-sample comparisons, experimental designs and basic programing for microcomputers.

An international workshop was held in October to discuss the implementation of ECF immunization programs in East and Central Africa, sponsored jointly by ILRAD and FAO. Eighty participants attended, including senior government officers concerned with the control of livestock diseases in Burundi, Kenya, Malawi, Mozambique, Rwanda, Sudan, Tanzania, Uganda, Zaire, Zambia and Zimbabwe. Other participants were staff members from international organizations and donor agencies, representatives of pharmaceutical companies and specialists in tick-borne diseases and their vectors. The workshop began with reports on the incidence and importance of theileriosis in different countries. The participants then discussed the results of field trials and presented papers on different approaches to immunization against ECF and related problems and needs. Participants from most of the countries represented agreed to initiate, or extend, field trials, and results will be compared through an information network which will promote the regular exchange of data, materials and expertise. A report on the workshop is being published by ILRAD in 1985.

Seminars are given by ILRAD scientists throughout the year, presenting the results of ongoing research projects. Seminars are also presented by other scientists carrying out research on related topics. Altogether, 30 seminars were given at ILRAD in 1984, and these were well attended by ILRAD staff members and scientists from other laboratories in the Nairobi area.

Two ILRAD scientists gave a series of lectures on trypanosomiasis and ECF to final-year veterinary students at the Universidade Eduardo Mondlane in Maputo, Mozambique in November 1984 as part of a seminar sponsored by FAO. ILRAD scientists also worked with staff from the Institut de Recherche Agronomique et Zootechnique in Burundi to assess the training needs of government officers from Burundi, Rwanda and Zaire in the field of veterinary epidemiology. Teaching material for parasitology courses was supplied to the University of Nairobi, the Veterinary Diagnostic Laboratory in Guyana and the Ministry of Agriculture in Togo.

[illegible] PUBLICATIONS

ILRAD's information services concentrate on publication and distribution of the annual report and the quarterly newsletter, *ILRAD Reports*. Both of these publications are prepared in English and French.

Four issues of *ILRAD Reports* were published in 1984, with major articles on in vitro cultivation of trypanosomes, electron microscopy studies focusing largely on *Theileria*, research on tsetse flies as trypanosomiasis vectors, and an intensive study on haemorrhagic *T vivax*. Shorter articles described other research activities and conferences held at ILRAD. Articles published by ILRAD staff members in scholarly journals are also listed in *ILRAD Reports* with information on how they may be ordered. ILRAD's quarterly newsletter received an award of excellence in 1984 from Agricultural Communicators in Education, a professional editors' association in the USA.

Members of ILRAD's scientific staff published 33 articles in international jour-

nals and 3 chapters in scientific books in 1984. These scholarly publications, plus numerous papers presented at international conferences, comprise the bulk of ILRAD's information output for specialized scientific audiences. Considerable effort has been directed towards ensuring the distribution of these publications to government officers, university lecturers and researchers in developing countries who may not have access to international scientific journals. To meet this objective, a brochure, *Scientific publications from ILRAD: 1982-83 update*, was produced in 1984, listing articles and chapters published by ILRAD staff members and giving information on how they may be obtained. This brochure has been widely distributed, along with *Scientific publications from ILRAD* which covers the years 1975 to 1981. Reprint requests are now received regularly, particularly from scientists and libraries in Africa.

The annual report and quarterly newsletter are intended for less specialized readers, with emphasis on animal health workers in laboratories and field situations around Africa. In addition, *ILRAD Reports* and occasional short press releases are distributed to journalists in many parts of the world. The distribution lists for ILRAD publications are amended and expanded on a continuing basis, as illustrated in Figure 22. Of all the institutions and individuals who have been sent information on ILRAD, 39% have responded expressing their interest in receiving ILRAD publications.

