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ANNUAL REPORT OF THE
INTERNATIONAL LABORATORY FOR RESEARCH
ON ANIMAL DISEASES

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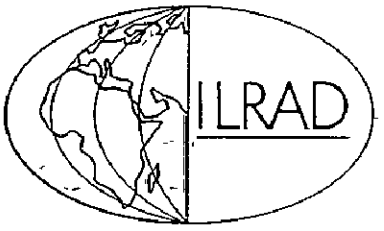

P O BOX 30709 NAIROBI, KENYA

FOREWORD

As ILRAD's incoming Director General, I have been given the unusual opportunity to write the foreword to a report recording the results of a year's scientific endeavour for which I can claim virtually no involvement, credit or responsibility. It is thus a privilege to introduce ILRAD's fifth annual report, which describes the activities and achievements of the laboratory in 1981.

The International Laboratory for Research on Animal Diseases—ILRAD—was established in 1973 in Nairobi, Kenya, with a mandate to develop effective control measures for livestock diseases which seriously limit world food production. ILRAD was initiated under the auspices of the Consultative Group on International Agricultural Research (CGIAR), an association of private foundations, national governments and regional and international organizations which supports a network of 13 agricultural research centres and programs around the world. The CGIAR is sponsored in turn by the Food and Agriculture Organization of the United Nations (FAO), the World Bank (International Bank for Reconstruction and Development—IBRD), and the United Nations Development Program (UNDP). ILRAD is one of two centres in the CGIAR network which focus on livestock production and the only centre with a mandate largely for basic scientific research.

ILRAD's research and support facilities have been developed on a 70 hectare site donated by the Kenya Government on the outskirts of Nairobi. The laboratory was inaugurated in April 1978 by President Daniel arap Moi, then Vice-President of Kenya. Two livestock diseases of major economic importance were chosen as the original focus of ILRAD's research program—trypanosomiasis and East Coast fever (ECF), a form of theileriosis. During 1981, more than 35 scientists and 300 supporting staff were engaged in the pursuit of knowledge which may lead ultimately to the control of these two diseases which affect the lives and welfare of millions of people and their domestic livestock in extensive areas of the world.

It will be apparent from the account in the following pages that substantial results were obtained in work on trypanosomiasis and East Coast fever in the past year. Exciting progress has been made, particularly in the case of ECF, but much more work will be required on both diseases before substantially improved control measures become available. Towards the end of the year, an internal review of ILRAD's programs was initiated with the objective of formulating a research plan encompassing activities during the next 5

years. This must take into consideration recommendations on research and training priorities put forward in a Quinquennial Review of ILRAD's activities carried out in 1980 by a Review Panel on behalf of the CGIAR's Technical Advisory Committee.

ILRAD's internal review is expected to continue into 1982, but it is already evident that in the coming years research on the ECF complex will emphasize at least three areas—the epidemiological aspects of the disease, the use of *Theileria* sporozoite antigens as immunogens, and the development of immunization procedures based on *Theileria* schizont antigens. Research on trypanosomiasis will include greater emphasis on the diseases caused by *Trypanosoma vivax* and *T congolense*, as well as basic work on the immune responses of domestic livestock and the mechanisms of parasitaemia control. New and encouraging findings in these areas, reported in this and previous annual reports, will be consolidated and developed. Comparative aspects of immunity in trypanotolerant livestock breeds and in wildlife will also be examined for factors which might be exploited for trypanosomiasis control, and a program component on the epidemiology of trypanosomiasis will be put into effect.

In addition to ILRAD's primary research programs, collaborative research projects are being conducted with other organizations in Kenya and elsewhere, and a vigorous research and technical training program encompasses participants from many developing countries. A major international conference was held at ILRAD in 1981 on Advances in the Control of Theileriosis, attended by 185 scientists from around the world. ILRAD has also played, and will continue to play, a major role in human and livestock health programs organized through the World Health Organization (WHO), FAO and the Organization of African Unity (OAU).

The scientists and other staff members at ILRAD work under the leadership of an international Board of Directors. The introduction to the 1980 annual report noted the retirement of five Board members who had served with distinction since ILRAD was founded in 1973. In 1981, ILRAD was particularly sorry to lose Sir Alexander Robertson as Chairman of the Board of Directors, but was pleased to welcome Dr K F Wells from Canada who has now assumed this responsibility.

Two Acting Directors General shared the tasks of managing the activities and development of ILRAD during much of

1981. Everyone at ILRAD is grateful to Dr K F Wells, Chairman of the Board of Directors, and Prof Dr D Zwart, Chairman of the Program Committee, who, in turn, guided the laboratory during the first 10 months of the year. Their leadership and concern for research progress and staff welfare have been appreciated by all concerned.

ILRAD was honoured to receive several distinguished visitors in the course of the year. Mr A W Clausen, President of the World Bank, came to the centre during a brief visit to Kenya in November. Other important visitors included Sir Dawada Jawara, President of the Republic of The Gambia, Dr Mukasa Mango, former Kenya Minister for Livestock Development, Sir John Crawford, Chancellor of the National University of Australia, and Their Excellencies the Ambassador of the Netherlands and the High Commissioners of Australia, Canada and Great Britain.

ILRAD is once again indebted to the national and international funding agencies which provide the financial support necessary to carry out the centre's research and training activities. These donors are the World Bank, UNDP, the Rockefeller Foundation and the governments of Australia, Belgium, Canada, the Federal Republic of Germany, Ireland, the Netherlands, Norway, Sweden, Switzerland, the United Kingdom and the United States of America. In addition, it is a pleasure to note the continuing warm support of the Kenya Government, whose citizens and officials have offered immeasurable assistance and friendship through the years.

The sections which follow contain an abbreviated account of ILRAD's research in 1981. Readers who would like a more detailed description of ILRAD's scientific work are referred to the list of publications near the end of this report.

A R Gray
Director General
ILRAD

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THEILERIOSIS

A number of theilerial diseases affect cattle, sheep and goats in Africa, the Middle East and Asia. The distribution of the three major parasite species which affect cattle is shown in Figure 1. Research at ILRAD concentrates on East Coast fever, a form of theileriosis which affects large numbers of cattle in Kenya, Uganda, Tanzania, Rwanda, Burundi, Zaire, Malawi, Mozambique and Zambia, over the area shown in Figure 2. ECF is caused by the protozoan parasite *Theileria parva*, which is transmitted by the brown ear tick, *Rhipicephalus appendiculatus*. Two subspecies of the parasite occur in Kenya, *T parva parva* maintained in cattle and *T p lawrencei* maintained in buffalo. Two related species, *T taurotragi* and *T mutans*, cause mild infections in cattle, but *T p parva* infections can lead to mortality rates approaching 100% in fully susceptible animals.

At present, ECF is controlled by dipping or spraying cattle to kill the tick vector. In areas of high infestation, animals must be dipped or sprayed at least twice a week. Drugs to cure ECF are being developed by commercial firms, but neither frequent treatment with acaricides nor regular drug treatment is feasible for many African livestock producers, and tick resistance to currently available acaricides is potentially a serious problem. However, the prospects for developing a vaccine appear promising, since cattle which recover from natural or experimentally-induced infections show long-lasting immunity to the same or similar *Theileria* strains, even when the initial infection is mild.

The complex life cycle of the *Theileria* parasite in the tick vector and the bovine host is depicted in Figure 3. Ticks feed on cattle three times during their life cycle—as larvae, as nymphs and as adults. Transmission of ECF is thought to occur most commonly when ticks feed on infected cattle as nymphs and then on disease-free animals as adults. ECF sporozoites which develop in the salivary glands of infected ticks are transmitted to cattle when the ticks feed. These sporozoites invade bovine lymphocyte cells where they develop into macroschizonts which multiply rapidly. The infected lymphocytes are transformed into enlarged lymphoblast cells, and many of these are induced to multiply synchronously with the parasites. The number of parasitized cells increases rapidly; large-scale destruction of lymphoid cells occurs, and often death of the animal.

During the infection, some of the macroschizonts change to microschizonts. These give rise to micromerozoites which are released and infect red blood cells.

