ILRAD 1986

ANNUAL REPORT OF THE
INTERNATIONAL LABORATORY
FOR RESEARCH ON ANIMAL DISEASES

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

ILRAD is one of 13 centres in a worldwide agricultural research network sponsored by the Consultative Group on International Agricultural Research (CGIAR). In 1986, core funding for ILRAD's research and training activities was provided by the World Bank (International Bank for Reconstruction and Development—IBRD), the United Nations Development Program (UNDP), the African Development Bank (ADB), the Rockefeller Foundation and the Governments of Australia, Belgium, Canada, Denmark, France, Germany (Federal Republic), Italy, Japan, the Netherlands, Norway, Sweden, Switzerland, the United Kingdom and the United States of America. Additional research activities were supported by special funding arrangements from the European Economic Community (EEC), the World Health Organization (WHO) and the Governments of Belgium, France, Germany (FR), Italy, Japan, the Netherlands, Switzerland and the UK.

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ANNUAL REPORT OF THE
INTERNATIONAL LABORATORY
FOR RESEARCH ON ANIMAL DISEASES

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FOREWORD

The International Laboratory for Research on Animal Diseases (ILRAD) was established in 1973 with a mandate to conduct intensive research leading to improved control of livestock diseases. ILRAD now occupies a modern complex of research laboratories and support facilities at Kabete on the outskirts of Nairobi, Kenya, and has a cattle breeding ranch at Kapiti, about 80 km from Nairobi. In 1986, ILRAD's full complement of staff included 49 senior scientific and administrative personnel, 24 specialized technicians, 48 technical support staff and 285 in the general support category. Research and training activities concentrate on immunological and related aspects of two diseases which seriously limit food production in Africa and other developing regions of the world—African animal trypanosomiasis and East Coast fever (ECF), a virulent form of theileriosis.

Despite intensive research efforts at ILRAD and in several other laboratories, the current situation regarding control of ECF and trypanosomiasis is not yet completely satisfactory. Control of ECF is still based primarily on control of the tick vector by regular acaricide treatment plus pasture management and exclusion of wildlife. Two new drugs have been marketed for the treatment of infected animals, but they are expensive and require early diagnosis if treatment is to be successful. Thus, ILRAD continues to work towards developing a practical means of immunization against ECF. Research is conducted in three areas: vaccination by infection and treatment, and the development of improved immunization procedures using either isolated components of the sporozoite or of the schizont form of the parasite.

Immunization by infection and treatment requires the simultaneous injection of live sporozoites plus a tetracycline antibiotic. ILRAD's research in this area focuses on improving the methods available for parasite strain characterization in order to gain a better understanding of the geographic distribution and immunogenicity of different strains. This type of immunization is also being tested in different field situations. Field trials have been completed in Kenya and are now in progress in Tanzania and Zimbabwe in close collaboration with national laboratories and veterinary services. These trials are providing information on the effectiveness and economic benefits of ECF immunization under a range of epidemiological conditions, both for small-scale farmers and for commercial ranching and dairy operations. If successful, immunization by infection and treatment could be adopted as a routine measure for ECF control.

The sporozoite is the form of the *Theileria* parasite transmitted to cattle by infected

ticks. ILRAD scientists have identified antigens on the sporozoite surface which stimulate neutralizing antibodies in cattle. These antigens appear to be common to sporozoites of all strains of the parasite studied so far, suggesting that they might be used to confer resistance to ECF over a broad geographic area. A partial amino-acid sequence for one of the antigens has been determined and used to synthesize a polypeptide. Genes coding for this and other sporozoite antigens are also being identified. The objective is to synthesize antigens, either biochemically or using recombinant DNA technology, which can be used for immunization trials in cattle.

Protective immune responses in cattle are also known to be directed against the schizont stage of the parasite, which occurs inside the white blood cells of infected animals. The third area of ILRAD's ECF research program is the search for a vaccine based on schizont antigens, to be used alone or in conjunction with a sporozoite vaccine. Research has been in progress for several years to define the immune responses of cattle to *Theileria* schizonts, and now the immunology, biochemistry and molecular biology techniques which have been developed at ILRAD are being used to identify the schizont antigens which induce protective responses, to determine their structure and to isolate the associated genes.

Once a protective antigen is identified, more research will be required to find out how best to present it in a vaccine. ILRAD is beginning to explore the potential use of viral vaccine delivery systems and other more conventional immunization systems in order to design the best possible ECF vaccine for widespread use. The successful development of an ECF vaccine could also lead to vaccines against other forms of theileriosis.

Control of trypanosomiasis is largely based on insecticidal control of the tsetse vectors and the use of trypanocidal drugs to prevent infection and treat infected animals. New techniques have been developed to control some tsetse species, but overall the area of Africa infested by tsetse has increased rather than diminished. At the same time, no new trypanocidal drug has been introduced for general use for nearly 30 years, and the potential development of drug resistance remains a serious threat.

ILRAD is pursuing short- and long-term strategies for improving the control of trypanosomiasis. In the short term, a better understanding of the epidemiological situation will help national organizations to design improved control programs and monitor their effectiveness. ILRAD's work in this area includes the development of more sensitive and accurate diagnostic techniques for detecting trypanosome infection in livestock and tsetse. Other studies, on trypanosomiasis chemotherapy, include the development of improved techniques for measuring drug levels in treated animals and testing trypanosome isolates *in vitro* for drug resistance. Work in this area was expanded in 1986 with partial support from the World Health Organization (WHO).

In collaboration with the International Livestock Centre for Africa (ILCA) and the International Trypanotolerance Centre in the Gambia, ILRAD scientists are participating in the African Trypanotolerant Livestock Network, with work in progress in eight countries. Tsetse surveys and animal health and production studies are undertaken under different ecological and management conditions, primarily concentrating on N'Dama cattle and other indigenous African livestock which have demonstrated a degree of resistance to trypanosomiasis. The objectives and activities of the Network were thoroughly reviewed in 1986 and a full report on results from 1983 to 1985 was published by ILCA.

Longterm trypanosomiasis research aims at developing new methods of disease control by immunological, chemotherapeutic or genetic means. Basic research is required on the biology of the parasite, the mechanisms of resistance of host animals and the consequences of host-parasite interactions. Substantial advances have been made at ILRAD and elsewhere on the genetics and biochem-

istry of antigenic variation, revealing unique parasite gene rearrangement mechanisms and metabolic pathways not described in mammalian cells which represent potential targets for immunological or chemotherapeutic attack. Research has also been strengthened on the genetic and biochemical processes involved in the differentiation events which take place during the parasite lifecycle, again searching for new targets for attack. The development of *in vitro* culture systems for trypanosomes, plus steady advances in molecular biology, allow a great deal of research which was not technically possible when ILRAD was founded. WHO relies on ILRAD for research on potential immunization methods against trypanosomiasis, and an ILRAD scientist serves on the WHO Steering Committee on African Trypanosomiasis.

Turning to the biology of the mammalian host, resistant hosts, such as N'Dama cattle, are characterized by their ability to control parasite growth, resist the pathological effects of infection—such as anaemia—and mount protective immune responses. Work on the mechanisms of resistance has been facilitated by the development of reagents at ILRAD for the analysis of different immune responses in resistant and susceptible cattle. This work will receive major attention in coming years. If research fails to identify new means of immunological or chemotherapeutic control for trypanosomiasis, a better understanding of the mechanisms of resistance will at least make it possible to identify resistant, productive livestock for introduction in the tsetse-infested areas of Africa.

Work on immunological and other aspects of trypanosomiasis is conducted with links to the Kenya Trypanosomiasis Research Institute (KETRI), the Uganda Trypanosomiasis Research Organization (UTRO), the Centre de Recherches sur les Trypanosomes Animales (CRTA) in Burkina Faso and the International Trypanotolerance Centre in The Gambia. ILRAD's Director General serves on the Executive Committee of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) of the Organization of African Unity's Inter-African Bureau of Animal Resources (OAU/IBAR). In Kenya, ILRAD is a founding member of the Nairobi Cluster, an association of institutes carrying out research on trypanosomiasis, theileriosis and other tick-borne diseases and their vectors. Cluster members include KETRI, the International Centre of Insect Physiology and Ecology (ICIPE), the Kenya Government's Veterinary Research Laboratory, the Veterinary Research Department of the Kenya Agricultural Research Institute, OAU/IBAR, ILCA, the Faculties of Science, Medicine and Veterinary Medicine of the University of Nairobi and the Clinical Research Centre of the Kenya Medical Research Institute. Collaborative ties involve the exchange of research materials, scientific visits and meetings.

Both the theileriosis and trypanosomiasis research programs are being enhanced by a new research group at ILRAD, initiated in 1986 with special funding from the Rockefeller Foundation. This group will be examining the social and economic benefits of improved control measures against livestock diseases.

ILRAD's training responsibilities are viewed at three levels. The first is to train scientists through the Ph.D. and post-doctoral programs who can develop the research base in their own countries and with whom ILRAD can collaborate in future. At the second level, young field officers must be identified who will be responsible for the practical implementation of new control measures for trypanosomiasis and ECF. This is being done through the provision of suitable courses, at ILRAD and elsewhere, and periods of individual laboratory training. The third component is training of technical staff who are required to support both the researchers and the field workers. In addition to its own training activities, ILRAD regularly supplies staff and a laboratory base to support regional training courses and seminars organized by the Food and Agriculture Organization of the United Nations

(FAO), the International Atomic Energy Agency (IAEA) and other organizations.

As one of 13 international agricultural research centres supported by the Consultative Group on International Agricultural Research (CGIAR), ILRAD was subject to a second external program and management review, completed in January 1986. The review panels expressed general and widespread approval of the quality of scientific research in progress at ILRAD. The associated training program and information activities were recognized as incomparably better than those in place at the time of the first review in 1981, the scientific and supporting staff measured up well in terms of ability and dedication, and the research support facilities and operations were rated as excellent. Following full consideration by the review panels and later in the year by the Technical Advisory Committee (TAC) of the CGIAR, ILRAD's long-term objectives continue as before—to develop safe, effective and economically sound measures for the control of diseases in livestock. ECF and trypanosomiasis remain the principal disease targets.

The review teams recommended construction of additional laboratories at ILRAD to solve problems of overcrowding and to achieve better use of specialized facilities. The construction of one new laboratory is nearing completion and research staff are being regrouped for a better focus on new research directions. Changes in the organization and management of research projects suggested in the external reviews are also being implemented.

The review teams urged ILRAD to strengthen collaborative research activities with national organizations in Africa. To meet this need, an additional staff member is being recruited who will be responsible for identifying and establishing mutually beneficial contacts with groups of scientists working in Africa and arranging more exchanges of scientists through collaborative research projects and the Visiting Research Fellow program. A new building will be constructed in 1987 to house ILRAD's expanded training and outreach activities with special funding provided by the Government of the Netherlands.

In concluding this foreword, I would like to express appreciation to the Board of Directors, the scientific and supporting staff and the donor organizations and nations who collectively support ILRAD's research and training activities. Four members of the Board of Directors retired in 1986. These were Dr L.L. Callow, Professor K. Eichmann, Mr J.S. Mburu and Dr K. Wells. Four new members joined the Board: Professor P.C. Doherty of the John Curtin School of Medical Research in Australia, Professor H.E. Jahnke of the Technical University of Berlin in the Federal Republic of Germany, Dr C. L'Ecuyer of the Canadian Ministry of Agriculture and Dr A.R. Njogu, Director of the Kenya Trypanosomiasis Research Institute. Also during the year, one of ILRAD's senior scientists, Dr Hiroyuki Hirumi, was awarded the 30th Noguchi Memorial Medical Prize for work on the *in vitro* cultivation of trypanosomes.

We acknowledge with gratitude the interest and financial support ILRAD receives from many countries and organizations. In 1986, ILRAD continued to receive core funding from the World Bank (International Bank for Reconstruction and Development —IBRD), the United Nations Development Program (UNDP) and the governments of Australia, Belgium, Canada, Denmark, France, Germany (FR), Italy, Japan, the Netherlands, Norway, Sweden, Switzerland, the United Kingdom (UK) and the United States of America (USA). The Rockefeller Foundation and the African Development Bank (ADB) added their support during the year. Additional research activities were supported by the European Economic Community (EEC), WHO and the Governments of Belgium, France, Germany (FR), Italy, Japan, the Netherlands, Switzerland and the UK.

During 1986, ILRAD was honoured to receive visits from a number of important

guests. These included Ms M. Catley-Carson, President of the Canadian International Development Agency (CIDA) and Dr E. Shuh, Director of Agriculture with the World Bank. Visitors from the Rockefeller Foundation were Ms N. Robinson, Vice-President for Administration, and Dr K. Prewitt, Vice-President for the Africa Program. Three important visitors from the Government of Kenya came to ILRAD during the year: the Honourable W.O. Omamo, Minister for Agriculture and Livestock Development, the Honourable W. Saina, Assistant Minister for Agriculture and Livestock Development and Mr B. Kiplagat, Permanent Secretary in the Ministry of Foreign Affairs. ILRAD also received visits from Their Excellencies, the Ambassadors of Belgium, Denmark, Finland, Italy, Norway, Sweden, Switzerland and the USA and the High Commissioners of Australia and Canada.

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

A.R. Gray
Director General
ILRAD

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THEILERIOSIS

Theileriosis is a complex of tick-transmitted diseases caused by protozoan parasites of the genus *Theileria*. The parasites infect cattle and other domestic animals in many parts of the world and pose a major constraint on the expansion and improvement of livestock production.

The most important *Theileria* species are *Theileria parva*, the cause of ECF which affects cattle in East and Central Africa, and *T annulata*, which causes tropical theileriosis in cattle in the Mediterranean littoral, extending through the Middle East, India, southern Russia and Asia. Other important *Theileria* species include *T sergenti*, *T orientalis* and *T mutans* in cattle and *T hirci* in sheep and goats.

Work at ILRAD concentrates on *T parva*, which causes a virulent form of theileriosis that threatens some 25 million cattle in Kenya, Tanzania, Uganda, southern Sudan, Rwanda, Burundi, Zaire, Malawi, Zambia, Zimbabwe and Mozambique. The term ECF is used to describe the classic disease, caused by *Theileria parva parva* and transmitted between cattle by ticks. Corridor disease is caused by *T p lawrencei*, transmitted by ticks to cattle from wild buffalo (*Syncerus caffer*). A mild form of theileriosis is caused by *T p bovis*.

The two subspecies *T p parva* and *T p lawrencei* share a complex lifecycle in the mammalian host and the arthropod vector—the brown ear tick. *Rhipicephalus appendiculatus*. This tick feeds on cattle three times—as a larva, a nymph and an adult. The parasites are transmitted transtadially, most commonly when ticks feed on infected cattle as nymphs and then on disease-free animals as adults.

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

During the course of infection, some of the *Theileria* schizonts differentiate into forms called merozoites which are released from the lymphocytes into the bloodstream. These invade red blood cells where they develop into forms called piroplasms. Feeding ticks become infected when they ingest red blood cells containing piroplasms. In the tick gut, the parasites undergo other transformations, move through the gut wall into the body cavity and migrate to the salivary gland. Here they form an elaborate intracel-

lular sporoblast. Stimulated by tick feeding, the sporoblast gives rise to 30,000 to 50,000 infective sporozoites. These are introduced with tick saliva into a new mammalian host, initiating a new cycle of parasite development.

At present, ECF is controlled principally by dipping or spraying cattle with acaricides to kill the tick vectors. However, frequent dipping or spraying is expensive, and cattle remain fully susceptible if there is any interruption of the acaricide regime. Tick resistance to the available acaricides is also a potential problem. Two curative drugs have been developed for the treatment of ECF, but drug treatment is expensive and infection must be diagnosed at an early stage for treatment to be most effective. Thus, there is an urgent need for alternative methods of ECF control.

Cattle which recover from ECF often show long-lasting immunity. However, different strains of *T parva* are present in most field situations and immunity against one strain does not necessarily protect cattle against another. The total number of ECF strains is unknown, but the number may be limited in some geographic areas and certain isolates or combinations of isolates appear to provide a broad protection. Thus, there are good prospects for controlling ECF by vaccination, and ILRAD places particular emphasis on this approach.

EPIDEMIOLOGY AND EXPERIMENTAL IMMUNIZATION

Early studies on ECF, initiated in southern Africa and further developed at the former East African Veterinary Research Organization in Kenya, have led to experimental immunization procedures based on infection with *T parva* sporozoites and simultaneous treatment with a long-acting oxytetracycline antibiotic. This method, if properly administered, offers the most practical and effective form of immunization currently available and it is being tested in extended field trails in several countries. The disadvantages are that infection with live parasites must be carefully controlled and full protection may not be achieved in areas where several strains of the parasite are present. In addition, cattle may become carriers of the parasites used for immunization.

The most urgent requirement for a better understanding of ECF epidemiology and for improving and extending control by infection and treatment is a reliable method to characterize parasite strains and identify patterns of cross-protection. The only fully reliable method available at present is cross-immunity testing in cattle, but this approach is time consuming and extremely expensive. For this reason, several *in vitro* methods are being developed for use in parasite characterization.

Field immunization trials were initiated in 1986 in Tanzania and Zimbabwe, using locally isolated parasites. In combination with earlier results from Kenya, this work should provide important information on the effectiveness of infection and treatment immunization in a variety of epidemiological situations.

STRAIN CHARACTERIZATION

Because protective immune responses against ECF appear to be strain specific, effective immunization must stimulate responses against the ECF strain(s) encountered in a particular field situation. For this reason, considerable research has been undertaken at ILRAD to develop precise and practicable techniques for identifying *Theileria* subspecies and strains.

Intensive studies were initiated in 1986 to develop a formal system of parasite characterization using a range of techniques. The goal is to develop a characterization system which can be used to identify parasites isolated in field situations throughout the ECF endemic region. Focusing initially on six *Theileria* stocks, several techniques are being tested, including the use of DNA

probes, analysis of schizont proteins by two-dimensional gel electrophoresis and examination of monoclonal antibody profiles. Considerable effort is also directed towards developing techniques to obtain more homogeneous populations of parasites.

The Muguga, Marikebuni, Mariakani and Uganda stocks of *T p parva* were selected for this work, as well as the Boleni stock of *T p bovis* and the 7014 stock of *T p lawrencei*. From reference stabilates of these stocks, bulk working stabilates have been prepared, both from ground up whole ticks and from dissected tick salivary glands. The six stocks, which may or may not be homogeneous, are being expanded, titrated and established in the same bovine cell lines so that parasites can be compared against a common background cell.

Use of DNA probes

Studies continued in 1986 in order to identify repetitive and single-copy DNA sequences which could be used as probes to distinguish *T parva* subspecies and strains. DNA probes are required which are sensitive enough to hybridize to *Theileria* schizont DNA in preparations of infected bovine lymphocytes, distinguishing parasite DNA from that of the host cell. The goal is to ascertain whether genomic differences detectable with DNA sequence probes can provide information about the relationship between different *T p parva* stocks and their ability to induce a cross-protective immune response in cattle. This would be an important tool for the identification of *T p parva* stocks which could be used in vaccine development.

A genomic expression library constructed from *T p parva* Muguga piroplasm DNA was screened with radiolabeled total DNA from *T p parva* Muguga piroplasms and with total DNA from uninfected bovine lymphocytes. Ten clones showed strongly positive, parasite-specific reactions and three were selected for further characterization. All of these probes hybridized to *T p parva* piroplasm DNA and to schizont DNA in infected lymphoid cells, but not to uninfected cells. Different Kenyan stocks of *T p parva* could also be distinguished based on the hybridization of the three selected probes to piroplasm or infected lymphoblastoid cell DNA (Figure 1).

These three probes did not hybridize to DNA from three other *Theileria* species—*T mutans*, *T taurotragi* and *T annulata*. Although more isolates need to be tested, this suggests that these probes may be species specific. Tests are now in progress to determine whether they are specific for the subspecies *T p parva*, or whether they also recognize *T p lawrencei*.

Two of the probes have been subcloned into plasmids and are now being tested to see whether they can be used to identify parasites in infected ticks and in tissue samples from infected cattle. They are also being used to determine if genotypic differences in parasites from the same stock, detected by DNA probes, correlate with differences in their ability to induce cytolytic T-cell responses *in vitro*. Additional DNA probes isolated from a *T p parva* Mariakani genomic library are currently being characterized.

Analysis of schizont proteins

Research was initiated in 1986 to characterize *T parva* subspecies and stocks by analysing schizont protein differences by two-dimensional gel electrophoresis. First, a technique was developed to purify *Theileria* schizonts from infected lymphoid cells by treating parasitized cells with aerolysin. Schizonts purified by this technique were essentially free from host cell material and appeared intact when examined by light and electron microscopy (Figure 2). They also appeared to retain their antigenicity. When electrophoretic patterns were compared from purified schizonts and infected and uninfected lymphoid cells, none of the schizont proteins was seen in uninfected cells. When purified schizonts from different theilerial

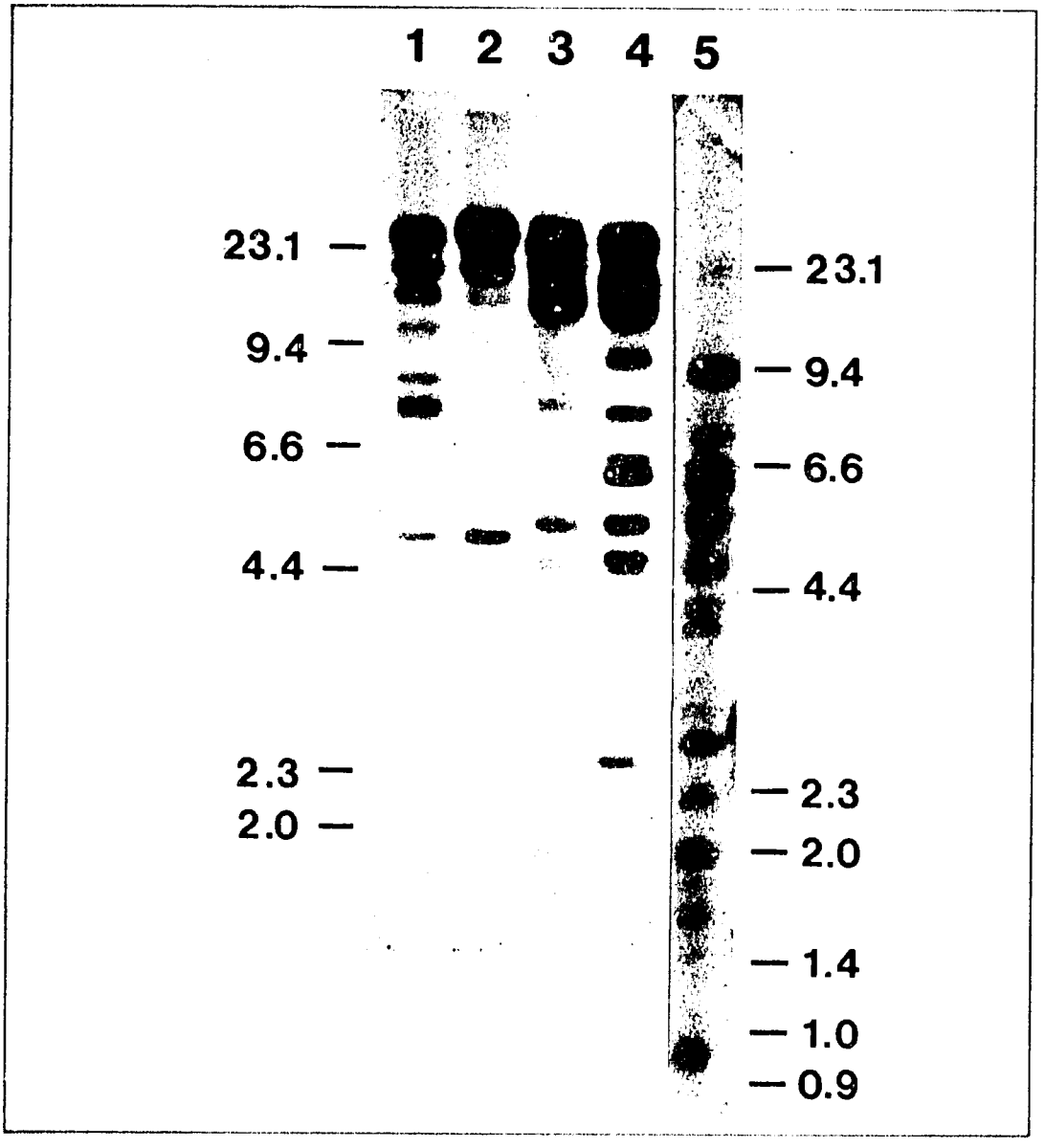

Figure 1. Autoradiograph showing hybridization patterns of *Eco*RI-digested piroplasm DNA from five *T parva* stocks probed with 1gTpM-23, one of three *T parva* DNA probes produced at ILRAD. The hybridization patterns showed clear differences among the stocks tested, (1) Muguga, (2) Kilifi, (3) Mariakani, (4) Kibarani and (5) Marikebuni.

stocks were compared, the same major proteins were seen in all stocks, but minor protein differences were detected. *T p parva* Muguga schizonts from two different cell lines showed identical patterns. The reproducibility of these results is now being tested using several representative *Theileria* stocks in different lymphoid cell lines.