In addition, an *Internal Newsletter* is published every week in English and Kiswahili for distribution to staff members and the Board of Directors. The *Internal Newsletter* contains messages from the ILRAD directors, lists of recent accessions in the library, announcements of seminars and meetings and other notices of interest to the staff.

In September 1984, ILRAD participated in the Nairobi International Show, which is sponsored every year by the Agricultural Society of Kenya. This was the third time ILRAD has sponsored an exhibit at the show, and thousands of people from all over Kenya visited the exhibit to learn about ILRAD's research and training activities.

The ILRAD Library

The ILRAD library is used primarily by staff members, visiting scientists and participants in the training program, but the collection is also made available to staff of other research institutes, government departments and universities. A brochure, *The ILRAD library: user's guide*, was published in 1984, describing the organization of the library and the collection of books and journals available. This brochure has

Region	Number of Countries	1984 Increase	Number of Institutions Individuals	1984 Increase
Anglophone Africa	26	3	749	322
Francophone Africa	24	4	334	133
Anglophone & other Europe	17	4	270	70
Francophone Europe	2	0	39	16
North & South America	27	6	304	87
Near & Far East	34	10	334	183
Total	130	27	2030	811

Figure 22. Summary of *ILRAD Reports* distribution at beginning of 1985, showing increases during 1984.

been widely distributed, particularly to appropriate institutions in the Nairobi area, in order to make this valuable resource more readily available to scholars and students working in Kenya.

The specialized collection covers topics related to ILRAD's research program. Emphasis is placed on scholarly journals and reports, with subscriptions to about 200 journals covering fields such as parasitology, immunology, biochemstry, entomology, cell biology and veterinary medicine. The library is also building a collection of scientific reference books, comprising almost 3000 volumes at the end of 1984, with 344 new books received during the year. New books are ordered on the recommendation of the scientific staff, while journal subscriptions are reviewed towards the end of each year and titles are added or dropped according to staff recommendations.

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. Requests for back issues of journals are often filled by older libraries in the network, while ILRAD can supply current issues of many periodicals which are not available anywhere else in Kenya. In 1984, ILRAD received 470 documents through interlibrary loans and supplied 960 documents to other libraries in Kenya and overseas. The ILRAD library also donated 40 journal volumes and 11 books to other libraries; most of these were given to the Faculty of Agriculture library of the University of Nairobi.

During the year, a major effort was made to improve current awareness services to ILRAD staff. New accessions are listed every week in ILRAD's *Internal Newsletter*, and monthly selective dissemination of information services have been initiated in cooperation with ILCA and the International Information System for the Agricultural Sciences and Technology (AGRIS) of FAO. Beginning in 1985, the library will also receive information from Biosciences Information Service (BIOSIS). Computer-based literature searches are carried out using specialized agents in Europe.

RESEARCH SUPPORT

TSETSE LABORATORY

Seven tsetse breeding colonies were maintained at ILRAD during 1984, representing the three major taxonomic groups—*morsitans, palpalis* and *fusca*. The species and subspecies maintained were *Glossina morsitans centralis* originating from mainland Tanzania, *G austeni* from Zanzibar, *G palpalis palpalis* from Nigeria, *G p gambiensis* from Bourkina Faso, *G fuscipes fuscipes* from the Central African Republic, *G tachinoides* from Chad and *G brevipalpis* from Kenya. All are important vectors of trypanosomiasis.

All tsetse flies are maintained under controlled climatic conditions at 25°C, with 12 hours of darkness and 12 hours of subdued indirect light. Tsetse in the *morsitans* group are kept at 70% relative humidity, and the others are kept at 85%. For some experiments, tsetse are fed artificially through membranes, but the breeding colonies are maintained by feeding on rabbits. In March 1984, higher mortality and lower fecundity were observed in all tsetse colonies, caused by the presence of coccidiostat in the rabbit diet. When this was changed, all the tsetse colonies fully recovered. Overall performance is shown in Figure 23.