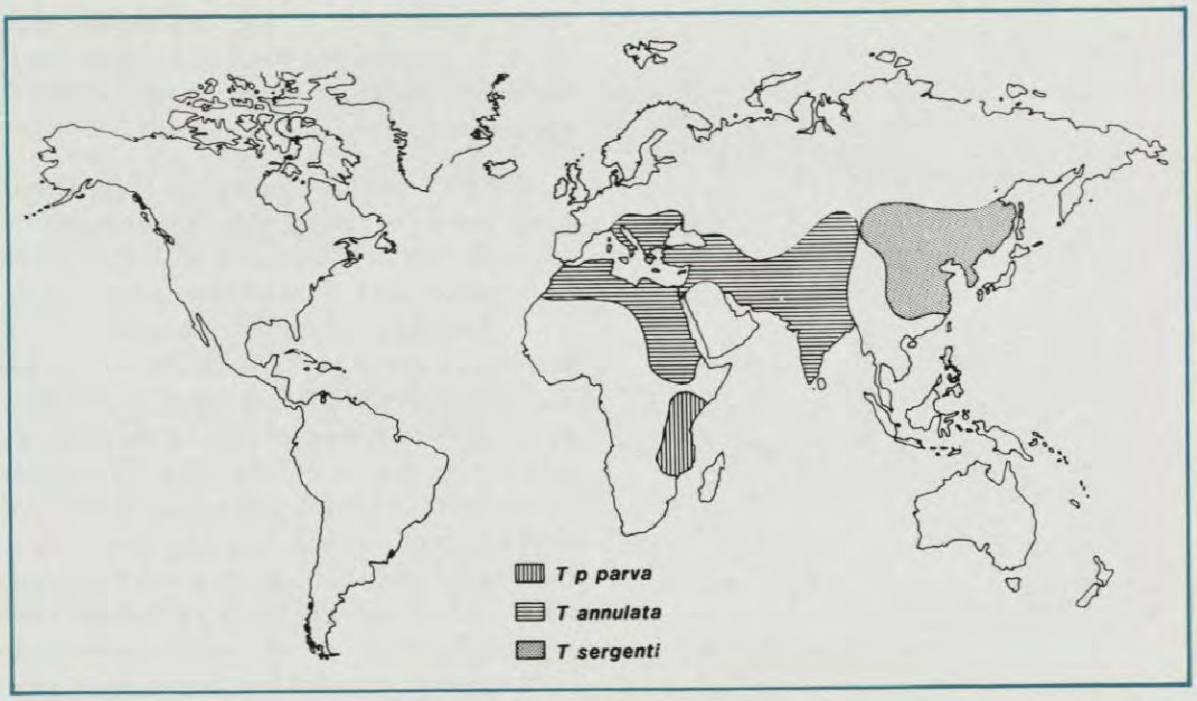


Figure 1. World distribution of Theileria *parasites.*

Transmission of the infection back to ticks occurs when the ticks ingest the infected red blood cells.

Scientists at ILRAD are investigating different stages of ECF, the immune responses of infected cattle and the ways in which the parasites evade host defense mechanisms. The aim is to identify phases in the disease cycle which are most vulnerable to immunological control. Among these stages, both the sporozoite and the macroschizont forms show promise as starting material for the development of a vaccine. Sporozoites are harvested from infected ticks and macroschizont-infected cells can be grown in culture as continuous cell lines.

ILRAD scientists are also focusing on the epidemiology of ECF in a well-defined field situation in the coastal region of Kenya, in cooperation with the Ministry of Livestock Development. Strains of the parasite are being isolated and identified for potential use in pilot immunization trials, which could eventually extend to immunization programs in Kenya and elsewhere. In collaboration with the Kenya Veterinary Research Laboratory, scientists are also investigating the role of buffalo and other wild animals which act as disease carriers.

THE DEVELOPMENT OF SPOROZOITES IN THE TICK

A method has been developed for rapidly screening tick salivary glands to assess the infection rate with *T p parva* sporozoites, based on a methyl green pyronin staining technique originally used at the University of Edinburgh. Factors affecting tick infection rates have been analysed, including the age and sex of the tick and the level of parasitaemia in the animal on which it fed. It has been found that female ticks are generally more heavily infected with parasites than males. The maturation rates of the parasites have also been determined: parasites in ticks artifi-

cially stimulated in the laboratory take about 1 day longer to mature than parasites in ticks stimulated by natural feeding.

The electron microscope at ILRAD has been used to carry out a comparative study on the development of *T p parva*, *T p lawrencei* and *T taurotragi* sporozoites. The findings obtained substantiate a previous impression that kinetes of *Theileria* which develop in the gut of the tick enter only one type of cell in the salivary gland, the E-cell of the type III acinus. Additional evidence was obtained on the development in the salivary gland of a ramifying mass of parasite protoplasm with increasing numbers of nuclei (see Figure 4), which develop into sporozoites during terminal fission. It is estimated that the number of sporozoites produced in a single salivary gland cell ranges from about 50 000 in *T p parva* to 150 000 in *T taurotragi*. Preliminary studies suggest that the ultrastructure of these sporozoites may be sufficiently distinctive to identify the species of parasite carried by feeding ticks collected from cattle.

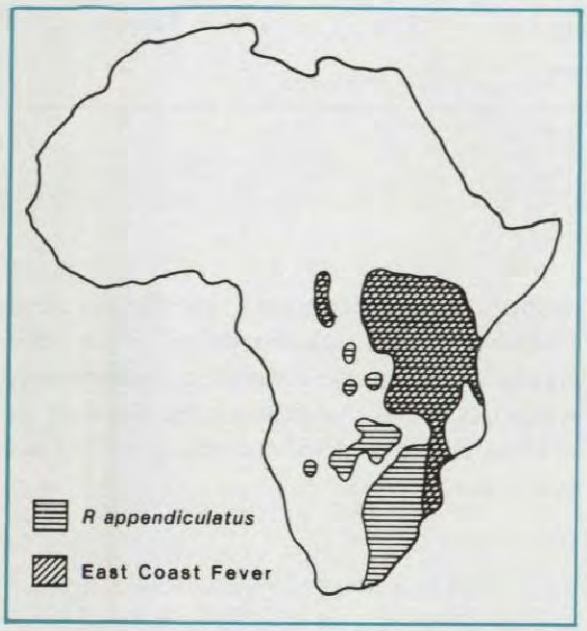


Figure 2. Distribution of R appendiculatus *ticks and* T p parva *in East and Central Africa.*

Developing sporoblasts of *T p parva* in tick salivary glands incorporate ^{3}H or ^{14}C hypoxanthine during nucleic acid synthesis, and the sporozoites which then develop are labeled and can be identified by radio-tracing techniques. This work has provided information about the timing of nucleic acid synthesis by developing parasites. Because the parasites may be more sensitive to the effects of ionizing radiation during nucleic acid synthesis, knowledge of this process could contribute to the development of a radiation-attenuated vaccine which would overcome the drawbacks of present immunization methods involving the use of virulent material. Further work is also in progress to improve techniques for harvesting and quantifying sporozoites from tick salivary glands. These sporozoites can be used for immunization trials.