One protein was also identified in infected lymphoid cells, but not in uninfected cells or in purified schizonts. Further studies are planned in order to characterize this protein,

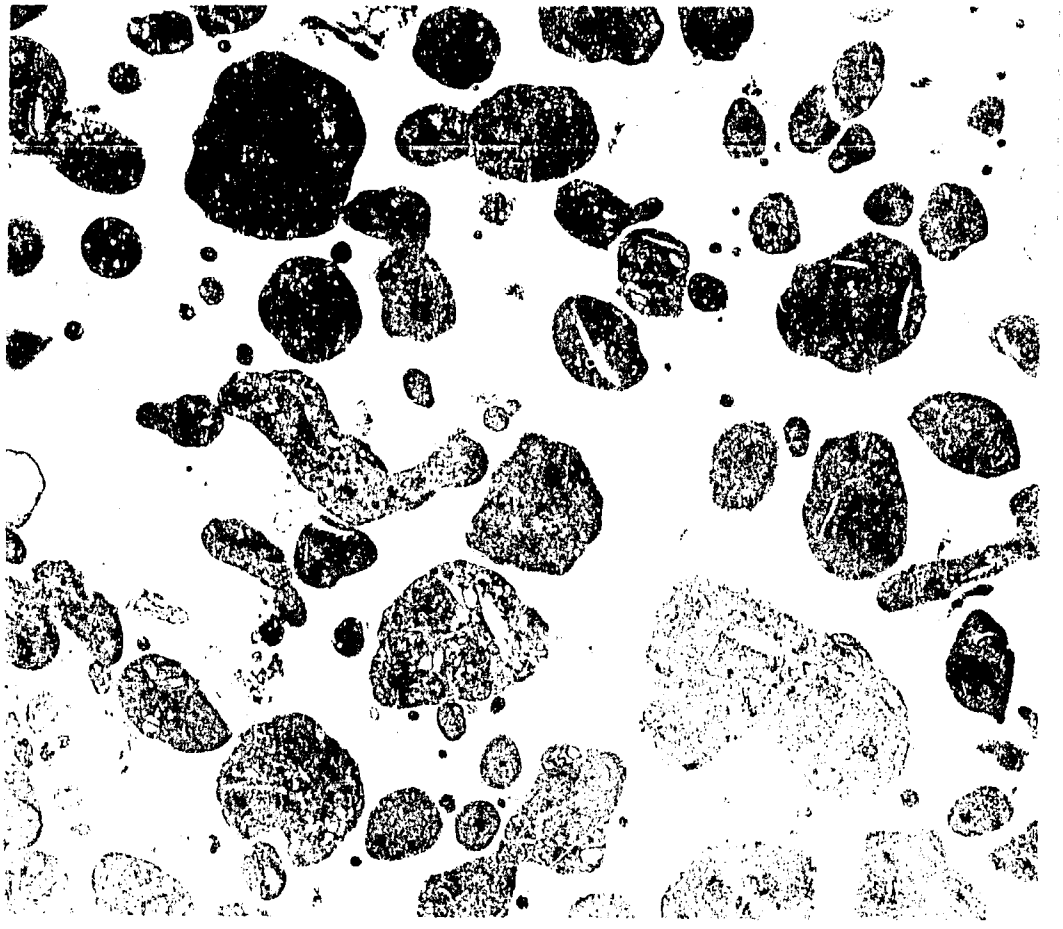

Figure 2. Electron micrograph of *Theileria* schizonts purified from infected lymphocytes by lysing the infected cells with aerolysin and separating the parasites from the lysed host cells by discontinuous density gradient centrifugation. Schizont fractions produced by this technique are essentially free from host cell material and the schizonts appear intact, with normal ultrastucture.

which appears to be specific to infected cells. Such a protein might be of interest because protective immune responses in *Theileria*-infected animals appear to be directed against surface antigen(s) which occur specifically on infected cells.

Lymphoid cells from cattle or buffalo are readily infected with *Theileria* sporozoites and maintained as cloned cell lines *in vitro*. However, the schizonts contained in these cells are not necessarily homogeneous cloned populations because single host cells may have been infected initially by more than one sporozoite. Cattle have also been infected successfully with sporozoites derived from an individual acinus taken from one infected tick. Yet parasites derived from these cattle cannot be assumed to be homogeneous because there is no evidence that a salivary gland acinus can only be infected by a single *Theileria* kinete.

Thus there remains an urgent need for *Theileria* clones derived unequivocally from single parasites. Without cloned parasites, it is impossible to obtain definitive results on

strain characterization or a variety of other *Theileria* research problems. In an effort to obtain sporozoite stabilates composed of more homogeneous, if not cloned, parasite populations, small numbers of sporozoites obtained by titration are being used to infect bovine lymphoid cells. The parasites are then further purified by limiting dilution cloning of the infected cells. The next step will be to use these cells to infect cattle. The objective will be to obtain relatively homogeneous sporozoite populations derived from ticks fed on these cattle. Attempts to infect ticks with single kinetes are also being re-examined as an approach to cloning.

Work in 1986 concentrated on infecting cattle with *Theileria*-infected lymphocytes obtained by cloning. Although cattle are readily infected with uncloned *T parva*-infected lymphoid cell lines, these experiments showed that it is more difficult to infect cattle with cell lines which have been cloned 2 or 3 times *in vitro*. Cattle inoculated with cloned lymphoid cells infected with *T parva* Muguga or a *Theileria*-infected cell line derived from a buffalo showed positive antibody responses to schizont and piroplasm antigens, but none developed clinical infection or detectable parasitaemia. Two animals inoculated with a different cell line derived from the same buffalo developed clinical infections and *Theileria* schizonts were demonstrated in their lymph nodes and blood. However, ticks fed on these cattle failed to develop detectable sporozoites or to transmit infection to other cattle.

Monoclonal antibody profiles: focus on *T p lawrencei* and *T p bovis*

In vitro characterization of *Theileria* stocks has been based primarily on the recognition of schizont antigens by specific monoclonal antibodies. Monoclonal antibodies are produced from hybrid cells formed by the fusion of mouse spleen cells, primed to produce specific antibodies, and mouse tumour cells capable of growing and multiplying *in vitro*. Although monoclonal antibody responses alone are probably not sufficient to classify *Theileria* isolates into groups which are cross-protective *in vivo*, this technique remains the most widely used method for characterizing *Theileria* isolates. ILRAD's battery of anti-theilerial monoclonal antibodies was used in 1986 to characterize over 40 cloned and uncloned bovine lymphoid cell lines infected with *T parva* Muguga or *T parva* Marikebuni.

T p lawrencei, the *Theileria* subspecies almost invariably carried by wild buffalo, causes high mortality in cattle. Several studies have suggested that cattle coming into contact with buffalo are exposed to a large number of different parasite strains. For this reason, the anti-theilerial monoclonal antibodies raised at ILRAD are being used increasingly to characterize *Theileria* parasites originating from buffalo. In 1986, in collaboration with the Kenya Agricultural Research Institute and the Kenya Government's Veterinary Research Laboratory, ILRAD scientists characterized isolates taken at intervals from seven naturally infected captive buffalo. Cattle were infected with sporozoites derived from ticks which fed at different times on these buffalo, and parasite isolates taken from the cattle were also characterized. Results of this work support earlier observations of marked antigenic diversity among isolates taken from different buffalo in the same area, and even among different isolates taken from the same buffalo at weekly or monthly intervals.

There has been considerable interest in theilerial parasites isolated in Zimbabwe which appear to cause mild infections in cattle. These have been grouped as *T p bovis* and the Boleni isolate has been investigated as a potential key immunizing stock. Fourteen infected cell lines isolated in Zimbabwe have been characterized at ILRAD using monoclonal antibodies. The Zimbabwe isolates were taken from infected cattle during an epidemiological study conducted by the national Veterinary Research Laboratory

with support from FAO. The monoclonal antibody profiles of two of these isolates were similar to those of *T taurotragi* isolates from Kenya, while the profiles of the other isolates resembled *T p parva*. There was considerable antigenic diversity between isolates, even when taken from cattle on the same farm, and there was evidence of mixed parasite infections in isolates from individual cattle. Overall, there did not appear to be a specific pattern of monoclonal antibody responses which could be considered characteristic of *T p bovis*-type parasites.

IMMUNIZATION BY INFECTION AND TREATMENT

ILRAD is helping to implement an ECF immunization project on Zanzibar and Pemba Islands in Tanzania in collaboration with the Tanzanian Government, FAO and an Irish Development Project. Two *T parva* isolates were made on each of the islands, and these have been characterized at ILRAD to determine their suitability for use in immunization trials by infection and treatment.

Two of the four isolates, one from each island, were found to be pathogenic, causing severe ECF. Immunization with stocks derived from these isolates—*T parva* Zanzibar South and *T parva* Pemba/Mnarani—provided protection against two pathogenic stocks from the Coast Province of Kenya—Mariakani and Marikebuni. Screening with monoclonal antibodies indicated that the two Tanzanian stocks were antigenically distinct and suggested the existence of antigenic heterogeneity within the Pemba/Mnarani stock. Infection with these stocks can be controlled by long-acting oxytetracycline (Terramycin LA) and appears to provide safe, effective and broad immunity. They are now being used in immunization trials on the two islands.

The characterization of *T parva* parasites isolated in Zimbabwe has been completed in collaboration with the Zimbabwe Veterinary Research Laboratory and FAO. The first immunization trial using these stocks was initiated in October 1986, with exposure of the immunized cattle to field challenge planned for the rainy season beginning in December.

Improving immunization: infection

Although nearly every buffalo in Kenya appears to be a carrier of *T p lawrencei*, very little work has been done using infection and treatment immunization with this parasite. In collaboration with the Kenya Government's Veterinary Research Laboratory and the Kenya Agricultural Research Institute, ILRAD scientists have been preparing and testing parasite stabilates derived from ticks fed on captive buffalo. These studies have used naturally infected buffalo captured at two widely separate locations in Kenya—Ol Pejeta ranch in the Nanyuki area and the Transmara. Different levels of stabilate dilution are being tested, as well as different drugs for use in an infection and treatment regime. The buffalo-derived stabilates are being used experimentally to induce immunity in cattle exposed to *T p parva* and *T p lawrencei* isolated in the respective locations where the two buffalo were captured.

Improving immunization: treatment

Although oxytetracycline is widely used in ECF immunization trails, the mode of action of this drug is not fully understood. The objective of work in this area is to improve the effectiveness of drug use in infection and treatment immunization and also to allow anticipation of situations where the drug may not be effective.

In vitro studies have shown that oxytetracycline reduces the growth rate of infected cells, both in the initial stages of infection and once infection is established. Inhibition of proliferation was also demonstrated in uninfected lymphoid cells stimulated by the mitogen concanavalin A. An effect on lym-

phocyte mitochondrial protein synthesis was detected within 24 hours of oxytetracycline treatment. This effect may be responsible for inhibiting the development and establishment of the parasite in the infected cell.

SPOROZOITE ANTIGENS AND IMMUNE RESPONSES

Cattle which survive in ECF endemic areas appear to develop a degree of immunity to the *Theileria* parasites. Sera from cattle in these areas have been shown to contain antibodies which neutralize *Theileria* sporozoites *in vitro*, thus potentially conferring protection against infection. The second major component of ILRAD's theileriosis research program focuses on the possibility of developing an immunization procedure based on stimulating host responses against sporozoites. The major objective of this work is to establish unequivocally whether protective responses against *Theileria* sporozoites do occur and, if so, how they can be exploited to the maximum benefit of animals at risk.

Early studies showed that antibodies raised against sporozoites of one *T p parva* strain recognized sporozoites of other strains of *T p parva* as well as strains of *T p lawrencei* which had been passaged through cattle. This suggested that anti-sporozoite antibodies might offer broad protection against ECF infection over a wide geographic area. Experiments were conducted in 1986 to expand these results using a strain of *T p lawrencei* obtained directly from a buffalo. Sera obtained from cattle and rabbits immunized with *T p parva* were shown to neutralize sporozoites of the *T p lawrencei* strain *in vitro*, and sera raised in rabbits against the *T p lawrencei* strain neutralized sporozoites of *T p parva*. Further experiments showed that antisera against both *Theileria* subspecies recognized a number of the same or similar sporozoite antigens. A 67,000-dalton antigen was the most prominent on sporozoites of both subspecies. Monoclonal antibodies raised against this antigen have also been shown to neutralize *Theileria* sporozoites *in vitro*.

Intensive research is now in progress to produce the 67,000-dalton antigen *in vitro* and test whether it might be capable of stimulating protective immune responses in cattle. Biochemical analysis has shown that the antigen is a glycoprotein (protein-carbohydrate compound), with antibody responses apparently stimulated by the protein moiety. A partial amino acid sequence of this protein has been obtained and research now concentrates on making synthetic peptides based on this sequence for experimental immunization of cattle. The synthetic peptides are being coupled with different carrier proteins to test whether they can stimulate neutralizing antibodies against sporozoites in cattle. This work is being conducted in collaboration with the Wistar Institute (USA).

Following another approach, scientists at the Wistar Institute have used the partial amino acid sequence of the 67,000-dalton protein to synthesize several oligonucleotides, DNA sequences which potentially code for parts of the protein. These are now being used as probes to screen whole *Theileria* DNA and DNA libraries in order to identify the gene which codes for the protein component of the antigen. Once identified, this gene can be inserted into an expression vector to produce fusion proteins containing the relevant antigen and these can be tested in cattle.

Efforts to identify genes which code for sporozoite antigens have included the screening of ILRAD's *T p parva* Muguga sheared genomic DNA library with antisera from immunized cattle. Five clones were identified in this way which code for the 67,000-dalton protein.

Scientists are also looking for genetic sequences which code for a number of other sporozoite antigens recognized by immune cattle. Apart from efforts to develop a sporozoite vaccine, this work could potentially lead to the identification of sequences useful

for characterizing *Theileria* species and strains.

SPOROZOITE INVASION OF HOST CELLS

Research on sporozoites at ILRAD includes studies on the mechanism of sporozoite binding to host cells, with the possibility of finding a way to intervene in this process and block infection. The binding and invasion of host cells by irradiated sporozoites has also received attention.

Sporozoites from different *Theileria* strains appear to bind to the same receptor molecule(s) on bovine lymphocytes, suggesting that parasites bind host cells by a common receptor/ligand mechanism. Studies have indicated that the receptor molecule(s) on host cells is a glycoprotein which is utilized by *Theileria* sporozoites during the process of recognition, attachment and invasion.

Monoclonal antibodies were raised which inhibited sporozoite invasion of bovine cells *in vitro*. These antibodies appeared to recognize a 44,000-dalton molecule on the surface of different bovine lymphocyte populations. One of the blocking antibodies also recognized a 200,000-dalton molecule. When this antibody was added to target cells which had been pre-incubated with saturating concentrations of sporozoites, it recognized the 44,000-dalton, but not the 200,000-dalton, molecule. This suggests that the 200,000-dalton component of the receptor molecule on the surface of bovine cells may be cleaved during the process of parasite attachment.

Early research on the effects of irradiation on *T parva* sporozoites showed that sporozoites attenuated by irradiation induced a variety of reactions in cattle. This variation suggested that irradiated sporozoites would not provide the basis for a broadly effective ECF vaccine. However, these studies did not examine in detail the structural changes which occur when irradiated sporozoites enter bovine target cells or the immune response which irradiated sporozoites induce in infected cattle.

Recent findings, indicating that *T parva* sporozoites pass through a phase in the tick vector during which they are sensitive to irradiation, have revived interest in the possibility of immunization with irradiated sporozoites. Experiments conducted in 1986 showed that irradiated sporozoites bind to bovine lymphocytes *in vitro* as efficiently as normal sporozoites, indicating that the binding molecule on the surface coat of the sporozoite is not affected by irradiation. Irradiation does not prevent sporozoites from entering lymphocytes, but very few irradiated sporozoites develop into schizonts.

These studies are being extended to investigate the ultrastructure of the schizonts which do develop from irradiated sporozoites and whether these schizonts are capable of stimulating bovine lymphocytes to proliferate. Scientists are also taking lymphocytes from cattle, infecting them with irradiated sporozoites *in vitro* and inoculating the infected cells back into the same animals to see whether they will establish infections and stimulate immune responses.

IMMUNE RESPONSES AND SCHIZONT-INFECTED CELLS

The third major component of ILRAD's theileriosis research program concentrates on the schizont stage of parasite development. Cattle which have developed immunity against ECF have been shown to produce cytolytic (cell-killing) lymphocytes which kill cells infected with *Theileria* schizonts. The killer cells appear to recognize a schizont-induced antigen on the surface of infected cells. By contrast, susceptible cattle suffering from acute ECF produce cytolytic cells which are capable of killing both infected and uninfected cells. These killer cells probably play an important role in the

destruction of the immune system which is a prominent feature of the disease.

The immunity stimulated in cattle by the infection and treatment procedure may be due primarily to cytolytic responses against schizont-infected cells. The goal of ILRAD's research in this area is to gain a better understanding of cell-mediated immune responses and the schizont-induced antigenic changes which trigger them. Besides possibly leading to the development of improved immunization procedures against ECF, a better understanding of immune responses in cattle should contribute towards the improved control of other diseases.

CHARACTERIZATION OF BOVINE CELL TYPES

In order to understand immune responses in cattle, it is important to be able to identify different types of bovine cells which play a role in immunity. Reagents available at ILRAD have been used extensively in studies to identify the cell types that become infected with *T parva*, to define the cells involved in the immune responses to *Theileria*, and to distinguish various cell subsets in order to examine their functional properties.

Broadly speaking, cattle and other mammals have two types of immune response—antibody and cell-mediated—which are mediated by different types of lymphocytes. B lymphocytes are responsible for the antibody responses directed against *Theileria* sporozoites. The cell-mediated immune responses directed against schizont-infected cells are mediated by T lymphocytes which mature in the thymus. T lymphocytes recognize foreign antigens in combination with molecules on the surface of the animal's own cells, known as major histocompatibility complex (MHC) molecules. Individual animals of the same species do not necessarily possess the same MHC molecules. For this reason, T lymphocytes generally only recognize antigens presented on cells from the same animal or from another animal with matching MHC molecules. This phenomenon is known as MHC restriction.

There are two main types of MHC molecules—class I and class II—each with different biochemical and functional characteristics. Correspondingly, T lymphocytes can be subdivided into cells which recognize foreign antigens in combination with class I MHC molecules (class I restricted) and cells which recognize foreign antigens together with class II MHC molecules (class II restricted). These two subpopulations of T lymphocytes generally have different immunological functions.

In 1986, the search continued for antigenic markers which can be used to improve the definition of important subpopulations of T lymphocytes and other cell types involved in immunity. Several monoclonal antibodies have been produced which react with the majority of T lymphocytes. These fall into two groups. One group reacts with about 80% of all cells found in the thymus, including mature and immature T lymphocytes. The antibodies recognize molecules of 55,000 daltons on the surface of the cells: these have been designated BoT2 because their properties are similar to those of CD2 molecules on human T lymphocytes. The other monoclonal antibodies only react with mature T lymphocytes in the medulla of the thymus. It has not yet been possible to identify the molecule(s) they recognize, but when these monoclonal antibodies are added to resting T lymphocytes *in vitro* the cells are stimulated to proliferate. This suggests that the molecule they recognize may be similar to the CD3 molecule on human T lymphocytes, so the bovine molecule has tentatively been designated BoT3.

Several other monoclonal antibodies define two major subpopulations of mature T lymphocytes found in the bloodstream and in peripheral lymphoid tissues. The target molecules on these T-lymphocyte subsets have been designated BoT4 and BoT8 because of similarities with CD4 and CD8 in man. In contrast with mature T lympho-

cytes, a population of immature cells in the thymus displays both the BoT4 and the BoT8 molecules. Staining of thymic lymphocytes by two-colour immunofluorescence, in conjunction with staining of tissue sections, has shown that the majority of these immature cells are located in the cortex of the thymus. Other minor populations of T lymphocytes that do not express BoT4 or BoT8 are also detected in the thymus and the bloodstream (Figure 3).

Extensive studies with cloned and uncloned T lymphocytes have demonstrated that the bovine cells displaying the BoT4 molecule are the T lymphocytes which recognize foreign antigen on antigen-presenting cells in the context of class II MHC molecules, whereas the cells with the BoT8 molecule recognize antigen in the context of class I MHC molecules. Analysis of cell functions has shown that the T lymphocytes displaying the BoT4 molecule act as 'helper' cells, while those displaying the BoT8 molecule act as cell 'killers': the BoT4 cells produce soluble growth factors which enable the BoT8 cells to proliferate and acquire a cytolytic function.

In 1986, these reagents were used to

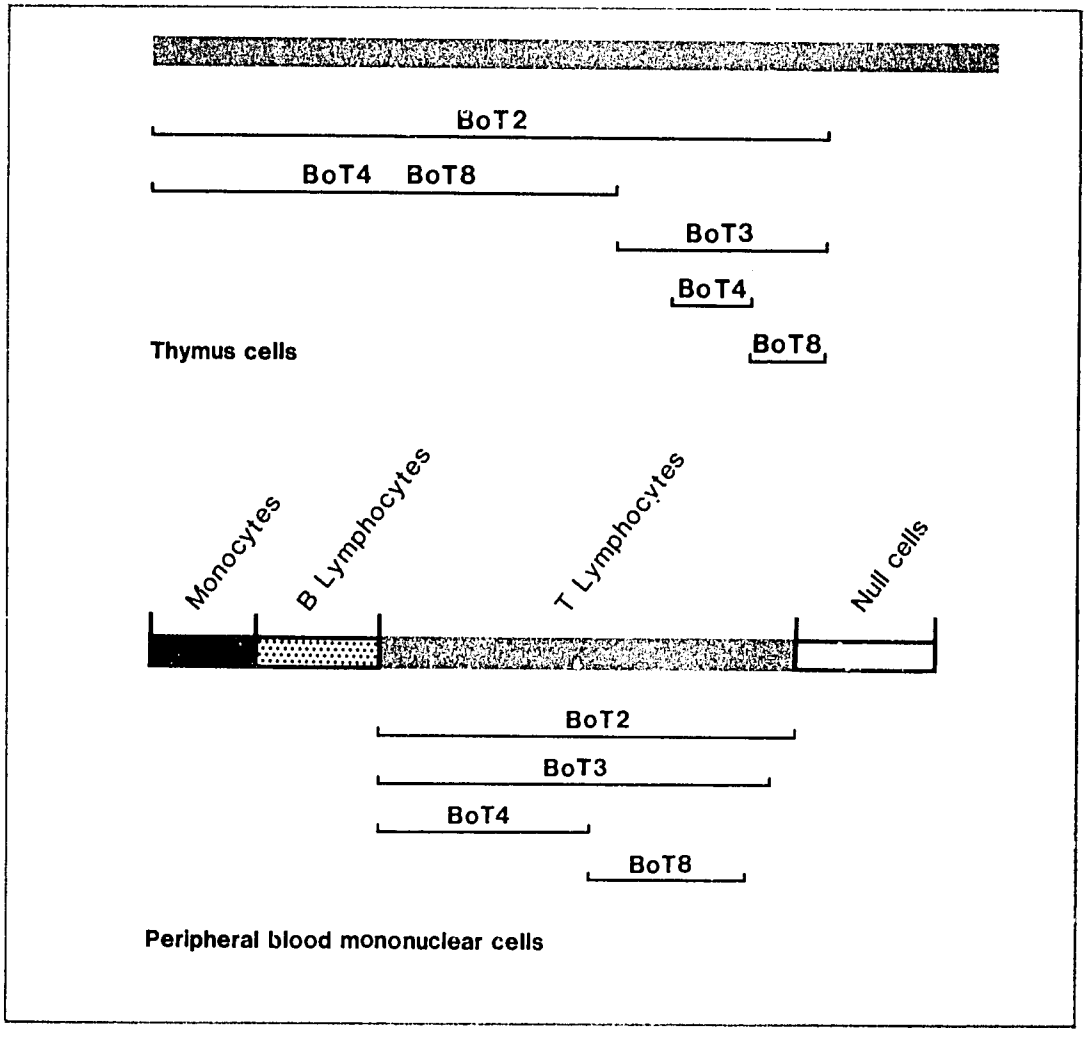

Figure 3. Schematic diagrams of T-lymphocyte populations in the thymus and in the bloodstream, showing the cell populations recognized by monoclonal antibodies raised at ILRAD.

investigate which cell types are parasitized when cattle are infected with *Theileria*. Results indicated that the majority of parasitized cells are T lymphocytes. Among normal peripheral T lymphocytes, the BoT4 and BoT8 molecules are expressed on separate populations of cells. However, in infected animals 10 to 20% of all T lymphocytes express both molecules (Figure 4), and among parasitized cells the proportion expressing both molecules is significantly higher. *In vitro* studies indicated that some T lymphocytes originally expressing only the BoT4 molecule acquired the BoT8 molecule after infection with *T parva*. While different subpopulations of T lymphocytes can be infected with *T parva in vitro*, these findings suggest that a major component of the infected cell population *in vivo* is derived from cells originally expressing the BoT4 molecule. The large increase in parasitized cells which express both molecules may reflect their state of activation.