Tsetse flies are supplied for a variety of trypanosomiasis research projects at ILRAD. In addition, tsetse puparia and adult flies were supplied in 1984 to colleagues at the Kenya Trypanosomiasis Research Institute, the Kenya Agricultural Research Institute, the University of Nairobi, the National Council for Scientific Research in Zambia, the Institut de Médecine Tropicale in Belgium and the Swiss Tropical Institute.

TICK LABORATORY

The tick laboratory provides infected and uninfected ticks, tick salivary glands and other organs and tick-derived *T p parva* sporozoites for a wide range of *Theileria* research activities at ILRAD. These include ECF epidemiology studies, immunization trials based on sporozoite infection and treatment, electron microscopy studies of parasite development and research on sporozoite antigens as possible starting material for an ECF vaccine.

Breeding colonies of six tick species were maintained in 1984. The most important of these was the theileriosis vector, *Rhipicephalus appendiculatus*. Three laboratory strains of *T p parva*, plus several *T p parva* isolates derived from cattle in the field and *T p lawrencei* derived from buffalo, are all maintained in the tick laboratory by continuous passage through *R appendiculatus* and

	Breeding Females (mean)	Daily Female Mortality (mean %)	Weekly Pupae per Female (mean)	Puparial Weight (mean mg)	Annual Number Puparia
G m centralis	8167	0.62	0.58	31.89	248,515
G austeni	2447	0.38	0.58	25.74	74,238
G p palpalis	1804	1.46	0.41	30.41	39,884
G p gambiensis	2289	1.22	0.48	29.36	58,046
G f fuscipes	1894	1.12	0.42	34.90	42,358
G tachinoides	2001	1.30	0.43	20.63	46,294
G brevipalpis	3531	1.30	0.31	74.11	59,556

Figure 23. Performance of seven tsetse breeding colonies maintained at ILRAD in 1984.

rabbits or cattle. Breeding colonies of 400,000 to 600,000 *R appendiculatus* nymphs are maintained. These include populations of *R appendiculatus* (Muguga), a well-established laboratory colony, *R appendiculatus* (Uganda), and several populations derived from ticks isolated in different parts of Kenya.

Investigations are carried out on a continuous basis to determine the best dietary and environmental conditions for producing large, healthy ticks which will supply the maximum number of *Theileria* sporozoites. Studies also concentrate on factors required for the optimal development of the parasites, such as individual and breed characteristics of cattle, the environment of cattle during tick attachment and the environment of ticks during different stages of their development. As a result of these studies, *T p parva* infection rates in ticks bred at ILRAD were raised substantially.

A colony of about 150,000 to 200,000 *Amblyomma variegatum* nymphs is also maintained, derived from ticks isolated in various parts of Kenya. This species, with a wide geographical range in Africa, is the vector of *Theileria mutans*, *T velifera* and *C ruminantium*. Smaller colonies were maintained of *Amblyomma gemma*, *Boophilus decoloratus*—which transmits *B bigemina* and *A marginale*—, *Rhipicephalus evertsi evertsi* and *R pulchellus*.

About 112,000 ticks were dissected in 1984 for the extraction of salivary glands and other organs, and *Theileria* sporozoites were taken from infected ticks and provided as stabilates for ECF research. Ticks collected in the field were identified and populations were maintained for study at ILRAD, while ticks bred at ILRAD were used to pick up parasites from infected cattle and buffalo in the field. Altogether, more than 3.5 million ticks were used in the course of the year. In addition to supporting research projects at ILRAD, the tick laboratory provided assistance and supplied tick material to the Kenya Government's Veterinary Research Laboratory, the Kenya Agricultural Research Institute, the Kenya Trypanosomiasis Research Institute, ICIPE, the ILCA/ILRAD trypanotolerance research network and an acaricide resistance project sponsored by FAO.