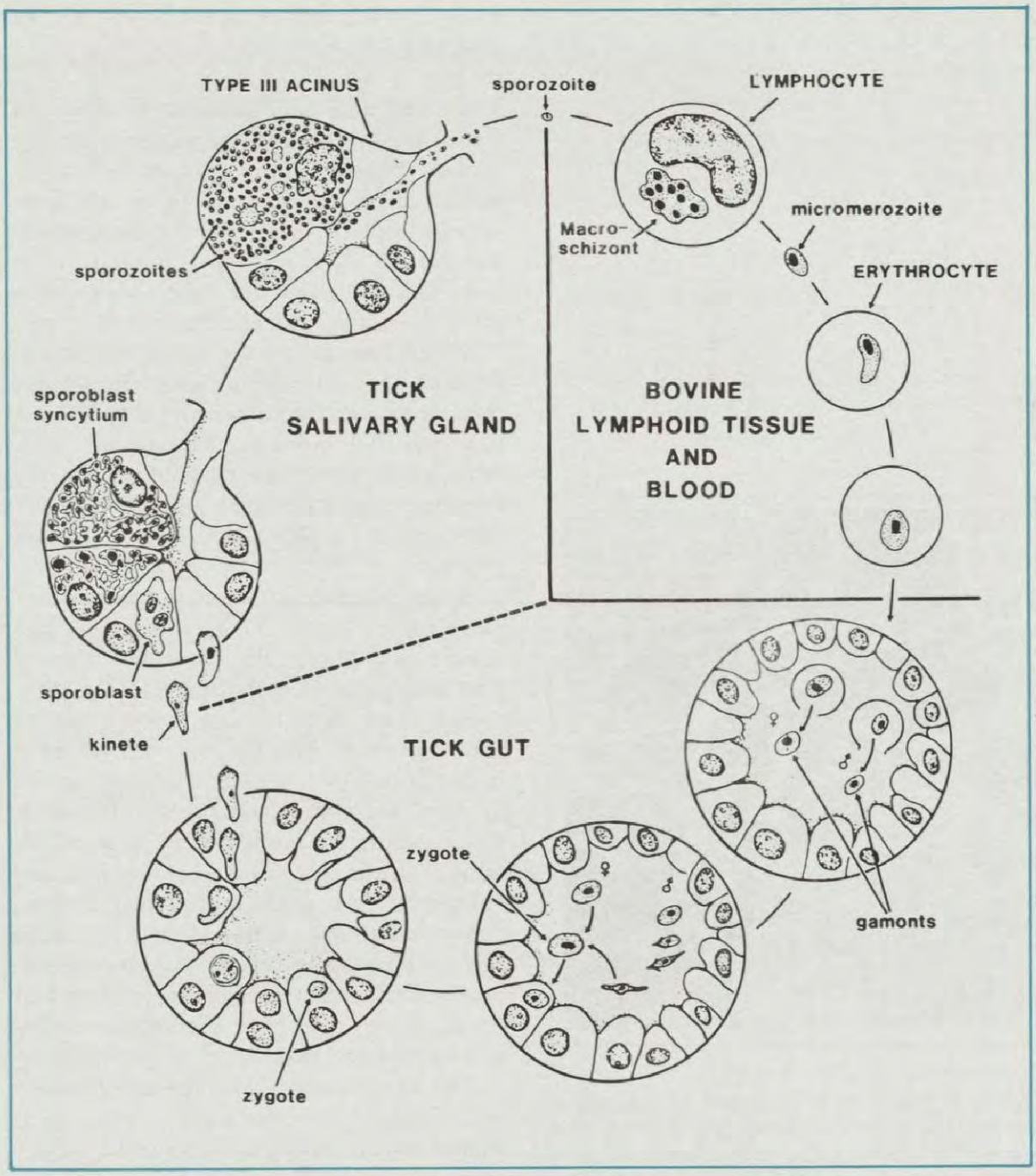


Figure 3. Life cycle of T p parva. *Sporozoites are transmitted to the bovine host in tick saliva. They enter the lymphocytes and develop into macroschizonts. After a period of nuclear division, these give rise to numerous micromerozoites which escape from the lymphocytes and enter the erythrocytes where they are ingested by feeding ticks. In the tick gut the micromerozoites differentiate into male and female gamonts which fuse to form zygotes. These enter the cell lining of the gut where they differentiate into kinetes. The kinetes traverse the gut wall and become free in the tick's body cavity. They move to the salivary gland and enter one cell type, the E-cell of acinus III. Here they form an elaborate intracellular sporoblast syncytium which undergoes segmental fission and gives rise to 30 000 to 50 000 sporozoites. These are introduced with the saliva into a new mammalian host, initiating a new cycle of parasite development.*

THE ENTRY OF SPOROZOITES INTO BOVINE LYMPHOID CELLS

The entry and establishment of *Theileria* sporozoites in bovine lymphocytes are important phases in the parasite life cycle which might be vulnerable to interference. In collaboration with colleagues at the Kenya Agricultural Research Institute, ILRAD scientists have investigated this process with the electron microscope.

When sporozoites are added to suspensions of bovine lymphocytes in the laboratory, they are found within the cells in less than 10 minutes. In contrast with other cells which are capable of actively injesting microorganisms, the lymphocyte cells appear to play a passive role in this process.

A thin surface coat on the sporozoite membrane appears to recognize and adhere to the host cell. The membranes of host and parasite cells bind closely from the point of contact, causing the spherical parasite to sink into the host cell where it is enveloped by a detached portion of the host cell membrane, as shown in Figure 5. Entry occurs as quickly at 1° as at 37°C, which indicates that it requires no energy: it is suggested that this process depends mainly on a ligand on the parasite surface binding to receptors on the membrane of the host cell. Further studies will focus on the effects of metabolic inhibitors on this process.

Figure 4. Electron micrograph of T p parva *sporoblast in tick salivary gland. At a late stage of sporogony, the sporoblast is a highly branched, multinucleate syncytium presenting an enormous surface area for interchange of metabolites with the host cell. A portion of the sporoblast has been opaqued on the micrograph to illustrate its elaborate ramifications.*

The lymphocytes invaded by *Theileria* sporozoites do not normally contain lysosomes, which are organelles capable of destroying ingested microorganisms, but these defensive structures are formed in the hours following entry of the parasite. However, the lysosomes must fuse with their own cell membrane in order to carry out their digestive function. During the time required for their formation the sporozoite disperses the host cell membrane which enveloped it at entry and thus evades the cell's principal defence mechanism. The sporozoite cytoplasm contains