Scientists are also searching for monoclonal antibodies which can be used to identify bovine B lymphocytes at different stages of maturation. One antibody was derived in 1986 which specifically recognized a monomorphic determinant on immunoglobulin (IgM) of mature B cells. Another monoclonal antibody was derived which reacted with a small population of lymphocytes that do not display B or T cell markers. These null cells were found in peripheral lymphoid tissues and in the medulla of the thymus. In mixed leucocyte reactions *in vitro*, the null cells appeared to proliferate in response to class II MHC antigens derived from the same animal. Other studies suggested that they might play a role in the non-specific

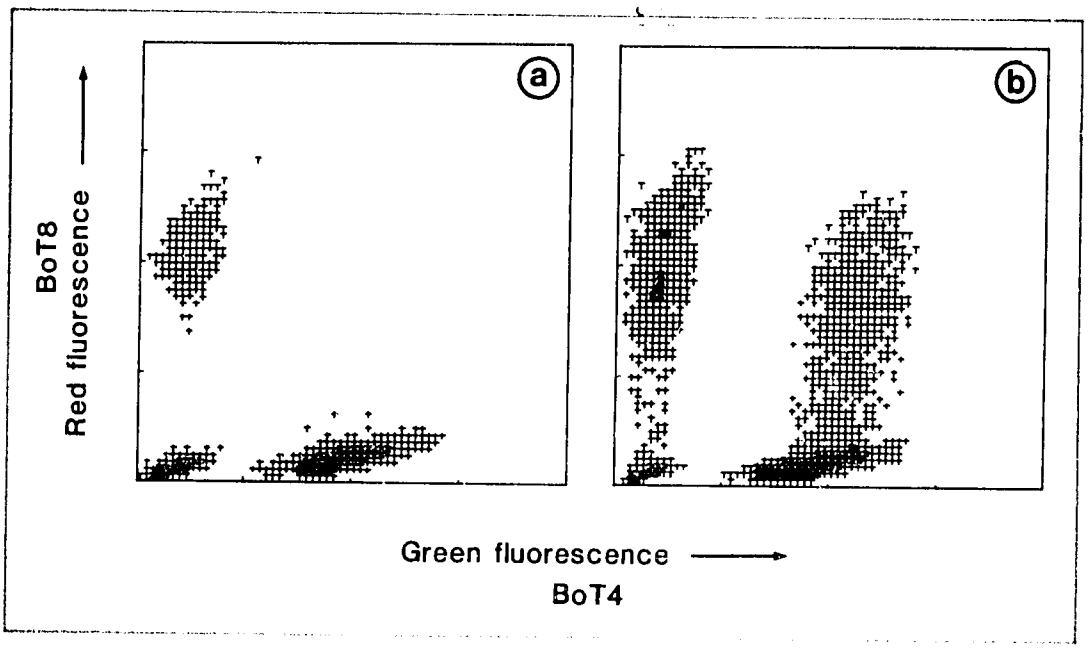

Figure 4. Fluorescence dot plot profiles of lymphocytes analysed on ILRAD's fluorescence-activated cell sorter (FACS) following staining by two-colour immunofluorescence with monoclonal antibodies specific for the BoT4 and the BoT8 molecules. A. In a population of lymphocytes from a normal uninfected animal, most of the T cells display either the BoT8 molecule (along verticle axis at left) or the BoT4 molecule (along horizontal axis at bottom). B. In a population of lymphocytes from an animal infected with *T p parva*, many T cells display both molecules (extending up from the middle of the horizontal axis). A large proportion of these double-positive cells are parasitized.

cytolytic activity characteristic of fatal ECF infections. Further work is being undertaken to elucidate the role of these cells in immune responses to *Theileria* infection.

Two monoclonal antibodies were derived which reacted with the majority of macrophages and granulocytes, including immature macrophages (called monocytes) in the bone marrow. These antibodies were used to identify macrophages in populations of cells from which the granulocytes were removed. The results of several experiments indicated that one of these antibodies inhibited the antigen-induced proliferation of T lymphocytes; inhibition resulted from interference with the processing and/or presenting of antigen, suggesting that the molecule recognized by this antibody is involved in regulating antigen presentation by macrophages. Two other monoclonal antibodies were derived which reacted with monocytes/macrophages: one reacted with a variable proportion of monocytes and mature macrophages, while the other appeared to react specifically with pulmonary macrophages.

Finally, a monoclonal antibody was derived which reacted with immature T cells in the thymus, interdigitating/dendritic cells in peripheral lymphoid tissues and a proportion of large macrophage-like cells in afferent lymph. Further experiments are planned to examine the functions of these accessory cells.

CLASSIFICATION OF MHC TYPES

In view of the important role of MHC antigens in immune responses, ILRAD is making considerable efforts to characterize MHC antigens in the Boran cattle used for the research program. Cattle are typed according to class I MHC antigens using a panel of alloantisera and monoclonal antibodies. This allows selection of suitable animals for experimentation and for breeding groups to produce offspring of desired MHC types.

In 1986, an automated fluorescence assay was successfully developed which can be used to type bovine cells for either class I or class II MHC antigens. Both the microscopic reading of the test and the analysis of results are now fully automated, producing results quickly and with a high level of reliability. Computer programs have been written for reading and analysing data in collaboration with the University of Strathclyde (UK), and these will be made available to a number of interested laboratories.

Bovine MHC typing has also been a component in the use of embryo transfer to produce multiple progeny from particular breeding pairs. For successful embryo transfer work, ILRAD specialists have optimized hormonal treatment regimes to synchronize oestrus and induce superovulation in *Bos indicus* cattle. In 1986, these procedures yielded 51 calves in MHC-identical and haplo-identical groups from single matings, plus one set of identical twin calves produced by embryo splitting. Among other experiments, MHC-identical cattle produced by embryo transfer will be used in attempts to confer immunity by transferring activated, *Theileria*-specific T lymphocytes from an immunized to a naive animal.

All of the antibody reagents currently available for defining polymorphic bovine MHC antigens react with class I molecules; most, and possibly all, react with the products of only one class I gene locus. It is important to be able to identify all of the other MHC antigens in order to define their roles in immune responses and to ascertain possible MHC-related influences on susceptibility to disease. A combination of several techniques is being used to tackle this problem.

One goal is to ascertain how many genetic loci are present in the class I region of the MHC and how many of these have functional products. This problem is being approached by producing mutant cell lines in which genes coding for some surface molecules are deleted while others remain. The resultant mutants are screened with a

large panel of monoclonal antibodies and cloned T lymphocytes which recognize specific class I MHC antigens. Reagents which recognize antigens in intact cell lines may or may not respond to mutant lines, depending on whether the antigens they recognize have been lost. If two reagents respond differently to a mutant cell line, they probably recognize different antigens.

Less is known about the bovine class II MHC than about class I MHC molecules. One constraint has been the difficulty of deriving monoclonal antibodies against class II MHC molecules by standard procedures using mouse cells. To overcome this problem, the possibility of producing bovine monoclonal antibodies using mouse-bovine or bovine-bovine hybrid fusions has been investigated. Initial efforts at ILRAD were not successful, but this approach is now being explored again in collaboration with the Agriculture and Food Research Council's Institute of Animal Physiology (UK).

Another aspect of work with mutant cell lines has involved efforts to produce mutant cells which do not express any MHC antigens. Success in this area might make experimental ECF immunization procedures more effective and practicable. At present, immunity can be stimulated in cattle by inoculating schizont-infected cells produced *in vitro*. However, very large numbers of cells must be used because many of the 'foreign' cells are rejected before the parasites can transfer to the cells of the recipient animal. This rejection is stimulated by the MHC antigens presented on the 'foreign' cells; many fewer cells might be rejected if parasitized cells could be used which lacked MHC antigens.

PROLIFERATION OF INFECTED CELLS

An understanding of how *Theileria* parasites stimulate host cells to proliferate might lead to new approaches to control infection or reduce the severe pathogeneic effects of ECF. Research conducted in 1985 demonstrated that *Theileria*-infected T lymphocytes produce a growth factor, similar to the T-cell growth factor interleukin 2 (IL-2), which may contribute to maintaining their proliferative state. In 1986, a probe for the DNA which encodes human IL-2 was used to localize and isolate the homologous genomic DNA encoding IL-2 in bovine cells. This DNA probe is being characterized and is being used, together with a human IL-2 cDNA probe, to define the IL-2-like growth factor produced by cloned T lymphocytes infected with *T parva*. Work now concentrates on producing anti-IL-2 monoclonal antibodies which can be used to extend these studies.

Several monoclonal antibodies have been raised that recognize antigens which occur specifically on proliferating bovine cells, but are absent or in very low concentration on resting cells. Work is now in progress to elucidate the functions and biochemistry of these antigens. Possible functions might include increased uptake of nutrients for rapid growth or binding with specific growth-controlling hormones or factors, such as IL-2.

In collaboration with the Kernforschungzentrum laboratory in Karlsruhe (F R Germany), ILRAD scientists are also investigating the possibility that oncogenes might be involved in the transformation of *Theileria*-parasitized cells to a proliferating state. Oncogenes have been implicated in the transformation and growth of cancer cells. Preliminary findings indicate that oncogenes are transcribed and translocated in *Theileria*-infected cells and that they are of host cell origin.

When oncogenes stimulate cells to multiply and transform into tumours, enzymes called protein kinases have been shown to play a role. These enzymes trigger a process called phosphorylation—changes in the molecular structure of proteins due to the attachment of phosphates. Comparison of a number of *Theileria*-infected and uninfected cloned populations of T lymphocytes has demonstrated that infection results in a

marked increase in the phosphorylation of host cell proteins. At least five proteins are altered. Present investigations focus on whether the observed phosphorylation activity is due to the activation of a specific enzyme in the host cell or to the presence of an enzyme of parasite origin. The host cell proteins which are targets for phosphorylation are also being characterized, as well as the genes encoding for protein kinases and other transforming activities.

ANTIGENIC CHANGES ON INFECTED CELLS

Although monoclonal antibodies have been identified which react with proliferating bovine cells, no antiserum or monoclonal antibody has been produced which detects surface antigens specifically on schizont-infected cells. Current efforts concentrate on indirect methods for identifying such antigens, using cloned and uncloned *Theileria*-specific T lymphocytes.

Soluble antigens prepared from *Theileria*-infected cells have been shown to stimulate proliferation of mixed cell cultures derived from immune cattle. This response required the presence of irradiated leukocytes taken from the same animal, suggesting that it involved the presentation of antigens by macrophages. The cells which proliferated were nearly all T lymphocytes displaying the BoT4 surface antigen and lacking a cell-killing function. In a preliminary experiment, freeze-thawed purified schizonts also induced proliferation.

Theileria-infected cells were fractionated and subcellular components were used to stimulate proliferation in mixed cell cultures. Strong proliferation was stimulated by microsomal fractions, which included plasma membranes, rough endoplasmic reticulum and Golgi. These preparations are now being fractionated further in an attempt to localize and identify the specific antigen(s) which stimulated proliferation. Similar experiments are planned to search for antigens that stimulate cell-killing responses.

Once the antigen(s) which stimulate proliferation or cell killing are purified, specific antibodies can be produced and used to screen *Theileria* DNA libraries for the associated genes. In order to demonstrate that any *Theileria* genes identified by these procedures play a role in the stimulation of immune responses, the genes will have to be transfected into non-parasitized cells bearing the relevant bovine MHC molecules and these cells will then be used as a source of antigen in the appropriate assay. Work is therefore in progress to develop a system for gene transfection, testing the suitability of various bovine cell types. An alternative approach is also being considered involving the transfection of a bovine class I MHC gene into a mouse cell line suitable for further transfection.

Another approach to identifying the antigen(s) which stimulate cell-mediated immunity involves attempts to identify parasite genes specific to the schizont stage of development. This is being done using schizont-specific monoclonal antibodies to screen a *Theileria* genomic DNA library.

IMMUNE RESPONSES TO INFECTED CELLS

Studies continued in 1986 on the cytolytic T lymphocytes responsible for killing *Theileria*-infected cells. This work has confirmed that *Theileria*-specific cytolytic T cells express the BoT8 molecule and are restricted by class I MHC molecules. Cytolytic responses were associated predominantly with three out of six class I molecules examined, suggesting that the parasite-induced antigen on the surface of infected cells may associate preferentially with certain MHC molecules or may stimulate immune responses more effectively when presented with some MHC molecules rather than others. This may result in MHC-related differences in susceptibility to infection or differences in the quality of immunity engendered by infection and treatment in different animals.

A limiting dilution assay has been developed, in collaboration with the Kenya Government's Veterinary Research Laboratory, to investigate the proliferation of *Theileria*-specific cytolytic T lymphocytes in immunized cattle. Preliminary results showed that cytolytic cells occurred in a population of peripheral blood lymphocytes at a frequency of about 1:5,000 in immunized animals, whereas in naive cattle cytolytic cells were undetectable to a level of 1:100,000.

Another study has investigated the possible role of lymphotoxin—a cell-killing factor secreted by T lymphocytes—in the cell destruction characteristic of the late stages of *Theileria* infection. Preliminary results showed that both *Theileria*-infected lymphocytes and T lymphocyte clones stimulated by *Theileria*-infected cells produced lymphotoxin *in vitro*.

Strain specificity

Parasite strain specificity of the cytolytic response was examined in three cattle immunized against *T p parva* Muguga by infection and treatment and then rechallenged with the Muguga stock. Cells from these animals were tested for cytolytic activity against cloned T cells taken from the same animals and infected *in vitro* with *T p parva* Muguga or *T p parva* Marikebuni. The cytolytic activity detected in cells from two of the cattle appeared to be completely specific for *T p parva* Muguga-infected cells, while cells from the third animal also showed low levels of cytotoxicity against target cells infected with the Marikebuni stock. This is consistent with results of cross-immunity testing showing that a proportion of cattle immunized with *T p parva* Muguga are susceptible to infection with *T p parva* Marikebuni. These findings also provide the first clear evidence for the parasite strain specificity of cytolytic responses generated *in vivo*.

Cross-immunity trials have shown that cattle immunized with the Marikebuni stock are protected against infection with both *T p parva* Marikebuni and *T p parva* Muguga. In 1986, 16 *Theileria*-specific cytolytic T cell clones were derived from an animal immunized with *T p parva* Marikebuni. All killed target cells infected with both parasite stocks, though unlike the cytolytic cells generated against the Muguga stock, they only killed 15 to 70% of parasitized cells over a period of 4 hours (Figure 5). These and other findings suggest that the antigen recognized by Marikebuni-specific cytolytic cells is expressed on the surface of parasitized cells in a cyclical manner and is probably different from the antigen recognized by the Muguga-specific cells.

Future plans include testing various cloned T-cell populations on a wider range of parasite stocks to determine whether their specificity correlates with patterns of cross-protection. Cloned T lymphocytes which recognize specific *Theileria* strains could become useful reagents for characterizing *Theileria* species and strains more precisely than is now possible using monoclonal antibodies.

Research on the strain specificity of cytolytic responses will also continue to focus on cells generated from immunized cattle. Experiments are planned to determine whether the specificity of the cytolytic response correlates with susceptibility to subsequent infection. It is possible that specificity of the response correlates with particular MHC types, so experiments will be conducted with cattle in which the MHC type is well defined.

Comparison with immune responses in buffalo

An understanding of how buffalo control infections with *T p lawrencei* might provide clues to how *Theileria* infections can be better controlled in cattle. ILRAD scientists are studying immune and nonimmune responses in infected buffalo in collaboration with the Kenya Government's Veterinary Research Laboratory and the Kenya Agricultural Research Institute, supported by the Gov-

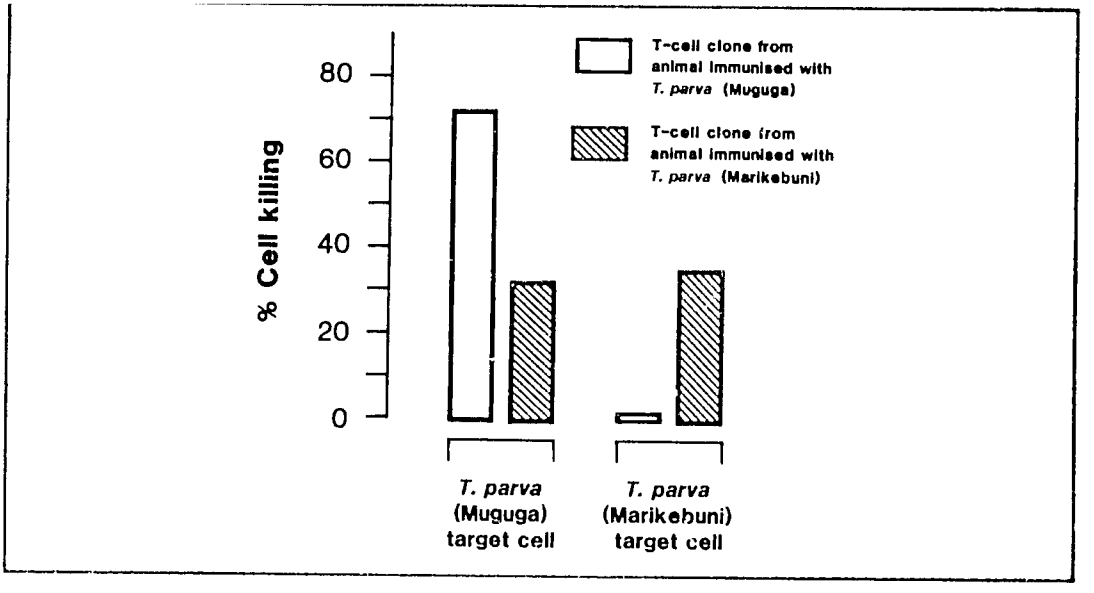

Figure 5. Parasite strain specificity of two cytolytic T-cell clones, one derived from an animal immunized with *T p parva* Muguga and the other from an animal immunized with *T p parva* Marikebuni. The bar chart shows the levels of cell killing obtained when the clones were tested on lymphocytes from the same animals which had been infected *in vitro* with parasites of the same or the other strain. The clone derived against *T p parva* Muguga is specific for the target cell infected with the same strain, whereas the clone generated against *T p parva* Marikebuni kills target cells infected with either parasite strain.

ernment of the Netherlands.

Earlier work showed that sporozoites of *Theileria* stocks derived from buffalo— but not of stocks derived from cattle—enter both buffalo and cattle cells readily *in vitro* and stimulate proliferation. In 1986, studies concentrated on cell-mediated immune responses in infected animals. Proliferative responses to autologous (taken from the same animal) *Theileria*-infected cells were detected in cells from 3 naive and 12 infected buffalo, though the responses were stronger in cells from the infected animals. Cytolytic responses were observed when cells taken from 8 of the 12 infected buffalo were stimulated with autologous *T parva*-infected cells *in vitro*, but not with cells taken from the naive buffalo or cells taken from infected buffalo and stimulated with *T parva*-infected cells from different animals. This indicates that the cytolytic response is parasite specific and genetically restricted. These findings are similar to those observed in cattle and thus suggest that this mechanism alone is probably not responsible for the greater resistance of buffalo to *Theileria* infection.

The strain specificity of cytolytic and proliferative responses was also tested. A mixed population of white blood cells (peripheral blood mononuclear cells) was taken from a buffalo infected with buffalo-derived *T p lawrencei*. These cells demonstrated cytolytic responses against four *T p lawrencei* isolates—including one which differed antigenically from the others according to monoclonal antibody reactivity with intracellular schizonts—and also against the cattle-derived *T p parva* stock, Kiambu-4. However, the proliferative responses appeared to be at least partially strain specific. Further studies of these responses might result in an assay which would usefully complement other methods for preselecting *Theileria* strains potentially suitable for immunization trials.

IMMUNIZATION AGAINST TICKS

Cattle have been shown to develop immune responses to feeding ticks after multiple tick infestations in the field. Laboratory animals also develop strong delayed-type hypersensitivity responses when sensitized by tick feeding or by inoculation with tick salivary gland extract. The development of resistance is demonstrated by a reduction in the number of ticks attaching to sensitized animals and by lower engorgement weights in the ticks that do attach and feed.

Several researchers have tried to enhance resistance to tick infestation by immunization with tick antigens, generally using complex tick extracts. These procedures have not improved upon the limited resistance attained naturally and are also impractical for field application. Research has been conducted at ILRAD to identify specific tick antigens which would be more effective in different adjuvant systems as a vaccine against ticks.

Rabbits and guinea pigs which have developed immune responses to feeding ticks generate antibodies that recognize a number of tick antigens. Antisera from these animals were used to identify 12 major tick antigens, most of which were found in tick salivary glands or tick cement. Some of these antigens appeared to be common to several tick species. One tick-cement antigen, which appeared to be common to *Rhipicephalatus appendiculatus*, *R pulchellus* and *R evertsi*, induced delayed-type hypersensitivity reactions in rabbits when the purified antigen molecule was inoculated intravenously.

TRYPANOSOMIASIS

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

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

Trypanosomiasis also occurs in Central and South America, the Middle East and Asia. In these areas, the parasites of veterinary importance are transmitted mechanically by biting flies. *T evansi* affects camels, horses, cattle and domestic buffalo in Africa, the Middle East, Asia and South America.

Programs attempting to control trypanosomiasis have been in operation for nearly 100 years. Initial efforts involved control of the tsetse vector, either by destroying the wild animal populations on which tsetse feed or clearing the bushy vegetation that tsetse require as breeding and resting sites. More recently, most tsetse control programs have been based on the application of insecticides, in some instances in connection with tsetse traps and targets or screens. Considerable success has been achieved where control measures using insecticides have been properly implemented—notably in Nigeria, Botswana, Zambia and Zimbabwe. However, in spite of control efforts, in many parts of Africa the area infested by tsetse flies is expanding.

Regular treatment of infected animals with trypanocidal drugs has been an important control method since the early 1920s. This approach has been successful in situations where livestock management has been competent and consistent over time. Disease control regimes based on chemotherapy must be implemented indefinitely because domestic animals are exposed to tsetse flies that have fed on wild animals, which act as an extensive reservoir of infection. The possibility of drug resistance developing in local parasite populations is also a serious consideration as no new trypanocidal compound has been introduced for general use in the last 25 years.

The goal of ILRAD's research program is to develop improved measures to control trypanosomiasis—measures which are effective, safe and economically sound. Research activities are broadly based, but can be con-

sidered under three headings: improved control of the parasites, enhanced host resistance to infection and trypanosomiasis epidemiology.

IMPROVED CONTROL OF THE PARASITES

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

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

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

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

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

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

Research at ILRAD aimed at improving control of the parasites concentrates on the mechanism of antigenic variation—its genetic basis and the way VSGs are synthesized in the trypanosome and moved to the surface of the parasite. Studies also focus on nonvariable proteins associated with specific stages of the trypanosome lifecycle and on various aspects of trypanosome metabolism. The goal is to identify factors which could be manipulated to disrupt the development of the parasites or make them more vulnerable to host defences.

VARIABLE SURFACE GLYCOPROTEINS

Considerable research has been conducted at ILRAD on the genes which code for try-

panosome VSGs and the gene rearrangements which occur when new VSGs are produced on the surface of the parasite. Because there are so many trypanosome genes encoding VSGs, attention has concentrated not on identifying all VSG genes, but on elucidating the mechanisms involved when expression switches from one VSG gene to another, as well as the pathways of VSG synthesis and transport to the surface of the cell.

A repetitive sequence was cloned from the genome of *T b brucei* for evaluation of its potential use as an epidemiological tool. This 5200-base pair sequence was analysed in some detail. About 200 copies of the sequence are dispersed throughout the genome of members of the subgenus *Trypanozoon*. It appears to belong to a class of mobile genetic elements called retroposons that have been described in mammalian and insect genomes. These elements probably become dispersed by insertion of retro-transcribed copies of an initial RNA transcript at new sites in the genome. The presence of such an element in trypanosomes raises some interesting questions concerning the evolution of retroposons and the possibility of genetic exchange between the trypanosome and its hosts.

Experiments were carried out to determine whether the *T b brucei* element coded for a protein. The results suggested that there was indeed an encoded protein present in bloodstream-form trypanosomes and a related protein in procyclic trypanosomes, forms which occur in tsetse flies and are not coated with variable antigen. Experiments are also in progress to determine whether the dispersed sequences may be involved in some of the genetic rearrangements that are involved in the switching of VSG expression.

The transcripts of all the genes active at any particular developmental stage should be represented in the mRNA of parasites at that stage. Under efficient cloning conditions, cDNA libraries, constructed with mRNA obtained at a given stage as a template, should contain all or most of the transcripts for that stage. *T congolense* cDNA libraries have been constructed using template mRNA purified from bloodstream and procyclic culture forms of two serodemes. Comparison of libraries from different developmental stages revealed transcripts that were present, or abundant, at one stage only.

Several recombinant clones were identified which were present, or abundant, in bloodstream parasites only. A number of these contained sequences coding for parasite VSGs. Clones represented only or primarily in procyclic forms are now being analysed to determine the nature of the proteins encoded, the location of these proteins within the parasite and the regulation of their genes during parasite differentiation. These studies will be extended to other developmental stages, such as epimastigotes and metacyclics, and to the other *T congolense* stocks and clones available at ILRAD. Particular attention focuses on gene sequences which code for the VSGs displayed by trypanosomes at the metacyclic stage of development.

Previous studies at ILRAD and elsewhere suggested that *T b brucei* parasites might shed their VSG coat by enzymatic cleavage during transition to the uncoated procyclic stage. This suggested that pharmocological stimulation of the enzyme responsible for cleavage—phospholipase-C (Enzyme-X)—might be exploited to develop new chemotherapeutic interventions. Work in 1986 concentrated on identifying the actual subcellular location of this enzyme, using recently developed procedures for separating trypanosome cell fractions and organelles. Enzyme-X was located within *T b brucei* bloodstream forms in the flagellar pocket and possibly in membrane organelles associated with or derived from the flagellar pocket, with smaller quantities also present in the Golgi apparatus. Cultured procyclic forms of *T b brucei* and bloodstream forms of *T vivax* showed little or none of the enzyme, while *T congolense* bloodstream

forms showed an intermediate amount. These studies suggested that Enzyme-X may play a role in the trypanosome's VSG-membrane recycling system. Present work focuses on the recycling of VSG components during synthesis of new VSG, as well as the uptake of mammalian host molecules into the cell.

There has been less research on *T vivax* VSGs than on those of the other major trypanosome species largely because the majority of *T vivax* stocks are restricted to ruminants, making it difficult to obtain sufficient antigenically homogeneous parasite material. However, studies on parasite antigens and host immune responses might be particularly useful for this species because—at least in East Africa—a significant proportion of infected livestock manage to control the parasites and recover.

Earlier work at ILRAD revealed a VSG coat on metacyclic forms of both East and West African stocks of *T vivax*. The surface coat on this fragile organism may be less dense than the surface coat on *T b brucei* or *T congolense*, suggesting that the *T vivax* VSG coat may provide a less effective protective covering than the VSG coats of other species.