FARM ANIMAL PRODUCTION

Because *Theileria* parasites do not infect small ruminants or laboratory rodents, nearly all research on ECF is carried out in cattle. In 1984, 70% of the cattle required for experimental work at ILRAD were used for ECF research, 20% were used for trypanosomiasis research and 10% were used to raise antigens for the diagnosis of different livestock diseases.

ILRAD's ranch at Kapiti provides most of the cattle required for the research pro-

gram. In 1984, the ranch supplied 242 Boran calves, 49 Guernsey calves and 45 Boran steers. At the end of 1984, the breeding herd on the ranch comprised 899 mature Boran cows and 816 Boran heifers, plus 96 Guernsey cows and 66 Guernsey heifers. The number of heifers of both breeds increased substantially over the year.

Scientists used 373 goats and 10 sheep in 1984, almost all for trypanosomiasis research. Most of the small ruminants were purchased from farms in different parts of Kenya—around Thika, Nanyuki and Gilgil.

Livestock production was difficult throughout Kenya in 1984 because of the severe drought which affected the entire country. The herd at Kapiti was maintained by supplementation with purchased feeds, which was expensive, but nearly all the animals survived. Development of the ranch continued during the year, with the construction of staff houses and spray races and the installation of improved fencing.

In addition to the cattle supplied by the Kapiti ranch, 34 Boran calves were born on the ILRAD farm at Kabete, and 27 Friesian calves and steers were purchased from outside farms. A few sheep and goats were also born at ILRAD. Drought conditions made it difficult to purchase animal feeds; animals were maintained in good condition on the farm, but at a greater expense than normal.

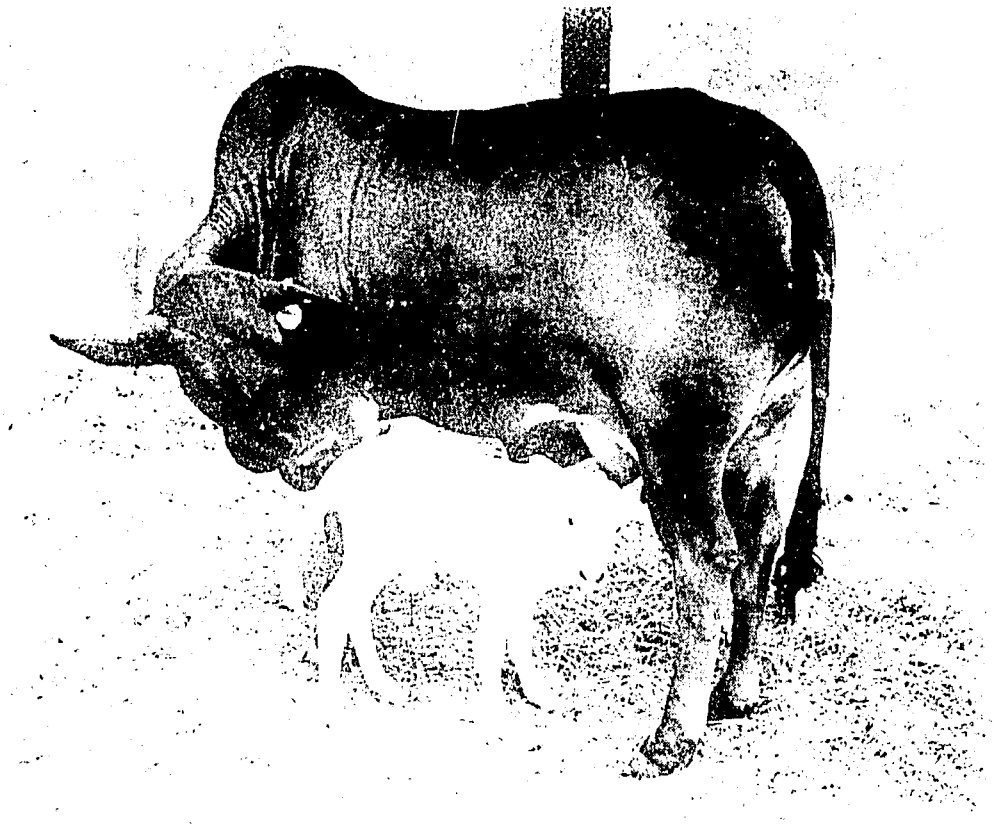
An embryo-transfer project was initiated at the end of 1982, aimed at bringing a small number of N'Dama cattle from West Africa to ILRAD for intensive research on trypanotolerance. Purebred N'Dama cows were superovulated by treatment with hormones and mated with bulls selected by the Livestock Marketing Board in The Gambia. The embryos obtained were deep frozen and shipped to East Africa. These were surgically transferred into Boran heifers in June 1983, and 10 purebred N'Dama calves were born on the ILRAD farm in March and April 1984 (see Figure 24).