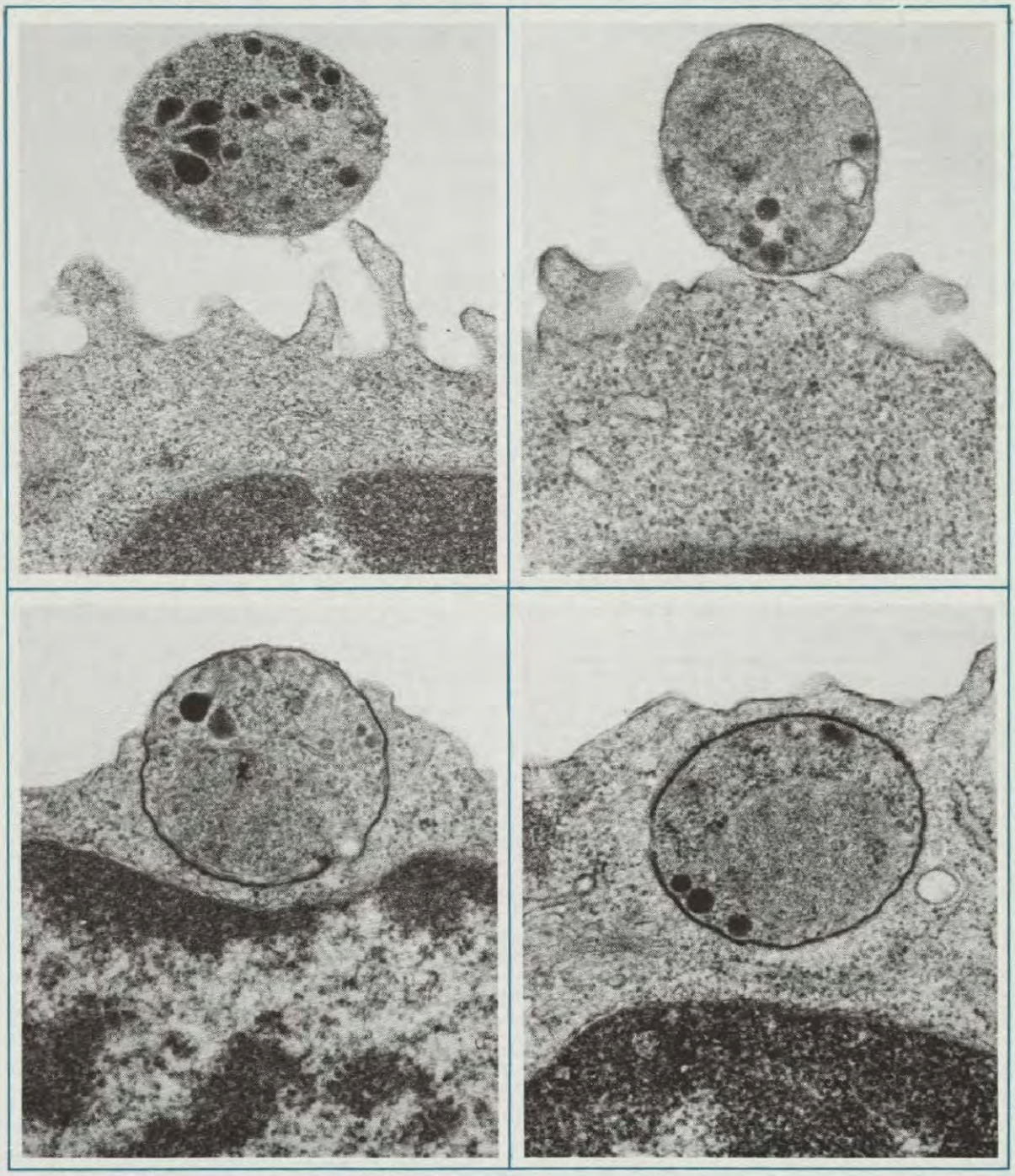


Figure 5. Electron micrographs of a T p parva *sporozoite entering a bovine peripheral blood lymphocyte. The sporozoite near the surface of the lymphocyte contains a cluster of rhoptries and numerous micronemes. The initial event in the entry process is a loose adherence of the sporozoite surface coat to the lymphocyte membrane. At a more advanced stage, the sporozoite membrane is in close contact with an invaginated region of the lymphocyte membrane. Unlike the entry process of other parasites, the sporozoite has not made initial contact at the point of attachment of the rhoptries to its membrane, and the host cell has not extended enveloping pseudopods. Finally, the lips of the invagination of the lymphocyte surface have met and fused behind the sporozoite, which is now in the lymphocyte cytoplasm but still enclosed in a detached portion of the host cell membrane.*

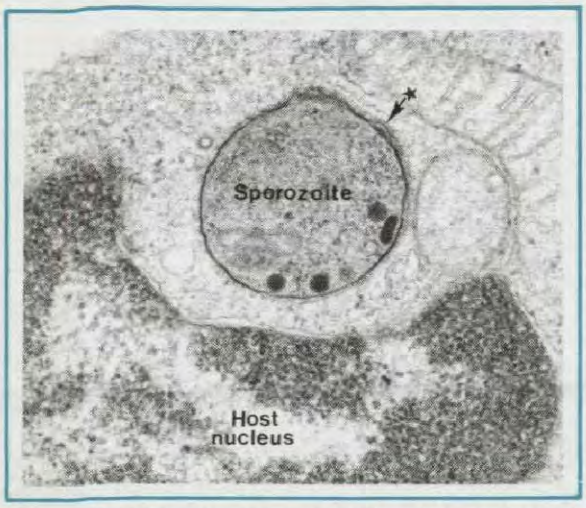


Figure 6. Electron micrograph of a portion of a bovine lymphocyte containing a recently entered T p parva *sporozoite. The dense boundary between the sporozoite and the surrounding cytoplasm consists of the closely opposed membranes of the parasite and the host cell. This can be seen most clearly at the arrow where the membranes have separated slightly.*

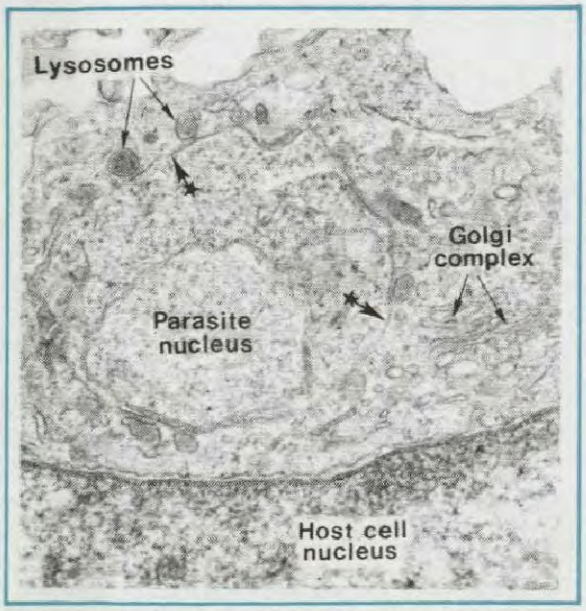


Figure 7. Electron micrograph of a bovine lymphocyte about 20 hours after the entry of a T p parva *sporozoite. The Golgi complex has been activated and newly formed lysosomes have gathered around the intracellular parasite. The boundary of the parasite is now a single membrane, as seen clearly at the arrow: the host-cell membrane which initially enveloped the sporozoite has been dispersed. The lysosomes are ineffective as a defence because they cannot fuse with the parasite membrane.*

small secretory granules called micronemes which disappear during the dissolution of the host cell membrane (see Figures 6 and 7): this suggests that dissolution is accomplished by releasing the contents of the micronemes.

THE TRANSFORMATION OF LYMPHOID CELLS INVADED BY PARASITES

Methods have been developed for identifying different types of bovine cells, including macrophages, B-lymphocytes and T-lymphocytes, using monoclonal antibodies specific for each cell type and lectins which identify different subsets of T-cells. Monoclonal antibodies are produced experimentally by creating hybrid cells from the fusion of mouse spleen cells, which produce antibodies, and mouse myeloma cells, derived from bone-marrow. These hybrid cells are capable of growing and dividing in culture.

Scientists elsewhere have observed that when bovine peripheral blood lymphocytes (PBL) are incubated with *Theileria* sporozoites, a morphologically identifiable schizont develops in approximately 25% of the PBL cells. ILRAD scientists, using monoclonal antibody techniques and electron microscopic examination, have shown that sporozoites enter bovine B-cells, T-cells, macrophages and perhaps other cell types, but only T-cells—and possibly only a small subset of T-cells—are transformed into lymphoblasts which enlarge and divide. When saturating concentrations of *Theileria* sporozoites were added to preparations of bovine lymphocytes in tissue culture, only 1 per 1000 to 1 per 10 000 of the T-cells present in the preparation were transformed into lymphoblasts.

It has also been found that the frequency of lymphocyte transformation by sporozoites is increased more than tenfold if the cells are already multiplying in re-

sponse to the mitogen Concanavalin A. This could indicate either that sporozoites only transform T-cells which are already multiplying due to some other stimulus, or that only a specific subset of T-cells can be transformed by sporozoites and this subset is also stimulated to multiply by the mitogen, so that it becomes a higher proportion of the total T-cell population which is then exposed to the parasites. This suggests that the susceptibility of cattle to ECF might be modified by reducing the number of T-cells capable of transformation into lymphoblasts, or by suppressing the multiplication of T-cells prior to the entry of the sporozoites. For instance, one approach might involve the suppression of the lymphocyte proliferation which occurs at the site of the tick bite in response to tick saliva.

The development of the parasite macroschizont in the transformed lymphoblast has also been studied in detail. ILRAD scientists have shown that the synthesis of DNA by the host and the parasite cell nuclei is synchronized, though not coincident, with the synthesis of macroschizont DNA occurring during the early mitosis phase of the host cell (see Figure 8). When the macroschizonts complete DNA synthesis, they divide immediately. The host cell divides just afterwards, and the parasite nuclei are distributed to the daughter cells. If this difference in timing reflects the operation of different mechanisms between host cell and parasite, it may be possible to identify drugs which act selectively on the parasite without disrupting host cell function.

These observations support the premise that the presence of the macroschizonts regulates and maintains the transformed state of the parasitized cells. As other workers have shown, when transformed cells multiply in vitro, 2 to 15% of the daughter cells produced do not contain parasites. These parasite-free cells do not continue multiplying, which is further evidence that the parasites or their secretions must be present to maintain the proliferative state of the host cells.

Detailed studies of surface changes on parasitized cells are also under way. Earlier work at ILRAD indicated that when parasites enter lymphocyte cells, changes are induced on the cell surface. This is shown by the ability of *Theileria*-infected cells to induce proliferative and cytotoxic (cell-killing) responses in lymphocytes taken from the same animal. Work now focuses on the identification of the specific surface antigens that trigger these responses.

EXPERIMENTAL IMMUNIZATION WITH *THEILERIA* SPOROZOITES

The work of others has shown that cattle simultaneously inoculated with *T p parva* sporozoites and treated with the antibiotic oxytetracycline develop only mild symptoms and subsequently resist infection by the same strain of the parasite. This 'infection and treatment' approach to immunization is currently being explored in a number of field situations by scientists at ILRAD and elsewhere. However, this technique has several disadvantages.

