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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, 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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ANNUAL REPORT OF THE
INTERNATIONAL LABORATORY
FOR RESEARCH ON ANIMAL DISEASES

P O BOX 30709 NAIROBI, KENYA

FOREWORD

This annual report for 1983 presents a general account of ILRAD's research and training programs during the past year. These activities are directed towards the improved control of animal theileriosis and trypanosomiasis in Africa.

Disease in livestock continues to exert a serious constraint on agricultural development in many parts of the world. ILRAD's target diseases, theileriosis and trypanosomiasis, are believed to be responsible for the death of 3 million cattle a year. Such losses, and the associated effects on food production and economic development, call for increasingly urgent attention. This is particularly true on the African continent where the human population is growing rapidly and agricultural production in many areas is showing a decline.

In undertaking research on theileriosis and trypanosomiasis, scientists at ILRAD are tackling two intractable animal health problems. Both diseases have been recognized since the last century, and scientists from many countries have worked for decades to develop effective control measures. Their work has led to a number of improvements, but no fully satisfactory vaccine or other control method has yet been produced for either disease. Major problems remain which require a continuing research effort. It is therefore reassuring to note the degree of cooperation and interest shown by many organizations working on different aspects of trypanosomiasis and theileriosis control.

Several national, regional and international agencies are involved in theileriosis and trypanosomiasis research and are carrying out field programs designed to improve disease control based on existing methods. The approach of these agencies is essentially practical, field-oriented and related to immediate needs. Generally speaking, they do not have the mandate or the capacity to carry out substantial basic research, but they are in a position to benefit directly from results achieved in the laboratory.

ILRAD is the only major international institution concerned with theileriosis and trypanosomiasis which devotes the greatest part of its resources to basic research. Collaboration with international, regional and national programs ensures the efficient transfer of ILRAD's research findings and expertise to organizations directly involved in disease control in the field.

Among international agencies, the Food and Agriculture Organization of the United Nations (FAO) coordinates the largest disease control programs. The FAO Worldwide Program for the Control of Animal Trypanosomiasis was initiated in 1974, and FAO activities aimed at the control of theileriosis and other tick-borne diseases began even earlier. Since ILRAD was founded, cooperation with FAO research and field

programs has been close. ILRAD scientists serve on FAO's Panel of Experts on the Ecology and Technical Aspects of the Program for the Control of Animal Trypanosomiasis and Related Development and on the Expert Panel on Ticks and Tick-borne Diseases.

The United Nations' World Health Organization (WHO) includes African trypanosomiasis as one of the six refractory diseases covered under its Special Program for Research and Training in Tropical Diseases. ILRAD collaborates closely with WHO, particularly in research on immunological aspects of African trypanosomiasis and in training. ILRAD scientists contribute to WHO's Scientific Working Group on Trypanosomiasis and to the Steering Committee on Immunology and Pathology of African Trypanosomiasis. ILRAD staff members have also served on expert panels of the International Atomic Energy Agency (IAEA) on the use of radioisotopes in disease research.

Several regional and bilateral organizations concerned with agricultural development are involved in research and field programs aimed at improving control of livestock diseases. These include the Organization of African Unity's Interafrican Bureau for Animal Resources (OAU/IBAR), the United Nations Economic Commission for Africa (ECA), the Cooperation for Development in Africa (CDA) association of principal bilateral donors and the European Economic Community (EEC). The effectiveness of their efforts will depend largely on the results of basic research, and ILRAD therefore maintains close contact with all these groups and conducts collaborative research projects in several areas.

ILRAD's research programs are designed to support the work of other organizations and to follow promising avenues for disease control, particularly immunological approaches, which have not been fully pursued elsewhere. The emphasis on immunological research, specified in ILRAD's mandate, is based on evidence that immunity to trypanosomiasis and theileriosis does occur in the field. Occasionally, cattle recover from theileriosis and they are subsequently immune to similar strains of the disease. Trypanotolerant varieties of livestock and many species of wild animals also show a degree of natural immunity to trypanosomiasis. The successful development of vaccines, which would stimulate immunity without the hazards of exposure to infection, would be a major advance in the control of these diseases.

The annual report for 1983 describes the approaches ILRAD's scientists have pursued in research on theileriosis and trypanosomiasis, their progress over the year and the highlights of their results. Further details may be found in the original research papers and reviews published during the year and listed in this report. The complexity of the problems and the high quality of the work which has been conducted will be apparent.

During the year, the Director of Research and members of the scientific staff participated in a thorough internal review of ILRAD's research programs. In response to requests from the Technical Advisory Committee of the Consultative Group on International Agricultural Research (CGIAR), a working report was prepared on how ILRAD's research programs on theileriosis and trypanosomiasis are likely to develop over the next 10 years, the resources likely to be required and what the results of ongoing activities might be. This report, *ILRAD 1984-1993: plan for the second decade*, is to some extent speculative, since the direction and results of any basic research program are uncertain at best. Yet these projections will provide a framework for the formulation of biennial program and budget proposals and a guide for monitoring the progress of ILRAD's research.

Training and conference activities continued to expand in 1983. Nearly 200 participants came to ILRAD from 42 countries to attend workshops, seminars and courses, or to learn laboratory techniques by work-

ing closely with the scientific staff. The distribution of information on ILRAD activities has also expanded, with the successful launching of a quarterly newsletter, *ILRAD Reports*, in English and French. Requests for copies of the newsletter and the annual report are received daily, from all over Africa and around the world.

As ILRAD is recognized more widely as a centre of scientific expertise, requests for specialized advice and training have also increased. Eleven staff members provided assistance to other agencies during the year, serving on specialized panels and missions or assisting with training courses. International organizations requesting assistance from ILRAD staff included FAO, WHO, OAU, ECA, IAEA, the Organisation Internationale des Epizooties and the CGIAR. In the course of the year, ILRAD scientists served on field missions in Benin, Ethiopia, Gabon, Ivory Coast, Kenya, Malawi, Mozambique, Nigeria, Somalia, Sudan, Tanzania, The Gambia, Uganda, Upper Volta, Zaire, Zambia and Zimbabwe. One of ILRAD's senior staff members was singled out for a special honour during the year: Professor D.W. Fawcett, Coordinator of ILRAD's Electron Microscopy Laboratory, received the Henry Gray Award, the highest award presented by the American Association of Anatomists.

ILRAD was honoured to receive many visitors in 1983, including Their Excellencies, the High Commissioners of the United Kingdom, Canada and Australia and the Ambassador of the Federal Republic of Germany. Members of the International Commission of Protozoologists also visited ILRAD. The research programs benefitted substantially from a steady flow of visiting scientists who contributed their special skills and experience during the year. Their names are included in the staff list towards the end of this report.

Finally, it is a pleasure to note with gratitude the financial support ILRAD received in 1983 from 15 donor governments and agencies. During the year, ILRAD continued to receive funds from the World Bank (IBRD), the United Nations Development Program (UNDP) and the governments of Australia, Belgium, Canada, the Federal Republic of Germany, Norway, Sweden, Switzerland, the Netherlands, the United Kingdom and the United States of America, and three new donors added their support—France, Italy and Saudi Arabia. In addition, scientists have been provided through special funding from the Governments of Belgium, the Netherlands, France, the United Kingdom and Japan. We also offer our thanks to the Government and people of Kenya for their continuing generosity in serving as hosts for ILRAD.

A.R. Gray
Director General
ILRAD

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THEILERIOSIS

Theileriosis is a complex of parasitic diseases which affects cattle, and sometimes sheep, goats and domestic buffalo, in Africa, the Middle East and Asia. Research at ILRAD concentrates on East Coast fever (ECF), a particularly virulent form of theileriosis which affects cattle throughout Eastern and Southern Africa—in Kenya, Tanzania, Uganda, Rwanda, Burundi, Zaire, Malawi and Mozambique. Cattle losses attributed directly to ECF are estimated to be as high as half a million animals a year out of a total cattle population of 25 million in the endemic area.

ECF is caused by the protozoan parasite *Theileria parva*, and is transmitted by the brown ear tick, *Rhipicephalus appendiculatus*. Two subspecies of the parasite occur in Kenya, *T parva parva* maintained in cattle and *T parva lawrencei*, which normally occurs in buffalo but can cause severe infections in cattle. Three related species—*T taurotragi*, *T velifera* and *T mutans*—may cause mild infections in cattle.

Another related *Theileria* species, *T annulata*, is a major disease problem in cattle in North Africa, the Middle East and Asia, causing chronic anaemia, reduced productivity and often death. *T sergenti* occurs in Asia, causing an important cattle disease in affected areas, and *T hirci* affects sheep in the middle East.

The *Theileria* parasite undergoes a complex lifecycle in the tick vector and the bovine host, as depicted in Figure 1. Ticks feed on cattle three times during their lives—as larvae, as nymphs and as adults. Transmission of ECF is thought to occur most commonly when ticks feed on infected cattle as nymphs and then on disease-free animals as adults. *Theileria* sporozoites which develop in the salivary glands of infected ticks are transmitted to cattle when the ticks feed. These sporozoites invade white blood cells in the bovine circulatory system, where they develop into schizont forms and multiply rapidly. Some of the infected lymph cells are transformed into enlarged lymphoblasts and are induced to multiply along with the parasites. Thus, enormous numbers of parasitized cells are produced, leading in the final stages of infection to large-scale cell destruction and often to death of the animal.

During the course of infection, some of the macroschizonts change to parasite forms called merozoites, which are released from the host's lymphoid cells and infect red blood cells. Feeding ticks become infected when they ingest these red blood cells, and the transmission cycle is complete.

The first sign of ECF in cattle is enlargement of the lymph nodes under the ears. Fever develops and the animal loses condition. Milk production declines and preg-

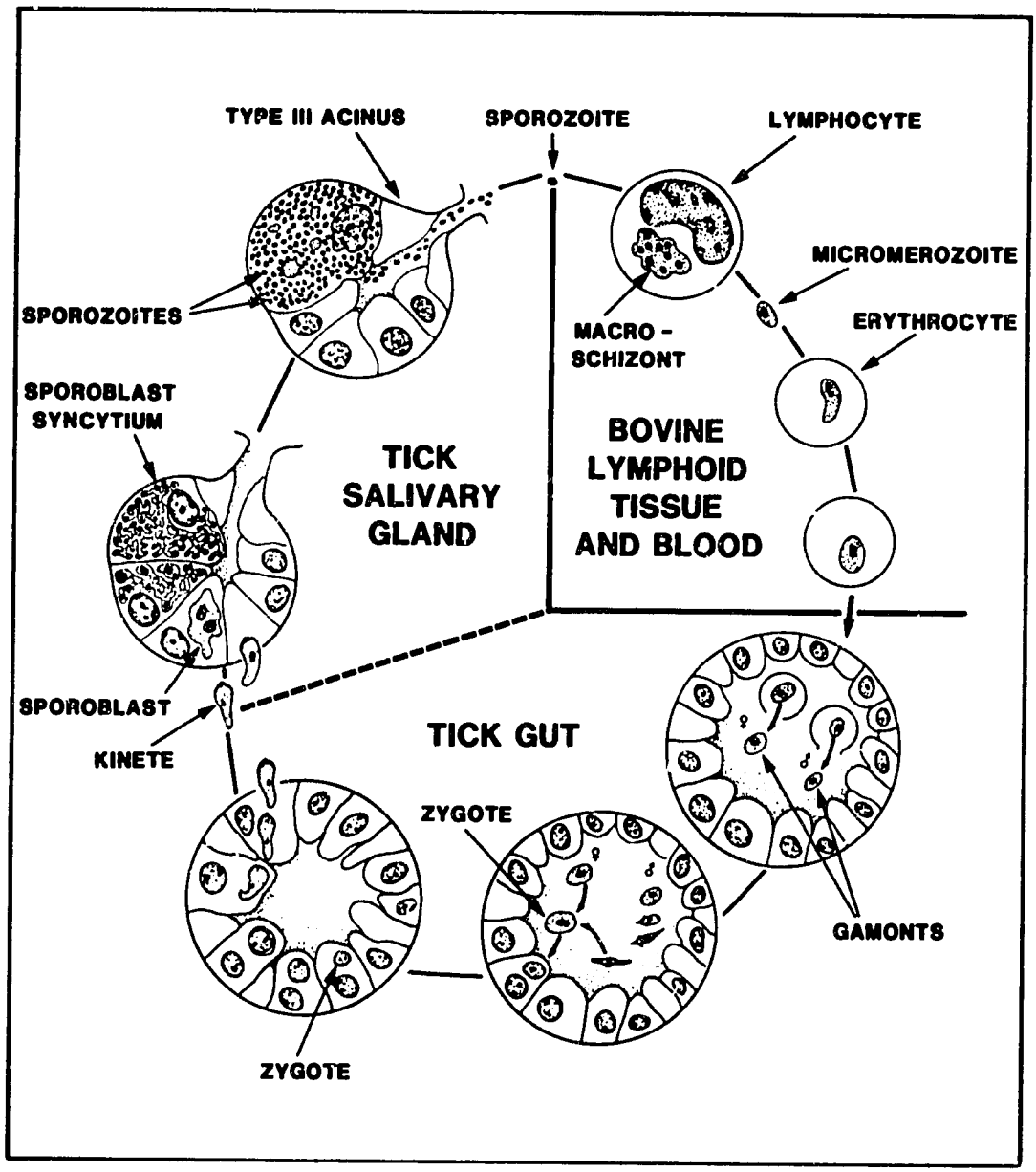

Figure 1. Lifecycle of *T p parva*. Sporozoites are transmitted to the bovine host in tick saliva. They enter the lymphocytes and develop into macroschizonts. After a period of nuclear division, these give rise to numerous micromerozoites which escape from the lymphocytes and enter the erythrocytes where they are ingested by feeding ticks. In the tick gut the parasites differentiate into male and female gamonts which fuse to form zygotes. These enter the cell lining of the gut where they differentiate into kinetes. The kinetes traverse the gut wall and become free in the tick's body cavity. They move to the salivary gland and enter one cell type, the E cell of the type III acinus. Here they form an elaborate intracellular sporoblast syncytium which undergoes segmental fission and gives rise to 30,000 to 50,000 sporozoites. These are introduced with the saliva into a new mammalian host, initiating a new cycle of parasite development.

nant cows may abort. Death occurs in acute cases 3 to 4 weeks after infection. In fully susceptible animals, such as valuable imported stock, mortality rates may be as high as 100%.

At present ECF is controlled primarily by dipping or spraying susceptible animals with acaricides to kill the tick vectors. In areas of heavy tick infestation, cattle must be dipped or sprayed at least twice a week. Tick control can be effective, but requires a level of capital investment and supervision often beyond the reach of small farmers. The development of acaricide resistance is also a serious problem. A drug for the treatment of ECF, Clexon (Wellcome), was first marketed in Kenya in 1983. This is an important development, but effective treatment requires early diagnosis and a level of veterinary supervision that is often not available.

Cattle which recover from ECF show long-lasting, strain-specific immunity. The precise mechanisms of immunity have not been fully clarified, but it is likely that both antibody production and cell-mediated immune responses are involved. The acquisition of immunity in some animals suggests that it should be possible to develop an effective ECF vaccine. This is the goal of ILRAD's theileriosis research program, though the immunological emphasis does not preclude studies on other promising methods of disease control.

Both the sporozoite and the macroschizont stages of parasite development are being studied as potential sources of vaccine material. In addition, epidemiological studies cover the identification and classification of ECF strains, the improvement of experimental immunization procedures based on infection and treatment, a collaborative study on the role of buffalo and other wild animals as disease carriers, and studies of tick biology and ECF transmission.

BIOLOGY OF THE PARASITE

The origins and functions of the sporozoite stage of *Theileria* development have received considerable attention at ILRAD, with a view to identifying ways to block infection and improve disease control. A thorough knowledge of sporozoite development could also enable scientists to produce sufficient quantities of parasite material to provide the basis for immunization trials.

Research carried out in 1981 and 1982 using electron microscopy shed light on the formation of sporozoites in the tick salivary gland. Work also concentrated on the origins of parasite cellular organelles which play a role during entry into bovine lymph cells. In 1983, electron microscopists at ILRAD concentrated on other phases in the complex *Theileria* life cycle.

Theileria sporozoites enter host lymphocytes by a process called passive endocytosis: ligands on the surface of the parasite bind to receptors in the membrane of the host cell. This mode of invasion requires little or no energy. Immediately after entry, the sporozoite is closely enveloped in a portion of the lymphocyte membrane, as shown in Figure 2a. Within 2 hours, the sporozoite is surrounded by a layer of dense material with poorly defined outer limits (Figure 2b), apparently the residue of the enveloping host-cell membrane. This dense halo is gradually dispersed over the next 6 hours. During entry into the lymphocyte, the sporozoite contains micronemes—minute organelles in the cytoplasm. These disappear during the dissolution of the host-cell membrane, sug-

Figure 2. High magnification micrograph of a portion of a *Theileria* sporozoite 10 minutes (2a) and 1 hour (2b) after entry into a bovine lymphocyte. After 10 minutes, a well-defined structure surrounds the sporozoite comprising the parasite and host-cell membranes. After 1 hour, the host membrane has been dissolved and only its remnants appear as a fuzzy dense material around the sporozoite.

gesting that they may play a role in this process.