In collaboration with colleagues from the University of Victoria and the University of Western Ontario in Canada, ILRAD scientists continued research in 1986 to identify and purify *T vivax* VSGs, concentrating initially on a well characterized clone (ILDat 1.2) originating from West Africa. Evidence obtained during the year suggested that the VSG expressed by this clone was a relatively small glycoprotein with a membrane anchor similar to that of VSGs in other species. Comparative studies using other clones from the same serodeme suggested that their VSGs were also relatively small basic molecules which may exist in different glycosylation states. The amino-acid sequence was determined for the N-terminal end of the ILDat 1.2 VSG: it appeared quite unlike the N-terminal end of VSGs from other trypanosomes. Work now concentrates on structural studies of VSGs from other clones of the ILDaR 1 serodeme and from newly rodent-adapted clones from other serodemes.

Another project concentrated on developing improved labeling techniques for the identification of VSGs and, perhaps, other cell surface molecules, on metacyclic forms of *T congolense* and *T vivax*. Live cells, whole cells broken up by sonication and crude fractions of cell membranes were covalently labeled with chemically reactive biotin, and the incorporation of the label into protein components was assessed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. As expected, suspensions of whole cells and isolated membranes of biotin-treated cells displayed essentially the same molecular bands, while cells sonicated before treatment with biotin, whose contents would also be exposed for labeling, displayed a much larger number of labeled proteins. These techniques are being refined to make it possible to analyse trypanosome surface molecules in greater detail.

NONVARIABLE TRYPANOSOME ANTIGENS

The major obstacle to the development of an effective vaccine against African trypanosomiasis is the phenomenon of antigenic variation. However, trypanosome antigens exist which are not variable and these could be of considerable importance for diagnosing infections, identifying parasite species and serodemes, perhaps interfering with parasite development at different stages of the lifecycle or even developing a vaccine. Two types of nonvariable antigen were investigated in 1986: surface antigens on procyclic trypanosomes and nonvariable antigens specific to bloodstream forms.

Cell surface glycoproteins were purified from procyclic forms of 17 trypanosome stocks, representing the *T brucei* subgroup, *T congolense* and *T simiae*. Total glycoprotein profiles were similar for all stocks

except the *T congolense* stock isolated at Kilifi, Kenya. Two major glycoproteins were present on all the parasites examined and a doublet of 86,000 daltons was present on all except Kilifi-type *T congolense*. The most important common surface glycoproteins are now being analysed in detail.

Preliminary studies on nonvariant *T vivax* antigens which might be exposed on the surface of the parasites led to the derivation of monoclonal antibodies against antigens on the surface of *T vivax* procyclic forms. Similar antibodies have been raised against *T congolense* and *T b brucei* procyclic antigens. Incorporated into tsetse bloodmeals, these antibodies might serve to block parasite development. Preliminary evidence suggested that the monoclonal antibody against *T b brucei* procyclic forms might induce agglutination of procyclics in the tsetse midgut. When this monoclonal antibody was added to *T b brucei* procyclics *in vitro*, parasite clumping and reduced growth rates were observed. Similar investigations on *T congolense* and *T vivax* procyclic antigens are in progress.

In collaboration with the University of Massachusetts at Amherst (USA), studies have been conducted in search of nonvariant antigens specific to bloodstream forms. Initial work with *T congolense* indicated the presence of several proteins specific to this developmental stage and preliminary observations suggested that some might be located on the surface of the parasites, making them possible candidates for immunization studies. Further work in 1986 concentrated on developing improved techniques for purifying these proteins, and the presence of at least two, distinct, nonvariant antigens specific for bloodstream forms was confirmed. Present efforts focus on determining their location, either on the surface or within the parasite.

One aspect of the trypanosome lifecycle which has aroused considerable interest is the transition in the mammalian bloodstream from rapidly dividing to nondividing forms. Transition to the nondividing stage helps to limit the severity of infection and is also thought to prepare the trypanosomes for transmission to tsetse. A better understanding of the mechanisms involved in this and other lifecycle transitions may suggest specific ways to disrupt parasite development.

In a study initiated in 1986, rapidly dividing and nondividing bloodstream forms of *T b brucei* were fractionated into different subcellular components which were analysed to identify any proteins specific for either of the bloodstream stages. Trypanosomes or trypanosome homogenates from the two stages were incubated with radiolabeled phosphate and analysed to identify any changes in phosphorylated proteins. One phosphorylated protein, which was much more abundant in nondividing parasites, was identified and partially purified. The phosphorylation process and the enzymes responsible for it are now being investigated in more detail. A marked increase in one enzyme, aconitase, was also associated with nondividing forms: a stain for this enzyme is being developed which could be used to differentiate rapidly dividing from nondividing parasites.

ASPECTS OF TRYPANOSOME METABOLISM

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

Trypanosomes are believed to ingest biologically important molecules, such as nutrients and differentiation signals, through a process of endocytosis mediated by coated vesicles. In a continuation of experiments begun in 1985, bloodstream forms of *T congolense* were incubated in culture medium containing colloidal gold particles coupled to bovine serum albumin, then chemically fixed and examined by electron microscopy.

Gold particles, ingested by the parasites *in vitro*, were observed in a number of intracellular organelles: the flagellar pocket, discrete spherical and cylindrical vesicles located near the flagellar pocket, networks of large and small cysternae, lysosomes and small lysosome-like structures (Figure 6). This observation of an interconnected network of endocytosis is unique. Work is now in progress to confirm whether the pathway occurs in live parasites or is related to trypanosome death.

Experiments conducted with bloodstream forms of *T brucei* have suggested that VSG from the surface of the parasite is endocytosed through the same pathways as those used for the uptake of nutrients (Figure 7). Presumably VSG is endocytosed for eventual breakdown or reprocessing.

Coated vesicles from bloodstream forms of *T b brucei* have been purified and partially characterized. By using antisera prepared against purified vesicles, scientists were able to detect a number of molecules found in normal and infected rat plasma which appeared to have been ingested by the parasites and taken up in the coated vesicles.

Lysosomes were isolated from blood-

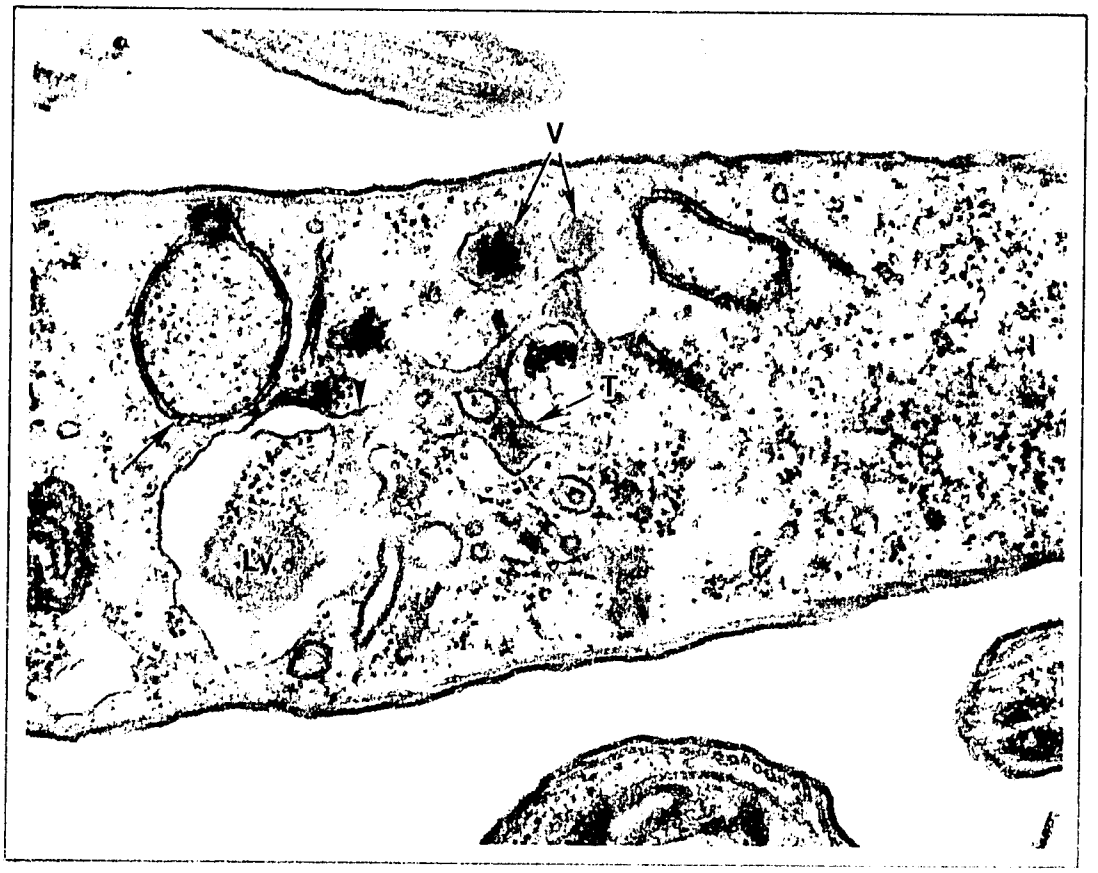

Figure 6. One of a series of epoxy resin sections through a *T congolense* cell incubated for 1 hour prior to fixing in bovine transferrin coupled to colloidal gold (5 nm particle size). A tubular network (T) containing the gold marker can be seen connecting (arrowhead) to a lysosome-like structure (Ly). A smaller tubular structure (t) and two vesicles (V) also contain gold marker. Transferrin is a protein which brings iron into cells; it is normally recycled through the early parts of the endocytotic pathways. The series of sections reveals a number of prelysosomal compartments, containing bovine transferrin, which all appear to be connected to the lysosome.

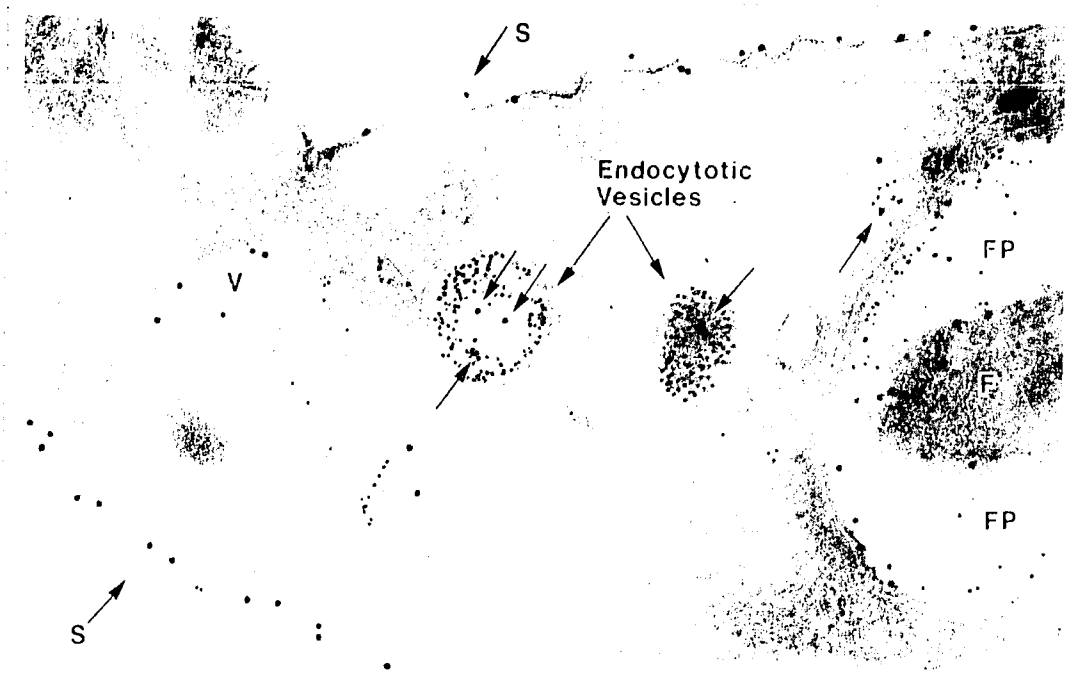

Figure 7. A thawed, thin frozen section through a *T brucei* cell which had been incubated for 1 hour prior to fixing in bovine serum albumin coupled to colloidal gold (5nm particle size). The section was labeled with rabbit anti-VSG antibody coupled to protein A-colloidal gold (10 nm particle size). The small particles label endocytotic pathways, presumably used for the uptake of nutrients. The larger particles (indicating the presence of VSG) label the trypanosome surface (S), the flagellar pocket (FP) containing the flagellum (F) and several structures within the cell. Many endocytotic vesicles contain both small and large particles, indicating that VSG from the surface of the parasite is being endocytosed through the same pathways as those used for the uptake of nutrients. One vesicle (V) contains the VSG label only

stream forms of *T b brucei* and their predominant proteolytic (protein-splitting) enzyme was identified and partially purified. When diluted mammalian serum was added to the purified lysosomes an increase in total proteolytic activity was observed. The activating factor is present in rat, rabbit and bovine serum and is now being characterized and purified. This factor, on entering the lysosomes of living trypanosomes, might act as a mammalian-derived regulator of parasite proteolysis.

Research in 1986 also concentrated on enzymes which might be released by trypanosomes into the host bloodstream, causing damage to host tissues and thus contributing to the pathology of trypanosomiasis. A serine peptidase was partially purified from *T b brucei* which appears to be released into the culture medium when parasites are damaged by improper handling, suggesting that it might also be released into the host bloodstream by dying trypanosomes. Another peptidase was identified in the plasma of rats infected with *T b brucei* and cattle infected with *T congolense*. This enzyme appears to be of parasite origin and work is in progress to characterize it more precisely.

In the tsetse vector, *T vivax* parasites develop entirely within the proboscis. An interesting phenomenon associated with *T vivax* cultivation *in vitro* is the attachment of late-stage bloodstream forms to Amicon Matrix Gel Green A beads. Work in 1985 indicated that *T vivax* attachment to beads *in*

vitro closely resembles parasite attachment to the proboscis of the tsetse. Biochemical studies showed that binding was by a receptor-ligand interaction. Isolation of either the *T vivax* or the tsetse molecules involved might provide a novel method to control parasite development and disease transmission.

Efforts to identify the precise configuration of dye molecules on the Green A beads to which the parasite binds have involved attempts to block parasite/bead binding by incubation with antibodies raised in rabbits against the Green A dye or with a number of lectins and sugars. Results have been inconclusive.

Related studies focus on identification of the ligand molecule on the surface of the parasite. Using the biotin-labeling technique described earlier, cell surface molecules from late bloodstream forms of *T vivax* were covalently labeled with chemically reactive biotin. The proteins identified by this technique will be investigated for their possible role in the *T vivax*/Green A bead interaction.

TRYPANOSOME CULTIVATION *IN VITRO*

Culture systems have been established at ILRAD which support the development of all three major African trypanosome species throughout their lifecycles. *In vitro* cultivation techniques are being improved in order to provide adequate numbers of trypanosomes at various lifecycle stages for biochemical and immunological research. Trypanosomes produced *in vitro* are also used to study the action of trypanocidal drugs and to investigate drug resistance. The emphasis in 1986 was on improving the production and characterization of metacyclic trypanosomes.

Although *T b brucei* was the first trypanosome species to be maintained in culture throughout its lifecycle, it has proven difficult to produce large numbers of metacyclic forms of this species. A system was introduced in 1986 based on passaging parasites cyclically between *in vitro* culture and mice. This method has enhanced the production of infective metacyclics considerably and has shortened the time period until their first appearance in culture.

A stock of *T congolense* isolated from the Trans Mara region of Kenya has proven to be the most prolific of the various *T congolense* stocks and clones available at ILRAD for the *in vitro* production of metacyclic forms. Thirty billion metacyclic parasites were produced from this *T congolense* stock in 1986 alone. However, some clones of this stock do not transform to metacyclic-producing epimastigote forms *in vitro*. For this reason, several new clones were derived in 1986 and are being tested to select those which will generate the largest possible number of animal-infective metacyclics.

T congolense parasites isolated from ILRAD's epidemiological study at Kilifi, Kenya, appear to differ in several respects from *T congolense* isolated elsewhere in Africa, including their requirements for *in vitro* cultivation. Earlier efforts to cultivate stocks and clones from Kilifi using systems successfully applied to other *T congolense* stocks failed to produce consistent results. In 1986, a new system was established which has made it possible to produce infective metacyclic forms of Kilifi-type *T congolense* in fairly large numbers. Unlike the system for producing *T congolense* metacyclics, the system for producing Kilifi-type metacyclic forms relies on the presence of mammalian feeder layer cells.

The antigenic stability of *in vitro*-derived metacyclic forms of Kilifi-type *T congolense* was tested by infecting mice with *in vitro*-derived metacyclics, treating them with diminazene aceturate (Berenil: Hoechst) and challenging them with metacyclics 14 days later: eight out of nine mice resisted infection, suggesting a high level of antigenic stability. In another experiment, five mice were infected, treated with diminazene aceturate and given a 'booster' challenge of *in vitro*-derived metacyclics 11 days later. When challenged with tsetse-transmitted

metacyclics derived from the same cloned parasites 3 weeks after drug treatment, four out of five resisted infection and in the other infection was delayed until 42 days after challenge.

It is possible to produce large numbers of *T vivax* metacyclics in culture, but the infectivity of these *in vitro*-derived forms has been in doubt. More than 10,000 *in vitro*-derived parasites are required to establish infection in mice, although the stock used (IL1392) is known to be infective to mice by tsetse transmission. Experiments were conducted in 1986 to test the infectivity of *in vitro*-derived *T vivax* metacyclics in goats. Four out of five goats became infected when inoculated with 5,000 parasites, and two out of four goats were infected with only 50, demonstrating that this *T vivax* stock is much more infective for goats than for mice.

In an experiment to test the antigenic stability of these parasites, goats were infected with *in vitro*-derived *T vivax* metacyclics, treated with diminazene aceturate and then challenged with similar parasite forms. All experimental animals became infected. This methology—which confers protection when used with other trypanosome species—also failed to protect experimental animals against *T vivax* metacyclic forms derived from tsetse. This phenomenon is being investigated further.

IMPROVED HOST RESPONSES

EARLY EVENTS IN THE SKIN

The development of a local skin reaction, or chancre, at the site of an infected tsetse bite is often the first host response to trypanosome infection. Previous studies on the dynamics of cell populations in the chancre showed dramatic increases in polymorphonuclear leukocytes and lymphocytes, as well as increases in plasma cells, macrophages and mast cells. Mast cells are of particular interest because they are located at all potential portals of parasite entry—including skin, lungs and gastro-intestinal tract—and appear to serve sentinel functions in the host defence system.

Mast cells secrete a large number of chemical mediators and enzymes, initiating immediate and delayed hypersensitivity reactions as well as cytolytic and immunoregulatory responses. Mast cells may also play a central role in attracting other host cells to the chancre. Because of their possible importance in the chancre reaction, work in 1986 focused on developing biochemical markers to identify bovine mast cells and their products.

Recent studies have suggested that aprotinin, a bovine protease inhibitor, is present in mast cells, so experiments were conducted to determine whether this well characterized molecule might be used as a mast-cell marker. Aprotinin was purified from bovine lung and the dimers were cross-linked and used to raise antibodies in rabbits. These anti-aprotinin antibodies were shown to recognize mast cells in tissue sections of bovine intestine, nasal mucosa, skin and lung. Examination by electron microscopy revealed aprotinin staining exclusively in mast cell granules (Figures 8 and 9).

The largest and most intensely inflamed chancre lesions often occur in animals that are most resistant to trypanosome infection. This suggests that a resistant animal may be able to release mast cell granular contents, attract neutrophils and lymphocytes, generate inflammatory mediators and thus limit the rate of parasite growth in the skin more efficiently than susceptible animals. The aprotinin staining technique developed in 1986 will make it possible to study the role of mast cell degranulation in response to trypanosomes present in the chancre and to compare the responses of relatively resistant and susceptible cattle. It will also be possible to study the effects of mast-cell secretions—including histamine, leukotrienes, prostaglandins and aprotinin—on trypanosome growth *in vitro*.

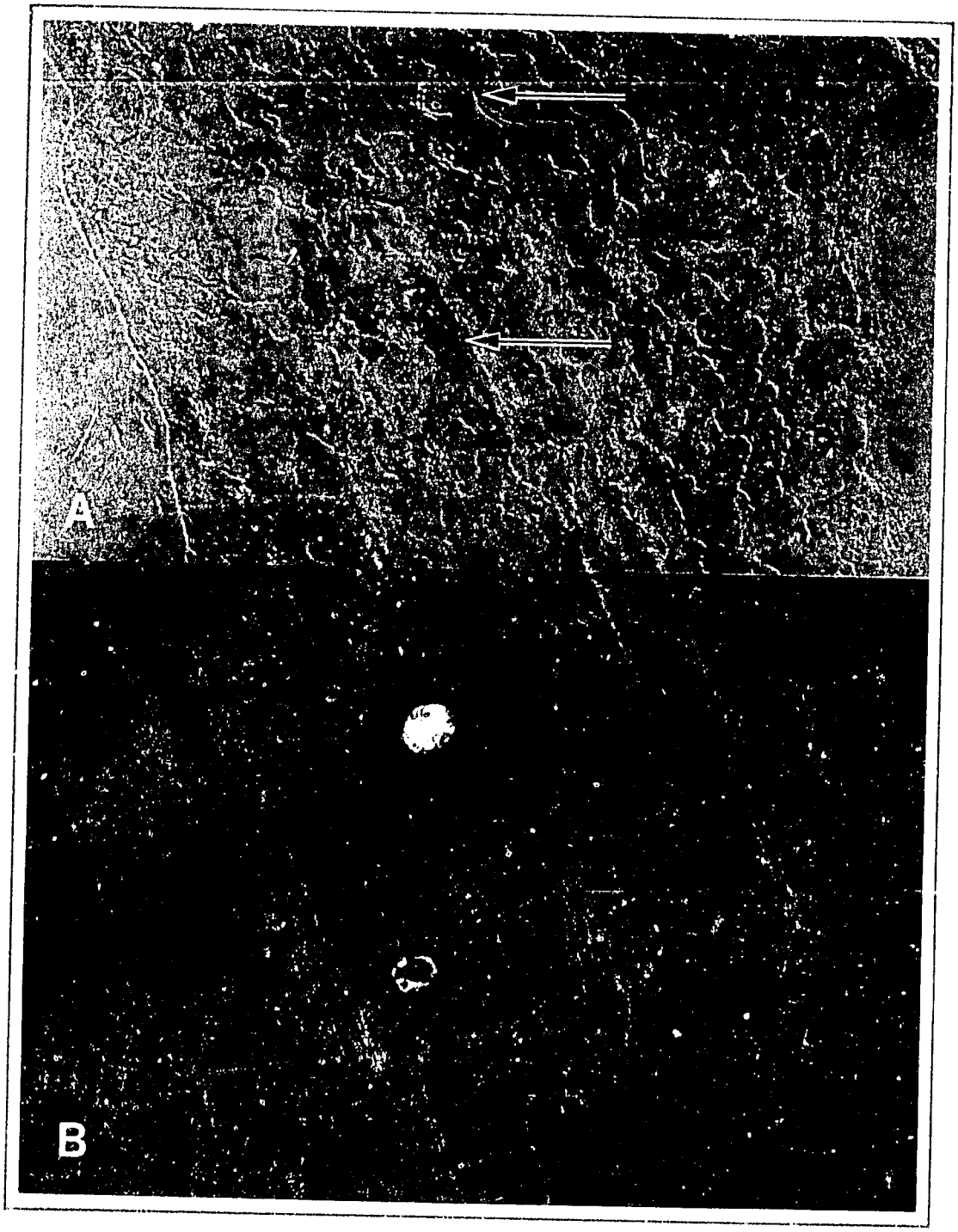

Figure 8. Indirect immunofluorescence antibody labeling of bovine intestine using rabbit anti-aprotinin antibody followed by rhodamine-conjugated sheep anti-rabbit antibody. A. A semi-thin thawed, frozen section (0.5 μm) through part of a villus of normal bovine intestine viewed with Nomarski differential interference contrast illumination. Two granular mast cells are visible (arrows). B. The same field of view illuminated with incident fluorescent light. Fluorescent label can be seen in association with the two granular mast cells shown by arrows in A.