In the first place, some isolates of *T p parva* are not cross-protective: immunity to one strain will not necessarily ensure protection against another. Secondly, not all strains can be controlled by oxytetracycline, so the initial infection with live parasites may be fatal, particularly in a young animal. An animal infected and treated in this way may also become a carrier of the infection. Finally, this type of immunization requires close veterinary supervision, which makes it difficult for widespread application. Given these drawbacks, ILRAD scientists are examining the feasibility of developing improved methods of immunization with *Theileria* sporozoites.

Recent work has shown that cattle which have recovered from ECF produce antibodies against the sporozoites of *T p*

parva. Further work will concentrate on the production of monoclonal antibodies to identify different *T p parva* strains and the isolation and characterization of sporozoite antigens to be used for immunization. The development of a suitable antigen preparation to detect monoclonal antibodies to *T p parva* sporozoites has proven difficult, but earlier problems may now have been overcome. The antigen preparations used are cryostat sections of salivary glands derived from heavily infected ticks. The assay system is based on a highly specific autoradiography technique, with ^{125}I labeled Protein A detecting specific antibody activity.

In an attempt to overcome the risks of infecting cattle with live parasites, some animals have been inoculated with irradiated sporozoites. Past studies suggest that irradiation kills a substantial proportion of the parasites, so that immunization with irradiated material may not be effective. Recent work, however, in collaboration with the Kenya Agricultural Research Institute and the International Atomic Energy Agency (IAEA), indicates that much lower doses of irradiation will inactivate parasites if they are exposed at the sporoblast stage in the tick. Further research is under way in this area, with the aim of producing an effective vaccine based on sporozoites attenuated by irradiation.

Figure 8. Autoradiograph of a bovine lymphoid cell parasitized with T p parva, *taken from a culture exposed to a half-hour pulse of tritiated (^{3}H) thymidine. The cell is in the metaphase stage of division, and the ^{3}H label is localized in the macroschizont nuclei. The chromosomes of the dividing cell nucleus are unlabeled. This indicates that the macroschizont is synthesizing DNA during host-cell metaphase, in contrast to the synthesis of DNA by the host cell nucleus which occurs during the S phase of interphase.*

DEVELOPMENT OF A VACCINE BASED ON SCHIZONT-INFECTED CELLS

It is possible to immunize cattle against ECF by injecting them with bovine lymphoblast cells infected with *Theileria* macroschizonts, but several problems need to be overcome before this approach can be broadly applied. For one thing, immunization is based on infection with live parasites, and a small proportion of the animals inoculated develop the disease at

clinical levels and may die. Also, for successful immunization, the parasites must enter the host's own cells, but before this can happen the animal's immune system tends to kill the injected lymphoblasts. This problem can be overcome by using lymph cells taken from the same animal and infected with sporozoites in vitro, so that the animal's system will not reject the cells before the parasites are transferred. Another approach would be to use very large numbers of lymph cells (about 100 million) from another animal, so that at least a few would survive the initial rejection effect. However, both of these approaches are impracticable for widespread application.

The detection of cytotoxic responses in cattle being immunized with *T p parva* suggests that these responses are involved in the mechanisms of immunity. Recent work has concentrated on examining the specificity of these immune responses, with the aim of identifying antigens which might be used for immunization. In fatal cases of ECF, cytotoxic cells are generated which indiscriminately kill parasitized and non-parasitized lymphoid cells from autologous and allogeneic hosts. By contrast, following successful immunization, cytotoxic cells are generated which kill only autologous parasitized cells. Because cytotoxic responses can be detected only very briefly in cattle following immunization, studies have been conducted in vitro to elucidate the mechanisms involved in more detail. Lymphocytes were taken from cattle with no previous exposure to ECF and from others which had been immunized; these were used as responder cells in a mixed lymphocyte reaction (MLR) with *Theileria*- infected autologous cells. The latter (stimulator) cells were treated with gamma irradiation or mitomycin C to prevent them from multiplying. Lymphocytes cultured in the MLR for 5 days were also monitored for their activity as effector cells in a cytotoxic assay against target cells from different sources. Lymphocytes from naive and immunized cattle killed a range of parasitized and non-parasitized target cells from autologous and allogeneic cattle as well as from xenogeneic hosts. The specificity of these responses was, therefore, similar to that generated in cattle undergoing fatal ECF infections. Thus, neither the MLR nor the cytotoxic assay currently shows the required specificity in vitro needed to differentiate between naive and immune cattle. This is further shown by the fact that when parasitized target cells were pretreated with inhibitors of carbohydrate synthesis, cytotoxic activity of cultured lymphocytes against these target cells was unaffected, indicating that target antigens on the cell surface do not contain carbohydrates. However, similar treatment of target cells blocked the cytotoxic response generated by lymphocytes taken from immunized cattle. Thus, the cytotoxic lymphocytes stimulated in cell cultures recognize different target antigens from those recognized by similar lymphocytes from immunized cattle.

These results indicate that the conditions for inducing cell-mediated responses in the laboratory require further manipulation in order to obtain a response which mimics the responses elicited in cattle during immunization. This work will also help in the identification of the specific surface antigens involved in successful immunization.

Because current experimental methods of immunization involve establishing infection in the host, it is difficult to pinpoint the antigens involved in induction of immunity. Since immunity appears to be induced by infected cells from the same animal, experiments have been initiated to explore methods of immunization with non-infective preparations of these cells. Cattle which were repeatedly inoculated with cell membranes taken from their own parasitized cells developed immunity, while animals inoculated with

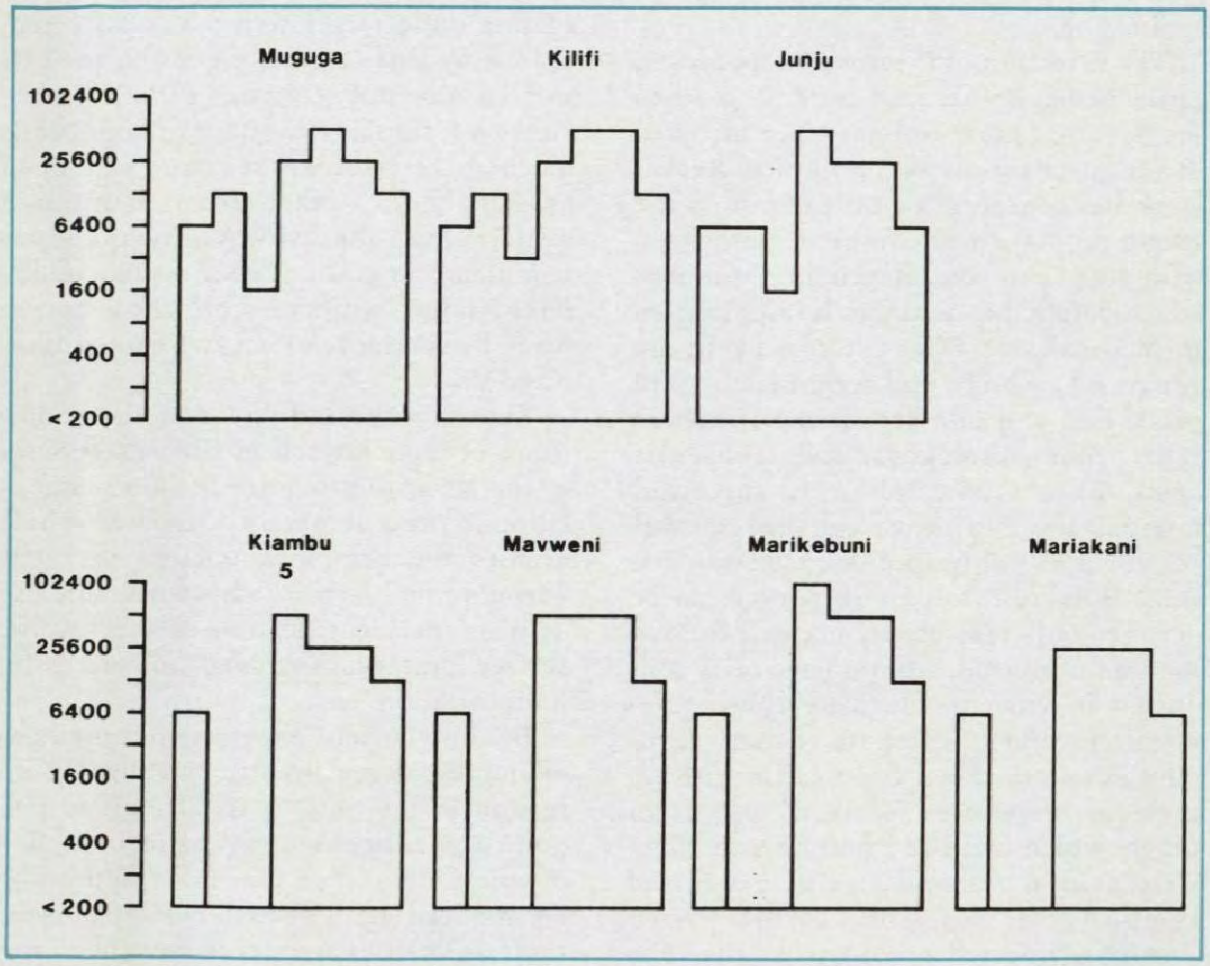


Figure 9. Bar charts showing the titres of 7 monoclonal antibodies screened against macroschizont-infected cells derived from different T p parva *isolates using the indirect fluorescent antibody test. Each bar represents the highest dilution of the respective antibody which gave fluorescence. Two basic profiles are seen: these appear to reflect strain differences, since cattle immunized against one isolate resist challenge with another isolate of similar profile, but may react and die if challenged with an isolate showing a different profile.*