Mammals possess phagocytic cells which contain a variety of digestive enzymes in organelles called lysosomes. These cells engulf invading bacteria or parasites and enclose them in a membrane within their own cytoplasm. The lysosomes then fuse with the membrane and discharge their enzymes, which digest the invader.

Theileria sporozoites enter lymphocytes, which are not phagocytic cells and normally do not contain lysosomes. However, in response to invasion, the lymphocytes form small lysosomes and multivesicular bodies. These organelles gather around the sporozoite between 12 and 48 hours after entry, as shown in Figure 3. However, they function by fusing with the invaginated portion of the host-cell membrane that envelops ingested material. By the time the lysosomes are formed in the lymph cell, the enveloping host membrane has already been dissolved. To evade the lymphocyte's potentially destructive response, the sporozoite has acquired the ability to synthesize and secrete a substance which dissolves the host-cell membrane without dissolving its own.

Bovine lymphocytes are normally capable of only limited proliferation in response to antigenic or other stimuli. However, infection with *Theileria* sporozoites results in the transformation of some lymphocytes into lymphoblastoid cells which divide rapidly. The sporozoite changes into a multinucleated schizont which is partitioned during host cell division, with a portion usually going to each daughter cell of the host. This process creates a rapidly expanding population of infected cells. Detailed electron microscopic studies are now in progress comparing the process of transformation in parasitized cells to that of uninfected lymphocytes which have been induced to transform by mitogens, such as concanavalin A and phytohemagglutinin, derived from certain plants. A study of the ultrastructural changes associated with the parasitized state will also provide a baseline for analysing the effects of chemotherapeutic drugs which may act by inhibiting lymphocyte transformation.

Studies also concentrate on the process by which *Theileria* schizonts give rise to merozoites which are released from the lymphocytes and invade red blood cells. This process begins in the schizont with the assembly of cellular organelles called rhoptries. The rhoptry complexes become associated with subplasmalemmal dense plaques spaced at intervals along the parasite membrane. Spherical merozoites, each containing one nucleus and a set of rhoptries, are then budded off at these sites along the periphery of the schizont, as shown in Figure 4.

Before formation of the merozoites, the schizont membrane develops a conspicuous surface coat. The merozoites which later bud off from the schizont are covered by this thick coat. Its function is probably to serve as protection in the extracellular environment and enable the parasites to recognize and attach to red blood cells.

SPOROZOITE ANTIGENS AND IMMUNE RESPONSES

Earlier research at ILRAD indicated that cattle infected and recovered from ECF produce antibodies which are capable of neutralizing the infectivity of *T p parva* sporozoites. Present work concentrates on identifying and characterizing sporozoite antigens which will induce the formation of protective antibodies and producing such

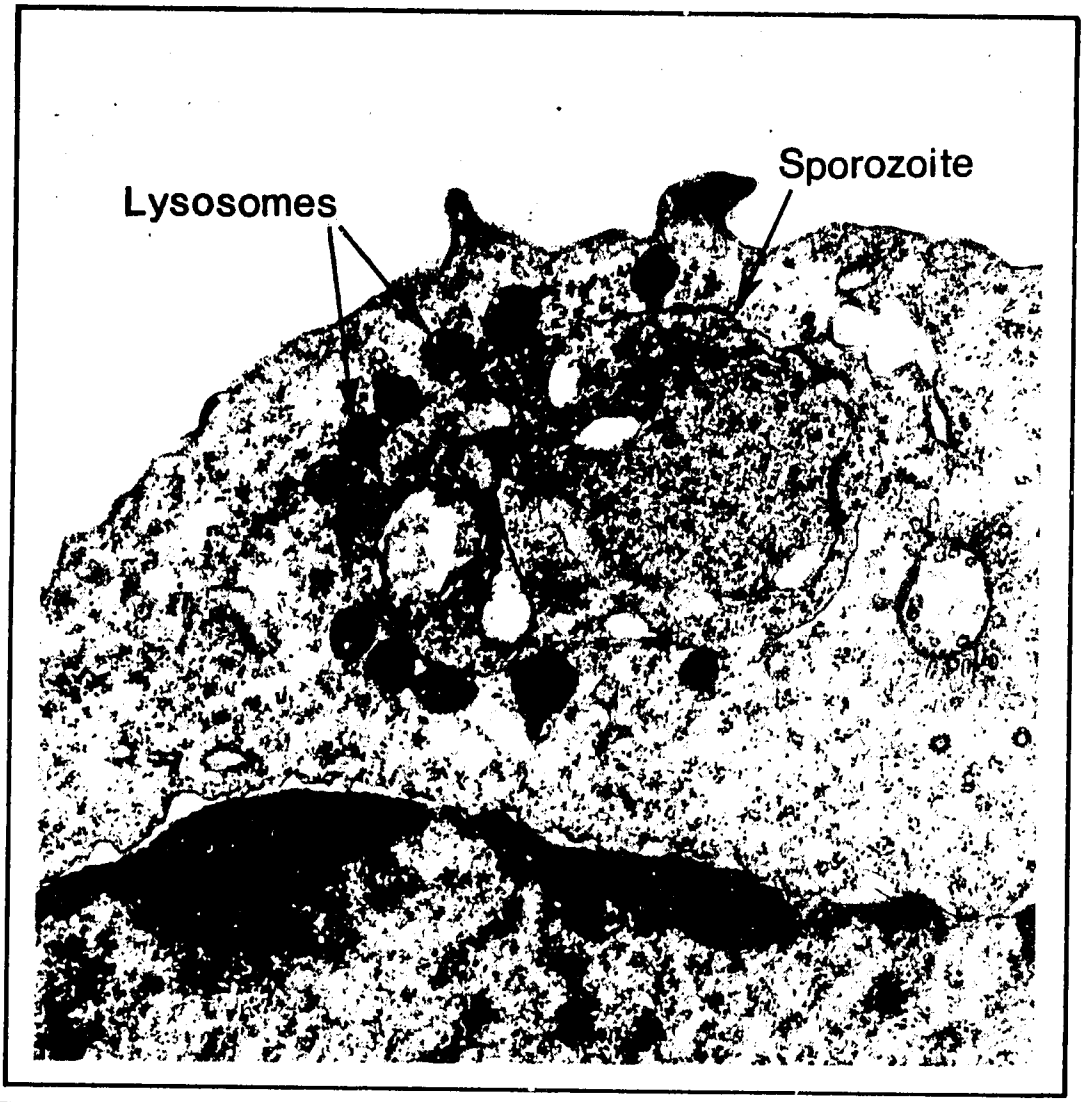

Figure 3. Electron micrograph of a *Theileria* sporozoite 48 hours after entry into a bovine lymphocyte The parasite is enclosed in its own membrane: the host-cell membrane has been completely dispersed. The host cell has made numerous small, dense lysosomes which have assembled around the sporozoite, but they need to fuse with the host membrane to carry out their digestive function.

antigens in sufficient quantities to provide starting material for an experimental vaccine. The objective is to find out if it will be practicable to develop a vaccine based on this stage of parasite development.

Three antigens have been identified from *T p parva* sporozoites, using antibodies from infected cattle, antibodies from rabbits immunized with isolated sporozoites, and monoclonal antibodies produced in mice by hybrid cells. Results indicate that at least one of these antigens is located on the surface of the sporozoite. Two of the antigens are capable of inducing the formation of antibodies in rabbits which neutralize the infectivity of sporozoites. Work continues to characterize all three antigens more precisely.

Salivary gland sections from infected, feeding ticks have been studied to determine when and where one of the antigens is synthesized during parasite development. This antigen, recognized by a monoclonal antibody, is synthesized primarily from the second day after the tick starts feeding, as shown in Figure 5. At this stage, sporoblasts in the tick already contain the antigen and/or its precursor.

Electron microscopic studies using ultracryomicrotomy techniques suggest that this antigen is closely associated with parasite entry into the bovine lymphocyte. This is indicated by the fact that the antigen appears on the surface of the host cell as well as on the parasite just before and during parasite entry (Figure 6). During entry, it appears to be shed from the parasite's surface coat. The antigen cannot be detected on sporozoites already within the host cell, but is seen on fragments of surface coat material that remain attached to the lymphocyte membrane at the site of sporozoite entry.

THE SCHIZONT-INFECTED CELL

ILRAD scientists are investigating host responses which regulate the growth of cells infected with *Theileria* schizonts. The goal is to develop a vaccine or other disease control measure directed at this stage of parasite development. Studies are aimed at defining the types of host cell which are infected and transformed by the parasite, the changes induced in host cells, and host responses which kill or control the growth of parasitized cells.

As a prerequisite to defining the cell types which become infected with the parasite, it is essential to have reagents which can be used to distinguish functionally significant subpopulations of mononuclear leukocytes. The approach taken by scientists at ILRAD is to derive monoclonal antibodies which recognize cell surface determinants specific to certain subpopulations of cells. Monoclonal antibodies were produced originally which recognize B-lymphocytes and T-lymphocytes, and in 1983 a monoclonal antibody was produced which identifies macrophages. Thus, it is now possible to purify these cell types and subtypes using a fluorescence-activated cell sorter (FACS).

In 1983, ILRAD gained the capability of typing cattle leukocytes for class I major histocompatibility (MHC) antigens. This work is supported by the British Government's Overseas Development Administration (ODA) through the Agricultural and Fisheries Research Council and the Animal Breeding Research Organization in the United Kingdom. MHC antigens are cell surface molecules which show marked differences between individuals of a species. In addition to being the major antigens against which graft rejection responses are

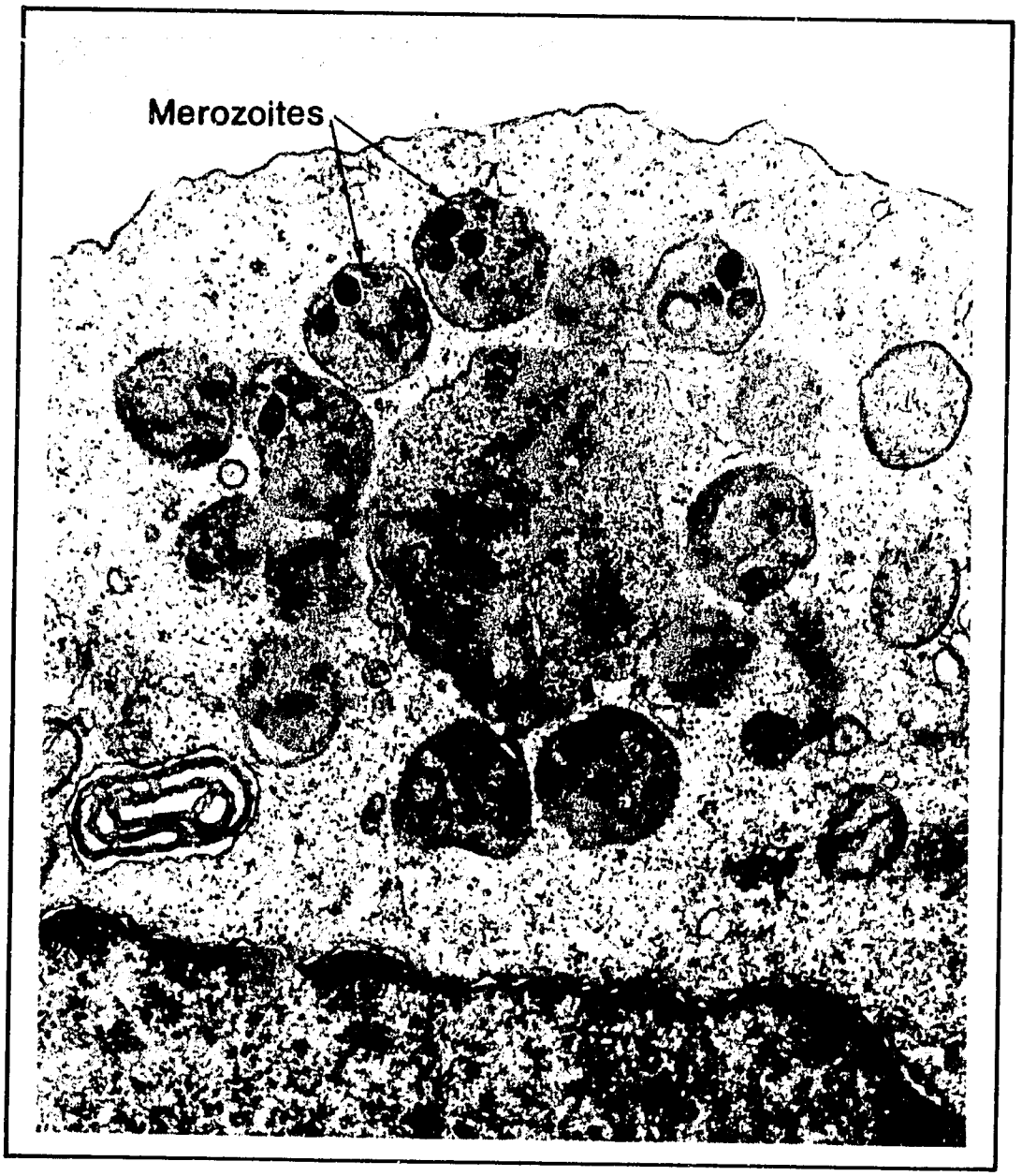

Figure 4. Merozoites budding off from the periphery of a *Theileria* schizont in an infected bovine lymphoblast.

directed, they are important structures in the communication between different cell types during generation of an immune response. Thus, the capability to type animals for MHC antigens provides both valuable information on the genetic relationship between different animals within experimental groups and a means of studying the role of these antigens in regulating immune responses.

Several approaches have been taken to identify the bovine cell types invaded by *Theileria* sporozoites and transformed into proliferating lymphoblasts. Populations of cells enriched for B-lymphocytes, T-lymphocytes or macrophages have been isolated from bovine blood. The B-lymphocyte- and T-lymphocyte-enriched populations could be infected and transformed by *Theileria* infections in vitro at a similar frequency. By contrast, the macrophage-enriched population could not be transformed.

The infected and transformed cells were cloned and their surface antigens were examined with monoclonal antibodies. The populations enriched with infected B-lymphocytes and T-lymphocytes gave rise to a number of clones, with antigens differing from those displayed by the uninfected precursor cells. Surface antigens also differed among individual clones. However, it was possible to distinguish between clones arising from infected T- or B-cells. Clones of *Theileria*-infected cells were then isolated from cattle and their surface antigens were examined using the monoclonal antibodies. The surface antigenic phenotypes were often different from each other and different from those of the cells infected in vitro. This may indicate that while the parasite has the ability to infect a wide variety of bovine lymphocyte types in vitro, only certain lymphocyte populations are capable of uncontrolled proliferation in cattle.

In some instances, such as when cells are collected from infected cattle, only a small proportion of lymphocytes containing *Theileria* schizonts develop into proliferating cell lines in vitro. In many of the parasitized cells, the *Theileria* schizont multiplies rapidly without stimulating a synchronous pattern of host-cell multiplication, so that the host cell becomes full of parasites and bursts. This might suggest that some cells in infected animals which contain schizonts require additional growth factor(s) to maintain proliferation. The effects of extrinsic growth factors on host-cell infection and proliferation rates are now being investigated.

Studies have started on possible differences in the ability of particular infected cell types to proliferate in cattle. In one experiment, populations of cells from different cattle were enriched for B-lymphocytes or T-lymphocytes. These were isolated using monoclonal antibodies and the FACS and infected with *T p parva* in vitro. The cells were then inoculated back into the animals from which they were originally obtained, and the cattle were monitored for the development of parasitaemia. It was found that the T-lymphocyte-enriched populations induced much more severe infections than the B-lymphocyte-enriched populations, suggesting that the T-cells were able to proliferate more easily in cattle than the B-cells.