The 5 N'Dama heifers and 5 bull calves remained with their Boran 'foster mothers' for 5 to 7 days and were then transferred to ILRAD's calf rearing unit. They have remained healthy and showed good growth rates, with liveweights ranging from 58 to 83 kg at 6 months of age. These calves will be used for studies on the mechanisms of resistance to trypanosomiasis.

Experiments are now in progress to produce Boran calves by embryo transfer from selected cows and bulls in the herd at Kapiti. These will be used for studies on the bovine MHC.

ILRAD's laboratory animal unit maintains breeding and production colonies of mice, rats and rabbits, plus small colonies of guinea pigs, gerbils and meadow voles (*Microtus montanus*). All laboratory animals are housed in plastic or metal cages in compliance with the Council of Europe's *Draft convention for the protection of vertebrate animals used for experimental and other scientific purposes*, and methods used for euthanasia are in line with international standards. The goal is to provide all the laboratory animals required for ILRAD's research programs with a minimum of wastage. Figure 25 shows the total numbers of rats, mice and rabbits produced and used at ILRAD in 1984.

Random-bred rats maintained at ILRAD originate from the Sprague Dawley strain. They are used primarily for the production of trypanosomes. In 1984, the rat breeding system was changed from monogamous pairs to a harem system, with 10 females allocated to each male. Females are isolated to individual cages when palpably pregnant and remain isolated until their litters are weaned. This system makes it possible to select future breeding stock for maximum productivity. The average monthly productivity rate



Species and Strain	Number Weaned	Number Used
Rats	25,476	17,632
Mice		
BALB.c	17,648	17,172
C3H.He	7,226	4,174
C57B1.6	5,996	3,484
AJ	7,676	3,586
Swiss	6,440	2,397
Rabbits	525	1,326

Figure 25. Rats, mice and rabbits produced and used at ILRAD in 1984. Most of the excess rats and mice produced during the year were donated to research organizations and schools in Kenya. The rabbits used above the number produced were purchased from local sources.

bils to several other organizations in Kenya. In 1984, these included ICIPE, the University of Nairobi's Departments of Zoology, Biochemistry, and Pharmacology and Toxicology, the African Medical Research Foundation, The Kenya Medical Research Institute, the Kenya Trypanosomiasis Research Institute, the Institute of Primate Research, the National Museums of Kenya, the Kenya Society for the Protection and Care of Animals and 53 Kenyan secondary schools.

CLINICAL AND DIAGNOSTIC SERVICES

The diagnostic laboratory provided 30,082 analyses in 1984, as required by ILRAD's research programs and animal production facilities. These comprised 21,532 serological analyses, 3,721 bacteriological analyses, 3,602 haematological analyses and 1,227 helminothological analyses—altogether an increase of 11% over the number of samples analysed in 1983. A considerable proportion of this diagnostic work was carried out in support of East Coast fever immunization trials and studies on trypanosomiasis epidemiology.