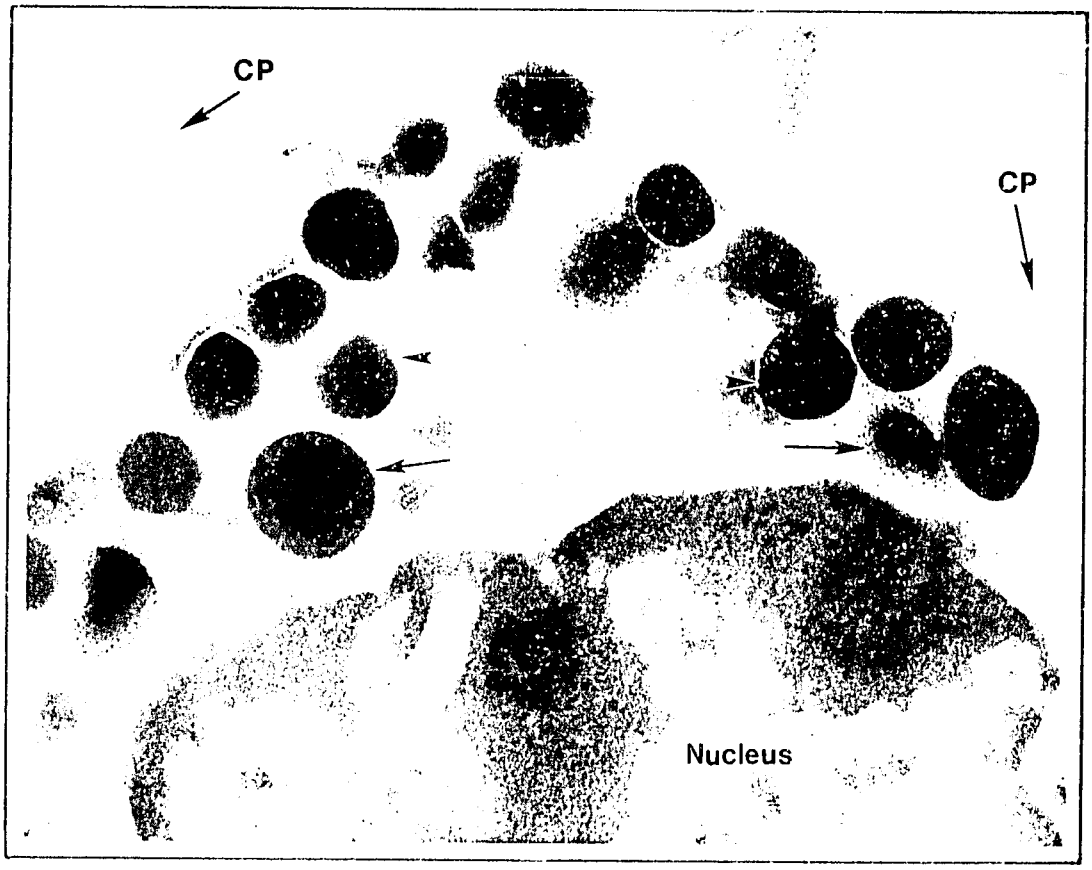

Figure 9. A thawed, thin frozen section through a bovine intestinal mast cell, characterized by the presence of cytoplasmic processes (CP) and granules (arrows and arrowheads). The section was incubated with rabbit anti-aprotinin antibody coupled to protein A-colloidal gold (5 nm particle size). Large numbers of particles, indicating the presence of aprotinin, can be seen over some granules (arrows), but few or none over others (arrowheads). Some particles are present over the cell cytoplasm, but only negligibly over the nucleus.

PARASITE-HOST INTERACTION IN THE BLOODSTREAM

Early studies on *T b brucei* infections in cattle, eland and African buffalo showed that two responses are involved in host control of parasitaemia in the bloodstream—control of parasite growth by nonimmunological processes and destruction of parasites by host antibodies. More detailed studies on both of these mechanisms have been in progress for several years, concentrating on trypanoresistant and susceptible strains of mice. The goal is to identify mechanisms which might be enhanced by chemotherapeutic or immunological means.

Control of parasite growth

In vitro studies in 1986 confirmed previous observations that bloodstream forms of *T b brucei* multiply in response to host-derived growth nutrients present in trace amounts in the sera of infected animals. The proliferation of bloodstream forms of *T b brucei* in a feeder-cell-free culture system was shown to correlate with the amount of serum provided and preliminary studies suggested that the

serum-derived growth-promoting molecules were of a molecular weight greater that 100,000 daltons.

The serum molecules which permitted multiplication of the parasites may have been proteins: they were not, however, immunoglobulins, lipoproteins or lipids. They were extremely heterogenous in terms of size and other factors, which strongly suggests that growth promotion was associated with molecules of more than one type. Studies are now being conducted to characterize these growth-promoting serum molecules more precisely.

Previous work showed that injection of mice with dead *Corynebacterium parvum*, a bacterium which acts as a nonspecific immunostimulator, enhanced their ability to control *T brucei* infection. This effect resulted from an acceleration in the rate of transition from dividing to nondividing parasite forms (Figure 10). *In vitro* studies conducted in 1986 indicated that large molecules in the serum of *C parvum*-treated mice inhibited the capacity of normal mouse serum or foetal bovine serum to support the growth of *T brucei* parasites. The inhibitory molecules were hydrophilic and ranged in molecular weight from 100,000 to 1 million daltons.

Molecules with similar characteristics could also be generated by feeding dead *C parvum* organisms to viable murine macrophages for 2 days *in vitro*, and at a 4000-fold lower efficiency by permitting *C parvum* organisms to degenerate *in vitro* in the absence of other cells. This suggests that macrophages might process dead *C parvum* organisms and release degradation products. The released products are likely to bind to *T b brucei* growth-nutrient receptors, but without stimulating growth. Work is now in progress to identify these *C parvum*-derived growth inhibitors.

Host antibody production

Mice which are highly susceptible to trypanosomiasis do not produce detectable antibodies against the parasites. Previous studies suggested that antibody secretion by B lymphocytes might be inhibited by the presence of trypanosomes. This possibility was further investigated in 1986 using trypanoresistant (C57Bl/6) and susceptible (C3H/He) mice.

After infection with *T b brucei* or *T vivax*, resistant mice controlled the first parasitaemic wave by producing antibody specific for the dominant parasite antigenic type. By contrast, susceptible mice failed to produce parasite-specific antibody and did not control the first parasitaemic wave, although their B lymphocytes were extensively activated and transformed into antibody-containing plasma cells. *In vitro* analysis of spleen cells from susceptible mice infected with either *T b brucei* or *T vivax* demonstrated that the parasite-induced antibody-containing cells were defective in their capacity both to synthesize and to secrete antibody (Figure 11).

Analysis of resistant mice demonstrated defective plasma cell function during the second parasitaemic wave. This correlated with an inability to produce antibody and a prolonged second-wave parasitaemia. However, antibody synthesis and secretion diminished and recovered in a cyclical manner, permitting the eventual remission of the second parasitaemic wave and suggesting the presence of a contrasuppressive mechanism which can lead to recovery of plasma cell function. Currently, these studies are being extended to cattle to assess whether the phenomenon observed in mice is of adequate significance to warrant deeper analysis.

Research on *T vivax* infection in goats was conducted in 1986 to determine the type and level of antibody response produced in infected animals and the degree of protection achieved. In an experiment initiated in 1985, goats were immunized—by tsetse-transmitted infection followed by drug treatment—with an East African *T vivax* stock (IL2133) or a clone (IL2710) derived from the stock. The goats infected with the

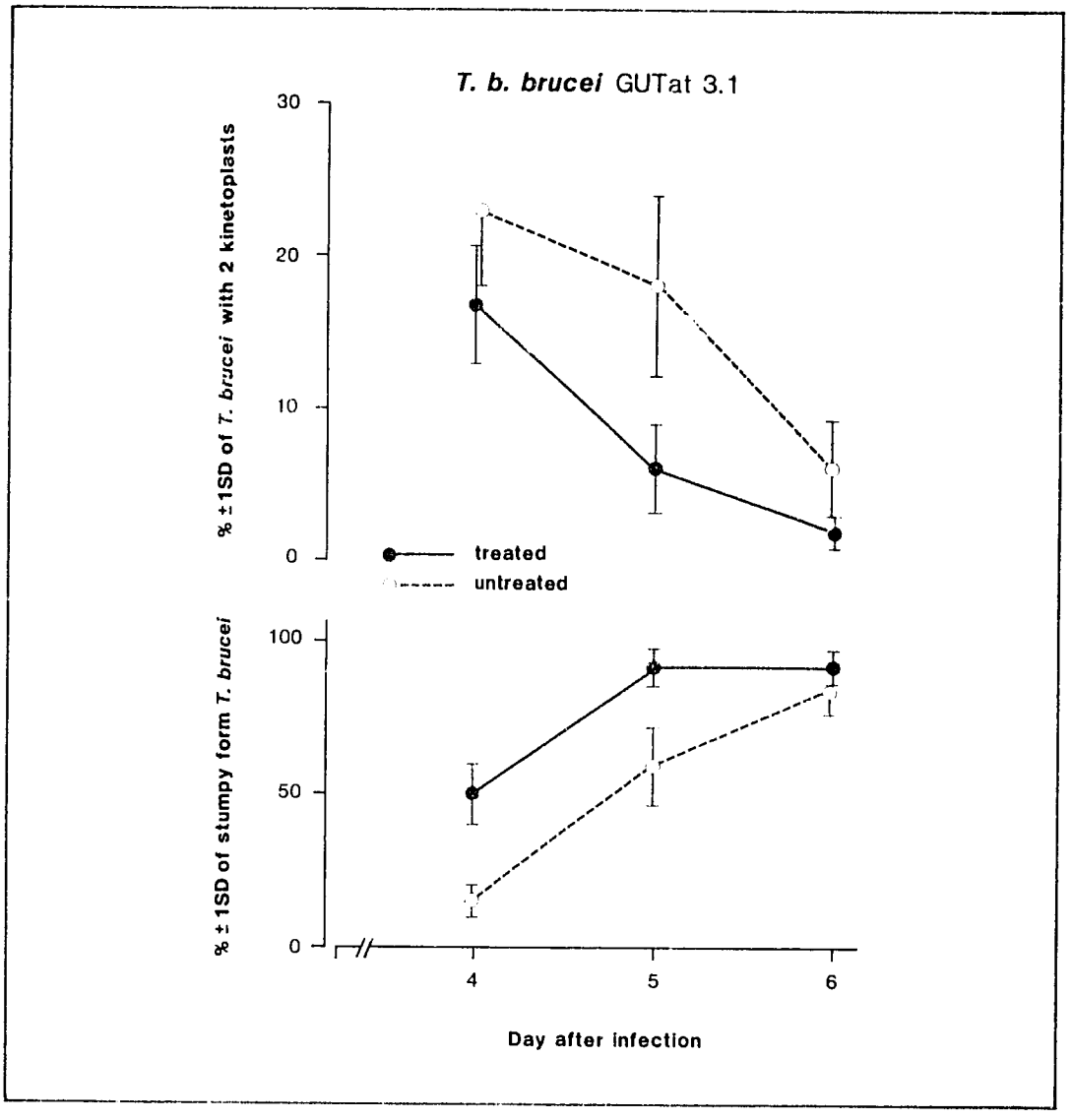

Figure 10. Influence of pretreatment with *C parvum* on the transition of *T b brucei* parasites to nondividing forms in the blood of infected mice. Groups of 10 C57Bl/6 mice were treated with 1.4 mg of heat-killed *C parvum* and infected 2 days later with *T b brucei* GUTat 3.1. Thin smears of tail blood were prepared at daily intervals, air dried, methanol fixed, Giemsa stained, and examined by light microscopy for the presence of organisms with two kinetoplasts (dividing forms) and for the presence of stumpy-form organisms (nondividing forms).

cloned parasites became resistant to challenge after two to three immunizations (cycles of infection followed by treatment), while the goats immunized with the parent stock remained susceptible to challenge but their infections were delayed and transient. A similar result was obtained when goats were allowed to 'self-cure' after chronic infection with the same stock and then challenged.

The number of metacyclic parasites extruded by infected tsetse in this experiment may have been too small to induce an adequate immune response or the parasites

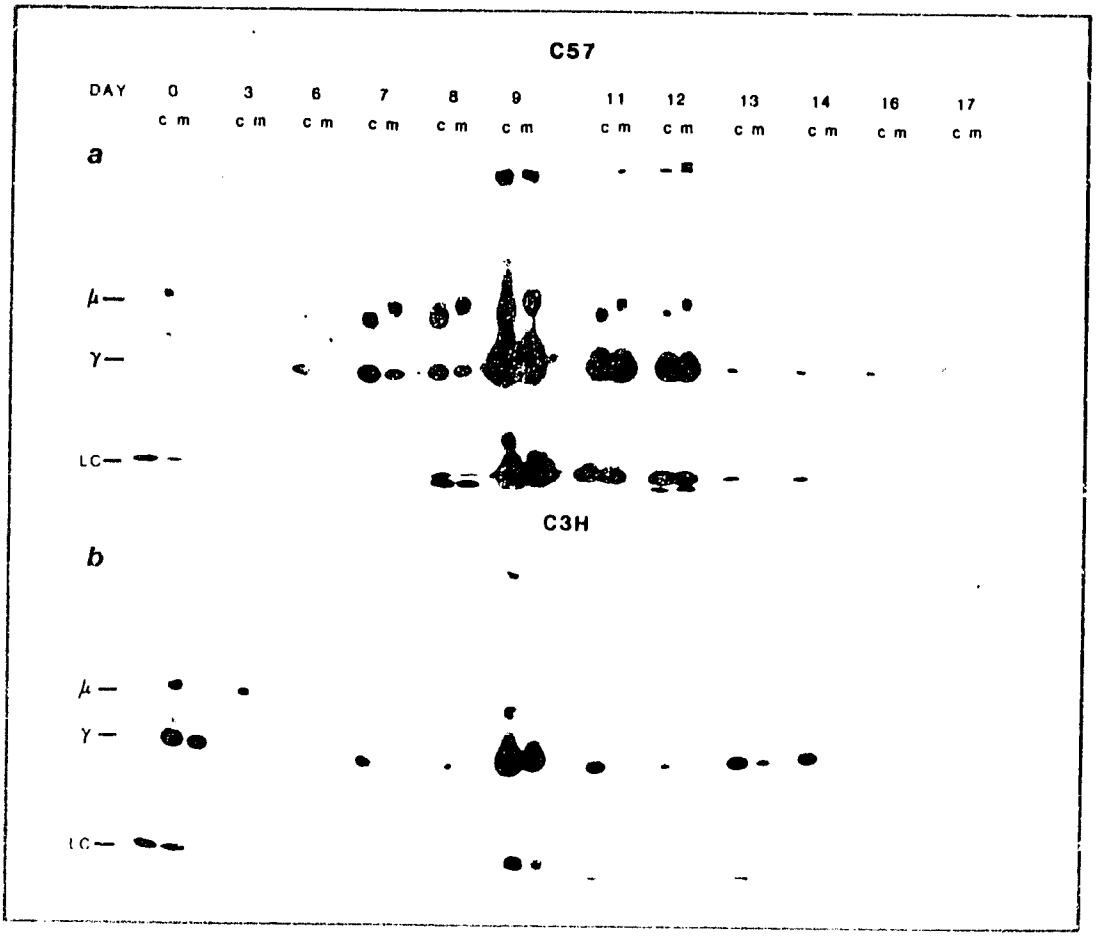

Figure 11. Immunoglobulin synthesis and secretion by spleen cells collected from resistant (C57Bl/6) and susceptible (C3H/He) strains of mice on various days after infection with *T b brucei* GUTat 3.1. From day 6 onwards, 7 to 18% of all spleen cell populations contained immunoglobulin (Ig). Cells were labeled by *in vitro* incubation with ^{35}S-methionine and cell-associated (c) and secreted (m) immunoglobulin was precipitated, analysed by SDS-PAGE and the dried gels were autoradiographed. μ denotes IgM heavy chain; the secretory form (m) is larger than the intracellular form (c). γ denotes IgG heavy chain. LC denotes Ig light chain. Clearly the susceptible mice synthesize and secrete considerably less IgM or IgG during the course of infection than the resistant mice, although they have similar numbers of spleen cells containing Ig.

may not have represented the full repertoire of variable antigen types which the animals encountered on challenge. For this reason, a second experiment has been initiated in which goats are being infected by exposure to very large numbers of infected tsetse.

Techniques have been developed to identify and quantify the different types of antibodies produced by goats infected with three different stocks of *T vivax*. Preliminary results showed that serum concentrations of IgG_2 did not change during the course of infection, while concentrations of IgG_1 increased in some but not all infected goats and concentrations of IgM rose about 10 days after infection. Antibodies to *T vivax* common antigen(s) and trypanolytic antibodies also appeared around this time. Further research will concentrate on measuring antibody responses and identifying the anti-

gens recognized by goats and cattle during *T vivax* infection.

Detection of an acute-phase reactant

Acute-phase reactants are host plasma components whose concentration significantly increases in the presence of some disease processes. These reactants are proteins, often glycoproteins, produced by liver parenchymal cells, and many have recognized biological function.

A project was initiated in 1986 to examine differences in levels of acute-phase reactants between trypanoresistant (C57Bl/6) and susceptible (C3H/He) mice infected with *T b brucei*. One protein present in much higher concentration in the plasma of resistant mice was found to be a subunit of haptoglobin, an acute-phase protein which binds haemoglobin that has become free in the plasma, thus preventing kidney pathology and loss of iron from the body. The higher level of haptoglobin in the plasma of the resistant mice was shown to be due to increased synthesis of this molecule in the liver. Susceptible mice appeared to produce as much of the molecule as resistant mice in response to other injuries, so it is not clear why they should differ specifically in their haptoglobin response to trypanosome infection.

CONTROL OF INFECTION IN TRYPANOTOLERANT AND MORE SUSCEPTIBLE CATTLE

Field studies have established that the N'Dama (*Bos taurus*) cattle of West Africa are more capable of controlling trypanosome infection and resisting clinical signs of disease than Zebu (*B indicus*) cattle or the European taurine breeds. ILRAD scientists began intensive research on the mechanism(s) of N'Dama resistance to trypanosomiasis in 1985, using 10 N'Dama cattle brought from The Gambia by embryo transfer and born at ILRAD. Experiments completed in 1986 examined the effects of repeated infection with different serodemes of *T congolense* on trypanotolerant N'Dama and more susceptible Boran.

Two groups of eight 13-month-old N'Dama and Boran were challenged with tsetse-transmitted *T congolense* (IL1180) and treated with diminazene aceturate 164 days later. Animals which developed severe anaemia, demonstrated by a drop in packed cell volume (PCV) to 15%, were treated sooner. At the time of final drug treatment, a group of 8 age-matched, uninfected Boran were also treated, and all 24 cattle were then challenged 25 days later with a second tsetse-transmitted *T congolense* serodeme (IL2642). All animals were treated with diminazene aceturate 127 days after challenge, and those showing a drop in PCV to 15% were again treated sooner. As before, a group of 8 age-matched, uninfected Boran were treated at the same time as the infected animals. The original 16 cattle plus the 8 uninfected Boran were then challenged with a third tsetse-transmitted *T congolense* serodeme (IL1587) and treated with diminazene aceturate 149 days later, and also sooner if their PCVs dropped to 15%. Preliminary analysis concentrated on the reactions of the N'Dama to the three consecutive challenges. A comparison of the reactions of the naive groups of Boran to each challenge was used as a measure of the virulence of the three *T congolense* serodemes.

None of the infected N'Dama required early drug treatment, whereas most of the Boran required early treatment after each challenge. This showed that the N'Dama were significantly more resistant than the Boran, and also that the three *T congolense* serodemes were of similar virulence.

Control of parasitaemia and anaemia

The early development of parasitaemia was very similar in both breeds in terms of both the level and timing of the first parasitaemic peak. The N'Dama began controlling parasitaemia immediately after the first peak, and most demonstrated low levels of parasitae-

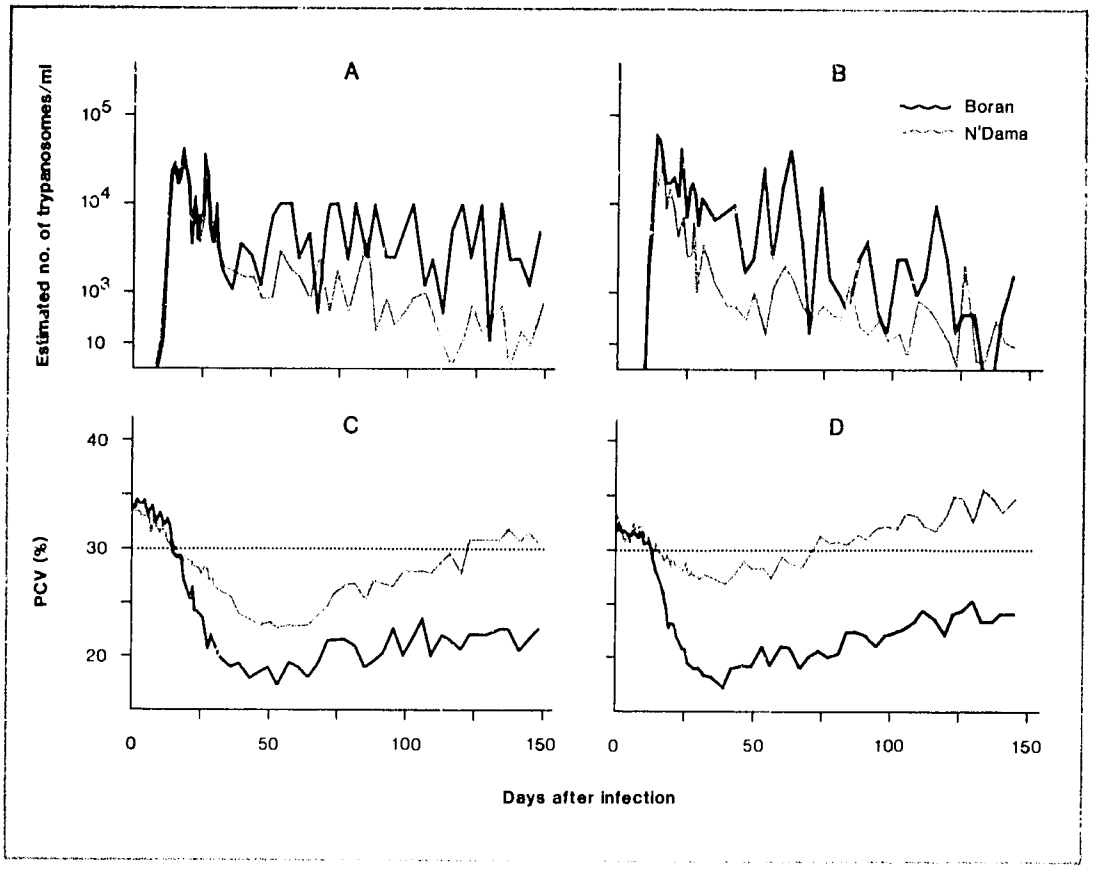

Figure 12. Development of parasitaemia and anaemia in N'Dama and Boran cattle during repeated infections with different serodemes of *T congolense*. Groups of eight 13-month-old N'Dama and Boran were challenged successively with tsetse-transmitted *T congolense* IL1180, IL2642 and ILI587 and treated with diminazene aceturate 4 to 6 months after each challenge. Graphs show estimated average number of trypanosomes per ml of jugular blood during the first (A) and third (B) infections and average PCVs after the first (C) and third (D) infections. Six Boran were treated with diminazene aceturate around day 41 after the first infection and five were treated around day 39 after the third infection. None of the N'Dama required treatment. The N'Dama demonstrated improved control of anaemia, but not of parasitaemia, over the three consecutive infections.

mia until the time of treatment (Figure 12). On 5 out of 24 possible occasions, individual N'Dama exhibited self-cure before drug treatment, as confirmed by subinoculation of their blood into mice. Statistical analysis of levels of parasitaemia in the N'Dama did not reveal any improvement in parasite control over the three consecutive infections.

By contrast, the N'Dama demonstrated improved control of anaemia by the third infection (Figure 12). They showed some evidence of anaemia during all three infections, but the lowest average PCV was 22% during the first and second infections and 26% during the third. Recovery of PCV after an initial drop was also faster after each consecutive infection. The Boran group subjected to all three infections did not demonstrate any improvement in the control of anaemia. Improved control of

anaemia in the N'Dama may be related to greater resistance of their red blood cells to the pathological effects of trypanosomiasis or to faster production of new red blood cells in the bone marrow.

Individual N'Dama controlled parasitaemia or anaemia consistently better or worse than other individuals throughout the three infections. However, the individuals which demonstrated good control of anaemia were not the same as those with good control of parasitaemia. This suggests the existence of two control mechanisms which operate independently, with larger numbers of parasites not necessarily causing a more severe anaemia.

This initial study will be followed by more detailed analysis of the mechanisms of resistance to trypanosomiasis. Studies on the control of anaemia will include an examination of any changes in the production of red blood cells which might occur in response to trypansome infection. To make this research possible, techniques were identified in 1986 for obtaining samples of bovine bone marrow and maintaining bone marrow stromal cells *in vitro*.

Changes in white blood cell populations

While the basis of trypanotolerance remains elusive, it has been suggested that superior anti-trypanosomal immune responses may make a significant contribution. To begin investigating this possibility, changes in white blood cell populations were monitored in the eight N'Dama and eight Boran cattle during their second infection with *T congolense*. Total and differential white cell counts were taken daily for 30 days from the time of infection and twice weekly for the next 60 days, and numbers of lymphocytes, neutrophils, monocytes and eosinophils were estimated. Lymphocyte populations were analysed using monoclonal antibodies raised at ILRAD to provide information on T-lymphocyte subsets, B lymphocytes and null cells. The T-lymphocyte subpopulations identified were those expressing the pan-T cell marker, BoT2, 'helper' cells expressing the BoT4 molecule and 'killer' cells expressing BoT8.

Prior to trypanosome infection, the N'Dama had more total white blood cells, T and B lymphocytes, neutrophils and null cells than the Boran, but no significant differences were detected for other cell types. Up until the point when the Boran required drug treatment, total white cell and total lymphocyte counts ran parallel for the two breeds: both cell populations decreased significantly from the time of infection to the first peak of parasitaemia, with values for the N'Dama consistently higher (Figure 13). Parallel decreases were observed in the two subpopulations of T lymphocytes positive for the BoT4 or the BoT8 antigen. Neutrophil counts remained the same during the period, while monocyte and eosinophil populations changed over time but without an apparent trend.

B lymphocyte levels were significantly higher in the N'Dama than the Boran from the time of infection until 22 days later when the first Boran required drug treatment. Monocyte numbers fluctuated considerably during the first 22 days after infection, but there was no general decrease or increase and no significant difference between the breeds. During the same period, the number of null cells decreased in both groups but remained higher in the N'Dama than in the Boran. These findings should provide a basis for further examination of white cell responses to trypanosome infection and their possible role in mediating the superior resistance of trypanotolerant cattle.