Studies in previous years have shown that host cells which develop into lymphoblasts require the continued presence of *Theileria* schizonts to maintain their proliferative state. Comparative studies of parasitized and non-parasitized cells continued in 1983, using the drug cytochalasin B which at low doses inhibits parasite multiplication without inhibiting the multiplication of the host cell. Thus, when host cells divide after treatment with this drug, the *Theileria* schizont does not divide, so only one daughter of the host cell receives a parasite. Parasitized and non-parasitized cells in a culture treated with cytochalasin B are shown in Figure 7. In these cultures, daughter cells which receive schizonts continue to proliferate, whereas non-parasitized daughter cells do not. This shows that cell transformation and prolif-

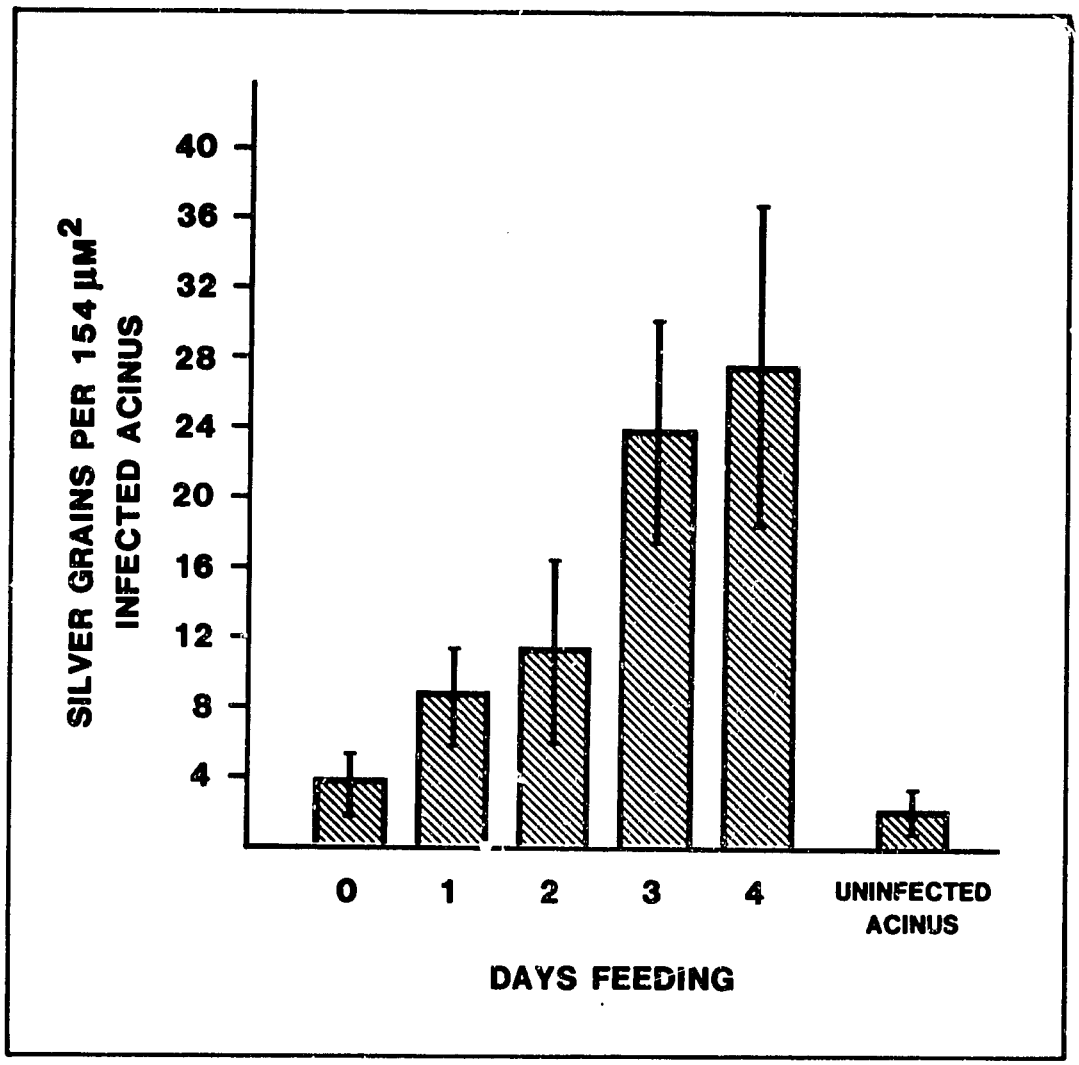

Figure 5. Development of a sporozoite surface antigen in the salivary glands of infected, feeding ticks. Cryostat sections were taken of infected salivary glands from adult ticks fed for 0, 1, 2, 3 or 4 days. These were incubated with a tritium-labeled monoclonal antibody which detects a sporozoite surface antigen. Autoradiography was carried out to reveal binding of this antibody to antigen, as demonstrated by the presence of silver grains. The amount of antigen in infected acini was compared by counting the number of silver grains per 154 μm^2 of section. A significant increase in silver grains, reflecting increased antigen synthesis, could be observed in ticks fed between 2 and 3 days, suggesting that the surface antigen is synthesized primarily at this time.

eration is a parasite-dependent, reversible process.

A comparative analysis of non-parasitized and parasitized daughter cells might provide a means of identifying surface antigenic changes induced by the *Theileria* schizont. Different approaches have been tested for isolating non-parasitized daughter cells, including separation by the FACS according to light-scattering properties, isopycnic centrifugation and gravity sedimentation, but so far none has proven wholly successful. Present attempts involve the use of drugs which selectively destroy dividing, but not non-dividing, cells.

In fatal cases of ECF, cytolytic (cell-killing) cells appear in the peripheral blood which are capable of killing parasitized and non-parasitized lymphocytes from different cattle. The appearance of these killer cells coincides with the development of extensive cell destruction in the lymphoid tissues during the terminal stages of the disease. By contrast, in animals undergoing immunization, cytolytic cells are generated which kill only the animal's own parasitized cells, but not parasitized cells from most other animals.

Research conducted in 1983 showed that cytolytic T-lymphocytes from immune cattle do kill infected cells from other cattle which share the same class I MHC surface antigens. These results suggest that cytolytic cells are generated in immune cattle which recognize class I surface antigens determined by the MHC along with surface antigenic changes induced by the parasite.

Considerable effort has been directed towards developing in vitro cell cultures which mimic the immune response in cattle. When leucocytes were cultured with cells from the same animal which had previously been infected with *Theileria* in vitro, cytolytic cells were generated which killed a variety of cells from the same and different animals. This resembles the situation in fatal infections, rather than in animals undergoing immunization. However, preliminary results suggest that it is possible to generate cytolytic cells in vitro which are specific only for infected cells of the same animal. First, an autologous mixed leucocyte reaction is generated, using blood leucocytes depleted of monocytes as responders against intact irradiated blood leucocytes as stimulators. This results in strong proliferation, but without any generation of cytotoxic cells. If membrane antigen is added from *Theileria*-infected cells of the same animal, cytotoxic cells are generated which are specific for that animal's infected cells.

More than 100 monoclonal antibodies were screened in 1983 in an effort to identify any surface antigens characteristic of *Theileria*-infected cells. The cell binding patterns of the most promising monoclonal antibodies are shown in Figure 8. A few antigenic determinants were detected on the surface of all infected cells. These antigens were not expressed on the surface on normal T- or B-lymphocytes taken from uninfected cattle. However, they were detected in smaller quantities on the surface of all T-lymphocytes exposed to the mitogen concanavalin A and maintained for 3 weeks or longer in the presence of bovine T-cell growth factor. The presence of these antigens on non-parasitized cells indicates that they are of bovine, not of parasite, origin. The relationship of these antigens to regulation of the proliferation of normal and *Theileria*-infected cells is being investigated.

Despite considerable effort, no parasite-specific antigens have been identified by monoclonal antibodies on the surface of infected cells. Yet cytolytic T-lymphocytes which killed *Theileria*-infected cells did not kill cells proliferating in response to concanavalin A. This indicates that the relevant antigens are expressed only on parasitized cells. New experimental protocols are being developed to try to identify these antigens and determine whether they are of parasite or host-cell origin.

One approach involves the propagation of cytolytic T-lymphocytes in culture. Four populations of bovine T-cells have been

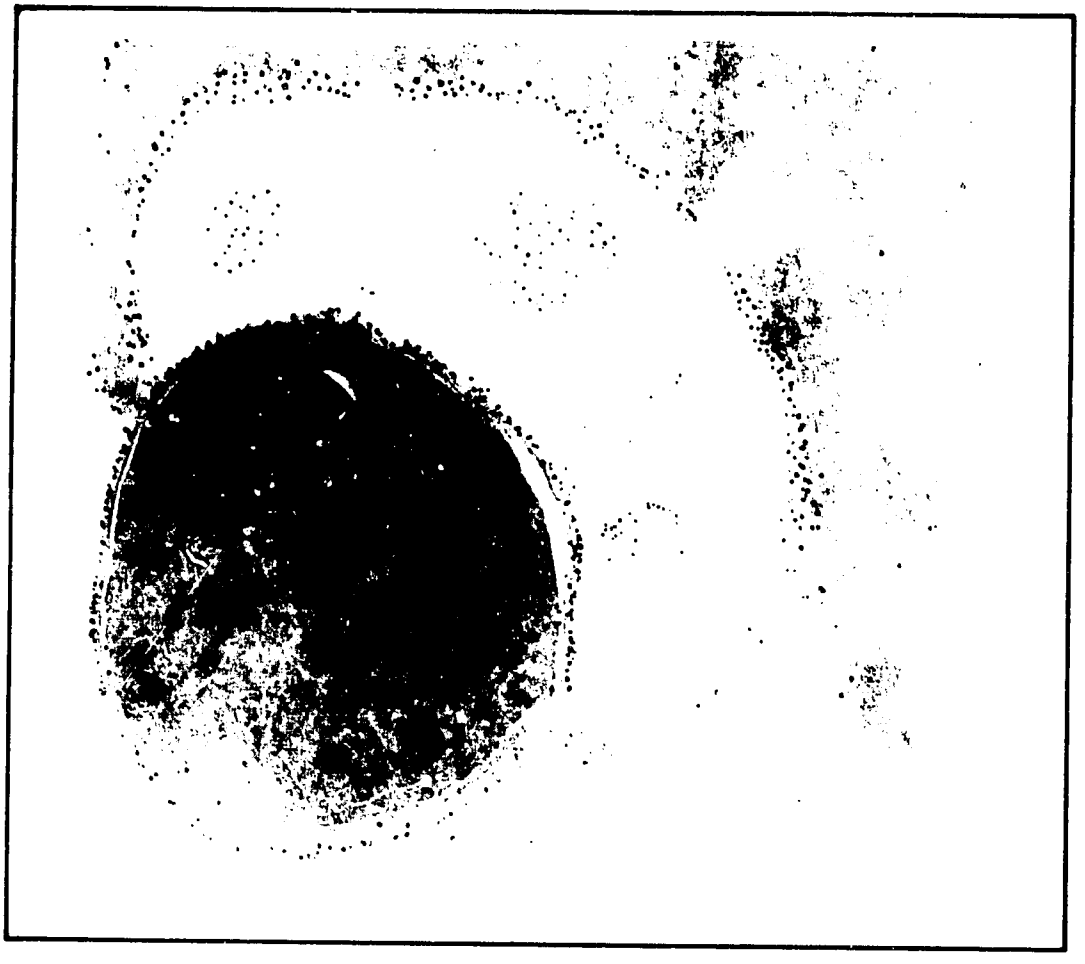

Figure 6. Electron micrograph showing antigen on the surface of a *Theileria* sporozoite and on a bovine lymphocyte. Using a recently developed technique called ultracryomicrotomy, the specimen was cut at a very low temperature (-110°C). A monoclonal antibody was used to bind to the surface antigen. An electron-dense colloidal gold label was combined with protein A, and this complex bound in turn to the antibody. The dense particles of gold appear as grains on the micrograph, indicating the location of the antigen.

maintained in vitro for up to 6 months which are cytolytic for normal lymphocytes of unrelated cattle. Efforts are currently being made to clone these cytolytic cells and to apply the same techniques to culturing anti-*Theileria* cytolytic cells.

Investigations were also carried out in 1983 to determine whether Theileria-infected and transformed cells secrete any biologically active molecules which stimulate or inhibit growth in other cells. Bovine interleukin-2, a factor which maintains T-cell proliferation, has been partially purified from populations of uninfected bovine peripheral blood lymphocytes stimulated with the mitogen concanavalin A. Some clones of *Theileria*-infected T-lymphocytes secrete a factor with a similar biological activity, and work is in progress to ascertain whether this factor is interleukin-2. Another factor, produced by the interaction of lymphocytes and macrophages, has been identified which suppresses the proliferation of lymphocytes stimulated by concanavalin A and, to a less extent, of *Theileria*-transformed lymphocytes. Investigations of these two factors are continuing, and new assays are being developed to detect biologically active molecules in smaller quantities than has been possible in the past.

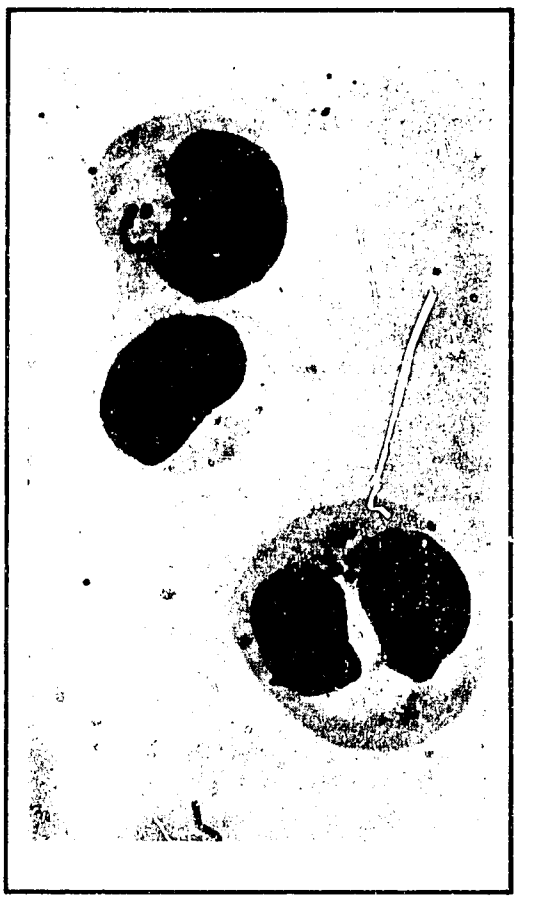

Figure 7. *Theileria*-infected cells treated with 1 µg/ml of cytochalasin B for 24 hours and stained with Giemsa. Cytochalasin B inhibits cell division, but not mitosis, so that double-nucleated cells are regularly seen (at bottom of figure). At the low concentration of the drug used here, division continues in most host cells, but division of the *Theileria* schizonts is impaired, resulting in large numbers of non-parasitized cells (centre).

EPIDEMIOLOGY AND EXPERIMENTAL IMMUNIZATION

Important aspects of ECF epidemiology research at ILRAD are the characterization of disease strains from different field locations and the identification of any cross-protective patterns between different *T parva* stocks. Epidemiologists are also conducting ECF immunization trials in cooperation with the Kenya Ministry of Agriculture and Livestock Development and

	Monoclonal Antibody				
	J3	J4	J5	J6	J7
Normal cells	±	+	–	–	–
lymphocytes from peripheral blood					
lymphocytes from lymph nodes	±	+	–	–	–
Non-*Theileria*-infected proliferating cells					
stimulated with concanavalin A and maintained with bovine T-cell growth factor	+	+	+	+	–
tumour line BL 3	–	–	–	–	–
tumour line BL 20	+	+	+	–	–
infected with bovine leucosis virus	–	+	–	–	–
Theileria-infected cells					
T p parva (cattle)	+	+	+	+	–
T p lawrencei (cattle)	+	+	+	+	*
T p lawrencei (buffalo)	+	+	+	+	–
T taurotragi (cattle)	+	+	+	+	–

– antigen absent
± antigen present on a proportion of cells
+ antigen present
* antigen present on some lines only.

Figure 8. Responses of 5 monoclonal antibodies raised at ILRAD which recognize bovine lymphoblastoid cells.

the Agricultural Development Corporation. Experimental immunization techniques are based on infection with live sporozoites and simultaneous treatment with a long-lasting oxytetracycline antibiotic (terramycin LA—Pfizer). This approach offers the most immediate possibility of practical immunization against ECF while work continues on the development of a safer and more effective vaccine.

STRAIN CHARACTERIZATION

Nineteen monoclonal antibodies have been raised at ILRAD to characterize *Theileria* strains. Parasite antigens are screened in schizont-infected cells established in culture from field isolates. By the end of 1983, 36 *T p parva* stocks had been screened. These were isolated from Kenya, Malawi, Rwanda, Uganda and Zambia. Cross-immunity trials in cattle have given good correlation with monoclonal antibody screening for the characterization of ECF strains.

Monoclonal antibody responses are measured by the indirect fluorescent antibody (IFA) test. Responses have also been measured using the immuno-peroxidase (IPO) test, but IFA procedures proved to be more sensitive and precise for the characterization of ECF strains.

ECF isolates have been characterized to identify appropriate strains which could be used for immunization in different field locations. All isolates tested so far fall into one of four groups, labelled A, B, C and D. Isolates from Kenya and Malawi fall into groups A, B and C, while isolates from Uganda and Rwanda fall into groups B and C and isolates from Zambia have been categorized in group D. A routine screening service has now been established to test isolates from different countries where ECF is

endemic. Detailed information on all isolates is recorded on computer. As further isolates are characterized, the situation could be expected to become more complex, but initial indications are that the total number of *T p parva* antigenic types may be small.