membranes of parasitized cells derived from other animals did not. Similar work is in progress using intact parasitized cells from the same animal rendered non-infective by gamma-irradiation. Such studies should generate information on the number of cells required to induce protective immunity and confirm whether or not immunity is effected primarily through the action of cytotoxic cells.

STRAIN DIFFERENTIATION

A major problem in the development of an effective ECF vaccine is the existence of different parasite strains in the field. Animals immunized against one strain, whether based on a sporozoite or a macroschizont preparation, may be only partly protected against other strains or fully susceptible.

Tests to identify specific strains by cross-immunity trials in cattle are expensive and imprecise, so ILRAD scientists are trying to develop a laboratory test. A battery of 14 monoclonal antibodies has been raised which can be used to screen macroschizont-infected cells. Profiles are obtained of the reactions of these monoclonals to different *Theileria* isolates, as shown in Figure 9. Preliminary work indicates that immunization against one isolate protects cattle against other isolates with similar profiles, but not against isolates with different profiles.

The monoclonal antibodies can be screened against cells taken from animals or from cell lines grown in the laboratory. The specific reactions of the monoclonals do not appear to change when a given isolate is tested in different host-cell backgrounds or when it is passaged sequentially through different cattle. Using this technique, it may be possible to identify and substantially reduce the number of strains which must be used for effective immunization in any field situation.

EPIDEMIOLOGY

A field program was initiated in 1980 to investigate the epidemiology of ECF in Kenya's Coast Province, in cooperation with the Ministry of Livestock Development. Early emphasis has been on Kilifi District, where the disease situation appears relatively simple and amenable to control.

Ticks and blood samples have been collected from about 100 farms and other sites in the district (see Figure 10) and seven *Theileria* isolates have been made. Stabilates of the different isolates have been prepared from ground-up ticks, and macroschizont-infected cell lines have been developed and cryopreserved. The five *T p parva* isolates fall into two profile types, resembling strains isolated and identified earlier at the former East African Veterinary Research Organization — *T p parva* (Muguga) and *T p parva* (Kiambu 5). These profiles are depicted in Figure 9. The identification in Kilifi of these two basic parasite types is also borne out by the results of cross-immunity trials. If these findings are substantiated, an immunization program based on the two basic types can be considered. Pilot immunization trials are now under way.

THEILERIA STUDIES IN WILDLIFE

Previous work in Kenya has shown that buffalo are chronic carriers of *T p lawrencei* which can cause high mortality in cattle. In areas where buffalo are present, the pattern of disease strains found in cattle becomes much more complex than the situation in Kilifi where cattle do not come into contact with buffalo.

Theileria parasites carried by buffalo appear to change antigenically, so that cattle immunized against parasites isolated from a buffalo may be fully susceptible to another isolate from the same buffalo only a few months later. Work has begun,

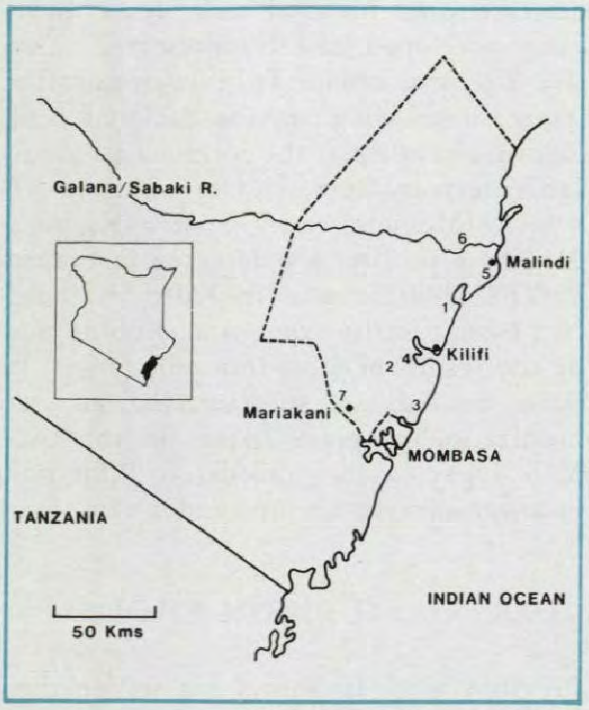
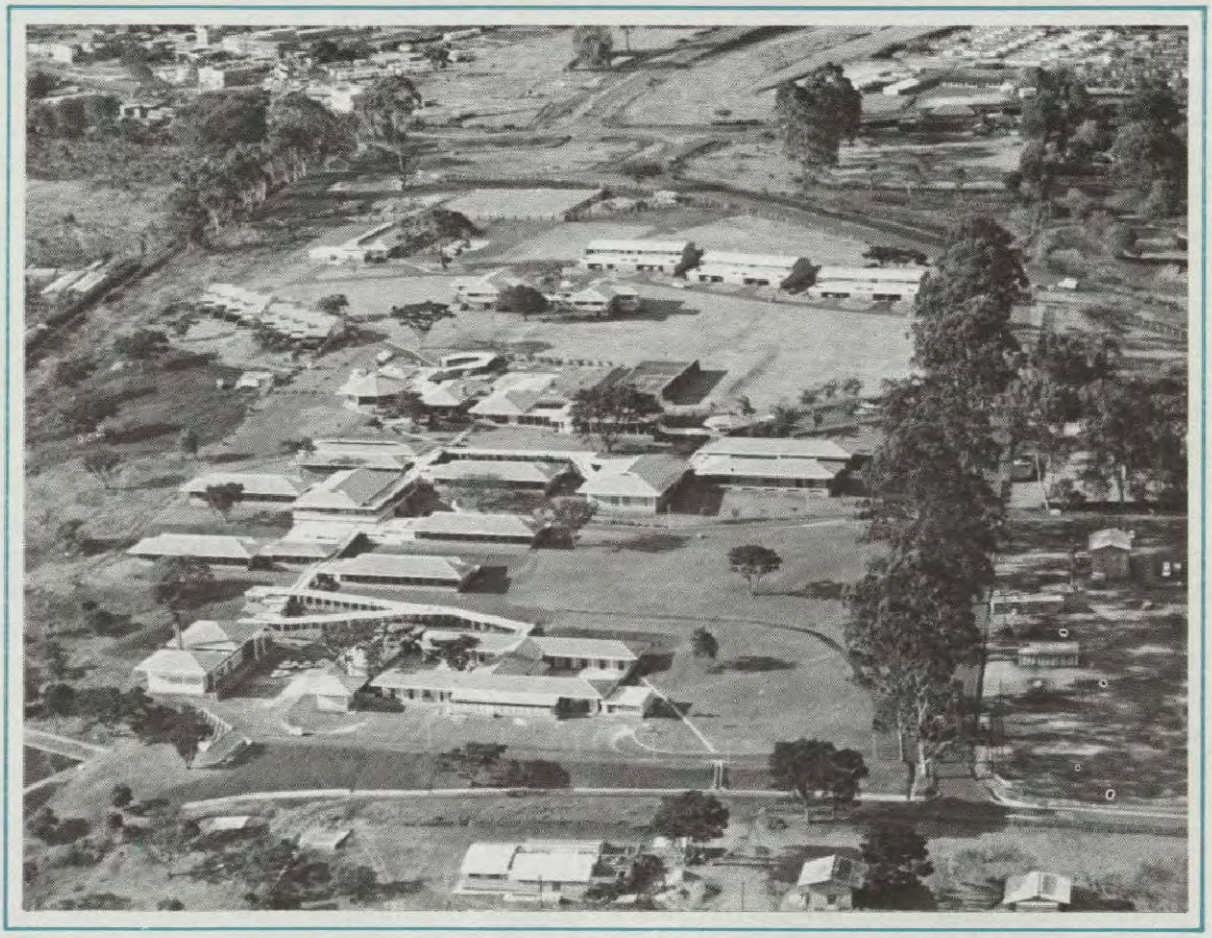