Bodyweights, liveweight gains and reproductive performance

At the time of the first infection when the eight N'Dama and eight Boran were 13 months old, average bodyweight was 154 kg for the N'Dama and 139 kg for the Boran. Because nearly all the Boran required early drug treatment after each

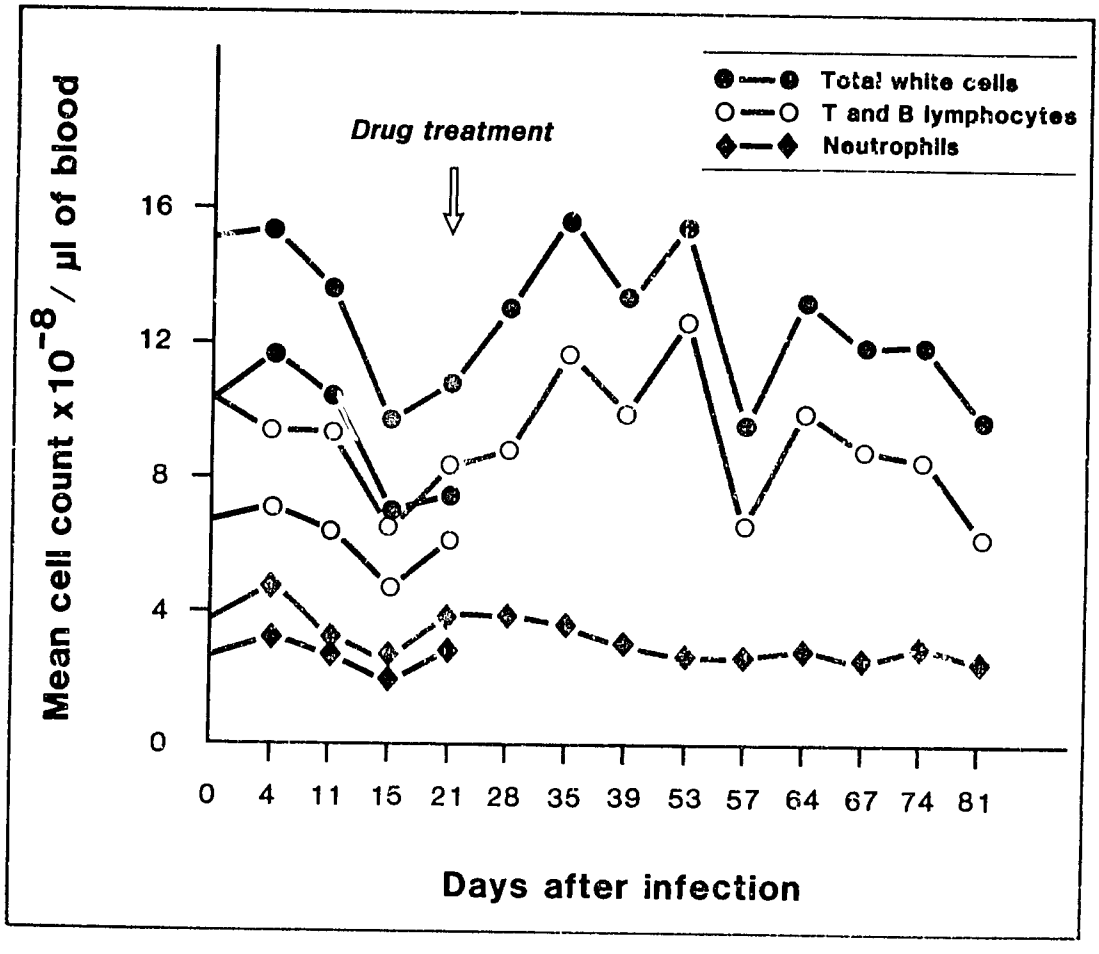

Figure 13. Sequential analysis of the total number of white blood cells, lymphocytes and neutrophils per µl blood in 8 N'Dama (blue) and 8 Boran cattle (black) during the course of infection with *T congolense* (IL2642). In both groups a leukopenia, characterized by significant decreases in the absolute number of white blood cells and lymphocytes, developed around day 15, while the number of neutrophils did not change significantly. Before challenge and throughout the course of infection, the N'Dama had significantly higher numbers of total white blood cells and lymphocytes. Values for the Boran are presented only up to 21 days after challenge when the first Boran received drug treatment.

infection, they were infected for an average of only 190 days over the 567-day study period, while the N'Dama were all infected for 432 days. In spite of this, at the end of the third infection when the cattle were 32 months old, average bodyweights were similar for both breeds—319 kg for the N'Dama and 309 kg for the Boran. Daily liveweight gains averaged monthly were also similar for the N'Dama and the Boran over the 567-day period (297 and 302 g respectively).

Among both breeds, males showed higher daily liveweight gains and higher final bodyweights than females. Trypanosome infection did not appear to affect bodyweights and liveweight gains among the N'Dama: three infected male N'Dama had similar bodyweights at 13 and 32 months and similar daily liveweight gains as two uninfected N'Dama males used as controls. By contrast, the average daily liveweight gain of the infected and treated Boran was 22%

lower than that of uninfected controls.

The infected N'Dama heifers demonstrated oestrus behaviour for the first time at 23 months of age, during the second *T congolense* infection. After treatment and throughout the third infection period, plasma samples were taken daily and progesterone levels were determined for the N'Dama and for nine infected Boran heifers. Among the N'Dama, no differences in cyclical activity were observed before and during infection: the cycle length was 20.4 days (± 0.2), the luteal phase was 12.5 days (± 0.3) and growth, function and regression of luteal tissue were not affected. By contrast, only three of the Boran were cycling at the time of the third infection and all three stopped cycling 2 to 3 weeks later. Their cyclical activity resumed, although irregularly, on average 40 days after drug treatment.

EPIDEMIOLOGY

TRYPANOSOME CHARACTERIZATION

Research has been in progress to develop simple, accurate and sensitive assays to detect trypanosomes in infected animals and tsetse and to identify parasite species and serodemes. Work on diagnostic methods is important for diagnosis *per se* and for evaluation of the results of prophylaxis and treatment. Better means of detecting trypanosomes in tsetse would also be useful, for instance to predict the level of trypanosomiasis risk in an area before introducing susceptible livestock.

Serological typing

As reported previously, monoclonal antibodies have been produced at ILRAD which distinguish the three major African trypanosome species—*T congolense*, *T vivax* and *T brucei*. These can be used to detect trypanosome antigens in the bloodstream of infected animals. In 1986, the monoclonal antibodies raised against *T brucei* and *T congolense* were also used successfully to detect trypanosomes in samples of tsetse bloodmeals. Work is in progress to isolate and purify the species-specific antigens recognized by these antibodies. The goal is to produce sufficient quantities of the antigens for diagnosis of trypanosomiasis and the development of simple tests for use in the field.

Research has also been in progress since 1982 to develop new methods for identifying trypanosome serodemes. This work has focused on *T congolense* stocks isolated from cattle at Kilifi in the Coast Province of Kenya. A technique that shows promise involves the use of chromosome electrophoresis. Parasite populations belonging to the same serodeme appeared to have identical patterns of chromosome bands, even when the populations expressed different VSGs at the time of testing.

This technique was also used to characterize 20 *T b rhodesiense* isolates taken from sleeping sickness patients in Busoga, Uganda. Results, confirmed by cross-neutralization assays using chronic infection sera, showed that the 20 isolates may belong to one serodeme. Studies are now in progress to confirm these findings by analysis of metacyclic antigens. This work was conducted in collaboration with the Uganda Trypanosomiasis Research Organization.

Use of DNA probes

The ideal method for identifying trypanosomes in the field should be species specific, sensitive, simple to use and should provide unequivocal results. Scientists at ILRAD have been using DNA hybridization probes to develop methods for the identification of African trypanosomes which will meet these criteria. Specific DNA probes have been prepared from *T b brucei*, *T congolense*, the *T congolense* parasites isolated at Kilifi, *T simiae* and East and West African populations of *T vivax*. Development of probes

for field use has concentrated initially on *T congolense*.

Highly repetitive DNA sequences were identified in the genomes of 15 *T congolense* isolates taken from 6 countries in Africa, in *T congolense* isolated at Kilifi, and in *T simiae*, a trypanosome species morphologically indistinguishable from *T congolense*. All three DNA sequences were individually cloned and were shown to hybridize specifically and exclusively with DNA from trypanosomes of the same species or subspecies: they did not hybridize with each other or with DNA from any of the other trypanosome species tested. The consistent distinction between *T congolense* and Kilifi-type *T congolense* was surprising, given that these trypanosomes are morphologically identical.

Work in 1986 concentrated on making these probes more sensitive in order to detect low levels of parasitaemia in infected animals. This was done by cloning the DNA sequences in multimeric form and by optimizing labeling and hybridization conditions. Both *T congolese* probes can detect 2.5 pg of parasite DNA after 12-hours exposure, equivalent to about 25 trypanosomes. After 1-week exposure, they each can detect the DNA equivalent of about 5 trypanosomes. The *T simiae* probe, which was obtained in monomeric form only, can detect about 1,000 trypanosomes after 12-hours exposure and 100 trypanomes after exposure of 1 week.

Because of the genomic diversity of *T congolense*, the two *T congolense* probes may not detect all *T congolense*-type parasites from various parts of Africa. Work now focuses on testing them using all the *T congolense* isolates available at ILRAD.

DNA probes have been developed to detect *T vivax* infection, first using a West African *T vivax* strain originating in Nigeria and more recently using an East African strain isolated at Galana, Kenya. In 1986, the specificity of these probes was tested using *T vivax* parasites from Kenya, Uganda and Nigeria plus *T b brucei*, *T congolense* and *T musculi* as negative controls. Although the two probes distinguished the *T vivax* isolates from parasites of other species, the distinction between the East and West African *vivax* types was not entirely clear. Work now concentrates on testing these probes with *T vivax* isolates from different parts of Africa and also on studies to determine their sensitivity.

DNA probes were originally developed for detecting trypanosomes in samples of blood taken from infected animals, but they have also proven useful for detecting trypanosomes in tsetse. Preliminary experiments showed that *T congolense* could easily be detected in tsetse midgut suspensions and proboscides, *T b brucei* could be detected in midgut and salivary gland suspensions and with some difficulty in proboscides, and *T vivax* could be detected in proboscides, though also with difficulty. This approach requires the dissection of tsetse organs and is thus not much simpler than traditional methods of tsetse dissection and microscopic examination. Studies were conducted in 1986 to find more convenient methods for use in the field. The simplest technique developed was to cut and discard the distal abdomen and squeeze the abdominal contents onto a nylon filter. Initial results with this 'abdominal touch-blot' method are promising and this technique is being considered for field testing, to be compared with results achieved from traditional methods.

Isoenzyme and protein analysis

Measuring differences in the electrophoretic mobility of enzymes is one method of assessing the genetic diversity of organisms. In 1985, scientists at ILRAD compared the electrophoretic mobility of enzymes from several stocks of *Nannomonas* trypanosomes isolated from Kilifi with that of enzymes from groups of *T congolense* parasites originating elsewhere in Africa. Analyses completed in 1986 showed that the *Nannomonas* trypanosomes isolated from

Kilifi formed a distinct genetic group which differed substantially from other groups of *T congolense*.

Comparison of radiolabeled parasite proteins on two-dimensional gels also showed that *T congolense* and Kilifi-type *T congolense* were two distinct genetic groups of organisms. These results suggest a wide spectrum of genetic diversity among trypanosomes currently classified as *T congolense* and it is possible that the taxonomic status of this group may have to be revised.

Proteins from procyclic forms of *T b brucei, T b rhodesiense* and *T b gambiense* were also analysed on two-dimensional gels. As expected, the three *T brucei* subspecies differed markedly from other trypanosome species, but subspecies discrimination was difficult. The radiolabeling of protein from *T vivax* has been achieved and analysis on two-dimensional gels showed differences in protein composition between different *T vivax* isolates.

Several laboratories have initiated studies on *in vitro*-derived procyclic trypanosomes and have tried to demonstrate that these are equivalent to the parasites which develop in the tsetse midgut. Using two-dimensional gel analysis, *T b brucei* proteins from *in vitro*-derived procyclic forms were compared with proteins in parasites derived from tsetse. Results of this work, along with immunological, biochemical and ultrastructural comparisons, demonstrated that *in vitro*-produced procyclics may be considered as equivalent to forms originating in the tsetse midgut.

Because of the problems involved in initiating and maintaining tsetse control programs and the absence of an effective trypanosomiasis vaccine, the livestock industry in the vast tsetse-infested regions of Africa depends almost entirely on trypanocidal drugs, both to prevent trypanosomiasis and to treat infected animals. No new trypanocidal compound has been introduced commercially for almost 30 years and the development of drug resistance in trypanosome populations has limited the choice of effective drugs available to veterinarians and livestock owners. One major constraint on the development of new trypanocidal drugs for human and veterinary use is the expense of drug testing in experimental animals.

Trypanosome culture systems developed at ILRAD are now being used to screen the effects of trypanodical drugs on different trypanosome species and stocks *in vitro*. Although the activity of a trypanocidal compound in the laboratory does not always correspond precisely with its effectiveness in the field, the results of drug screening assays provide important insights into possibilities for improved trypanosomiasis control.

One study completed in 1986 concentrated on simplifying the *in vitro* assay systems used for testing the effects of trypanocidal drugs. To date, drug screening *in vitro* has been conducted in the presence of a cell feeder layer. Workers outside ILRAD have described a culture system for *T brucei* which involves culturing the parasites on a layer of feeder cells for 10 to 20 days and maintaining them thereafter in a cell-free culture system. Attempts to cultivate *T vivax* and *T congolense* in such cell-free systems have not yet been successful.

Previous work indicated that the responses of *T vivax* and *T congolense* bloodstream forms to trypanocidal drugs were more predictable after the parasites had been transformed through their entire lifecycle *in vitro* into 'second-generation' bloodstream forms. However, many laboratories in Africa lack the facilities to maintain trypanosomes *in vitro* throughout their lifecycle. For this reason, work in 1986 concentrated on developing *in vitro* tests for drug activity using bloodstream trypanosomes isolated directly from mice or cattle.

Trypanocidal activity was measured in terms of parasite growth inhibition and the effect of drug treatment on the infectivity of trypanosomes for mice. When trypanocidal activity was examined in sera from cattle inoculated with isometamidium chloride (Samorin: May & Baker), results were complicated by the fact that sera from some animals naturally inhibited trypanosome growth and infectivity, even in the absence of the trypanocidal drug. No correlation could be found between this inhibitory property and the protein profile or the level of high- and low-density lipoproteins in sera from individual animals.

One experiment examined the inhibition of growth and infectivity of *T congolense* IL1180 (an isometamidium chloride-sensitive trypanosome population) after exposure of the parasites to sera from cattle inoculated with isometamidium chloride. The drug was administered to five Boran steers (0.5 mg/kg) which were challenged 30 days later with a *T vivax* isolate from Kilifi, Kenya, by tsetse transmission. Three animals became infected. Sera taken from these three cattle just before drug treatment, on the day of *T vivax* challenge, and 7 and 14 days after challenge were incubated with the *T congolense* population in culture for 24, 48 and 72 hours. Growth inhibition was observed. In all instances, longer incubation of the parasites with drug-containing sera resulted in more pronounced growth inhibition. When parasite samples were incubated for 24 hours with different sera and then injected into mice, it was found that only sera collected after drug administration inhibited parasite infectivity.

The two steers which did not become infected during the previous experiment were challenged a second time with the Kilifi *T vivax* 2 months after isometamidium chloride administration. Although the two animals became infected, their sera collected on the day of challenge inhibited the growth and infectivity of *T congolense* IL1180 after 24-hours *in vitro* incubation. It thus appears that Kilifi *T vivax* populations were multiplying in all five cattle in the presence of drug levels sufficient to inhibit the growth and infectivity of *T congolense*. Three months after administration of isometamidium chloride, the same five cattle were challenged with *T congolense* IL1180 by tsetse transmission. None became infected, confirming the *in vitro* findings.

A further experiment was designed to investigate the sensitivity of the *in vitro* screening technique. Sera were taken weekly from three calves inoculated with a low dosage of isometamidium chloride (0.25 mg/kg). Bloodstream forms of *T congolense* IL1180 were incubated in the sera and then injected into mice. When the parasites were incubated in the sera for 24 hours, only sera that had been taken 7 and 14 days after drug treatment completely inhibited infectivity. When the incubation was extended to 48 hours, trypanosome infectivity was inhibited in sera collected up to 42 days after drug administration. The three calves were refractory to syringe challenge with the same *T congolense* parasites 42 days after treatment.

When a bovine-derived *T vivax* from Nigeria was incubated in the same sera, the results, in terms of infectivity for mice, were extremely inconsistent. This strain of *T vivax* appeared to be much more sensitive than *T congolense* IL1180 to the growth-supporting, or inhibiting, properties of sera from individual animals, thereby masking any trypanocidal effects associated with the presence of the drug. When this *T vivax* population was expanded in mice and then incubated in the same calf sera, all sera collected up to 14 days following drug administration inhibited infectivity for further mice, whilst sera collected at a later date failed to do so.

Results from this work suggested that the growth and infectivity of trypanosomes cultured in sera from drug-treated animals could be used as an indirect indicator of drug resistance in parasite populations. Inhibition of parasite infectivity appeared to be a more sensitive parameter than inhibition of

parasite growth, and correlated closely with activity of the drug *in vivo*.

Trypanocidal drugs: detection and testing *in vivo*

Successful treatment of trypanosomiasis is very difficult once the parasites have penetrated the central nervous system, because very few trypanocidal drugs can pass through the natural physiological barriers that separate the central nervous system from the bloodstream. A better understanding of how trypanocides work and their distribution within the host could lead to the development of improved treatment systems. To address this problem, scientists at ILRAD have developed a cannulation system for monitoring the cerebrospinal fluid of goats, together with biological assays and high-performance liquid chromotography (HPLC) techniques for drug detection. This work is being conducted with support from WHO.

Goats infected with *T b brucei* develop a subacute disease which results in death after 5 to 8 weeks, with central nervous system involvement demonstrated by meningitis/choroiditis lesions in the brain. If infected animals are treated with diminazene aceturate (dosage 10 mg/kg), a relapse infection occurs associated with severe meningoencephalitis, identical to that seen in humans suffering from advanced sleeping sickness.

When uninfected goats were treated with diminazene aceturate by intramuscular injection, the drug could be detected in plasma but not in cerebrospinal fluid. The drug level reached a peak within 24 hours of treatment at about 1 μg/ml and rapidly declined over the following 5 days, with the lower level of detection (50 ng/ml) still present 8 days after treatment.

Similar studies were carried out with suramin (Naganol: Bayer). Plasma samples were chemically denatured to assist extraction and measurement of the drug, which binds strongly to plasma proteins. This resulted in an approximate 30% recovery of the drug. Twenty-four hours after uninfected goats were injected intravenously with a single dose (20 mg/kg) of suramin, a level of 10 to 20 μg/ml could be detected in plasma. Drug levels above the limit of detection (200 ng/ml) were still present after 10 days, but at no stage was suramin detectable in cerebrospinal fluid.

Cannulated goats were infected with *T b brucei* and treated with either diminazene aceturate or with suramin. Although suramin was not detectable at any time in the cerebrospinal fluid of uninfected goats, scientists are now analysing the cerebrospinal fluid of goats suffering relapse infections of *T b brucei* and treated with suramin. It might be possible for suramin to attain trypanocidal levels in the central nervous system once the blood/brain barrier is compromised by infection. Similar studies are in progress using melarsoprol (Arsobal: Specia), a trypanocidal compound which is capable of crossing into the central nervous system.

Present work to improve the sensitivity of drug measurement includes the application of enzyme digestion techniques to aid extraction of drugs from plasma and cerebrospinal fluid. Detection by fluorescence and by radiolabeling are also being tested as a means of increasing the sensitivity of measurement. The drug levels detected by such methods will be correlated with levels detectable by *in vitro* assay. Together, these appoaches should provide insights on the efficacy of treatment in infected animals.

Trypanocidal drugs: evaluation of protection

Despite the wide use of trypanocidal drugs, very little is known about their mode of action *in vivo* or the factors which influence the effectiveness or duration of protection afforded by drug treatment. Work in this area is being carried out at ILRAD with support from the British Government's Overseas Development Administration (ODA). Studies conducted in 1985 showed that cat-

tle treated with isometamidium chloride (1 mg/kg) were protected for at least 5 months against monthly challenge with two unrelated serodemes of *T congolense* transmitted by tsetse. This work also indicated that:

1. A direct relationship exists between drug dosage and the duration of protection.
2. The presence of a pre-existing infection at the time of drug treatment does not appear to affect subsequent protection.
3. The level of metacyclic challenge does not appear to affect the duration of protection.
4. Antibody production is unlikely to play a protective role in cattle maintained under a chemoprophylactic regime, as the size of metacyclic challenge required to stimulate an antibody response was much larger than the level likely to be encountered in the field.

Similar experiments were conducted in 1986 to investigate the duration of protection provided by isometamidium chloride against *T vivax*. A preliminary experiment confirmed the drug sensitivity of *T vivax* parasites originating from Kenya and Nigeria. Ten Boran steers were infected by tsetse transmission, 5 with a *T vivax* clone originating from Kenya (IL2969) and 5 with a *T vivax* clone from Nigeria (IL2968). Eleven days following infection, all 10 animals were treated with isometamidium chloride at a dosage of 0.5 mg/kg. Four of the steers infected with the Nigerian clone and all five steers infected with the Kenyan clone were completely cured. When different Boran steers were inoculated with a single prophylactic dose (0.5 mg/kg) of isometamidium chloride and subjected to monthly tsetse-transmitted challenge with the same *T vivax* clones, they were completely protected against the Nigerian clone for at least 2 months, but were protected for less than 1 month against the Kenyan clone (Figure 14).

Serological investigations showed a complete correlation between the onset of parasitaemia and the first detection of antibodies: none of the treated animals showed an immune response which might have contributed to the protection afforded by the drug. As described elsewhere, sera from the cattle susceptible to Kenyan *T vivax* challenge demonstrated trypanocidal activity against *T congolense* (IL1180) *in vitro*, even though the sera were taken after all animals had

T vivax strain	Animal number	Infection Detected Month	Day After Monthly Challenge
Nigerian (IL2968)	D335	none up to 5 months	
	D339	none up to 5 months	
	D343	3	11
	D359	4	11
	D363	3	11
Kenyan (IL2969)	D338	2	7
	D340	2	9
	D347	1	12
	D350	1	23
	D355	1	24

Figure 14. Effectiveness of isometamidium chloride administration against *T vivax* challenge in cattle. Ten yearling Boran steers were divided into two groups of five, inoculated with isometamidium chloride (0.5 mg/kg) and then challenged with a Nigerian (IL2968) or a Kenyan (IL2969) stock of *T vivax*. Challenge was by 10 infected tsetse, monthly beginning 1 month after drug treatment. Two untreated controls were also challenged with each stock each month.

become infected with *T vivax*.

In an attempt to detect isometamidium chloride in the five cattle in which Kenyan *T vivax* infections had broken through, the same animals were treated with a curative dose (3.5 mg/kg) of diminazene aceturate 75 days after the original isometamidium chloride administration. Diminazene aceturate was also given to four control cattle—two which had been inoculated with isometamidium chloride but not infected and two which had been infected with the same *T vivax* clone but not treated. All the cattle were challenged 18 days later with tsetse-transmitted *T congolense* (IL1180). All the controls became parasitaemic 17 days after challenge, but none of the cattle which had previously been susceptible to challenge with Kenyan *T vivax* developed *T congolense* infections, indicating that trypanocidal levels of isometamidium chloride were still present.

Conclusions may be summarized as follows:

1. No immune responses could be detected in cattle protected from infection by administration of isometamidium chloride.
2. Cattle challenged with the Nigerian *T vivax* were completely protected for 2 months, whereas cattle challenged with the Kenyan stock were completely protected for less than 4 weeks.
3. When isometamidium chloride was used to treat infections with either population of *T vivax*, both populations appeared fully sensitive to the drug at a dosage of 0.5 mg/kg.

In the past, isometamidiun chloride-resistant parasites have been demonstrated by a strategy of infection and treatment. The present work has described a Kenyan *T vivax* clone sensitive to the therapeutic activity of isometamidium chloride, as defined in the field situation, but resistant to the prophylactic activity of the drug. This suggests that future studies on putative isometamidium chloride-resistant trypanosomes should ideally be conducted using a prophylactic protocol.

TSETSE FLIES AS TRYPANOSOME VECTORS

In a research project partly funded by IAEA and completed in 1985, scientists measured the vectorial capacity of sexually sterile and fertile males of seven tsetse species for East and West African stocks of *T vivax*, *T congolense* and *T b brucei*. This study demonstrated that sterile males transmit trypanosomes just as efficiently as fertile tsetse, indicating that the release of large numbers of sterile males as part of a tsetse control program could increase the immediate risk of trypanosomiasis in the affected area.

To overcome this problem, research in 1986 concentrated on developing a method for reducing trypanosome infection rates in sterile flies. Teneral male tsetse (*G m centralis*) were sterilized by gamma irradiation, fed artificially on a bloodmeal containing isometamidium chloride at a rate of 8 μg/ml of blood and then fed 2 days later on goats infected with *T vivax*, *T congolense* or *T b brucei*. *T vivax* and *T b brucei* infection rates in mature flies were completely suppressed after this treatment, and *T congolense* infection rates were substantially reduced. When the drug concentration was increased to 12 μg/ml of blood and the flies were fed on infected goats 10 days after feeding on the treated bloodmeal, infection with all three species was completely suppressed. These results indicate that the problem of increased trypanosomiasis risk associated with the release of sterile male tsetse might be alleviated by feeding a bloodmeal containing isometamidium chloride prior to release.

Relatively little is known about the development of *T vivax* in the mouth parts of tsetse flies or the vectorial capacity of different tsetse species for this species. A study was carried out in 1986 to test the vectorial capacity of the seven tsetse species available at ILRAD for two East African and two West African stocks of *T vivax*. The East African stocks used were isolated from a

goat at Likoni and from a cow at Galana, both in Kenya; the West African stocks were both from Nigeria, one isolated from a cow and one from a tsetse fly.