EXPERIMENTAL IMMUNIZATION

In 1983, three groups of cattle were immunized against two ECF stocks isolated from the Kenya coast and characterized at ILRAD—*T p parva* Kilifi and *T p parva* Marikebuni. The cattle were then exposed to field challenge from infected ticks at different sites near the Kenya coast. Cross-immunity trials previously conducted at ILRAD had indicated that cattle immunized against these stocks would be protected against the other ECF stocks which had been isolated in the area.

Eleven Jersey calves between 2 and 12 months old were used for one field trial. This breed is highly susceptible to ECF. Eight calves were immunized by infection with the two parasite stocks. Fifty-four days after immunization, the eight calves and three non-immunized controls were taken to graze for 3 months in an endemic ECF area. The immunized calves showed only mild signs of disease or none at all and all survived, while the non-immunized calves showed much higher levels of parasitaemia and all died of acute ECF.

In another experiment, 16 Sahiwal/Red Poll crossbred calves were immunized by inoculation with the two ECF strains and treatment with oxytetracycline, and another two calves were inoculated with the Marikebuni strain only and treated with Clexon (Wellcome). Forty-nine days after the initial immunization, all 18 calves, plus 7 controls, were taken to graze for 3 months in fields infested with ticks at a second site. All of the immunized calves survived ECF challenge, but towards the end of the trial period three died of heartwater, a tick-transmitted rickettsial disease caused by *Cowdria ruminantium*. All seven of the controls became infected with ECF: five died and two recovered.

Seventeen Boran cattle raised at ILRAD's ranch on the Kapiti Plains were immunized against *T p parva* Marikebuni and exposed to field challenge 12 months later at a third site, along with 5 non-immunized Boran and 5 non-immunized local cattle as controls. Fifteen of the immunized cattle survived 42 days' exposure to infected ticks; one, which had been immunized at 1 month of age, died of ECF, and one died of unknown causes (not ECF). All 10 controls became infected with ECF and died.

The results of these three trials are summarized in Figure 9. This work demonstrates that, at least in some areas, stocks of *T p parva* can be isolated, characterized and used to immunize cattle against local strains. Several aspects of the infection and treatment method are being examined in order to improve and standardize this immunization technique. Investigations have shown that calves can safely be immunized at 1 month of age or less, but protection may not always be long-lasting when very young animals are immunized. Further studies will examine the possible effects of maternal factors in establishing protection in the offspring of immunized cows.

When cattle are immunized against ECF, it may be possible to relax dipping schedules. However, the animals may then be exposed to other tick-transmitted diseases and to the effects of the ticks themselves. To investigate the consequences of increasing dipping intervals, four groups of cattle were monitored for 6 months at one site on the Kenya coast. They comprised 8 cattle immunized against ECF and dipped twice a week, 8 non-immunized cattle dipped twice a week, 8 immunized cattle dipped once every 3 weeks, and 8 non-immunized cattle dipped every 3 weeks. The tick challenge over the period was high, and 9 of the 16 non-immunized cattle under both dipping regimes died of ECF, while none of the

	Number Exposed	Number Survived (%)	Number Died of ECF (%)
Immunized	43	42 (98)	1 (2)
Non-immunized controls	20	2 (10)	18 (90)

Figure 9. Summary of three field trials conducted at the Kenya coast testing the effects of exposure to natural ECF challenge in immunized and non-immunized cattle.

immunized cattle were affected. No other tick-borne diseases were apparent, and there were no measurable effects of tick burden. These results indicate that dipping even twice a week may be insufficient to control ECF in fully susceptible cattle in some areas, while immunized cattle can be left safely for up to 3 weeks without dipping.

Some research was also initiated in 1983 on other tick-transmitted diseases. *Theileria mutans* is routinely encountered in cattle during ECF immunization trials. Although this *Theileria* species normally causes only benign infections in cattle, it can cause anaemia and death when virulent strains are encountered, in mixed infections with other tick-transmitted diseases or in animals which are immunologically depressed. Strains derived from buffalo are more pathogenic than strains maintained in cattle. This parasite is being isolated from a buffalo for experimental transmission to cattle by the tick *Amblyomma variegatum*.

Stabilates have been made of two heartwater isolates from the Kenya coast. They have proved infective to sheep, goats and cattle, causing severe clinical signs and over 90% mortality in susceptible animals. Attempts are being made to establish these isolates in vitro, with the objective of obtaining antigen which can be used for epidemiological studies.

THEILERIA STUDIES IN WILDLIFE

African buffalo are frequently carriers of *T p lawrencei* which can cause serious infections in cattle. ILRAD scientists are studying the antigenic diversity of *T p lawrencei* infections in buffalo and cattle, and 10 *T p lawrencei* stocks have been screened using monoclonal antibodies. Their antigenic diversity appears to be more complex and variable than that of *T p parva*. One monoclonal antibody recognizes only *T p lawrencei*, but does not recognize every *T p lawrencei* stock.

Three *Theileria* isolates were made in 1983 from cattle in areas with substantial buffalo populations. All proved to be *T p lawrencei*. Currently, emphasis is placed on analysing parasite isolates taken from buffalo. This work is being carried out in collaboration with the Kenya Government's Veterinary Research Laboratory and the Veterinary Research Department of the Kenya Agricultural Research Institute, with funding provided by the Government of the Netherlands.

ASPECTS OF TICK PHYSIOLOGY AND THEILERIA TRANSMISSION

Tick salivary gland extracts (TSGE) and saliva have been shown to contain a number of biologically active molecules, including enzymes and anticoagulants, which could play a role in circumventing host defences against tick infestation and transmission of *Theileria*. Of the various activities detected, TSGE anticoagulant is of major interest because of its importance to tick feeding.

Several biochemical and biological assays were employed in 1983 to purify the anticoagulant molecule. It is a protein with an estimated molecular weight of 35,000 daltons. It does not appear to neutralize thrombin, but blocks the activation of prothrombin.

TRYPANOSOMIASIS

Trypanosomiasis is a serious, often fatal, disease caused by protozoan parasites. Different species of the genus *Trypanosoma* infect cattle, sheep, goats, pigs, horses, donkeys, camels, many wildlife species and man. The most important species in economic terms are those which infect domestic ruminants in Africa: *Trypanosoma congolense*, *T vivax* and *T brucei brucei*. Closely related subspecies of *T brucei*—*T b rhodesiense* and *T b gambiense*—cause human sleeping sickness. In Africa, trypanosomes are transmitted cyclically by about 30 species and subspecies of tsetse fly (*Glossina* spp) or occasionally mechanically by other biting flies.

Trypanosomiasis occurs in large areas of Africa, Latin America, the Middle East and Asia. In tropical Africa, the disease occurs over a vast area which appears to be expanding: estimated at 10 million square kilometres, or roughly one-third of the continent. It is impossible to assess, even approximately, the direct losses caused by animal trypanosomiasis, but the fact that livestock in Africa are treated with more than 25 million doses of trypanocidal drugs a year gives some indication of the magnitude of the problem.

The full economic consequences of trypanosomiasis go far beyond figures of mortality or disease occurrence, because few farmers in the region affected by trypanosomiasis are able to keep livestock at all. Out of a total cattle population of about 160 million in Africa, only 20 million are located in the tsetse-infested zone. It is estimated that effective control of trypanosomiasis would make it possible to raise a further 120 million cattle in this region without environmental stress, providing an additional 1.5 million tons of meat a year.

Outbreaks of *T vivax* have occurred in several South American countries, transmitted mechanically by biting flies. Originally introduced with animals imported from Africa, this parasite poses a serious threat to cattle populations in South America, though its epidemiology and economic impact are not fully understood.

WHO estimates that 45 million people are at risk from human trypanosomiasis, with 20,000 new cases reported every year. Trypanosomiasis in livestock may play a role in the epidemiology of the human disease: both *T b rhodesiense* and *T b gambiense* have been isolated from domestic livestock.

In addition to the trypanosomes normally transmitted by tsetse flies, *T evansi* is a serious problem in Africa, the Middle East, Asia and South America. This species is transmitted mechanically. It causes a severe disease in horses and camels and loss of productivity in cattle and domestic buffalo.

Regular treatment with trypanocidal drugs has been an important method of trypanosomiasis control since the early 1920s. However, drug-resistant trypanosome populations are being encountered with increasing frequency, while at the same time the cost of developing new drugs has escalated out of all proportion to the potential return from drug sales. No new trypanocidal drug has been introduced for general use for the past 20 years. The existence of an extensive reservoir of infection in the wildlife population also reduces the effectiveness of chemotherapy as a longterm solution to the trypanosomiasis problem.

For many years, major efforts to control trypanosomiasis have been based on spraying tsetse resting and breeding sites with insecticide. Yet tsetse flies are highly mobile, and different species occupy a variety of habitats, ranging from arid bushland to dense rain forest. Insecticide spraying is not entirely effective in areas of heavy tsetse infestation, dense vegetation or high rainfall, and there is a risk that repeated large-scale spraying might lead to serious environmental pollution.

The goal of ILRAD's trypanosomiasis program is to develop effective, safe and economic methods to control the disease—by immunological, chemical or genetic means. Research concentrates on measures to control the parasites themselves or to enhance the resistance of infected animals. Scientists are also studying aspects of trypanosomiasis epidemiology.

TOWARDS IMPROVED CONTROL OF THE PARASITES

Trypanosomes develop and multiply in the mammalian bloodstream. When a tsetse fly feeds on an infected animal, it ingests parasites along with blood, and these undergo a cycle of development within the fly. The three major trypanosome species all finish this phase of development as metacyclic forms in the tsetse mouthparts or salivary glands. When the infected fly next feeds, the metacyclic parasites are injected into the skin of another animal along with tsetse saliva.

When *T congolense* or *T b brucei* parasites are transmitted by an infected fly, a hard swelling, called a chancre, develops in the skin at the site of the bite. The metacyclic parasites develop further here, invade the local lymph vessels and move into the bloodstream. *T vivax* parasites are also transmitted from the skin to the bloodstream via the lymphatic system, but the local skin reaction, if it occurs, is less severe. In all three species, the infection which follows is characterized by successive waves of parasitaemia, as parasite populations multiply rapidly and die off. *T vivax* and *T b brucei* parasites may also invade the connective tissues, and, in later stages of infection, *T b brucei* is sometimes observed in the central nervous system.

Metacyclic trypanosomes and the forms which develop in the bloodstream are coated with a dense layer of antigen, 12 to 15 nm thick. This surface coat is composed of identical 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 mammals. 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 descendants of a single parasite, and scientists estimate that a single trypanosome clone could produce cells bearing several hundred different surface coats.

An animal produces antibodies in response to the VSG displayed by a wave of invad-

ing trypanosomes. However, before all the parasites can be eliminated, new trypanosomes appear coated with a different VSG, thus evading the host'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. The trypanosome's ability to change the antigenic composition of its surface coat is called antigenic variation. This is the primary mechanism which prevents most domestic livestock from developing an effective immune response to the parasite. It is also the main reason why it has not yet been possible to produce a trypanosomiasis vaccine.

Research continued in 1983 on the genetic basis of antigenic variation and on the way VSGs are synthesized in the parasite and moved to the surface coat. Progress was made in developing and standardizing techniques for maintaining different stages of the three main trypanosome species in vitro. Special attention has been given to *T vivax* in several research areas, as relatively little is known about the biology of this species. 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 defenses.

THE GENETIC BASIS OF ANTIGENIC VARIATION

Trypanosomes have a large number of VSG genes—probably several hundred—which code for the protein component of the surface glycoprotein. Only one of these genes is expressed at a time. To be expressed, a VSG gene must be located at an expression site near one end of a DNA molecule, called a telomeric site. From this site, the gene is transcribed from DNA to RNA, and from RNA it is translated into the protein of the surface coat.

Early results suggested that there were two distinct groups of genes coding for VSG in *T b brucei*. Expression-linked copy (ELC) genes required duplication before being expressed, while non-duplication activation (NDA) genes did not. Current research implies that this division of antigen genes may be misleading.

Research at ILRAD has focused on VSG genes derived from clones of two *T b brucei* stocks. A single cloned VSG gene from each of these stocks has been used to study the genetic rearrangements accompanying expression. By immunological criteria, the VSGs coded by the two genes appear to be identical. In the first stock (ETaR 1), four copies of the ETat 1.8 VSG gene are present in clones before the gene is expressed. When expression of the gene is switched on, a fifth copy appears which occupies a typical telomeric expression site. The VSG messenger RNA (mRNA) is transcribed from this copy, so the gene appears to undergo the ELC type of rearrangement. However, after subsequent switching to expression of different VSGs, the new copy is lost from some trypanosomes, but remains in others.

In the second stock (ILTaR 1), there are five copies of the ILTat 1.3 VSG gene before the gene is expressed, and no new copy appears when expression is switched on. Yet, when expression is switched off again, one copy may or may not be lost in different trypanosomes.

Thus behaviour characteristic of either gene class can be observed in a single system. If current work shows that the ILTat 1.3 and ETat 1.8 genes are indeed identical, as they appear to be, then all the results typifying either of the gene classes will have been observed with a single gene. The chance selection of trypanosome clones showing particular patterns of rearrangement may have led to an artificial division of VSG genes into two classes. It is more likely that observed differences among various clones reflect different aspects of a pathway of activation common to all VSG genes.

Current work aimed at elucidating a common activation process involves mapping the DNA sequences around the two VSG genes. The expression site occupied by the ILTat 1.3 gene has been shown to contain a region of DNA remarkably lacking in restriction enzyme cleavage sites, which has hampered the cloning of this and other expressed VSG gene copies. This 'barren region' consists of an array of contiguous repeats of a sequence of about 80 base pairs, which is probably the target of the recombination enzymes. The exact nature and number of these repeat units in different sites may be responsible for the different frequencies of recombinations at these sites, and thus may hold the secret of why some VSG genes are more likely to be expressed earlier than others during the course of an infection. The expressed copy of the ETat 1.8 gene has also been cloned and comparative studies are being started.

Studies on the genetic basis of antigenic variation have been extended to *T congolense*. By the end of 1983, ILRAD scientists had isolated mRNA from four different *T congolense* clones and had synthesized a library of complementary DNA (cDNA). From this library, four cDNAs have been isolated and characterized: three contain the entire coding sequence for three different VSGs, while the fourth contains more than half the coding sequence for a fourth VSG. These cDNAs are being used in genomic blot analyses to study antigenic variation in *T congolense*, and to determine whether cDNAs for *T congolense* VSGs are specific for different genetically distinct parasite populations (serodemes) and could thus be used for serodeme typing. In 1984, this work will be extended to *T vivax*.

A collaborative project has been initiated with the Vrije Universiteit Brussel to study antigen processing in transfected mammalian cells, using the characterized cDNAs which code for *T b brucei* and *T congolense* VSGs. This work involves placing a trypanosome gene in a well characterized mammalian cell and observing how the gene is activated in a foreign environment.

ANTIGEN SYNTHESIS AND TRYPANOSOME METABOLISM

The integrity of the trypanosome's surface coat is crucially important to the parasite's survival. In addition to evading host immune responses with its changing VSGs, the thick coat of surface antigen also prevents antibodies produced by the host from reaching other parts of the parasite. Biochemists at ILRAD are studying how trypanosome VSGs are synthesized, processed and exported to the surface of the parasite.

Previous studies have shown that *T b brucei* and *T congolense* VSGs contain at least two classes of carbohydrate side chains. The process of binding these side chains to the protein component of the VSG is called glycosylation. One side chain, referred to as the internal or core side chain, is located in the C-terminal third of the protein. It contains mannose and N-acetylglucosamine, and its addition can be blocked by the antibiotic tunicamycin. Some of the enzymes involved in this process have been characterized and show similarities to enzymes identified in several different eukaryotic (nucleus-containing) cells.

The second carbohydrate side chain is potentially more interesting in terms of disease control. It is located at the C-terminal amino acid and contains mannose, N-acetylglucosamine and galactose. The incorporation of this side chain into the VSG is not inhibited by tunicamycin. Its importance is evident from the fact that it appears to remain constant on all *T b brucei* and *T congolense* VSGs and is therefore probably a cross-reacting determinant (CRD), responsible for common antibody recognition among VSGs. Little information is available about carbohydrate side chains in *T vivax*, but recent findings suggest that some *T vivax* VSGs may also contain the CRD.

Figure 10. Electron micrograph of a frozen section of a trypanosome labeled with antibody which recognizes the cross-reacting determinant (CRD) bound with protein A and colloidal gold. The location of the CRD is shown by gold particles over the secretory face of the Golgi complex.

In order to identify the sites of major VSG glycosylation, ILRAD scientists have developed a technique for isolating rough endoplasmic reticulum (ER), smooth ER and Golgi fractions from *T b brucei*, *T congolense* and *T vivax* and have characterized the enzymes involved in the glycosylation process. One interesting finding reported in 1982 was that trypanosomes appear to synthesize the internal glycoprotein after the protein component has been transported to the smooth ER, rather than in the rough ER as in other eukaryotes.