Figure 10. Sites in Kilifi District where Theileria *isolates have been collected. 1 is at Kilifi, 2 and 4 are at Mavweni, 3 is at Junju, 5 is at Malindi, 6 is at Marikebuni and 7 is at Mariakani.* T mutans *was isolated at the second site, and* T taurotragi *at the fifth.* T p parva *was isolated at the other sites. The insert shows the location of Kilifi District in Kenya.*

in conjunction with the Kenya Veterinary Research Laboratory, to study the nature of this antigenic change, and monoclonal antibodies have been raised for use in a screening test against parasitized cell lines isolated sequentially from an infected buffalo. A correlation will be sought between changes detected in monoclonal profiles and the results of cross-immunity studies.

Cattle lymphocytes have also been infected with *T p lawrencei* taken from buffalo, and monoclonal antibodies have been raised which appear to recognize specific parasite antigens on the surface of the host cells. Although the presence of such antigens has been postulated for some time, this work provides evidence of their existence and has important implications for the mechanisms involved in the induction of the immune response■



ILRAD laboratories and support facilities at Kabete.

TRYPANOSOMIASIS

Different forms of trypanosomiasis occur over large areas of Africa, Latin America, Asia and the Middle East, as shown in Figure 11. In tropical Africa, trypanosomiasis is found over an area of about 10 million km^2, or roughly one-third of the continent (see Figure 12). The disease is caused by protozoan parasites which affect most types of domestic livestock, many wildlife species and man. In Africa, the major pathogenic species of trypanosomes for domestic ruminants and wildlife are *Trypanosoma congolense*, *T vivax* and *T brucei brucei*. These are transmitted by tsetse flies (*Glossina* spp) and very occasionally by other biting flies. *T evansi* also affects livestock in Africa and elsewhere, transmitted by biting flies. Outside Africa, pathogenic trypanosomes are transmitted by biting flies or occasionally sexually.

Trypanosomes are ingested by a tsetse fly when it feeds on the blood of an infected animal. The parasites undergo a cycle of development in various locations within the fly, depending on the trypanosome species, as shown in Figure 13. All three major African species finish this phase of their development as metacyclic forms in the mouth parts or salivary glands of the tsetse. The metacyclics are transmitted to mammals and initiate new infections when the tsetse feed.

In the case of *T congolense* and *T b brucei*, a swelling in the animal's skin, called a chancre, develops at the site of an infected tsetse bite. The trypanosome metacyclics develop further here, invade the local lymph vessels and then move into the bloodstream. The ensuing infection is characterized by successive waves of parasitaemia in the bloodstream, as parasite populations multiply rapidly and die off. The infected animal may die or eliminate the parasites and recover.

The parasites undergo further development in the mammalian bloodstream. In the case of *T b brucei* infections, rising waves of parasitaemia consist of long, slender forms of the parasite which divide rapidly. These are transformed into less rapidly dividing intermediate forms and finally into senescent short, stumpy forms which die off as the parasitaemic wave recedes. Tsetse flies become infected when they ingest blood containing the intermediate and stumpy forms of the parasite. *T congolense* and *T vivax* parasites also appear to undergo development in the bloodstream, but this is not characterized by clearly visible morphological changes as is the case with *T b brucei*. *T vivax* and *T b brucei* parasites may also invade an animal's connective tissues, and, in the final stages of the disease, *T b brucei* may invade the central nervous system.

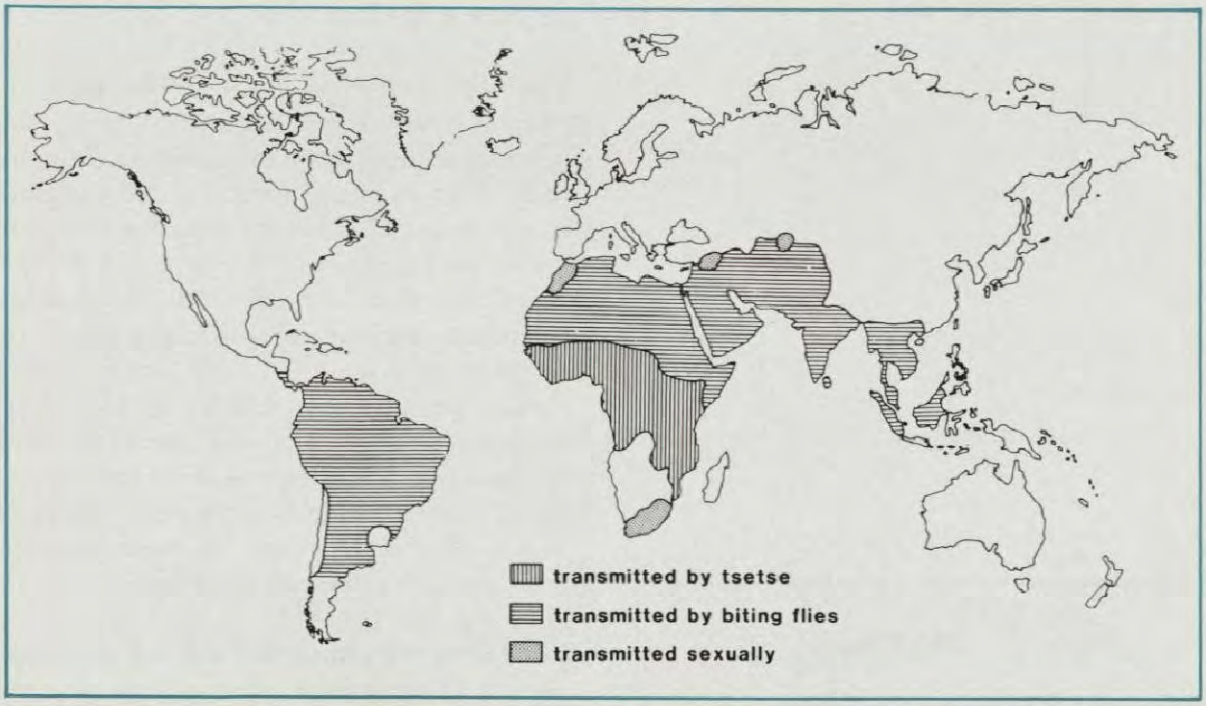


Figure 11. World distribution of animal trypanosomiasis (Derived from P T K Woo, 1977, in Parasitic Protozoa, *J P Kreier, ed, New York, Academic Press).*

Trypanosomiasis in livestock causes anaemia, poor growth, infertility, abortion and frequently death, or an animal may survive but with greatly reduced productivity. In addition to high mortality rates and productivity losses, a calculation of the effects of trypanosomiasis on livestock production in Africa must take into account:

- large areas of otherwise productive grazing land where practically no livestock can be kept,
- extensive farming areas where cultivation must be by hand because draft animals cannot survive,
- limited disease-free areas which are threatened by over-population and over-grazing, and
- constraints on the introduction of more productive livestock breeds because these are generally highly susceptible to the disease.

Considering all these factors, it is estimated that effective control of trypanosomiasis could double livestock production in Africa and have a substantial impact on arable farming.

At present, measures available to control trypanosomiasis include regular treatment with trypanocidal drugs and reduction of tsetse populations by insecticide spraying. However, both of these approaches are expensive and neither is completely effective in areas of heavy tsetse infestation. Further drawbacks include increasing resistance to trypanocidal drugs and the possibililty of environmental pollution due to large-scale insecticide spraying.

The goal of ILRAD's trypanosomiasis program is to develop an effective, safe and economic method of controlling the disease by immunological, chemical or genetic means. Work focuses on the parasites and on their interactions with the tsetse vectors and the mammalian hosts.