Teneral male tsetse were fed for 24 days on Boran calves infected with these stocks and then dissected to determine infection rates. For the two East African stocks, infection rates in *G m centralis* and *G brevipalpis* were high, ranging from 36.2 to 75.3%. In the other five tsetse species—*G austeni, G palpalis palpalis, G p gambiensis, G fuscipes fuscipes* and *G tachinoides*—infection rates were low, ranging from 0 to 5%. By contrast, infection rates with the two West African stocks were high in all tsetse species, ranging from 55.5 to 97.1%.

Fibrinolytic enzymes have been identified in the tsetse midgut which probably help protect feeding tsetse from the potentially harmful effects of blood clotting. In collaboration with the University of Nairobi, five tsetse fibrinolytic enzymes were identified and the two most active ones were purified and shown to be serine proteases. Rabbits immunized against one of the enzymes produced antibodies which crossreacted with the other, suggesting that the two enzymes share some common antigenic determinants. However, these antibodies failed to block the fibrinolytic activity of the enzymes on synthetic substrates, indicating that the antibodies may not be directed to the enzymes' active sites. Further work in this area will be conducted in collaboration with ICIPE.

AFRICAN TRYPANOTOLERANT LIVESTOCK NETWORK

ILRAD and ILCA participate in a research Network in West and Central Africa to study the health and productivity of trypanotolerant and non-trypanotolerant cattle, sheep and goats exposed to different levels of tsetse-transmitted trypanosomiasis. Research is being conducted at 11 sites—3 in Zaire, 2 in Togo, 2 in Ivory Coast, 2 in The Gambia, 1 in Gabon and 1 in Nigeria—plus 2 associated sites, 1 in Kenya and 1 in Ethiopia. Additional projects are being initiated in Senegal, Benin and Guinea. Research in all these countries is conducted in close cooperation with livestock owners and government livestock departments. ILCA published a report in 1986 providing extensive background information on the Network and on each of the sites.

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

Trypanosomiasis control strategies, such as regular preventive treatment with trypanocidal drugs, have been introduced at some of the Network sites and their effects are being monitored and analysed. Tsetse control programs will also be monitored within the Network.

ILRAD provides training and supervision for the monitoring of tsetse populations, trypanosome infection rates in tsetse and livestock and the health status of animals included in the study. ILCA assists with the collection of data on animal production and nutrition and is responsible for processing and analysing health and productivity data.

Because of the crucial importance of accurate data collection and standardization among the different sites, field staff working within the Network receive intensive training and careful supervision. Two 7-week training courses are conducted at ILRAD every year, one in English and one in French. By the end of 1986, 53 staff members working in the Network had received training at ILRAD.

Studies were completed in 1986 on trypanotolerant cattle, sheep and goats at eight sites in five countries. These included information collected over a 2-year period on

factors which influence the prevalence of trypanosome infections and the effects of infection on animal health and productivity. ILCA published a report of results obtained from 1983 to 1985 at the end of 1986.

Tsetse density and infection rates

Nine tsetse species were identified at the 11 sites included in the Network. These were *G fuscipes*, *G tachinoides*, *G palpalis*, *G tabaniformis*, *G nashi*, *G brevipalpis*, *G pallidipes*, *G austeni* and *G morsitans submorsitans*, representing the three major taxonomic groups of tsetse—*morsitans*, *palpalis* and *fusca*.

Tsetse density is measured at all Network sites for 5 to 10 consecutive days each month using biconical traps. Density is expressed as the average number of flies captured per trap each day. The figures recorded are expressed as relative density, using standard techniques at all research sites to facilitate comparison. Relative density is a function of real tsetse density, behaviour and nutritional status of the flies, position of the traps and weather conditions. Relative densities tend to be low at most Network sites, ranging from an overall average from 1984 to 1986 of 5.9 at Mushie Ranch in Zaire to 0.1 at Avetonou in Togo.

Network staff dissect and examine the proboscides, midguts and salivary glands of captured tsetse to determine trypanosome species and infection rates. Infection rates tend to be high at many Network sites, ranging from a 3-year average of 16.1% at the OGAPROV Ranch in Gabon to 0.4% at Kolo Ranch in Zaire. Generally, tsetse infection rates are high at sites where the recorded prevalence of trypanosome infection in livestock is high, which would be expected in situations where tsetse are feeding primarily on domestic animals.

Virtually all the trypanosome infections detected in tsetse were of the *congolense* or *vivax* type. *Vivax*-type infections were strongly predominant in tsetse species of the *palpalis* group (*G palpalis* and *G tachinoides*) and in *G tabaniformis* and *G nashi* of the *fusca* group. *Congolense*-type infections predominated in *G brevipalpis*, also of the *fusca* group, and in *G pallidipes* and *G austeni* of the *morsitans* group. These findings generally agree with results from other studies.

Estimates of tsetse challenge

Preliminary estimates of tsetse challenge have been calculated for the 11 study sites, based on relative tsetse density and mean trypanosome infection rates. Results are presented in Figure 15. Challenge levels appear to be low with one exception (Mushie Ranch in Zaire); this would be expected in locations where livestock production is economically viable.

Tsetse blood meals are also being analysed to determine feeding behaviour. This will make it possible to refine estimates of tsetse challenge by taking into account the proportion of tsetse feeds taken from the animals under study. In 1986, 659 blood meals were collected from Network sites in Gabon, The Gambia, Ivory Coast, Kenya, Togo and Zaire for analysis at ILRAD. Monoclonal antibodies have been derived for identifying tsetse blood meals originating from Bovidae (cow, buffalo), Alcephinae (wildebeest, hartebeest, kongoni, topi), Tragelaphinae (bushbuck, eland), Antelopinae (impala, Thompson's gazelle, Grant's gazelle), Caprinae (goat, sheep), Suidae (warthog, bushpig, domestic pig) and reptiles.

Comparisons of tsetse challenge between sites are complicated by several factors. These include differences in the vectorial capacities of different tsetse species and the possibility of mechanical trypanosome transmission by other types of biting flies.

Trypanosome infection in livestock

Across eight Network sites where studies were completed in 1986, trypanosome prevalence was similar in the two trypanotoler-

Country	Site	— Mean Tsetse Challenge — 1984	1985	1986	Overall
Zaire	Mushie	16.8	155.7	46.0	72.8
Ivory Coast	Boundiali	14.1	18.2	46.2	26.2
Ethiopia	Tolley	—	—	22.9	22.9
Kenya	Muhaka	30.4	10.2	6.8	15.8
Gabon	OGAPROV	7.5	11.8	5.9	8.4
Togo	Sokode	—	4.0	8.4	6.2
Ethiopia	Ghibe	—	—	3.9	3.9
Zaire	Idiofa	—	—	3.2	3.2
Ivory Coast	Tengrela	7.4	1.1	0.4	3.0
Togo	Avetonou	—	0.2	0.1	0.2
Zaire	Kolo	0.0	0.0	0.3	0.1

Figure 15. Mean tsetse challenge at 11 research sites in the African Trypanotolerant Livestock Network. The mean tsetse challenge is the product of relative tsetse density (number of tsetse/trap/day) and the trypanosome infection rate in the tsetse population.

ant cattle breeds (7.3 to 11.0%) and lower in these breeds than in the more susceptible breeds (25.9 to 30.6%), with crossbred cattle in an intermediate position (15.2 to 16.5%). Where different species of livestock were kept together, trypanosome prevalence tended to be similar in sheep and goats but higher in cattle.

Estimates of tsetse challenge are compared with the recorded prevalence of trypanosome infection and incidence of new infections in livestock at each Network site on a monthly basis. A general positive correlation was observed between annual averages for tsetse challenge and the prevalence of infection in trypanotolerant cattle at seven Network sites (Figure 16). A similar correlation was found between tsetse challenge and the prevalence of infection in trypanotolerant sheep. The methods for estimating tsetse challenge and detecting infection levels in livestock may not be sufficiently sensitive to allow correlations between monthly averages within sites except where major changes occur. A detailed analysis of results from the Boundiali and Tengrela areas in Ivory Coast revealed the importance of local factors, such as the extent of herd movements, the proximity of tsetse traps to herds, the frequency of recorded and unrecorded treatment with trypanocidal drugs, and the varying susceptibility of different breeds.

Trypanosome species are identified in infected livestock from direct examination of the buffy coat layer and from stained blood smears. Trypanosome infections in tsetse are designated as *vivax* type, *congolense* type or belonging to the *T brucei* complex. Limitations of available methods include some overestimation of *congolense*-type infections and underestimation of *T b brucei*. Over a 2-year period at seven research sites, *T congolense* was more prevalent in cattle, while *T vivax* was seen more frequently in sheep and goats.

Infection levels among cattle of different ages appeared to be related to management factors: prevalence of trypanosomes was similar when all age groups were kept under similar management. Trypanosome prevalence differed significantly in cattle from one year to the next, but clear seasonal differences were only observed at one site—Boundiali in Ivory Coast.

Levels of anaemia

Anaemia is one of the most important clinical signs of trypanosomiasis. Network staff measure PCV—a reliable indicator of anae-

mia—regularly in all cows and calves covered by the study. Regression analysis showed a consistent relationship between trypanosome infection and average PCV levels, with infection significantly linked to depressed PCVs. The extent of PCV depression was proportional to the duration of trypanosome infection, suggesting that the effect of continuous or repeated infections may, at least in part, be cumulative. Among cattle infected with *T congolense* or *T vivax*, there was no indication that one trypanosome species caused more severe anaemia than the other.

Reproductive performance and liveweights

Trypanosome infection was shown to depress female reproductive performance substantially at a research site in Gabon where breeding was restricted to a 3-month period. Some reduced fertility was also measured in infected cows at a site in Zaire where breeding took place throughout the year. Lower calving percentages could be attributed directly to infection and not to any associated loss of liveweight. There was a consistent association between good fertility and high PCV levels.

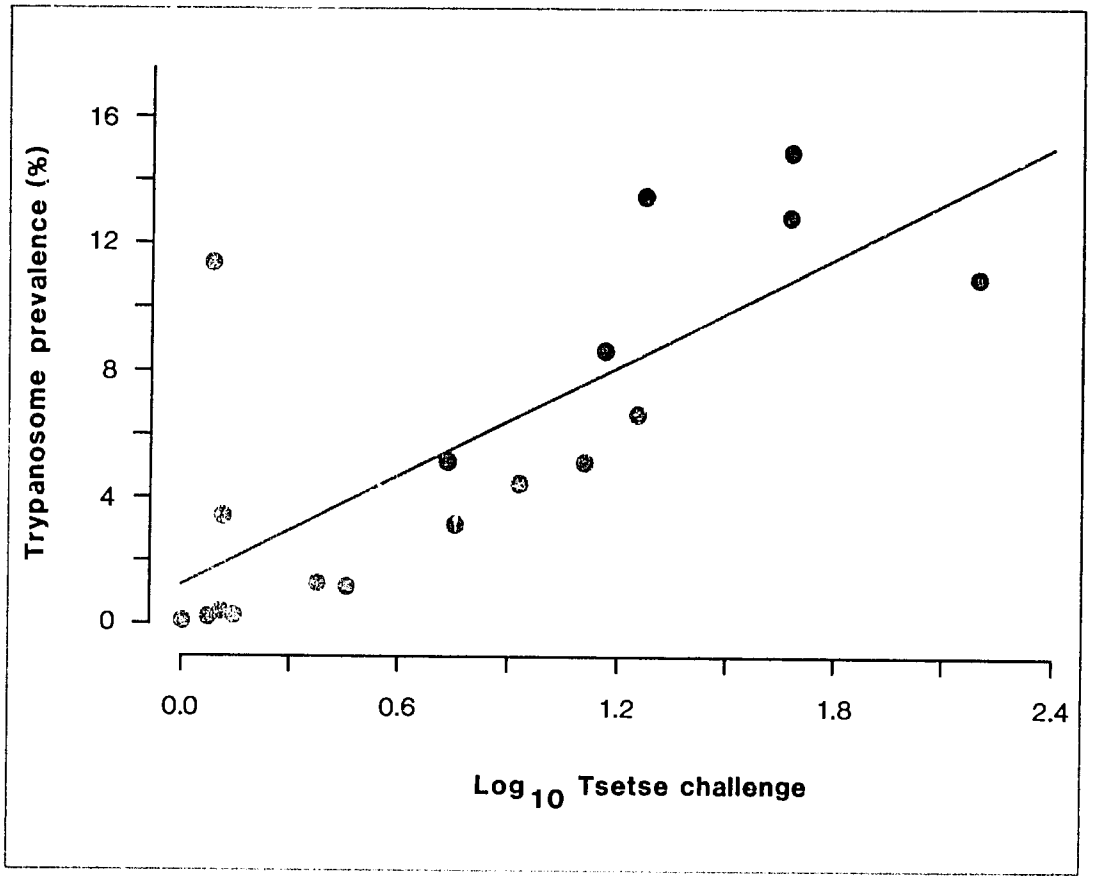

Figure 16. A comparison of tsetse challenge and the prevalence of trypanosome infection in trypanotolerant cattle at eight sites in the African Trypanotolerant Livestock Network, showing a positive correlation. Annual averages for 1984, 1985 and 1986 were used for Mushie and Kolo in Zaire and Boundiali and Tengrela in Ivory Coast. Annual averages were available for 1984 and 1985 only at the OGAPROV ranch in Gabon, for 1985 and 1986 at Avetonou in Togo, and for 1986 at Idiofa Plateau and Idiofa Forest in Zaire, giving a total of 18 situations for comparison.

Weight loss and stunting are generally reported among the major effects of trypanosomiasis in cattle. However, trypanosome infection did not have a significant effect on liveweight or liveweight changes among trypanotolerant cattle monitored at five of the Network sites.

Research on N'Dama productivity in The Gambia

In 1985, ILCA and ILRAD initiated a research project in The Gambia under the African Trypanotolerant Livestock Network with funding from the EEC. The objective is to study the productivity of trypanotolerant cattle under village conditions. The research team works in close collaboration with a tsetse research project funded by ODA and with a large-scale livestock development project funded by the ADB. All three projects will be based at the newly established International Trypanotolerance Centre.

A preliminary epidemiological survey was conducted at the end of 1985 and regular monitoring began in January 1986, covering a total of 1000 N'Dama cattle in two village locations. These are Gunjur, with low trypanosomiasis challenge transmitted by *G palpalis gambiensis*, and Keneba, which is moderately infested with *G morsitans submorsitans*. In June, the study was extended to cover 500 cattle in Pirang and Jattaba villages, where tsetse infestation is believed to be moderately high.

The ILCA/ILRAD team is investigating the health and productivity of the cattle included in the study by monitoring trypanosome prevalence, anaemia as indicated by PCV levels, productivity as indicated by calving rates, weight changes and milk production, the presence of anti-trypanosomal antibodies and the incidence of other infectious diseases. The ODA-funded team collects data on the distribution, density and trypanosome infection rates of tsetse in the four areas.

Preliminary results from the first 10 months of data collection in two areas are given in Figure 17. As would be anticipated from tsetse infestation rates, trypanosome infections are more prevalent at Keneba than at Gunjur. This coincides with lower PCV values and lower average body weights for all age groups. Young adult cattle, from 3 to 9 years old, showed the highest prevalence of trypanosome infection, while animals 10 years old and above had the lowest PCV values. Monthly variations in weight gains appeared to relate primarily to nutritional factors.

Milk production was calculated from a sample of 69 cows at the two sites over an 8-month period. Milk intake by calves was estimated from calf growth, based on the standard calculation of 9 kg of milk equivalent to 1 kg of calf growth, and this was added to measurements of milk extracted for human consumption. Total milk production was remarkably high in both villages, as shown in Figure 18. The average ratio of

Age Group (Average values)	Infection Rate (%)		PCV (%)		Body Weight (kg)	
	Gunjur	Keneba	Gunjur	Keneba	Gunjur	Keneba
Pre-weaners	0.4	3.0	26.3	25.4	58.8	55.5
Post-weaners	0.4	1.7	27.2	25.7	138.7	133.4
Young adults	2.0	5.1	29.2	26.6	237.4	218.3
Old adults	1.1	4 2	26.2	24.0	249.6	223.8

Figure 17. Average trypanosome infection rates, PCV levels and body weights among N'Dama cattle in four age groups at two sites in The Gambia—Gunjur with low tsetse density and Keneba with moderate tsetse density.

Milk and Calf Production (kg)	Gunjur	Keneba
Milk extracted for human consumption	272.4	260.3
Calf growth	39.9	27.7
Estimated milk consumed by calves	359.1	249.3
Total estimated milk production	631.5	509.6

Figure 18. Milk extracted for human consumption and estimated milk consumed by calves from N'Dama cows at Gunjur and Keneba villages in The Gambia. Milk and calf production data are averages from 69 cows monitored over an 8-month period in 1986.

milk extracted for humans to milk consumed by calves was 47:53.

Analysis of species-specific anti-trypanosomal antibodies in a sample of 1000 cattle from all four study areas revealed high antibody levels in most older animals, but very little evidence of species-specific anti-trypanosomal antibodies in animals under 5 years of age. Antibody levels varied at different sites, at different times of year and between different trypanosome species. Some animals with antibodies at one point during the study period showed no antibodies 6 months later.

TRAINING AND INFORMATION SERVICES

TRAINING

ILRAD's training activities include individualized training programs for scientists and technicians from laboratories and field programs in Africa and other developing areas; post-graduate training for students working towards master's and doctoral degrees; and post-doctoral positions to enhance the professional knowledge and experience of young scientists from many parts of the world. In addition to training for individuals, courses, conferences and workshops are held each year on topics closely related to ILRAD's research program. The emphasis in all these activities is to develop veterinary research and disease control capabilities in African countries where trypanosomiasis and ECF are important animal health problems. Training activities account for nearly 10% of ILRAD's total budget.

In 1986, 22 scientists and technicians came to ILRAD on study attachments for periods ranging from 1 week to 6 months. Their training programs were planned on an individual basis according to the needs of their home institutions. Nineteen came from African countries, as listed in Figure 19: two were sponsored by FAO, one by DSE/GTZ (Deutsche Stiftung für internationale Entwicklung/Gesellschaft für technische Zusammenarbeit) and the remainder by ILRAD. All were staff members of national universities, research centres or veterinary departments in their home countries. Three additional training participants were sponsored by universities in developed countries—two from Italy and one from the USA.

Some training program participants combined their work at ILRAD with training at other research centres in the Nairobi area, such as ICIPE, the Kenya Agricultural Research Institute and the Kenya Government's Veterinary Research Laboratory. This type of collaborative training activity is becoming more common among research centres in Kenya working on related disease-control problems.

Fifteen post-graduate students were working at ILRAD in 1986, on Research Fellowships lasting from 1 to 4 years (Figure 19). Three Visiting Research Fellows also worked at ILRAD for shorter periods. These students work closely with ILRAD scientists on projects related to the research program. The 14 Research Fellows from African countries all received support from ILRAD; 6 were also sponsored by their home governments, 1 by the Government of The Netherlands and 1 by WHO. Four Research Fellows from developed countries

Country	1985	1986	Country	1985	1986
Specialized technical training					
Burundi	2	2	Somalia	–	2
Burkina Faso	1	–	Tanzania	–	2
Central African Republic	–	1	Uganda	2	–
Ethiopia	1	2	Zambia	2	–
Kenya	2	5	Zimbabwe	2	2
Nigeria	2	1	Italy	–	2
Rwanda	–	2	USA	–	1
Post-graduate training					
Ghana	1	1	Belgium	1	–
Kenya	10	8	Italy	2	2
Nigeria	1	2	Germany (FR)	1	–
Sudan	–	1	Netherlands	1	1
Tchad	1	–	UK	1	1
Uganda	1	1	USA	1	–
Zaire	1	1			
Post-doctoral training					
Kenya	2	3	Germany (FR)	1	1
Rwanda	1	–	Ireland	–	1
Australia	–	1	Japan	1	–
Belgium	1	–	UK	1	2
France	1	1	USA	5	4

Figure 19. Individual training at ILRAD in 1985 and 1986: number of participants in different types of training by country of origin.

received outside support. In addition to Research Fellows working at ILRAD, one Kenyan staff member from the ILRAD/Veterinary Research Laboratory Wildlife Project was sponsored for post-graduate studies in the USA.

A Senior Research Fellowship program was initiated in 1985. Under this program, young scientists working at universities or research institutes, principally in Africa, come to ILRAD for several months to enhance their research experience. Three Senior Research Fellows worked at ILRAD in 1986, from Kenya, Uganda and Tanzania.

Post-Doctoral Fellows are selected on an international basis, though emphasis is placed on recruiting well qualified young scientists from African countries. They normally work at ILRAD for 2 years, and their projects are expected to contribute directly to ILRAD's research program. In 1986, 13 Post-Doctoral Fellows worked at ILRAD—4 from the USA, 3 from Kenya, 2 from the UK and 1 each from Australia, France, Ireland and Germany (FR).

In addition to training activities for individuals, five training courses were held in 1986. A 1-month course was conducted in April on 'The Diagnosis of Haemotropic Cattle Diseases, with Emphasis on East Coast Fever'. Seventeen participants came from Burundi, Ethiopia, Kenya, Mozambique, Rwanda, Somalia, Sudan, Tanzania, Uganda, Zambia and Zimbabwe. One was on the staff of OAU/IBAR and one worked for a UNDP/FAO project; the others were staff members with national universities, research centres or veterinary departments.

In April and May, a 2-month course was

conducted on 'Trypanosomiasis and other Parasitic Diseases' for English-speaking field personnel in the ILCA/ILRAD African Trypanotolerant Livestock Network. Five participants came from Ethiopia, Kenya, Nigeria and The Gambia. A second 2-month course was conducted in October and November, primarily for French-speaking Network staff. Four participants came from Network sites in Gabon, Ivory Coast and Zaire and two other participants came from the Central African Republic and Rwanda.

A 3-month course was held at ILRAD from September through November on the 'Preparation and Use of Reagents for the Diagnosis of Haemoprotozoan Cattle Diseases'. Nine participants attended from national veterinary laboratories and universities in Burundi, Kenya, Mexico, Mozambique, Nigeria, Senegal, Somalia, Rwanda and Uganda.

In November, The Animal Production and Health Section of IAEA and FAO sponsored a 1-month regional training course on the 'Use of Radio- and Enzyme Immunoassay Techniques for Studies on Animal Reproduction and Disease Diagnosis'. This course was conducted at the University of Nairobi, the Kenya Agricultural Research Institute and ILRAD, with financial administration through ILRAD's Training Department. Fifteen participants attended from veterinary research departments in Benin, Burkina Faso, Burundi, Ghana, Kenya, Mali, Nigeria, Somalia, Sudan, Tanzania and Zambia.

ILRAD scientists gave lectures during the year for two courses held at ICIPE on ticks and tsetse flies, and participants in these courses visited ILRAD. ILRAD also provided training material to the University of Nairobi, the Veterinary Research Laboratory in Australia and GTZ.

Laboratory technicians on the ILRAD staff are selected for 2- and 3-year training programs at the Kenya Polytechnic leading to Ordinary and Advanced Diplomas in Medical Laboratory Technology. Six ILRAD staff members were sponsored for training in 1986, and three were awarded the Advanced Diploma at the end of the year.

MEETINGS

An 'International Workshop on Genetic Exchange in Trypanosomes' was held at ILRAD in October, bringing together researchers from KETRI, the Veterinary Research Laboratory, ICIPE and ILRAD as well as 22 distinguished specialists from around the world. The possibility of genetic exchange in trypanosomes, by sexual or other processes, has profound implications for trypanosomiasis epidemiology, especially in terms of the development of antigenic diversity and the spread of drug resistance. Recent success at the Swiss Tropical Institute in obtaining hybrid trypanosomes has allowed intensive investigations in several laboratories. Most of the scholars working in the field came to this workshop to report and discuss their most recent findings. A summary proceedings document has been published by ILRAD's Information Services.

ILRAD staff members and visiting scientists give seminars throughout the year, presenting the results of ongoing research projects. Scientists are invited from other research institutions in the Nairobi area. In 1986, 37 seminars were presented at ILRAD, 13 on trypanosomiasis research, 10 on ECF research and 14 on related topics.

ILRAD also makes its conference facilities available for meetings and courses sponsored by other organizations. In 1986, these gatherings included training courses sponsored by ILCA and by the International Potato Centre (CIP), a poultry seminar sponsored by the US Department of Agriculture, meetings of the Kenya Veterinary Association, the Animal Production Society of Kenya and the Kenya Government's Ministry of Livestock Development, a scientific meeting of the Kenya Veterinary Association and the Nairobi Cluster and a

workshop sponsored by the Small Ruminant Collaborative Research Support Program.

INFORMATION SERVICES

In 1986, the Information Services Department published the annual report, *ILRAD 1985*, four issues of the quarterly newsletter, *ILRAD Reports*, and the weekly *ILRAD Internal Newsletter*. The annual report and quarterly newsletter were published in English and French and the internal newsletter in English and Kiswahili.

Papers originally presented at an international conference held at ILRAD were published in 1986 by Cambridge University Press. This 570-page volume, *The Ruminant Immune System in Health and Disease*, reviews the immune system of domestic ruminants, with particular emphasis on mechanisms of immunity and resistance to infectious diseases. The book was edited by W.I. Morrison, a senior scientist at ILRAD, with support from the Information Services Department.

ILRAD published a second proceedings volume—*Immunization against East Coast fever: report of a workshop on collection, handling and analysis of performance and productivity data*—containing papers and discussions presented at an international workshop held in September 1985. An updated brochure was also published in 1986 listing publications by ILRAD staff which appeared in 1984 and 1985, plus a partial listing for 1986. In addition, the Information Services Department provided editorial and production support for the publication of ILRAD's annual program and budget document and the External Program and Management Reviews.