In 1983, scientists conducted experiments to clarify the intracellular pathway of newly formed VSG in greater detail. These studies have shown that the protein component of the VSG is synthesized in the rough ER in 6 to 8 minutes. Ten minutes later, the VSG is found in the Golgi. Using purified anti-IgG antibody which recognizes the CRD carbohydrate, preliminary data have been obtained which suggest that the CRD side chain is added to the VSG in the trans-Golgi region which faces the plasma membrane (Figure 10).

Studies have also focused on laminin, a large adhesive glycoprotein, which occurs primarily in basement membranes and may be involved in anchoring VSG to the para-

site's cell membrane. ILRAD scientists identified a laminin-like protein in *T b brucei* and *T congolense* in 1983, using radioimmunoassay techniques. This protein is found in coated bloodstream forms of the parasite, but not in the uncoated forms.

In collaboration with researchers at Yale University, ILRAD scientists have detected an important calcium-dependent regulatory protein, calmodulin (CaM), in *T vivax*. Scientists elsewhere have described this protein in *T b brucei*. There is evidence that trypanosome calmodulin may be located in the flagellum. Calmodulin in trypanosomes is biochemically and functionally similar to calmodulin in mammals, but the molecule in trypanosomes is smaller. Trypanosome calmodulin also appears to differ immunologically from calmodulin in mammalian tissue, as determined by radioimmunoassay. It might be possible to take advantage of this difference to inhibit parasite calmodulin without interfering with the function of the comparable protein in the host.

CULTIVATION OF TRYPANOSOMES IN VITRO

Major research efforts in 1983 focused on extending the culture systems developed originally for *T b brucei* to the cultivation of various stages of *T congolense* and *T vivax*. A standardized procedure was established for the cultivation of *T congolense* bloodstream forms, using feeder layers composed of bovine aortic endothelial cells. Longterm cultivation was improved by supplementing the culture medium with goat serum.

Using this improved system, bloodstream forms of *T congolense* were cultivated for 93 days. These parasites could not be distinguished from those which develop in the mammalian bloodstream; they had surface coats and retained their infectivity for mammals.

Scientists at the University of Edinburgh developed a system to cultivate metacyclic forms of *T congolense* in 1981. Using a modification of this system, cell biologists at ILRAD have established eight lines of metacyclic-producing cultures from five clones and three uncloned stocks. Twenty to 50 million metacyclic trypanosomes can be harvested 3 to 5 times a week. These parasites appear similar to metacyclic forms obtained from tsetse flies. They have surface coats and they induce a typical local skin reaction when inoculated intradermally in cattle or goats.

When one of the *T congolense* metacyclic lines was transferred to the culture system developed for propagating bloodstream forms, the parasites transformed to the bloodstream type and multiplied rapidly. They were coated with VSG and retained their infectivity for goats and mice. Analysis by immunofluorescence showed that these bloodstream-like forms display the same VSGs as metacyclic trypanosomes derived from tsetse infected with the same trypanosome clone.

Immunization trials in goats also indicated that metacyclic forms of *T congolense* maintained in culture retain the same VSG repertoire as the tsetse-transmitted parasites from which they are derived. Preliminary trials were initiated at ILRAD in 1983, using metacyclic forms of *T congolense* produced in vitro and broken down by ultrasonication. In the first experiment, eight goats were inoculated three times with in vitro-derived metacyclic forms of one *T congolense* serodeme plus adjuvant. Six goats received adjuvant alone as controls.

Two weeks after the second immunization, blood was collected from all the animals. Tsetse flies infected with the same *T congolense* serodeme were allowed to probe in wells containing these blood samples, and after 30 minutes' incubation each blood sample was inoculated into six susceptible mice. The blood taken from the goats used as controls did not neutralize the metacyclic trypanosomes transmitted by the infected flies: all mice inoculated with blood taken from the controls developed infec-

tions. By contrast, none of the mice inoculated with blood taken from immunized goats became infected, showing that the blood from the immunized goats had neutralized the parasites transmitted by the tsetse flies.

In a second experiment, the eight immunized goats, six goats inoculated with adjuvant only and five other goats with no pretreatment were all exposed to tsetse flies infected with the same *T congolense* serodeme. All of the controls developed infection, while none of the immunized goats became infected. The immunized animals were exposed to infected tsetse 2 months later, and again they all resisted infection. These results show, for the first time, that metacyclic trypanosomes produced in vitro can stimulate protective immunity against tsetse-transmitted parasites of the same serodeme.

In collaboration with a colleague from the Swiss Tropical Institute, the culture system developed in 1982 to maintain bloodstream and uncoated insect forms of a West African *T vivax* stock was extended in 1983 to support the transformation of uncoated insect forms to the metacyclic stage of development and back to bloodstream forms, completing the parasite lifecycle.

Research and testing are carried out on a continuous basis aimed at simplifying and standardizing the trypanosome culture systems currently in use. All the systems which have been developed require mammalian serum and feeder layer cells, which adds considerably to the expense of in vitro cultivation. Furthermore, variations between different batches of cells and sera make it difficult to standardize the cultures. Work in 1983 focused on the characterization of specific serum and cell components which stimulate trypanosome growth in vitro, so that these components can be added to culture media rather than whole sera or cells. Components of feeder layer cells which promote parasite growth have not yet been identified: when fractions of cell membranes or soluble cell components were added to *T b brucei* cultures in place of intact feeder layer cells, the parasites no longer multiplied.

To isolate serum factors which promote or inhibit trypanosome growth, sera were tested from cattle and goats, taken from fetal, young and adult animals. From batches of commercial fetal bovine serum, a protein fraction with a molecular weight of about 80,000 daltons was identified and partially characterized. Fetal goat serum was shown to contain less of this protein than fetal bovine serum. Among the bovine sera tested, those which contained less of this protein supported trypanosome growth more successfully. Research is now in progress to characterize such bovine and goat serum factors more precisely.

In collaboration with the Kenya Trypanosomiasis Research Institute and a Kenya Government project funded by the German (Federal Republic) Gesellschaft für Technische Zusammenarbeit (GTZ), ILRAD scientists have developed two simple in vitro tests to measure the activity of trypanocidal drugs based on the culture system developed to maintain bloodstream forms of *T b brucei*. 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} (minimum inhibitory concentration). Possible toxic side-effects are also indicated by observing effects on the bovine feeder cells used in the culture system. Figure 11 summarizes results for 17 compounds tested in 1983. These values can be compared with MIC_{50}s measured in 1982 of 1.00 for diminazene aceturate (Berenil) and 10.00 for isometamidium (Samorin), the two most widely used trypanocidal compounds. Thus, pentamidine, a drug used to treat human sleeping sickness, is a hundredfold more active than diminazene aceturate under these conditions. Sinefungin shows the most potent trypanocidal activity in vitro of all the drugs tested, though research in mice suggests that this drug must

Compound	Supplier	MIC_{50} (µg/ml)
Pentamidine	May & Baker	0.010
Aromatic diamidines		
150/49	University of Erlangen/Nuremberg	30.000
261/115	University of Erlangen/Nuremberg	10.000
313/40	University of Erlangen/Nuremberg	10.000
192/188	University of Erlangen/Nuremberg	0.300
225/144	University of Erlangen/Nuremberg	3.000
98/202	University of Erlangen/Nuremberg	0.100
S 766495	Hoechst	0.030
S 785704	Hoechst	30.000
S 786834	Hoechst	10.000
1530	Hoechst	0.100
18349	Hoechst	0.300
Nr. 363 (Ca 838)	Hoechst	10.000
Arsanilic acid	Animedica	>10.000
Chlorpromazine	Sigma	3.000
Alloxan monohydrate	Sigma	1000.000
Sinefungin	Lilly	0.003

Figure 11. Trypanocidal activity of 17 compounds, indicated by the minimum drug concentration (MIC_{50}) required to inhibit in vitro growth of *T b brucei* by more than 50% during 24 hours' exposure.

be administered in repeated doses to be effective in vivo. Chlorpromazine, which showed some trypanocidal activity in vitro, might be effective against infections in the central nervous system as it is known to cross the blood-brain barrier. In concentrations 10 times higher than the required MIC_{50}, this drug caused severe damage to the supporting feeder layer cells.

The second test being developed is a mammalian-cell-free assay based on the inhibition of trypanosome protein synthesis. Trypanosomes are incubated in a methionine-free medium. Then radioactively labelled methionine is added, and its incorporation into trypanosomal protein is measured in the presence and absence of the drug being tested. Trypanosomes exposed to sinefungin in this assay at a concentration of 1 µg/ml stopped protein synthesis completely after 1 hour.

TOWARDS IMPROVED HOST RESPONSES

A key factor in resistance to trypanosomiasis appears to be host capacity to control parasite growth. Scientists at ILRAD are studying host reactions which might be manipulated to limit trypanosome growth and development in the skin at the site of an infected tsetse bite, in the bloodstream and in the central nervous system. Increasing attention is directed towards the pathogenesis of trypanosomiasis, including anaemia and the severe haemorrhagic syndrome which occurs in some *T vivax* infections. A better understanding of the mechanisms involved might lead to interventions which could alleviate the more severe effects of the disease.

THE LOCAL SKIN REACTION

The hard swelling, or chancre, which develops in the skin at the site of an infected tsetse bite represents the host's first response to trypanosome infection. Interest has focused on the local skin reaction because resistance to trypanosomiasis appears to depend in part on the ability to restrict parasite development in the skin. Resistant wild animals develop smaller chancres than susceptible livestock when bitten by infected tsetse, and the period before trypanosomes appear in the bloodstream is longer in resistant animals, suggesting that parasite development has been slowed down during the initial stages of infection.

Detailed studies of the local skin reaction were conducted in 1983, using metacyclic forms of *T congolense* cultivated in vitro. Intradermal inoculation of metacyclic parasites produced local skin reactions in goats which were similar to chancres associated with infected tsetse bites. Metacyclic preparations at different dilutions resulted in chancres of corresponding sizes, indicating that the local reaction depends, at least in part, on the number of parasites injected into the skin. When irradiated *T congolense* metacyclic forms were used, they did not produce a chancre or stimulate any antibody response.

In a preliminary histochemical survey, the activity of many different types of cells is being observed during chancre formation. A detailed biochemical analysis of chancre material revealed two biologically active molecules which increase as the chancre develops. These molecules are now being identified.

The presence of a trypanosome infection in goats can significantly interfere with the establishment of a second tsetse-transmitted infection. The mechanisms of interference are being investigated, focusing particularly on the inhibition of parasite multiplication in the skin. This work is being carried out in collaboration with the Centre for Tropical Veterinary Medicine at the University of Edinburgh supported by the British Government's Overseas Development Administration.

Goats were infected with a serodeme of *T congolense* and then challenged at different intervals with different *T congolense* serodemes. The first experiment focused on the role of the time interval between the initial infection and subsequent challenge. When goats were infected with two *T congolense* serodemes at the same time, they showed local skin reactions at all the sites of infection and they developed bloodstream infections with both serodemes. However, when goats infected with one serodeme were challenged with a second at intervals from 7 to 35 days after the initial infection, they developed very small local skin reactions, or none at all, and they did not become infected with the second serodeme, as shown by the absence of detectable antibody response. These results indicate that the response to the first infection was not sufficient to rid the animal of trypanosomes, but blocked infection by a second parasite population. This response was not effective until one week after the initial infection.

PARASITE-HOST INTERACTIONS IN THE BLOODSTREAM

Once parasitaemia is established in the bloodstream, resistance appears to be due largely to host factors which regulate parasite development and growth. Antibody-producing lymphocytes are activated soon after infection, but, under certain conditions in susceptible animals, the secretion of parasite-specific antibody appears to be blocked.

Control of parasite growth

All three major trypanosome species change from rapidly-dividing to non-dividing forms

in the course of infection in the mammalian bloodstream. This transition was first observed in *T b brucei*, where it is associated with a clear morphological change from a long-slender to a short-stumpy shape. In 1982, similar transitions from rapidly-dividing to non-dividing bloodstream forms were observed in *T congolense* and *T vivax*.

Little is known about the mechanisms within the parasite which regulate the transition from the rapidly-dividing to the non-dividing bloodstream form and then to the uncoated form which develops after ingestion by the tsetse fly. In 1983, a technique was devised for purifying *T b brucei* nuclei, and four nuclear proteins were identified which are characteristic of specific stages of parasite development. These proteins are currently being analysed more precisely to assess their role in the transition process.

Evidence has accrued that some host factor plays a role in the timing of parasite transition, both in livestock and in mice. It has been shown that this host factor is not parasite-specific antibody. Evidence has accumulated which suggests that host factors modify the growth rate of parasite populations, not by stimulating parasite transition to non-dividing forms, but rather by stimulating parasite multiplication and retarding their transition.

Research in 1983 concentrated on identifying these host factors. A cell- and serum-free culture system was developed for *T b brucei* parasites and then modified under controlled conditions to identify factors which stimulate parasites to multiply. It was found that cattle fibroblast cells, which occur in connective tissue, act together with components of cattle serum to stimulate parasite growth and inhibit transition to the non-dividing form. The serum components involved range in molecular mass from 100,000 to more than 1 million daltons and do not include lipoproteins. Attempts are being made to purify these factors in order to find out whether they stimulate bovine fibroblasts to produce molecules which in turn stimulate trypanosome growth, whether they carry growth-promoting molecules from the fibroblasts to the parasites, or both.

A variety of treatments have been shown to influence the growth rate of trypanosomes in mice. When mice are irradiated or treated with immunosuppressants, parasite growth is stimulated, while treatment with the adjuvant *Corynebacterium parvum* retards parasite growth. All three treatments affect trypanosome growth rates by modifying the rate of transition from rapidly-dividing to non-dividing forms. Culture systems are now being developed to explore how these treatments affect the action of mouse fibroblasts and serum components.

C parvum has been shown to inhibit parasite growth in irradiated mice, and hence is unlikely to exert its effect via the immune system. Preliminary research indicates that *C parvum* reduces parasitaemia in cattle, but the effect is less pronounced that in mice. Studies are in progress to determine how *C parvum* works and which host cell types it affects.

Production of antibodies

When mice are infected with monomorphic *T brucei* serodemes, which do not transform to non-dividing forms, the trypanosome population increases exponentially until the mice die. These monomorphic parasites do not stimulate antibody responses themselves, but they do not suppress the capacity of infected mice to make antibodies against other trypanosomes or antigens.

In contrast, when mice are infected with pleomorphic serodemes of *T brucei*, which transform to non-dividing forms, antibody responses are stimulated which lead to parasite wave remission. Antibodies are first detected which react with trypanosome plasma membrane antigens and with VSG accessible on the surface of living parasites, but not with isolated VSG. These studies suggest that antibody responses are stimulated by parasite fragments on which

VSG is present and plasma membrane antigens are also accessible. It is probable that the fragments arise from non-dividing parasites which die. This conclusion is supported by experiments which show that antibody responses are readily induced by lethally irradiated monomorphic *T brucei* parasites. A possibility arising from the studies is that the kinetics of the host protective antibody response are regulated by the rate at which non-dividing parasites are generated and die.

To pursue this idea, an investigation was made of the antibody responses of different inbred strains of mice in which the same pleomorphic *T brucei* parasites give rise to non-dividing forms at different rates. It was observed that parasite transformation is rapid in relatively resistant mice which mount efficient antibody responses, whereas parasite transformation is slow in susceptible mice which mount poor or no antibody responses. However, these differences in antibody response cannot be attributed solely to the rate at which non-dividing parasites arise. The higher parasitaemia which results from slow parasite transformation in some mouse strains eventually leads to the generation of very large numbers of non-dividing parasites, but this is not accompanied by the production of very large amounts of antibody.

Current studies show that B-lymphocytes are stimulated to produce parasite-specific antibodies in susceptible mice, but that somehow the cells do not release the synthesized antibodies. Similar results have been obtained in susceptible mice infected with *T vivax*, and the investigations are being extended to examine antibody responses to *T congolense*.

EFFECTS OF TRYPANOSOMIASIS IN THE CENTRAL NERVOUS SYSTEM

In the past it was difficult to evaluate the sequential events arising from trypanosome infection in the central nervous system because samples of cerebrospinal fluid (CSF) could only be taken from an infected animal by lumbar puncture. This technique involves repeated trauma and the possible mechanical introduction of blood elements and trypanosomes. A cannulation technique was developed at ILRAD in 1983 which makes it possible to study the composition of the CSF without repeated surgery so that any changes observed can be ascribed solely to the effects of trypanosome infection.

Previous work has suggested that normal CSF does not provide the required elements to support trypanosome growth, but CSF does provide a supportive medium for parasite development at a later stage of infection. CSF samples and other materials which affect trypanosome development are now being analyzed to identify specific factors which stimulate or inhibit parasite growth. In future, it should also be possible to monitor the penetration of trypanocidal drugs into the central nervous system using the cannulation technique.