Serum samples are screened for antibodies to six bloodstream parasites, most often by the IFA test. These are *Theilera parva*, *T mutans*, *A marginale*, *Trypanosome brucei*, *T congolense* and *T vivax*. The ELISA test is also used to screen bovine sera for antibodies to the *Trypanosoma* species. During the year, 205 post-mortem examinations were conducted, comprising 118 cattle, 70 goats and 17 sheep.

A new method was developed in 1984 to harvest and purify *A marginale* antibodies from the red blood cells of infected cattle. The purified antibodies have been used to prepare a soluble antigen which is now used with the ELISA to detect *A marginale* antibodies in bovine sera collected in the field.

In addition to collaboration and services provided to ILRAD staff, the diagnostic laboratory continues to provide reagents and training to other laboratories and projects in developing countries. In 1984, *Theileria*, *Trypanosoma* and *Anaplasma* antigens and antibovine conjugates were provided to the Laboratoire vétérinaire Lubumbashi in Zaire, an FAO/ILRAD collaborative project in Zimbabwe, FAO and German (Federal Republic) projects in Burundi, a USAID (United States Agency for International Development) project at the Kenya Agricultural Research Institute, FAO projects in Zambia and Mozambique and a WHO (World Health Organization) project in Zambia. ILRAD's diagnostic laboratory also analysed 1,283 serological samples for the Ministry of Agriculture and Livestock Development in Kenya and for FAO projects in Kenya and Tanzania.

The diagnostic laboratory conducted a 3-month training course in 1984 for veterinarians, field officers and senior technicians from Burundi, Tanzania, Uganda, Zaire and Zambia. The course covered the preparation of reagents and their use in the serodiagnosis of bloodstream parasites. Individual training was also provided to laboratory technicians from Burundi and Kenya.

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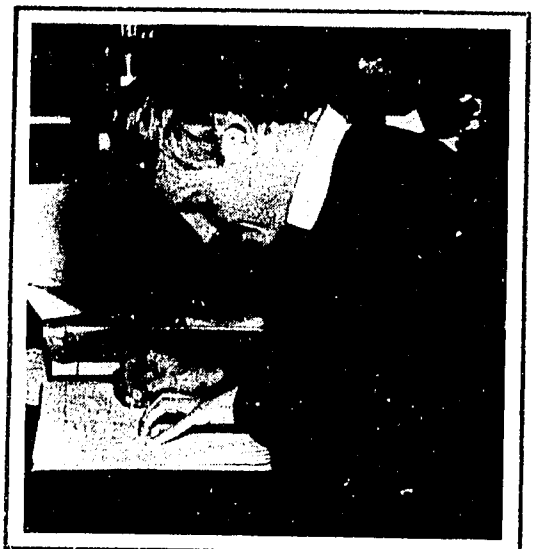

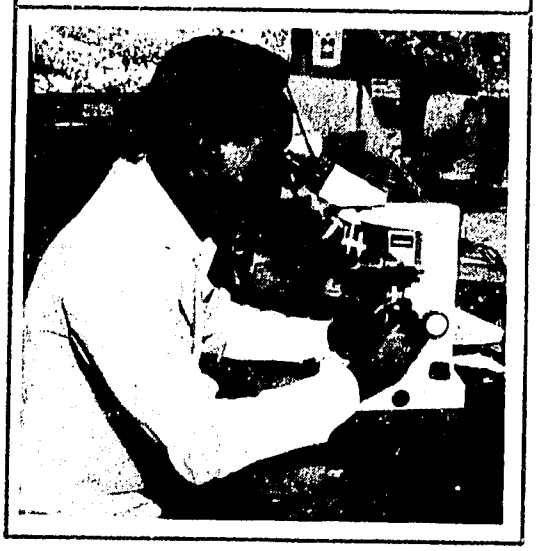

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REPORT TO THE MANAGEMENT OF THE INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES (ILRAD) ON INTERNALLY PREPARED ACCOUNTING INFORMATION RELEVANT TO THE FINANCIAL YEARS ENDED 31 DECEMBER 1983 AND 1984.