ILRAD's first audio-visual production was completed in 1986. This is a 20-minute slide-tape presentation describing ILRAD's establishment, development and research and training activities. It is now shown to many of ILRAD's visitors. The Government of Japan presented ILRAD with a video system in 1986, including cameras, recorders, microscopic filming equipment, editing facilities, players and monitors. Short video tapes have been produced on *in vitro* cultivation of trypanosomes, embryo transfer techniques in cattle and the pathology of heartwater.

The four issues of *ILRAD Reports* published in 1986 included major articles on trypanosomiasis epidemiology studies at the Kenya coast, ECF immunization trials, research on the role of cellular immunity in ECF and a summary of ILRAD's second External Program and Management Reviews. For the second time, ILRAD's quarterly newsletter received an award for excellence from Agricultural Communicators in Education, a professional editors' association in the USA.

ILRAD staff members published 39 articles in international journals in 1986, plus 23 chapters and 6 full-length reports or books. These scholarly publications, together with numerous papers presented at international conferences, comprise the bulk of ILRAD's information output for specialized scientific audiences. The Information Services Department directs considerable effort towards ensuring the distribution of these publications to researchers and field staff in developing countries who may not have access to a wide range of scientific literature. The agreement with Cambridge University Press to publish *The Ruminant Immune System in Health and Disease* also included the sale of copies to ILRAD for free distribution to scientists in developing countries.

The annual report and quarterly newsletter are intended for less specialized readers, with emphasis on animal health workers in Africa and the staff of donor organizations which support ILRAD's research and training programs. *ILRAD Reports* and occasional short press releases are also distributed to journalists in many parts of the world. A major effort to verify and update the distribution list for these publications was completed in 1986 (Figure 20).

Region	Countries 1985	Countries 1986	Institutions/Individuals 1985	Institutions/Individuals 1986
Anglophone Africa	24	25	851	745
Francophone Africa	24	23	378	268
Anglophone & other Europe	20	19	286	235
Francophone Europe	2	2	42	41
North & South America	27	25	317	277
Near & Far East	34	31	248	233
Total	131	125	2122	1799

Figure 20. Comparison of *ILRAD Reports* distribution at the end of 1986 and the end of 1985.

This has resulted in the more accurate identification of the appropriate audience for ILRAD's publications and considerable reduction in production and distribution costs. Virtually 100% of those on the mailing list are in active communication with the Information Services Department.

THE ILRAD LIBRARY

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

The specialized collection concentrates on topics related to ILRAD's research program. Emphasis is placed on scientific journals. Subscriptions to 210 journals cover fields such as parasitology, immunology, biochemistry, entomology, cell biology and veterinary medicine, including several indexing and abstracting journals. Journal subscriptions are reviewed every year according to the recommendations of the scientific staff. The collection of scientific reference books is steadily expanding, with 200 new books acquired in 1986.

The ILRAD library participates in an interlibrary loan network along with the University of Nairobi, the Kenya Government's Veterinary Research Laboratory, the Veterinary Research Department of the Kenya Agricultural Research Institute, the Ministry of Health and ICIPE. In 1986, ILRAD staff members borrowed 600 items from these other libraries and the ILRAD library loaned out 100 items through the network. The ILRAD library donated over 200 books and 30 journal volumes to other libraries in the Nairobi area.

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

The library orders copies of scientific articles which are not available in Kenya as requested by ILRAD staff members. About 200 articles were acquired in 1986, mainly from the British Library Lending Division.

Reprints of scientific articles by ILRAD staff are supplied on request to researchers

and field workers throughout the world. In 1986, 600 reprints were distributed free of charge, primarily to specialists in developing countries.

In September 1986, the library acquired an IBM PC/AT computer and a software package, INMAGIC, suitable for computerizing the library's catalog and acquisition system. By the end of the year, over 1000 items had been entered into the database. A complete computerized catalog should be available in mid-1987 and other library activities are also gradually being computerized. The ILRAD library has supplied information and advice on library computerization to several libraries in Kenya and one in Malawi.

RESEARCH SUPPORT

TSETSE LABORATORY

Seven tsetse breeding colonies were maintained at ILRAD in 1986. These were *Glossina morsitans centralis* originating from mainland Tanzania, *G austeni* from Zanzibar, *G palpalis palpalis* from Nigeria, *G p gambiensis* from Burkina Faso, *G fuscipes fuscipes* from the Central African Republic, *G tachinoides* from Tchad and *G brevipalpis* from Kenya. These represent the three taxonomic groups of tsetse—*morsitans*, *palpalis* and *fusca*.

Although it is possible to maintain trypanosomes *in vitro* and to infect laboratory animals and livestock by injection, the parasites normally pass through several stages of their lifecycle in tsetse. Trypanosomes maintained and transmitted artificially are different in some respects from parasites transmitted through the bite of an infected tsetse. For this reason, a great deal of the trypanosomiasis research conducted at ILRAD uses trypanosomes which have developed in tsetse flies. Work on parasite development and disease transmission is conducted in close collaboration with staff of the Tsetse Laboratory.

All breeding colonies are maintained at 25°C and fed 5 days a week on the ears of half-lop rabbits. Tsetse in the *morsitans* group are kept at 70% relative humidity, and the others at 85%. Overall breeding performance in 1986 is summarized in Figure 21.

The breeding colonies provided all the tsetse required for research work at ILRAD in 1986, as well as tsetse used during the two training courses conducted under the ILCA/ILRAD African Trypanotolerant Livestock Network. In addition, tsetse puparia and adults were supplied to colleagues at the University of Nairobi, KETRI, the Swiss Tropical Institute, the Prince Leopold Institute of Tropical Medicine (Belgium), the Institute of Tropical Medicine (Japan), the University of Alberta (Canada) and IAEA.

TICK LABORATORY

The ECF research program at ILRAD requires large numbers of the infective sporozoite forms of *T parva*. At present, *Theileria* sporozoites can only be obtained in sufficient quantities by continuous passage of the parasites between tick vectors and mammalian hosts. In ILRAD's Tick Laboratory, ticks are infected with *Theileria* parasites by feeding on infected cattle. They are then dissected and the sporozoites are isolated from their salivary glands. The Tick Laboratory provides sporozoites, ticks and

Tsetse Species	Breeding Females (mean)	Daily Female Mortality (mean %)	Weekly Pupae per Female (mean)	Puparial Weight (mean mg)	Annual Number Puparia
G m centralis	10,348	0.49	0.61	32.70	329,295
G austeni	1,151	0.39	0.64	26.50	38,198
G p palpalis	1,122	0.82	0.53	31.33	30,844
G p gambiensis	2,033	0 69	0.70	29.37	73,953
G f fuscipes	2,157	0.40	0.45	35.87	50,502
G tachinoides	1,201	0.71	0.63	19.88	39,433
G brevipalpis	2,363	0.95	0.53	73.68	65,370

Figure 21. Performance of seven tsetse breeding colonies maintained at ILRAD in 1986.

tick tissues for a variety of research projects.

Colonies of infected and uninfected ixodid (hard) ticks are maintained by feeding on cattle and rabbits. The most important species is *Rhipicephalus appendiculatus*, the principal vector of *T p parva* and *T p lawrencei*. Colonies of several *R appendiculatus* laboratory strains are maintained at ILRAD, as well as field isolates from different locations in East Africa. A number of other tick species are maintained in smaller colonies. These include *Amblyomma variegatum* and *A gemma*, vectors of *Cowdria ruminantium* (heartwater) and of two relatively benign *Theileria* species—*T mutans* and *T velifera; Boophilus decoloratus* and *B microplus*, which transmit *Babesia* spp and *Anaplasma marginale*; and two other *Rhipicephalus* species, *R evertsi evertsi* and *R pulchellus*.

Continuing studies are conducted to determine the factors which affect *Theileria* development in *R appendiculatus* ticks with the aim of ensuring uniformly high infection rates. An important advance has been the use of synthetic corticosteroids to supress the immune reactions of the infected cattle used for tick feeding. However, significant, unexplained differences persist in the infection rates of ticks fed on different individual animals. These may relate to variability in infectivity among *Theileria* stocks or differences in susceptibility among populations of ticks. Tick populations which appear to produce the highest infection rates are currently being selected in an attempt to produce tick stocks which are particularly susceptible to the *Theileria* strains used at ILRAD.

Studies also concentrate on developing methods to feed ticks artificially. Artificial feeding, by improving the control of variables, would allow more precise studies on tick development and parasite transmission and would reduce requirements for experimental animals.

To be successful, an artificial feeding system must attract ticks to probe a membrane and must provide an acceptable diet so that they remain attached until engorgement. However, ixodid ticks are highly selective feeders. Several species have now been fed successfully through a membrane of rabbit skin but they have rejected all artificial membranes tested so far, though various attractants are still being tested. Diets which have been tested consisted of defibrinated blood from different mammalian species and whole blood with various anticoagulants. In some cases, blood could be replaced by simple media for up to 2 days. Some ticks required the addition of an appetite stimulant, adenosine triphosphate.

Further trials involve the uptake of parasites, radioactive markers and other substances through artificial feeding systems. In one experiment, *A variegatum* nymphs were fed artificially on the blood of a cow infected with heartwater and successfully

transmitted the parasites to a susceptible calf. Similar experiments are being conducted to test the transmission of *Theileria* parasites by *R appendiculatus* ticks. Artificial feeding is also being used to investigate humoral immune responses to feeding ticks.

LARGE ANIMAL PRODUCTION

The livestock facilities at ILRAD were filled to capacity in 1986, with 557 cattle and 385 goats allocated for experimental purposes. Overall, 55% of the cattle were used for ECF research, 30% for trypanosomiasis research, 10% for bovine MHC characterization and 5% for other projects, such as antigen production. Most of the goats were used for trypanosomiasis research. The ILRAD ranch, Kapiti Plains Estate Ltd, supplied nearly all the cattle required, while goats were purchased from outside farms.

Cattle production has improved steadily at Kapiti since the ranch was purchased at the end of 1981. In 1986, 1,112 calves were born from a breeding herd of 1,194 cows and heifers, giving a calving rate of 93%. Increased production has resulted primarily from completion of a paddocking system which has improved the control of grazing and stock movement. Figure 22 shows how the gradual construction of fencing has allowed steady increases in stocking and calf production over the past 5 years.

In 1986, the Kapiti ranch supplied 337 Boran calves, 52 *Bos taurus* calves, 16 steers and 8 heifers for research at ILRAD and sold 626 cattle to other users. Twenty-five Boran cows from the breeding herd were accepted as foundation stock for registration in the Kenya Stud Book. Development of the ranch during the year included the construction of a fodder bank, pump house, additional water reservoir and staff housing.

Over 100 of the Boran calves born at Kapiti in 1986 were sired by bulls of selected MHC types, and 51 calves were produced by embryo transfer using selected, MHC-typed cows and bulls. The embryo transfer program is being steadily expanded with the testing and improvement of techniques for handling and manipulating embryos and synchronizing the ovulation of donor and recipient cows. Present plans include the production of N'Dama calves by embryo transfer: the five N'Dama heifers born at ILRAD in 1984 were mated in November 1986, and eight N'Dama embryos have been recovered and surgically transferred into Boran recipients.

A brief outbreak of lumpy skin disease occurred on the Kabete farm, but was quickly controlled. An outbreak of foot-and-mouth disease near the Kapiti ranch interrupted the supply of calves to ILRAD for a short period.

LABORATORY ANIMAL PRODUCTION

The Laboratory Animal Unit provides a regular supply of rats, mice and rabbits for experimental work at ILRAD and maintains

Year	Total Stock at Year End	Births	Offtake
1982	2,365	707	692
1983	2,406	858	759
1984	2,563	999	781
1985	2,813	1,029	715
1986	2,818	1,112	1,039

Figure 22. Cattle production at Kapiti Plains Estate, 1982-86.

small colonies of meadow voles (*Microtus montanus*) and guinea pigs, which are occasionally required for research projects. All laboratory animals are housed and maintained according to international animal welfare conventions.

All the rats used at ILRAD are random-bred, originating from the Sprague Dawley strain. They are used primarily for the production of trypanosomes. In an effort to match supply and demand more efficiently, rat breeding colonies were reduced by 25% in 1986 while the number of rats supplied to scientists remained the same.

ILRAD researchers use mice primarily to study the mechanisms of host resistance to trypanosomiasis, as different mouse strains vary in their susceptibility to trypanosome infection. Mice are also used for the production of monoclonal antibodies. Random-bred Swiss mice are used increasingly because they are more productive and faster growing than inbred strains. Colonies of three inbred strains—BALB/c, C3H/He and C57/Bl6—were maintained during the year to meet specific research requirements, along with C3H/He x C57Bl/6 and BALB/c x Swiss F_1 crosses. The C3H/He x C57Bl/6 F_1 cross is not currently used at ILRAD, but the BALB/c x Swiss F_1 cross is widely used for the production of ascites for monoclonal antibodies.

An isolation system for laboratory animals is now fully functioning, and isolator-reared pedigree pairs have been imported for the three inbred mouse strains used at ILRAD. All primary mouse colonies and pedigree pairs are maintained in isolators, and all other imported mice are also housed in isolators to prevent the risk of contamination to or from the main breeding colonies.

Rabbits are used primarily to produce antisera and to support the tsetse and tick colonies. ILRAD's rabbit colony has increased slowly through the years and rabbits have had to be purchased from outside breeders, with the associated risk of introducing disease. Previous high mortality rates were attributed partly to inadequate ventilation, and now most rabbits are maintained after weaning in outdoor pens. For the first time at the end of 1986, the rabbit breeding unit was able to produce all the animals required for the research program.

In 1986, WHO gave ILRAD a small foundation colony of Cotton rats (*Sigmodon hispidus*). It has been suggested that Cotton rats may possess natural antibody against *T vivax*, and this possibility is now being investigated at ILRAD.

Figure 23 shows the total number of rats, mice and rabbits produced and used in 1986. The demand for mice decreased by 9% from the 1985 level while the demand for rats and rabbits remained the same.

In addition to supplying nearly all the lab-

Species and Strain	Number Weaned	Number Supplied to Scientists
Rats	16,141	13,075
Mice		
random-bred Swiss	29,608	20,967
inbred BALB/c	18,189	10,751
inbred C3H/He	7,766	4,685
inbred C57Bl/6	5,729	2,412
BALB/c x Swiss F_1	6,835	4,760
Rabbits	820	1,301

Figure 23. Rats, mice and rabbits produced and supplied to ILRAD scientists in 1986.

oratory animals used at ILRAD, the breeding unit provides mice and rats to several other organizations. In 1986, these included ICIPE, KETRI, the Kenya Medical Research Institute, the Institute for Primate Research, the Biochemistry Department of the University of Nairobi, the Kenya Government's Veterinary Research Laboratory, the National Museums of Kenya, the Kenya Society for the Protection and Care of Animals, several Kenyan secondary schools and the National Veterinary Laboratory in Kigali, Rwanda.

CLINICAL AND DIAGNOSTIC SERVICES

The Diagnostic Laboratory carries out several types of routine analysis in support of the research program and animal production facilities. In 1986, a total of 37,008 samples were analysed. These included 26,362 s[illegible]ological analyses, 4,851 bacteriological analyses, 2,986 haematological analyses and 2,809 helminthological analyses.

Serum samples from farm animals are screened for antibodies to seven bloodstream parasites—*Theileria parva, T mutans, Trypanosoma brucei brucei, T congolense, T vivax, Anaplasma marginale* and *Babesia bigemina*. Most routine screening for *Theileria* and *Trypanosoma* species was by the indirect fluorescent antibody (IFA) test. The indirect enzyme-linked immunosorbent assay (ELISA) was also used to screen bovine sera for antibodies to [illegible] *B bigemina* and the t[illegible] species. Major achievements in 1986 were the development of the indirect ELISA for screening antibodies to *T mutans* and the introduction of a dot ELISA (ELIDOT) for the diagnosis of bovine trypanosomiasis.

In addition to services provided to ILRAD scientists, the Diagnostic Laboratory continued to provide assistance to other institutes and projects. Materials such as antigens, conjugates, lymphocyte lysates and control sera were provided to the International Trypanotolerance Centre in The Gambia, a GTZ project in Burundi, an FAO project in Zimbabwe and the University of Ibadan, Nigeria. Twenty serum samples were screened for antibodies to *T parva* for the FAO project in Zimbabwe and another 250 samples were screened for an FAO tick-control project in Kenya.

Staff of the Diagnostic Laboratory helped conduct the 3-month training course on the 'Preparation and Use of Reagents for the Diagnosis of Haemoprotozoan Cattle Diseases', with participants from nine developing countries. The Diagnostic Laboratory also contributed to the regional training course on the 'Use of Radio- and Enzyme Immuno-assay Techniques for Studies on Animal Reproduction and Disease Diagnosis'. In addition, six veterinarians from developing countries received training on an individual basis.

BIOSTATISTICS AND COMPUTING SERVICES

ILRAD's Biostatistics and Computing Sciences Unit, in collaboration with the University of Strathclyde (UK), advises research staff on statistical problems and installation and use of microcomputers. Major projects in 1986 included statistical support for an ECF field trial in Kenya's Coast Province, for trypanosomiasis experiments in N'Dama and Boran cattle, for tsetse surveys carried out in West Africa under the African Trypanotolerant Livestock Network and for isoenzyme analysis of different trypanosome species. Several databases have been created at ILRAD—for instance, on bovine MHC types, on tick production and infection rates and on all trypanosome and tick-borne disease stabilates available at ILRAD. Programs have been written for the analysis of MHC typing data and are now being written for the analysis of data generated by ILRAD's fluorescence-activated cell sorter (FACS).

An important development in 1986 was

the establishment of an improved telecommunications system. The installation of a new switchboard has made it possible to create links among the computers within ILRAD and, by utilizing external modems, has put ILRAD in touch with international databases as well as the CGNET of the CGIAR. Cataloging and ordering operations are also being computerized in the ILRAD Library.

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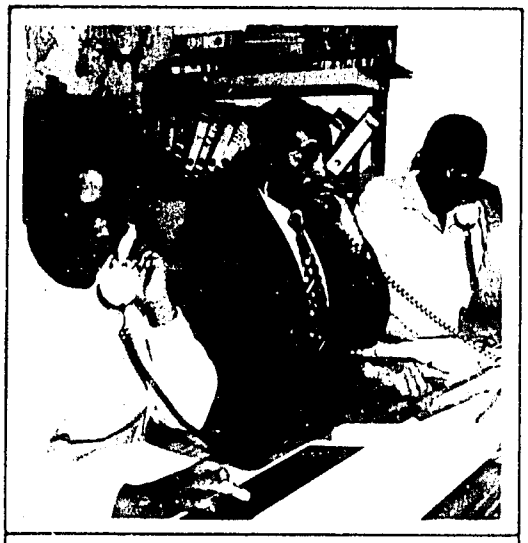

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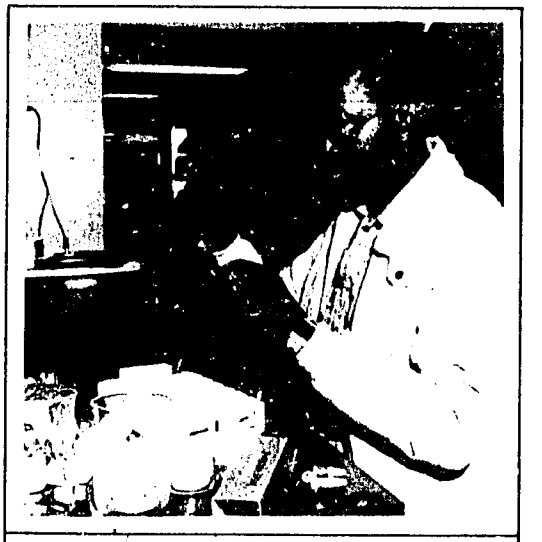

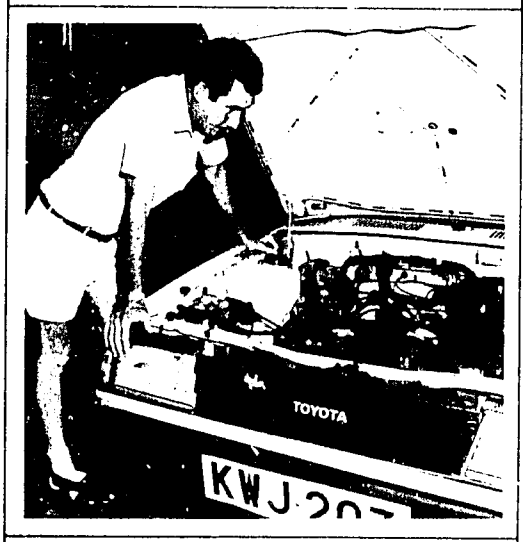

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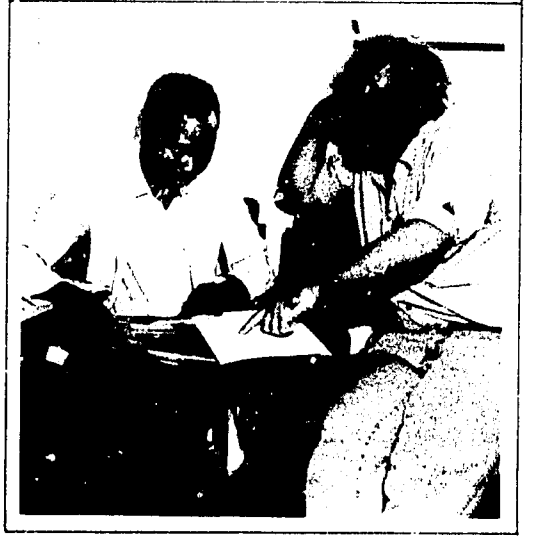

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Price Waterhouse

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

We have reviewed the abridged financial statements set out in Figures 24 to 27 which contain information extracted from the accounting records of ILRAD for the years ended 31 December 1985 and 1986.

We confirm that the information set out in the abridged financial statements is consistent with that contained in the audited financial statements for the years ended 31 December 1986 and 1985, on which we expressed an unqualified opinion.

Price Waterhouse

PRICE WATERHOUSE
Certified Public Accountants

20 March 1987

	1986	1985
Research		
Parasitology—Trypanosomiasis	484	432
Biochemistry	640	656
Cell Biology	490	466
Immunobiology	484	357
Parasitology—Theileriosis	563	516
Pathology	605	605
Tsetse Laboratory	314	297
Tick Laboratory	150	124
Electron Microscopy	131	148
Socio-economic	33	—
Total Research	3894	3601
Research Support		
Office of Director of Research	550	416
Farm Animal Production	597	614
Laboratory Animal Production	209	148
Clinical and Diagnostic Services	88	72
Radioisotope and Central Core Services	388	341
Total Research Support	1832	1591
Training and Conferences	793	752
Library and Information Services	394	369
Administration		
Board of Directors	86	83
Office of the Director General	373	394
Finance	342	332
Personnel	80	74
Purchasing	343	302
Total Administration	1224	1185
General Operations		
Engineering	661	660
Transport	190	214
Services	237	228
Food and Housing	58	5
Stores	53	48
Total General Operations	1199	1155
External Program Review	—	125
Total Core Costs	9336	8778

Figure 24. Summary costs by program and activity (US$'000).

	1986	1985
Unrestricted Funds		
United States Agency for International Development (USAID)	2525	2490
World Bank (IBRD)	1400	1200
United Kingdom	794	546
Canadian International Development Agency (CIDA)	730	686
Germany (Federal Republic)	510	528
Switzerland	474	415
Japan	351	201
Sweden	290	237
Netherlands	280	249
Belgium	255	135
Norway	250	234
Australia	230	281
African Development Bank	190	—
Italy	170	164
France	90	61
Denmark	60	47
Total Unrestricted Funds from Donors	8599	7474
Restricted Funds		
United Nations Development Program (UNDP)	766	690
Italy	500	500
Japan	150	—
Belgium	95	110
Rockefeller Foundation	33	—
Total Restricted Funds from Donors	1544	1300
Total Unrestricted/Restricted Funds from Donors	10143	8774

Figure 25. Summary of core operating funds from donors (US$'000).

	1986	1985
SOURCES		
Core Operating Funds		
Unrestricted		
Unrestricted Funds from Donors	8599	7474
Earned Income Applied in Year	583	289
Total Unrestricted Operating Funds	9182	7763
Total Restricted Operating Funds	1544	1300
Total Unrestricted/Restricted Operating Funds	10726	9063
Transfer to Capital Funds	(954)	(285)
Net Unrestricted/Restricted Operating Funds	9772	8778
Capital Funds		
Transferred from Core Operating Funds	954	285
Unexpended Balance from Previous Year	278	419
Balance of Working Funds	823	778
Balance of Revolving Fund from Previous Year	100	100
Total Capital Funds	2155	1582
Special Projects		
Wildlife	156	167
Trypanotolerance	296	137
Total Special Projects	452	304
TOTAL SOURCES	12379	10664
APPLICATIONS		
Core Operations	9336	8778
Capital	954	426
Special Projects	452	304
Unexpended Balance		
Unrestricted Core	714	278
Working Funds	823	778
Revolving Fund	100	100
TOTAL FUNDS	12379	10664

Figure 26. Summary of sources and application of funds (US'000).

	1986	1985
ASSETS		
Fixed Assets		
Land and Buildings	10671	10125
Research Equipment	5415	5040
Other Assets	1869	1943
Subsidiary Company		
Investment	1786	1786
Longterm Loan	10	—
Total Fixed Assets	19751	18894
Revolving Fund		
Loans	51	93
Cash	49	7
Total Revolving Fund	100	100
Net Current Assets	1537	1056
TOTAL ASSETS EMPLOYED	21388	20050
FUND BALANCES		
Capital Fund	19751	18894
Working Capital	823	778
Unrestricted Core Surplus	714	278
Revolving Fund	100	100
TOTAL FUNDS	21388	20050

Figure 27. Balance sheet as at 31 December 1986.