In a preliminary experiment, a cannulated goat was infected with bloodstream forms of *T b brucei*. Parasites were detected 10 days later in the CSF. Analysis showed dramatic alterations in the composition of the CSF on a day-to-day basis, suggesting that changes occur at earlier stages of infection than was previously believed.

When goats were infected with a West African stock of *T vivax*, by intradermal inoculation or by infected tsetse, trypanosomes were observed in many cases in the CSF and the aqueous humour of the eye. Relapse infections also occurred in several goats after chemotherapy, and in all these cases parasites were isolated from the eye and the CSF. In both primary and relapse infections, goats developed a diffuse meningoencephalitis and pronounced inflammation in the choroid plexuses of the brain.

These results suggest that the eye and/or the central nervous system may act as a privileged site where *T vivax* evades the

action of trypanocidal drugs, possibly explaining the occurrence of relapsing *T vivax* infections reported from the field. Similar results were obtained from studies of *T b brucei* infections in 1982. In the goats infected with *T vivax*, trypanosomes isolated from the eye showed a markedly different VSG profile, compared with parasites isolated from the CSF, lymph nodes or blood. Studies are now in progress to determine the interval after infection before *T vivax* parasites enter the central nervous system, the VSG profile of parasites which develop there, and the central nervous system responses, if any, mounted by the host.

PATHOGENESIS OF TRYPANOSOME INFECTION

Mechanisms of anaemia

Most investigators agree that anaemia in trypanosomiasis arises from the destruction of red blood cells outside the vascular system, largely in the spleen. Alterations must take place on the surface membrane of red blood cells which are signals for cell destruction by the host's reticulo-endothelial system. Work at ILRAD has focused on the identification of trypanosome enzymes which may play a role in this process.

A haemolytic factor, which destroys red blood cells, and phospholipases (fat-splitting enzymes) A_2 and B were detected in crude homogenates of freshly isolated *T b brucei* and *T evansi*. Phospholipase A_2 was found in homogenates of the non-pathogenic trypanosome, *T musculi*, but no measurable haemolytic activity or phospholipase B.

An antibody against crude trypanosome homogenate was raised in rabbits which blocked the haemolytic activity of parasite homogenates against rat red blood cells. However, it had no effect on the activity of the two phospholipases. Rats were infected with *T evansi* and parasitaemia, anaemia and haemolytic activity were monitored. Anaemia and haemolytic activity remained insignificant until a high level of parasitaemia was reached in the bloodstream (10 million trypanosomes/ml). As parasitaemia rose further, from 16 to 50 million/ml, there was a rapid increase in haemolytic activity and severe anaemia, indicated by a drop in packed red cell volume (PCV) of approximately 30%. The haemolytic factor in sera collected from heavily parasitaemic rats resisted heat and could be extracted by organic solvents, which strongly suggests that it is a lipid. This factor is not present in the blood of uninfected rats.

When crude extracts of *T b brucei* and *T evansi* were subjected to ultracentrifugation (100,000 × g), the haemolytic activity was restricted to the pellet, while the two phospholipases were found in comparable amounts in the pellet and in the supernatant. Mice were injected with trypanosome pellet, trypanosome supernatant, palmitic acid, commercial phospholipase A_2, or lysolecithin, a potential product of phospholipase action. Mild anaemia, with a 10 to 15% drop in PCV, was observed in mice injected with palmitic acid or the trypanosome supernatant. The trypanosome pellet produced an 18 to 20% drop in PCV over 16 to 20 hours. More severe anaemia, with a PCV drop of 40%, was produced by phospholipase A_2 and lysolecithin.

It thus appears that anaemia in African trypanosomiasis arises, at least in part, from the action of phospholipases, probably released by dying parasites. These enzymes have the capacity to generate a lipid from parasite substrates as well as from host red blood cells. Such 'processed' red blood cells would then become targets for removal by the host reticulo-endothelial system.

This work is currently being extended to *T congolense* and *T vivax*. Attempts are being made to purify trypanosome phospholipases and to identify their lipid products. Such an analysis might provide a basis for determining the pathogenicity of a particular parasite strain in a given host, as

well as identifying host factors which might contribute to resistance.

Trypanosome enzymes other than phospholipases may also contribute to host anaemia by disrupting proteins and carbohydrates on the surface of red blood cells. This could lead to cell destruction or to defects in cell function. ILRAD scientists developed a procedure in 1983 to identify these enzymes and determine their molecular weights and other characteristics. Protein-disrupting enzymes have been detected in highly pathogenic trypanosome species, including *T b brucei*, *T congolense* and *T vivax*. In each species, these enzymes have a different, but characteristic, molecular weight profile, which suggests that they might be used for species identification in the manner of isoenzyme typing. These enzymes are present in much lower amounts in the non-pathogenic parasite, *T musculi*.

The haemorrhagic syndrome in *T vivax* infections in cattle

Certain strains of *T vivax* are capable of producing a severe haemorrhagic syndrome in cattle which results in death, usually in the second week after infection. The syndrome is characterized by high fever and generalized haemorrhages, which are most severe in the gastrointestinal tract. Animals are often dead before the disease can be diagnosed or treated. While all breeds are susceptible, the European *Bos taurus* breeds are affected most severely. As a result, these productive breeds cannot be maintained in several areas of Africa with potentially highly productive pastures, particularly in the coastal regions of Eastern and Southern Africa.

This acute form of *T vivax* infection has not been well studied, largely because it has not been possible to reproduce the syndrome under experimental conditions. Early attempts were based on inoculating bloodstream *T vivax* forms derived from stabilates made during acute disease outbreaks. While coagulation abnormalities and some haemorrhages were produced, results were variable and the experimental cattle usually eliminated the parasite and recovered.

To test whether tsetse transmission plays a role in producing the syndrome, *G m centralis* flies were infected in 1983 with a *T vivax* strain isolated from a haemorrhagic cow at the Kenya coast. These tsetse were used to infect three Friesian and three Boran cattle. No chancres were detected, but all the animals became infected within 10 days and maintained high levels of parasitaemia throughout the 48 days of the study. The course of temperature, parasitaemia and anaemia in one of the Friesian cattle is shown in Figure 12. Although the Friesians were more severely affected than the Borans, all the cattle developed fever, with temperatures as high as 41°C, and all became severely anaemic, with PCVs averaging 15% by the third week of infection. Platelet levels also dropped dramatically. Five of the cattle showed signs of haemorrhaging in the intestinal tract, and three developed haemorrhages in the mucous membranes. All would probably have died in the third or fourth week after infection had they been maintained under field conditions. Further work is in progress to analyse the disruptions that occur in the bloodstream and to identify specific parasite or host products which contribute to the disease syndrome.

RESISTANCE TO TRYPANOSOMIASIS IN LIVESTOCK AND WILDLIFE

Comparative studies on host resistance to trypanosomiasis comprise an important research area at ILRAD. This work focuses on resistant wildlife species and on different livestock breeds which vary in their susceptibility to trypanosome infection. Factors being investigated include the ability to limit parasite establishment and growth in the skin and the ability to limit parasitaemia and

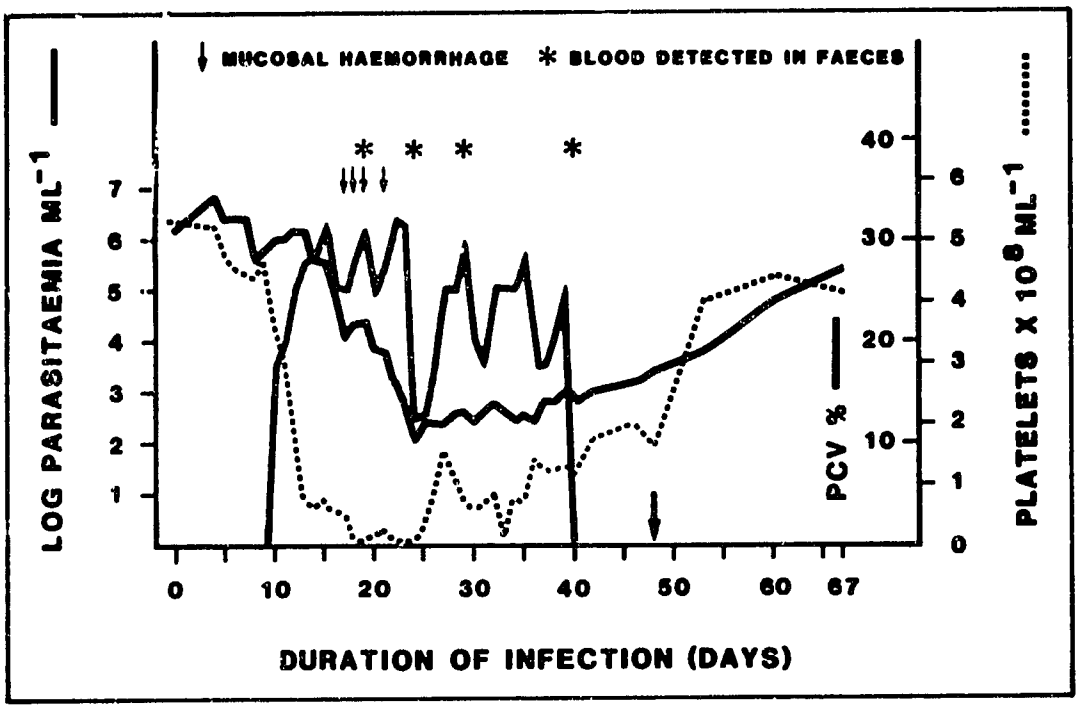

Figure 12. Graph illustrating the relationship between parasitaemia, PCV and platelet numbers in a Friesian cow bitten by tsetse flies infected with a haemorrhagic strain of *T vivax* (2241). The heavy arrow at day 48 indicated treatment with diminazene aceturate.

anaemia at later stages of infection. One goal is to find genetic markers which can be used to identify relatively resistant animals for livestock improvement programs. A better understanding of how some animals resist trypanosomiasis may also suggest promising interventions for increasing the resistance of others.

A short-term culture system has been developed which appears to reflect relative resistance to trypanosomiasis in different animal species and breeds. Results so far indicate that bloodstream forms of *T congolense* survive better in vitro when cultured with cells from susceptible animals. The system was originally developed using cells from resistant and susceptible mouse strains. In 1983, *T congolense* parasites were found to survive equally well when cultured with peripheral blood leucocytes from several susceptible breeds of cattle, sheep and goats. The parasites also survived well in culture with eland, wildebeest and waterbuck cells. In most cases, parasites did not survive well when cultured with buffalo cells, although some variation was observed, perhaps reflecting a spectrum of resistance among individual buffalo. Currently, *T congolense* parasites are being cultured with cells from two reputedly resistant cattle breeds—Orma and dwarf East African Zebu If results continue to show a positive correlation between the susceptibility of differerent hosts to trypanosomiasis and parasite survival in culture with host cells, then this system may prove useful in screening programs to identify relatively resistant animals.

One experiment carried out in 1983 concentrated on West African N'Dama and Baoulé cattle, two breeds which have demonstrated a significant level of genetic resistance to trypanosomiasis. Two N'Dama, three Baoulé and two Baoulé/N'Dama crossbred cattle were infected with *T congolense* transmitted by tsetse. The infected

flies were also allowed to feed on two West African Zebu cattle, a breed considered susceptible to trypanosomiasis. The responses of these four groups were compared, focusing primarily on the local skin reaction.

Chancres developed at 94% of all bite sites. The number of chancres and the course of their development were similar among all the breeds, though the chancres were somewhat larger in the susceptible Zebu. These results are shown in Figure 13.

Histologically, the chancres were most severe in the Zebu, as assessed by the early appearance of large numbers of neutrophils and other cells. Antibodies which neutralized parasite surface antigen appeared in the sera of all the cattle at about the same time. While the first peak of parasitaemia was similar in all the cattle—at about 10^5 trypanosomes per ml—subsequently levels were lower in the trypanotolerant breeds than in the Zebu, and only the Zebu became anaemic. In a related study, East African Boran and European taurine breeds were infected by tsetse with the same *T congolense* serodeme. Their responses were similar to those of the West African Zebu.

Plans for 1984 include a comparative study of *T b brucei* infections in susceptible European cattle breeds and dwarf East African Zebu. This work will focus primarily on host factors which limit parasitaemia in the bloodstream, possibly by influencing the rate of parasite transition from rapidly-dividing to non-dividing forms.

Another experiment in 1983 concentrated on responses to *T vivax* infection. Attempts were made to infect four eland, four waterbuck and two control Friesian cattle with *T vivax* transmitted by infected tsetse. The animals were exposed to as many as 100 infected flies, but none of the eland or waterbuck became infected. However, these same animals were susceptible to intravenous infection with bloodstream parasites. A group of buffalo, cattle and goats were also challenged with two *T vivax* stocks transmitted by infected tsetse. The *T vivax* stock originating from East Africa did not cause chancres in any of the animals, but they all became infected. The West African *T vivax* stock caused chancres in the goats and Friesian cattle, smaller reactions in the Boran cattle and none in the buffalo. The goats and both groups of cattle became infected, but the buffalo did not. Experimentation continues to determine the mechanisms of resistance to *T vivax* in the skin. This work is being carried out in collaboration with the Kenya Government's Veterinary Research Laboratory.

EPIDEMIOLOGY

An important goal of ILRAD's trypanosomiasis epidemiology program is to develop simple, accurate tests to identify trypanosome species and serodemes from samples collected in the field. The epidemiology program also includes studies on the role of tsetse flies as trypanosomiasis vectors. In addition, ILRAD scientists are participating in a collaborative research network to investigate livestock productivity and trypanotolerance at sites in several African countries.

Breed	Number of Chancres/ Number of Tsetse Bites	% Increase in Skin Thickness
N'Dama	10/10	60.0
N'Dama	7/10	66.7
Baoulé/N'Dama	7/7	45.8
Baoulé/N'Dama	10/10	77.3
Baoulé	10/10	58.3
Baoulé	8/10	47.8
Baoulé	8/8	47.8
West African Zebu	8/8	87.0
West African Zebu	5/8	66.7

Figure 13. Local skin reactions in relatively resistant and susceptible cattle breeds infected with tsetse-transmitted *T congolense* (ILR 1180).

CHARACTERIZATION OF TRYPANOSOME POPULATIONS

Species-specific monoclonal antibodies were prepared in 1982 against *T congolense*, *T b brucei* and *T vivax*, and the antigens recognized by these antibodies were isolated. Assays are now being developed which will detect trypanosome species in animals with active infections, as well as animals which have been exposed to trypanosomes in the past. These antibodies can also be used to detect and type trypanosome infections in tsetse flies.

Another objective is to identify specific serodemes within a trypanosome species by analysing parasite antigens. This work has focused on a site at Kilifi near the Kenya coast where an epidemiological survey was conducted in 1982. In 1983, 79 isolates of *T congolense* were collected from cattle which had been kept in a small tsetse-infested area at the site. Metacyclic parasites derived from these isolates are being analysed by immunofluorescence and by neutralization with antisera, and the results are being tested by crossimmunity studies in goats. Four stocks and four derived clones have been analysed so far: they appear to belong to two different serodemes. These preliminary results suggest that several different serodemes may be present, even in a small, isolated focus of infection.

Molecular biologists are using cDNA isolated from a *T congolense* VSG gene to probe *T congolense* parasites isolated from various locations in East Africa, primarily by Southern blot hybridization. This work is being conducted in collaboration with the Prince Leopold Institute of Tropical Medicine in Belgium, drawing on the extensive trypanosome bank maintained by the Institute in Antwerp. The aim is to identify *T congolense* serodemes and characterize their distribution throughout the region.

A spot test is also being developed to identify trypanosome species and subspecies based on the analysis of parasite DNA by molecular hybridization. This technique, designed for use in trypanosomiasis epidemiology studies, has been applied experimentally to identify *T b brucei*, *T b rhodesiense* and *T b gambiense* from field infections, as these subspecies cannot be distinguished morphologically. The development of accurate field tests for the three *T brucei* subspecies could help scientists to determine the importance of *T brucei* infections in livestock for the epidemiology of the human disease.

In vitro studies initiated in 1982 revealed differences among *T b brucei* populations

in terms of resistance to the cytotoxic effects of human serum. This resistance may be an indication of potential human infectivity, because only parasites which resist the cytotoxicity of human serum could maintain a viable infection in the human bloodstream.

Human-serum-resistant parasites have been isolated from goats and cattle originally infected with human-serum-susceptible clones. In all cases, trypanosomes were found in the central nervous system as well as in the blood of infected animals, suggesting that there may be some environmental factor in the central nervous system which favours the expression of human-serum-resistant parasite types. Susceptible parasites were observed in the bloodstream and resistant parasites were observed in the central nervous system of a cow which had been infected with a susceptible *T b brucei* serodeme 6 months previously. When the resistant trypanosomes were passaged cyclically through tsetse flies and goats, they were resistant as bloodstream forms in goats, but susceptible as metacyclic forms extruded from the tsetse. These findings could be significant for the characterization of field isolates of questionable human infectivity, but they are of particular interest in relation to mechanisms controlling gene expression for human-serum resistance.