From a detailed examination of the accompanying schedules (Figures 26 to 28) we can confirm that the information contained therein has been extracted from the books and records of ILRAD from which statutory accounts were prepared for the financial years ending 31 December 1983 and 1984.

Whereas the analysis of financial data in the accompanying schedules differs in some respects from that disclosed in the statutory accounts, the key figures remain consistent with the audited financial statements at 21 December 1983 and 1984.

Price Waterhouse

PRICE WATERHOUSE
Certified Public Accountants

Figure 26. Summary costs by program and activity (US$'000).

	1984	1983
Research		
Parasitology—Trypanosomiasis	315	293
Biochemistry	727	666
Cell Biology	541	340
Immunobiology	356	490
Parasitology—Theileriosis	527	497
Pathology	598	541
Tsetse Laboratory	279	317
Tick Laboratory	106	115
Electron Microscopy	150	178
Total Research	3599	3437
Research Support		
Office of Director of Research	562	281
Farm Animal Production	623	685
Laboratory Animal Production	133	133
Clinical and Diagnostic Services	86	67
Radio Isotope and Central Core Services	303	347
Total Research Support	1707	1513
Training and Conferences	685	695
Library and Information Services	313	337
Administration		
Board of Directors	91	70
Office of the Director General	348	357
Finance	342	358
Personnel	85	72
Purchasing	275	274
Total Administration	1141	1131
General Operations		
Engineering	572	726
Transport	252	208
Services	146	104
Food and Housing	14	127
Stores	52	48
Total General Operations	1036	1213
Contingency	10	31
Total Core Costs	8491	8357

Figure 27. Summary of sources and application of funds (US$'000).

	1984	1983
SOURCES		
Core Operating Funds		
Unrestricted		
World Bank (IBRD)	1570	800
Germany (Federal Republic)	556	603
United Kingdom	468	548
Canadian International Development Agency (CIDA)	699	615
Switzerland	437	383
Saudi Arabia	300	300
Norway	255	287
Belgium	229	—
Netherlands	264	250
Australia	208	286
Sweden	197	186
Italy	112	136
France	59	52
United States Agency for International Development (USAID)	2500	2500
Balance From Previous Year	—	881
Earned Income Applied in Year	144	85
Total Unrestricted Operating Funds	7998	7912
Restricted		
United Nations Development Fund (UNDP)	639	576
Italy	500	350
Belgium	105	151
Japan	150	—
Total Restricted Operating Funds	1394	1077
Total Unrestricted/Restricted Operating Funds	9392	8989
Transfer to Capital Funds	(482)	(654)
Net Unrestricted/Restricted Operating Funds	8910	8335
Capital Funds		
Transferred from Core Operating Funds	482	654
Unexpended Balance from Previous Year	—	181
Balance of Working Funds from Previous Year	687	709
Balance of Revolving Fund from Previous Year	100	—
Total Capital Funds	1269	1544
Special Project—Netherlands	236	203
Total Sources	10415	10082
APPLICATIONS		
Core Operations	8491	8357
Capital	391	735
Special Projects	236	203
UNEXPENDED BALANCE		
Unrestricted Core	419	—
Working Funds	778	687
Revolving Fund	100	100
Total Funds	10415	10082

Figure 28. Balance sheet at 31 December 1984 (US$'000).

	1984	1983
ASSETS		
Fixed Assets		
Land and Buildings	9882	9444
Research Equipment	4875	4642
Other Assets	1871	2005
Subsidiary Company		
Investment	1728	1651
Longterm Loan	58	77
Total Fixed Assets	18414	17819
Revolving Fund		
Loans	86	89
Cash	14	11
Net Current Assets	1197	687
Total Assets Employed	19711	18606
FUND BALANCES		
Capital Fund	18414	17819
Working Capital	778	709
Unrestricted Core Surplus (Deficit)	419	(22)
Revolving Fund	100	100
Total Funds	19711	18606

ILRAD

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