Research was initiated in 1983 on the epidemiology of *T vivax*. Questions relate to the number of serodemes present in the field, the number of metacyclic antigen types displayed by a given serodeme and the rate of antigenic variation. Studies have focused on a well characterized, rodent-infective *T vivax* stock. Nearly 30 clones of this stock are available, but antigenic studies have been complicated by the fact that these parasites appear to change their VSGs very rapidly, even in irradiated mice where they are not influenced by host antibodies.

When goats were infected with a clone of this stock transmitted by tsetse flies, the VSG of the original clone used to infect the tsetse was not represented in the first peak of parasitaemia in the goats. After drug treatment, the goats were still susceptible to tsetse-transmitted reinfection by the same *T vivax* clone. This may be because tsetse infected with *T vivax* transmit a small number of metacyclic parasites, so the host animal may be exposed to an insufficient number of metacyclic parasites to stimulate a protective immune response. More extensive analysis of *T vivax* antigens is now in progress, focusing on this and other clones of the same stock.

STUDIES ON TSETSE FLIES AS TRYPANOSOME VECTORS

Studies at ILRAD include the role of tsetse flies as trypanosome vectors and possible approaches to trypanosomiasis control through interference with the transmission cycle.

In one study carried out in 1983, groups of teneral *Glossina morsitans morsitans* and *G m centralis* were infected with the three major trypanosome species. The tsetse were then maintained on uninfected calves, goats or rabbits or were fed through membranes on heparinized or defibrinated goat or calf blood. Observed differences in infection rates were small and/or inconsistent among tsetse maintained on these different diets. This indicates that the diet of infected flies probably has no important effect on the cyclical development of the parasites, at least in the two tsetse species used in the experiment.

One approach to tsetse control which has been used experimentally in Tanzania, Nigeria, Upper Volta and Zimbabwe is based on the release of large numbers of male flies sterilized by gamma radiation or chemosterilant. Female tsetse normally mate only once, so if most or all females in an area mate with sterile males the next generation of tsetse flies will be substantially reduced. Research was initiated at ILRAD in 1980 on trypanosome infection rates and

	G m centralis	*G austeni*	*G tachinoides*
T vivax			
sterile	27.3 ± 9.5	4.0 ± 1.4	14.8 ± 7.0
fertile	27.7 ± 11.1	4.0 ± 2.6	12.2 ± 5.0
T congolense			
sterile	24.6 ± 5.2	0.7	0.3
fertile	30.3 ± 4.3	0.3	0.3
T b brucei			
sterile	22.7 ± 2.6	1.1	0.7
fertile	23.4 ± 3.1	0.2	1.7

Figure 14. Trypanosome infection rates (%) in mature male tsetse of three species: tsetse sterilized by gamma irradiation compared with fertile controls.

transmission characteristics of sterile male tsetse, partially funded by IAEA.

In 1983, groups of sterile and fertile male tsetse were fed as tenerals on goats infected with *T vivax*, *T congolense* or *T b brucei*, and their infection rates were assessed, as shown in Figure 14. Three important tsetse species were included in this experiment: *G m centralis*, *G austeni* and *G tachinoides*. Results indicated that *G m centralis* is the most efficient vector of the three species. Transmission rates were high, and did not differ significantly between sterile and normal males. There was also no difference in disease patterns in goats infected by sterile or normal males. These results confirm earlier studies indicating that release of sterile male tsetse flies will potentially increase the immediate risk of trypanosomiasis, and should be combined with other disease-control measures.

Trypanosomes lose their coat of VSG during cyclical development in tsetse flies, and there is evidence that the membrane which becomes exposed is the same antigenically for all parasites of a particular species. Thus, trypanosomes at this stage of development may be more vulnerable to antibody attack than the metacyclic and bloodstream forms coated with VSG.

In an experiment conducted in 1983, goats were immunized with uncoated forms of *T congolense*, *T brucei* or *T vivax* propagated in vitro. The goats were then infected with bloodstream forms of each trypanosome species and were exposed to uninfected tsetse. As shown in Figure 15, infections rates in flies maintained on immunized goats were substantially reduced, compared with controls maintained on infected goats which had not been immunized. The reduction in infection rates was significant for all three trypanosome species, but greatest for *T congolense*. This effect was species specific; when goats were immunized with uncoated forms of one trypanosome species and infected with bloodstream forms of another, no reduction in infection rates was observed among feeding tsetse.

Antibodies to uncoated trypanosomes have also been detected in the serum of infected cattle from 6 to 15 weeks after infection. Such antibodies could play a role in reducing transmission of trypanosomiasis in the field. They might also be used as the basis of a diagnostic test to identify trypanosome species.

The reduced infection rates given in Figure 15 were achieved when tsetse were

Trypanosome Species Used to Immunize and Infect Goats	Infection Rate in Tsetse (%)	Number of Tsetse Dissected
T congolense		
test	0	190
control	31	178
T vivax		
test	46	176
control	86	172
T b brucei		
test	10	174
control	18	171

Figure 15. Infection rates in *G m centralis* which were fed on goats immunized with uncoated forms of *T congolense*, *T vivax* or *T b brucei* and infected with bloodstream forms of the same trypanosome species.

allowed to feed on immunized goats during an entire 30-day period of parasite development. Another aspect of this study aimed at identifying any stage of development when uncoated trypanosomes might be particularly vulnerable to antibody attack. Tsetse infected with *T congolense* were maintained on immunized goats during the early, middle or late phase of parasite development. Infection rates were reduced, but not to the same extent as when tsetse were maintained on immunized goats for the full 30 days. The rate of reduction appeared to depend on the length of time tsetse were maintained on immunized goats, rather than on the particular stage of parasite development during which they were maintained.

An anticoagulant was detected in tsetse salivary gland extracts over 60 years ago, but little was known about its physical or chemical characteristics. Scientists at ILRAD, in collaboration with a colleague from Harvard Medical School, purified a thrombin inhibitor in 1983, first from tsetse saliva and then in larger quantities from dissected salivary glands. In an experiment to deplete this protein from feeding tsetse, flies were fed on blood to which thrombin had been added, and clots were demonstrated in their crops; 43% failed to empty the crop 60 minutes after feeding, compared with only 1.8% of the controls. Sixteen percent of the tsetse fed on thrombin-enriched blood died, while all controls survived.

A fibrinolytic enzyme was also identified in the tsetse crop which is now being characterized. This enzyme would function as a back-up defence mechanism to prevent the potentially harmful effects of coagulant activity in the tsetse bloodmeal.

Work continued in 1983 on the development of a serological system to identify the source of tsetse bloodmeals, using small quantities of residual blood obtained from the flies. Antisera were prepared in 1982 against 42 species of mammals and 4 species of birds. These have been used to analyse tsetse bloodmeals collected in Gabon, Togo and Zaire under the trypanotolerance research network organized in collaboration with the International Livestock Centre for Africa (ILCA). Analyses are now being carried out as requested by the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi. Species-specific mon-

oclonal antibodies are being derived to provide more standardized tests.

COLLABORATIVE TRYPANOTOLERANCE RESEARCH NETWORK

The limitations of current methods to control African trypanosomiasis and the lack of a field vaccine are focusing increasing consideration on the use of trypanotolerant livestock breeds in tsetse-infested areas of Africa. Attention is being given primarily to 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 scientists have been collaborating since 1977 with colleagues at ILCA and ICIPE in a research and training network comparing the productivity of different indigenous African livestock breeds at sites in 11 countries. The network is coordinated by ILCA, with full cooperation from national livestock organizations and support from several donor agencies. ILRAD staff are providing training, advice and supervision for the animal health and tsetse components of the program.

The objective is to evaluate the productivity of different breeds of domestic livestock living under different levels of tsetse challenge, under different management conditions and in different ecological zones. By carefully defining the parameters to be measured and thoroughly training and supervising all field staff, it is hoped to standardize the collection of data so that results from different areas can be critically compared.

These results should make it possible to evaluate genetic differences in susceptibility to trypanosomiasis among livestock breeds throughout Africa. They should also permit an assessment of the role of acquired resistance in different field situations, and an evaluation of factors which affect the stabililty of trypanotolerance. Once these baseline data are established and meaningful productivity indices are computed, it should be possible to predict the productive capacity of different breeds living under different levels of tsetse risk, as well as the potential economic impact of different management regimes, such as the strategic use of trypanocidal drugs. These results will provide essential information to economists planning livestock development programs in the tsetse-infested regions of Africa.

Comparative studies are in progress or are being initiated in Benin, Congo, Gabon, Ivory Coast, Kenya, Nigeria, Senegal, Tanzania, the Gambia, Togo and Zaire. In The Gambia, plans are well advanced for an intensive research project on animal health and productivity in trypanotolerant cattle. The ILCA/ILRAD component of this work will be carried out with financial support from the European Economic Community. In 1983, field staff from several participating countries were trained individually and in two 6-week courses conducted at ILRAD, and a training manual, *Livestock productivity and trypanotolerance*, was published in English and French. ILRAD staff members visited research sites in Gabon, Kenya, Nigeria, Ivory Coast and Zaire to advise field staff on all aspects of the project and to assess the reliability of the data being collected. Data covering the initial 12 to 18 months of operation are currently being analysed, with results expected to be available in late 1984. These will cover production situations involving trypanotolerant N'Dama and susceptible Nguni (*Bos indicus*) cattle and their crosses; Sahiwal and dwarf East African Zebu cattle, reported to exhibit some resistance to trypanosomiasis; susceptible Boran and Ayrshire cattle; and trypanotolerant Djallonké sheep and dwarf West African goats.

RESEARCH SUPPORT

TSETSE LABORATORY

Nine tsetse breeding colonies were maintained at ILRAD during 1983: *Glossina morsitans centralis* originating from mainland Tanzania, *G morsitans morsitans* from Zimbabwe, *G pallidipes* from Kenya, *G austeni* from Zanzibar, *G fuscipes fuscipes* from the Central African Republic, *G palpalis palpalis* from Nigeria, *G palpalis gambiensis* from Upper Volta, *G tachinoides* from Chad and *G brevipalpis* from Kenya. These species and subspecies represent the three main taxonomic tsetse groups—morsitans, fusca and palpalis. They are all important vectors of trypanosomiasis.

Eight colonies were carried forward from 1982. The colony of *G palpalis gambiense* was initiated in February 1983, based on 2000 pupae introduced from Upper Volta.

The *G m morsitans* colony was discontinued during the year, as this subspecies is available in many laboratories and is no longer needed for experimental work conducted at ILRAD. The *G p gambiense* colony reached the target of 2200 breeding females in October and produced pupae weighing an average of 29.6 mg. The *G brevipalpis* colony is still being expanded, with about 1600 breeding females. The average weight of pupae is 74.0 mg. The colony of *G pallidipes* dwindled in early 1983; a new colony of this species will be initiated in 1984. The performance of the other five colonies is shown in Figure 16.

Tsetse flies are used for a variety of trypanosomiasis research projects at ILRAD. In addition, adult *G m centralis* flies and puparia were supplied in 1983 to the Swiss Tropical Institute, the Institut de Médecine Tropicale in Belgium, the Kenya Trypanosomiasis Research Institute, the University of Nairobi and a Commonwealth Institute for Biological Control project being conducted at the Kenya Agricultural Research Institute.

TICK LABORATORY

ILRAD's tick laboratory provides researchers with infected and uninfected ticks, tick salivary glands and other organs and tick-derived *Theileria* stabilates. The tick unit was enlarged in 1983 and production of tick material more than doubled, with over 2 million ticks used and nearly 90,000 dissected in the course of the year. In addition to supplying the needs of scientists at ILRAD, the tick laboratory provided ticks to the Kenya Veterinary Research Laboratory and to three secondary schools in the Nairobi area.

Breeding colonies of five tick species and subspecies were maintained in 1983. The

	Breeding Females (mean)	Daily Female Mortality (mean %)	Weekly Pupae per Female (mean)	Puparial Weight (mean mg)	Annual Number Puparia
G m centralis	9280	0.72	0.56	32.64	269,163
G austeni	2399	0.56	0.54	26.69	67,402
G f fuscipes	1923	0.50	0.43	35.32	43,003
G p palpalis	2040	1.15	0.45	30.75	47,851
G tachinoides	2051	1.15	0.50	20.54	53,155

Figure 16. Performance of five tsetse breeding colonies maintained at ILRAD.

most important of these were *Rhipicephalus appendiculatus*, the vector for *T p parva* and *T p lawrencei*, and *Amblyomma variegatum*, the vector for *T mutans* and *Cowdria ruminantium* (heartwater). The largest number of *R appendiculatus* ticks were from a strain originally isolated in Muguga, Kenya: these were used for a variety of research activities. Increasing numbers of another *R appendiculatus* strain were maintained, isolated originally in Entebbe, Uganda. Smaller colonies were maintained of *R evertsi evertsi*, a two-host tick which is widespread in Africa, *R pulchellus*, which is found in large numbers in certain areas of eastern Africa, and *Hyalomma marginatum rufipes*, a possible *Theileria* vector at present not found in Kenya. In addition, *Boophilus decoloratus* ticks, which transmit babesiosis and anaplasmosis, were occasionally obtained for demonstration purposes.

Several species and strains of tick were collected in the field in connection with ECF epidemiology studies, particularly from sites at the Kenya coast. Many of these were identified and maintained in the tick laboratory for further study. Breeding colonies of uninfected ticks were maintained primarily on rabbits, but where feasible some stages were maintained on cattle, which resulted in increased tick growth.

Large numbers of infected *R appendiculatus* ticks were also maintained, supporting several strains of *T p parva*. Parasites were isolated in the field by applying ticks bred at ILRAD to cattle infected with ECF or by collecting ticks already infesting infected cattle. Established *T p parva* isolates were then maintained in the laboratory by continuous passage through ticks and cattle, with the cycle manipulated wherever possible to increase tick size and infection rates. *Theileria* sporozoites were collected from infected ticks and stored in liquid nitrogen as stabilates.

Factors such as temperature, humidity and the timing of tick attachment were investigated in order to improve the viability and growth of both ticks and parasites. By manipulating these factors, it was possible to increase tick sizes considerably: the average weight of engorged *R appendiculatus* nymphs increased by 33% over 1982 levels, resulting in correspondingly larger adults. In addition, much higher infection rates were achieved than normally occur in the field, resulting in larger harvests of *Theileria* sporozoites.

FARM ANIMAL PRODUCTION

In 1983, ILRAD scientists used 390 cattle for research projects—70% for ECF research, 18% for trypanosomiasis research and 12% for research in other areas. Most of these cattle were Boran (*Bos indicus*) supplied by ILRAD's ranch at Kapiti. The

Kapiti ranch also supplied 22 Guernsey (*Bos taurus*) calves for research projects. Thirty-five Friesian (*Bos taurus*) calves and steers and 28 Boran steers were purchased from other farms in Kenya. The Kapiti ranch provided an assured supply of Boran cattle throughout the year, but the supply of taurine cattle from other farms was interrupted several times when movement restrictions were imposed due to outbreaks of foot-and-mouth disease.

Most research work involving cattle was carried out at the ILRAD site at Kabete. In addition, 81 animals were kept in four locations at the Kenya coast for studies on ECF epidemiology and experimental immunization trials.

An N'Dama embryo-transfer project was initiated in 1983 to produce a group of trypanotolerant cattle at Kabete to be used for experimental purposes. Fertilized N'Dama ova were obtained in The Gambia and surgically transferred to Boran heifers at Kapiti in August 1983.

The herd at Kapiti has increased since the ranch was purchased at the end of 1981. By the end of 1983, the breeding herd included 875 mature Boran cows and 600 Boran heifers, plus 90 mature Guernsey cows and 30 Guernsey heifers. During the year, a spray race was constructed to improve tick control on the ranch, two staff houses were built, fencing was installed, and cattle holding facilities were completed. At Kabete, cattle holding facilities were expanded in 1982, and no major improvements were required in 1983.

During the year, 610 small ruminants were used for research projects, comprising 555 goats and 55 sheep. These animals are of local East African breeds, purchased from the area around ILRAD. As in previous years, about 95% of the small ruminants were used for trypanosomiasis research. Housing for sheep and goats was expanded in 1983 to provide accommodation for an additional 100 animals, bringing total capacity up to 400.

LABORATORY ANIMAL PRODUCTION

In 1983 as in previous years, ILRAD's laboratory animal unit maintained breeding populations of mice, rats and rabbits, plus small colonies of guinea pigs and gerbils. The goal is to supply all the laboratory animals required for ILRAD's research programs with a minimum of wastage. This requires close collaboration with the scientific staff to estimate the numbers and types of laboratory animals needed and to plan appropriate breeding programs.

The mouse breeding program achieved this goal in 1983, with 33,121 mice bred and 30,668 used—a difference of only 9.2%. The unit maintained 14 inbred mouse strains, used primarily to study the mechanisms of resistance to trypanosomiasis. A breeding colony of one random-bred strain was also introduced. Random-bred mice are used increasingly for research which does not require specific genetic traits because they breed more prolifically and grow faster than inbred mice and are thus less expensive to produce.

Three strains of nude mice were also maintained. These are hairless animals with no thymus glands. They are particularly useful hosts for studies of parasite infections because their immune responses are inhibited. One strain was introduced in 1982 bearing the nude gene on a random-bred background. In 1983, two additional strains were introduced, with the nude gene on a C3H.He and a C57Bl.6 background. A program was also initiated of backcrossing the imported nude gene onto ILRAD's own C3H.He and C57Bl.6 lines.

Rats are used primarily to produce trypanosomes. A random-bred rat strain (Sprague Dawley) was introduced in 1983 in order to increase production through higher fecundity and growth rates. During the year, 27,352 rats were produced, which exceeded the requirements of the trypanosomiasis research program. The rat breed-

ing program has been adjusted, with the aim of bringing production more closely into line with demand.

Rabbits are bred primarily to support the tsetse and tick colonies, and a few are used to produce sera for research and diagnostic purposes. A half-lop strain has been developed by crossing local breeds with full-lop-eared rabbits, which were introduced from the UK in 1982. Production increased to 646 in 1983, compared with 450 in 1982. The laboratory animal unit produced 58.6% of all the rabbits used at ILRAD during the course of the year, with the remainder purchased from local sources. Self-sufficiency in rabbit production has not yet been achieved, primarily because an outbreak of *Staphylococcus aureus* occurred in 1982 which was resistant to antibiotic treatment. ILRAD staff produced an attenuated form of the bacterium, and all breeding and young stock have been vaccinated, with encouraging results.

In addition to supplying nearly all the laboratory animals used at ILRAD, the breeding unit provides animals to several other research institutes working in Kenya. In 1983, these included ICIPE, the Kenya Trypanosomiasis Research Institute, the Kenya Veterinary Research Laboratory, the Veterinary Research Department of the Kenya Agricultural Research Institute, the University of Nairobi, the National Museums of Kenya, and a field project conducted by the London School of Hygiene and Tropical Medicine.

CLINICAL AND DIAGNOSTIC SERVICES

The diagnostic laboratory carried out several types of routine analysis in 1983 in support of ILRAD's research programs and animal production facilities. A total of 27,205 samples were received for analysis during the year, including 20,334 samples for serological, 4,304 samples for bacteriological, 367 samples for haematological and 2,200 samples for helminthological analyses. This represented a considerable increase in the number of serological analyses carried out over the 1982 level, due to repeated screening of cattle transferred to the Kenya coast for ECF immunization trials.

Sera were screened for antibodies to six bloodstream parasites: *Theileria parva*, *T mutans*, *Trypanosoma brucei*, *T congolense*, *T vivax* and *Anaplasma marginale*. Most routine serological screening for *Theileria* and *Trypanosoma* parasites was by the IFA test. The enzyme-linked immunosorbent assay (ELISA) was also used to screen bovine sera for antibodies to the three trypanosome species. The card agglutination test was used to screen sera for *A marginale* antibody.

A total of 306 post-mortem examinations were carried out during the year, comprising 140 cattle, 150 goats and 16 sheep. Several diseases were diagnosed in addition to trypanosomiasis and ECF. In cattle, these were salmonellosis, babesiosis, anaplasmosis, bacterial endocarditis, torsion of the abomasum, traumatic reticulitis/peritonitis and *Sphaerophorus necrophorus* causing multiple abcesses in the liver. Pasteurellosis, jaggziekte, haemonchosis, coccidiosis, urolithiasis and heartwater were found in sheep and goats.

In addition to services provided to ILRAD scientists, the diagnostic laboratory continued to provide assistance to other centres and projects in developing countries. In 1983, *T parva* schizont antigens, lymphocyte lysate, positive and negative control sera and goat anti-bovine IgG and IgM conjugates were provided to the Kenya Veterinary Research Laboratory, an FAO project in Burundi and the Veterinary Research Laboratory in Zimbabwe. The diagnostic laboratory also provided training in serodiagnosis of haemoparasites to veterinarians and technicians from Kenya, Burundi, Zambia, Somalia, Ethiopia, Nigeria and Mali, for periods ranging from 1 to 6 months.

TRAINING AND ...

TRAINING

Training activities at ILRAD are designed to support the development of scientific and field personnel who can extend research on trypanosomiasis, theileriosis and other pressing veterinary problems—primarily in Africa, but also in other parts of the world. Participants in the training program work closely with ILRAD's scientific staff. Many work on research projects within the scope of ILRAD's mandate. In addition to research training, scientists and technicians are trained to implement the most effective disease control programs possible with the methods now available. Training at ILRAD also prepares research and field personnel to implement new control programs when improved methods are ready for introduction.

In 1983, 22 scientists and technicians came to ILRAD for specialized training on an individual basis. Two worked for projects sponsored by FAO, and all the rest were employees of national universities, veterinary laboratories or research centres in their home countries. Seventeen came from 11 African countries, 3 came from India and 1 each from Sri Lanka and Australia. Their individualized training programs at ILRAD lasted for periods of 1 week up to 6 months.

Five of these participants were Kenyan. Two technicians from the Kenya Veterinary Research Laboratory learned techniques for rearing tsetse flies. One staff member from the Kenya Trypanosomiasis Research Institute was taught methods of trypanosome cryopreservation, and another staff member from the same institute learned serodiagnostic techniques related to trypanosomiasis. A technician from the Institute of Primate Research received training in parasitology.

From Zambia, a technician from the Central Veterinary Research Institute came to ILRAD to learn principles and techniques in serology, and an FAO specialist received training in parasitology. A staff member from the Uganda Trypanosomiasis Research Organization and a technician from the Tropical Pesticides Research Institute in Tanzania both spent several weeks at ILRAD learning tsetse rearing techniques. A scientist from a Tsetse and Trypanosomiasis Project in Somalia and a staff member from the Livestock Marketing Board in The Gambia both received training in protozoology. An FAO specialist from Rwanda, a scientist from the Veterinary Research Laboratory in Zimbabwe and a scientist from the Central Veterinary Laboratory in Malawi all received advanced training in ECF research. A scientist from the Laboratoire Central Vétérinaire in Mali came to ILRAD for training in trypanosomiasis immunology and parasitology, and a staff member of the Département du Laboratoire Vétérinaire

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in Burundi received training in parasitology and serology related to ECF.

Two staff members from the Haryana Agricultural University in India came to ILRAD to learn techniques for the production of monoclonal antibodies, and a scientist from the Indian National Disease Diagnostic Laboratory learned protozoology techniques. A scientist from the Tick Fever Research Centre in Queensland, Australia learned monoclonal antibody techniques at ILRAD, and a staff member from the Veterinary Research Institute of Sri Lanka received training in ECF parasitology.

Post-graduate students normally work at ILRAD from 1 to 3 years in partial fulfillment of their degree requirements. Fifteen post-graduate students were working with ILRAD scientists in 1983 on projects closely linked with ILRAD's research programs. Five were enrolled in M.Sc. programs and 10 were working towards their Ph.D. degrees. Seven were students from the University of Nairobi, while the others were enrolled in universities outside Kenya. Nine were from Kenya, two from Uganda, two from Belgium, one from Rwanda and one from Sudan.

Post-doctoral fellows are selected internationally, though emphasis is placed on recruiting well-qualified young scientists from African countries. They normally spend 2 years at ILRAD, and their work is expected to contribute directly to the research program. In 1983, 11 post-doctoral fellows were working at ILRAD. Two were from Kenya, two from the USA, two from Germany (Federal Republic), and one each from Uganda, Zaire, Japan, Belgium and the UK.

Altogether, 47 students, scientists and technicians received training at ILRAD in 1983 on an individual basis, 8 more than in 1982. Thirty-three of these trainees were from African countries, including 5 from francophone Africa.

In addition to training opportunities for individuals, five courses were held at ILRAD in 1983. The first, in February, was a series of lectures on data analysis and experimental design, given over a 3-week period by a senior lecturer from the Department of Mathematics at the University of Strathclyde, UK. The lectures were attended by 50 scientists from national and international research oreganizations in Nairobi. The visiting lecturer also helped ILRAD staff members with statistical questions on an individual basis.

A 4-week training course was held in February and March on the Diagnosis of Haemotropic Cattle Diseases, with Emphasis on East Coast Fever. Twelve participants took part in the course from nine African countries and one participant from Indonesia. The course covered the diag-

nosis of ECF and other haemotropic cattle diseases, current disease control methods and recent advances in ECF research.

In October and November, a 4-week course was conducted on the Use of Nuclear Techniques in Animal Parasitology, co-sponsored by ILRAD, FAO and IAEA. Nineteen participants came from 19 different countries around the world. The course focused on radioisotope labeling techniques used to study the immunology and pathogenesis of protozoa and helminth infections.

Two 6-week courses were conducted in 1983 to train field staff working in Nigeria, Togo, Gabon and Ivory Coast as part of the ILCA/ILRAD trypanotolerance research network. The courses concentrated on the diagnosis and control of trypanosomiasis, helminthiasis and tick-borne diseases, as well as tsetse trapping and analysis of animal production data.

An international conference was held in September on the Application of Ruminant Immunology to the Control of Bovine Diseases. The conference was designed to bring together specialists in the field of ruminant immunology and participants from developing countries actively engaged in research on the immunological aspects of bovine disease. It was conducted in English and French; 127 participants came from 12 countries in Africa and 10 countries in Europe, South America and Asia. Topics covered included structural arrangements and cellular components of the immune system, antibody responses, cell-mediated responses, soluble mediators in immune responses, the complement system, inflammatory mediators and the role of immune responses in the control of disease.

ILRAD's training program provided support for one participant from The Gambia to attend the Fourth International Conference of Institutions of Tropical Veterinary Medicine, held in the USA in May 1983. In addition, ILRAD provided funding for 16 participants from developing countries to receive the conference proceedings.

ILRAD also provided support for one Ugandan and one Somali to attend the Fourth FAO/OAU/WHO Seminar on Trypanosomiasis, which took place in Congo in November. An ILRAD scientist gave a series of lectures on trypanosomiasis to final-year veterinary students in Maputo, Mozambique, and another staff member spent 2 months in Zambia giving training in tsetse biology, ecology and control techniques to technical personnel from several African countries as part of a course sponsored by UNDP and FAO.

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Research and training activities carried out in 1982 were described in ILRAD's annual report, published in 1983 in English and French. A quarterly newsletter, *ILRAD Reports*, was also launched during the year; the first issue was published in July and the second in October. Aimed at a broad audience and published in English and French, *ILRAD Reports* includes articles on important events which take place at ILRAD, reviews of selected research projects, summaries of conferences and training activities and lists of recent scientific publications by ILRAD staff.

Considerable effort has been directed towards building up an appropriate distribution list for *ILRAD Reports* and the annual report series. Figure 17 shows the distribution of these publications by region, as of early 1984. Of all the institutions and individuals who have been sent information on ILRAD, 41% have responded, expressing their interest in receiving ILRAD publications.

In 1983, members of the scientific staff published 23 articles in international journals and made 8 contributions to scholarly books. An ILRAD scientist also coauthored a training manual, *Livestock productivity and trypanotolerance*, published by ILCA.

A brochure, *Welcome to ILRAD*, was produced at the beginning of 1983, provid-

Region	Number of Countries	Number of Institutions/ Individuals
Anglophone Africa	23	427
Francophone Africa	20	201
Anglophone & other Europe	13	200
Francophone Europe	2	23
North & South America	21	217
Near & Far East	24	151
Total	103	1219

Figure 17. Summary of *ILRAD Reports* distribution at beginning of 1984.

ing travel information for participants in the training program and other visitors. The brochures published in 1982 describing ILRAD's theileriosis and trypanosomiasis research and training activities are still widely distributed to visitors and others who do not require the detailed descriptions presented in the annual reports.

The ILRAD library maintains a specialized collection covering topics of relevance to ILRAD's research program. At the end of 1983, the collection included over 2500 books and subscriptions to 196 journals, as well as reports sent by other research institutes and copies of articles published by ILRAD staff members.

Current issues of scientific journals are received by air every week, covering fields such as parasitology, immunology, biochemistry, entomology, cell biology and veterinary medicine. Many journals have been collected since 1976 when the library was established. A few volumes of older issues have been sent to the library as gifts from individuals and other institutions. Requests for back issues are often filled through the network of interlibrary loans which includes several research institutes in the Nairobi area. ILRAD, in turn, holds current subscriptions to many periodicals which are not available anywhere else in Kenya.

In addition to serving staff members and participants in ILRAD's training program, the library provides material through the interlibrary loan network to researchers at the University of Nairobi, the Veterinary Research Laboratory, the Veterinary Research Department of the Kenya Agricultural Research Institute, the Kenya Trypanosomiasis Research Institute, the Ministry of Health and ICIPE. ILRAD staff also receive material from these institutions on request. Articles requested by ILRAD staff members which are not available in the Nairobi area are purchased from the British Library Lending Division. Staff members also carry out literature searches, most often of the databases held by the Commonwealth Agricultural Bureaux.

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FINANCIAL STATEMENTS

RATTANSI EDUCATIONAL TRUST BUILDING
HONROVIA/KOINANGE STREET
P.O. BOX 41968
NAIROBI, KENYA
TELEPHONES 20891, 20418, 335362
TELEX 22140
CABLES "CHUNGA NAIROBI"

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 1982 AND 1983

From a detailed examination of the accompanying schedules (Figures 18 to 20) 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 1982 and 1983.

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 31 December 1982 and 1983.

Price Waterhouse

PRICE WATERHOUSE
Certified Public Accountants

18 June 1984

Figure 18. Summary of costs by program and activity (US$'000).

	1983	1982
Research		
Parasitology-Trypanosomiasis	293	446
Biochemistry	666	483
Cell Biology	340	382
Immunobiology	490	285
Parasitology-Theileriosis	497	449
Pathology	541	448
Tsetse Laboratory	317	301
Tick Laboratory	115	93
Electron Microscopy	178	129
Collaborative Research	—	142
Total Research	3437	3158
Research Support		
Office of Director of Research	281	—
Farm Animal Production	685	573
Laboratory Animal Production	133	131
Clinical and Diagnostic Services	67	63
Radio Isotope and Central Core Services	347	400
Total Research Support	1513	1167
Training and Conferences	695	526
Library and Information Services	337	267
Administration		
Board of Directors	70	70
Office of the Director General	357	319
Finance	358	379
Personnel	72	73
Purchasing	274	257
Total Administration	1131	1098
General Operations		
Engineering	726	523
Transport	208	185
Services	104	275
Food and Housing	127	33
Stores	48	54
Total General Operations	1213	1070
Contingency	31	166
Total Core Costs	8357	7452

Figure 19. Summary of sources and application of funds (US$'000).

	1983	1982
SOURCES		
Core Operating Funds		
Unrestricted		
World Bank (IBRD)	800	280
Germany (Federal Republic)	603	717
United Kingdom	548	559
Canadian International Development Agency (CIDA)	615	545
Switzerland	383	391
Saudi Arabia	300	—
Norway	287	325
Belgium	—	185
Netherlands	250	295
Australia	286	286
Sweden	186	200
Italy	136	—
France	52	—
United States Agency for International Development (USAID)	2500	2400
Balance From Previous Year	881	2648
Earned Income Applied in Year	85	106
Total Unrestricted Operating Funds	7912	8937
Restricted		
United Nations Development Fund (UNDP)	576	799
Italy	350	—
Belgium	151	99
Total Restricted Operating Funds	1077	898
Total Unrestricted/Restricted Operating Funds	8989	9835
Transfer to Capital Funds	(654)	(1550)
Net Unrestricted/Restricted Operating Funds	8335	8285
Capital Funds		
Transferred from Core Operating Funds	654	1550
Unexpended Balance from Previous Year	181	45
Balance of Working Funds from Previous Year	709	709
Total Capital Funds	1544	2304
Special Project—Netherlands	203	60
Total Sources	10082	10649
APPLICATIONS		
Core Operations	8357	7452
Capital	735	1366
Special Projects	203	81
UNEXPENDED BALANCE		
Unrestricted Core	—	881
Capital	—	181
Working Funds	687	709
Special Projects	—	(21)
Revolving Fund	100	—
Total Funds	10082	10649

Figure 20. Balance sheet at 31 December 1983 (US$'000).

	1983	1982
ASSETS		
Fixed Assets		
Land and Buildings	9444	9390
Research Equipment	4642	4485
Other Assets	2005	1540
Subsidiary Company		
Investment	1651	1521
Longterm Loan	77	130
Total Fixed Assets	17819	17066
Revolving Fund		
Loans	89	—
Cash	11	—
Net Current Assets	687	1771
Total Assets Employed	18606	18837
FUND BALANCES		
Capital Fund	17819	17066
Working Capital	709	709
Unrestricted Core Surplus (Deficit)	(22)	1062
Revolving Fund	100	—
Total Funds	18606	18837

ILRAD

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