THE PARASITES

The failure of most domestic livestock to recover from trypanosomiasis or to acquire immunity to the disease is thought to be due to a phenomenon called antigenic variation. This means that the antigens on the surface coat of the parasites change rapidly so that the host cannot produce sufficient antibodies quickly enough to combat the disease. In 1981, studies continued on antigenic variation, including its genetic basis and the structure and synthesis of the antigens involved. Work also focused on other parasite antigens which do not vary and on their possible role in the course of the disease and in eliciting the host's immune response. Finally, work continued on the development and utilization of techniques to cultivate and maintain different trypanosome species in the laboratory at different stages of their life cycle.

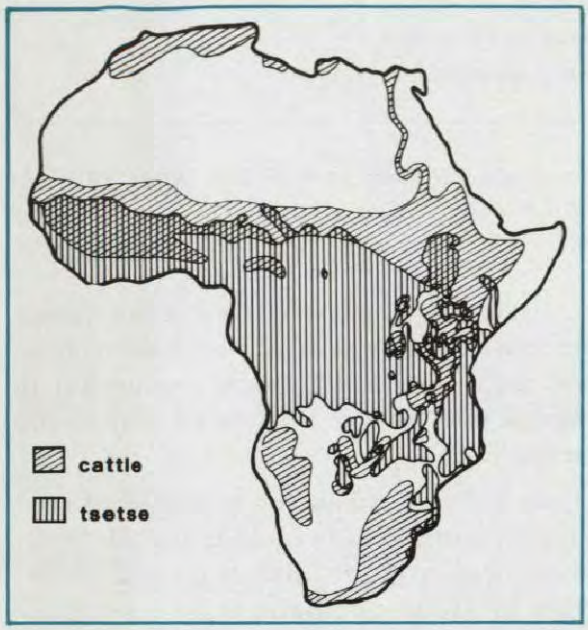


Figure 12. Distribution of major cattle-production areas and tsetse-infested zones in Africa. There is little overlap except in the regions of West and Central Africa where trypanotolerant N'Dama and West African Shorthorn cattle are kept.

VARIABLE ANTIGENS

Trypanosomes maintain a chronic infection in their mammalian hosts by antigenic variation: the molecules on their surface coats change rapidly, thus evading the antibodies produced by the host. Surface antigens vary widely between trypanosome species. Within a species, the surface antigens also vary between populations of similar genetic background (serodemes). Evidence to date suggests that even a single trypanosome in the bloodstream can give rise to populations which express a very large number of variable antigen types (VATs). However, the fact that some animals recover from chronic trypanosome infections suggests that the number of VATs may be finite. In the sequence of VATs in the course of an infection, similar or very closely related antigens may also reappear. This was shown earlier for *T b brucei*, and was observed in 1981 for *T congolense* infections in cattle.

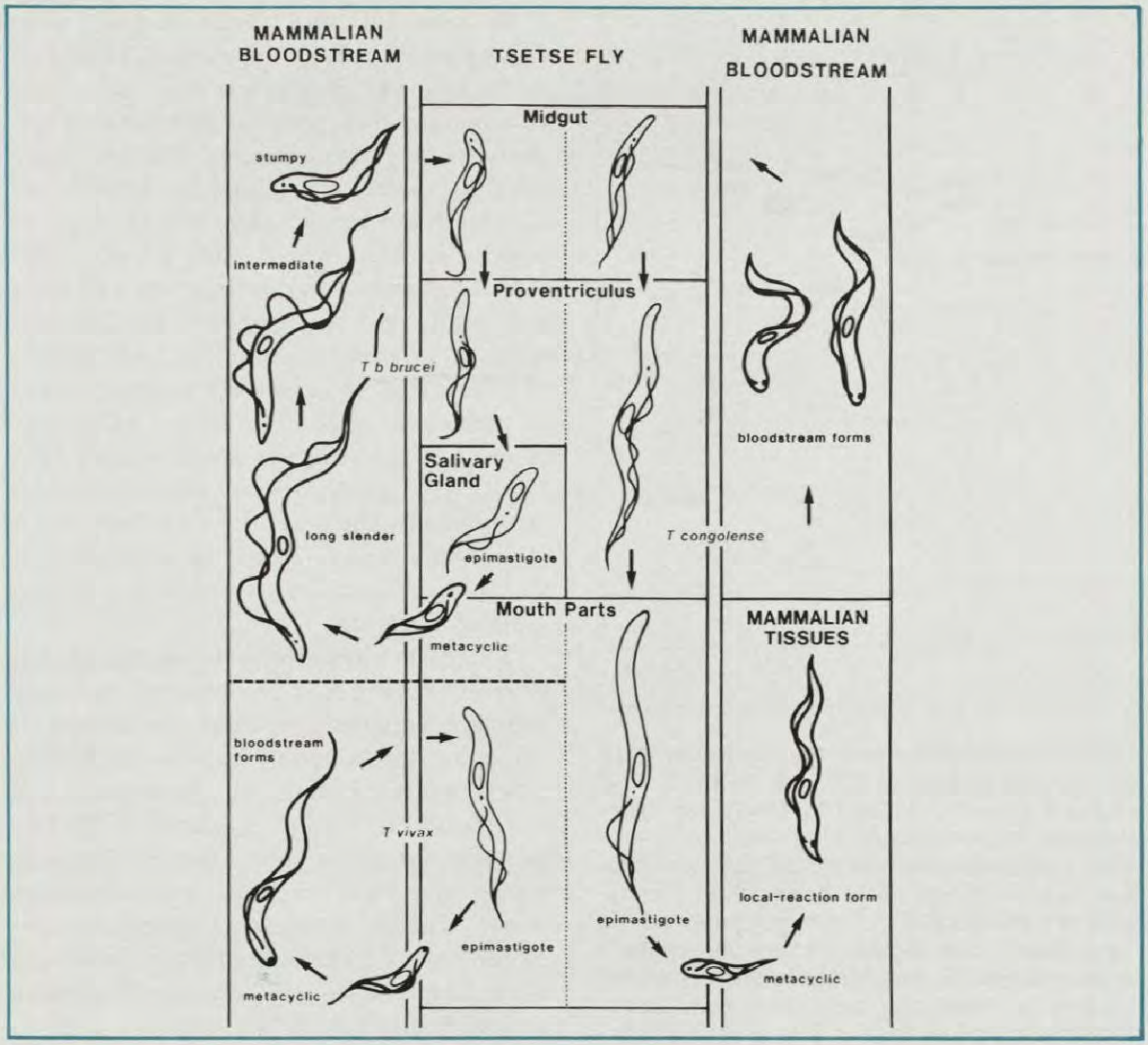


Figure 13. Life cycle of T b brucei, T congolense *and* T vivax. *Heavy outlines indicate parasite forms with surface coats containing variable glycoprotein antigens. Light outlines indicate uncoated forms which are not infective to mammals.* T b brucei *develops in the tsetse midgut, proventriculus and salivary glands, where infective metacyclic forms are produced. Tsetse most readily become infected if their first feed after emerging from the puparium is from an infected animal. Infection rates in flies are only about 0.1% in the field. Infection rates in flies in the laboratory vary from 1 to 15%, and thousands of metacyclic parasites are found in each feeding probe of an infected fly. Parasite development in the fly takes 2 to 4 weeks, and flies remain infective for life.* T congolense *parasites develop over a 2- to 4-week period in the tsetse midgut, proventriculus and mouth parts, where infective metacyclic forms are produced. Tsetse most often become infected during their first feed after emerging from the puparium, but flies can also become infected later on. In the field, infection rates in flies average about 2%, while infection rates in the laboratory range from 5 to 15%. Only 50 to 100 metacyclic parasites are found in the feeding probe of an infected fly.* T vivax *parasites develop entirely in the tsetse mouth parts. Flies of any age can become infected, and infection rates in the field are high, at 10 to 20%. In the laboratory, infection rates of up to 100% can be achieved. Apparently, relatively few metacyclic parasites are produced, and it is not yet known whether these have a surface coat. During the course of infection in the mammal,* T b brucei *undergoes morphological changes to forms more infective to tsetse.* T congolense *and* T vivax *may undergo differentiation in the mammal, but this is not accompanied by obvious morphological change.*

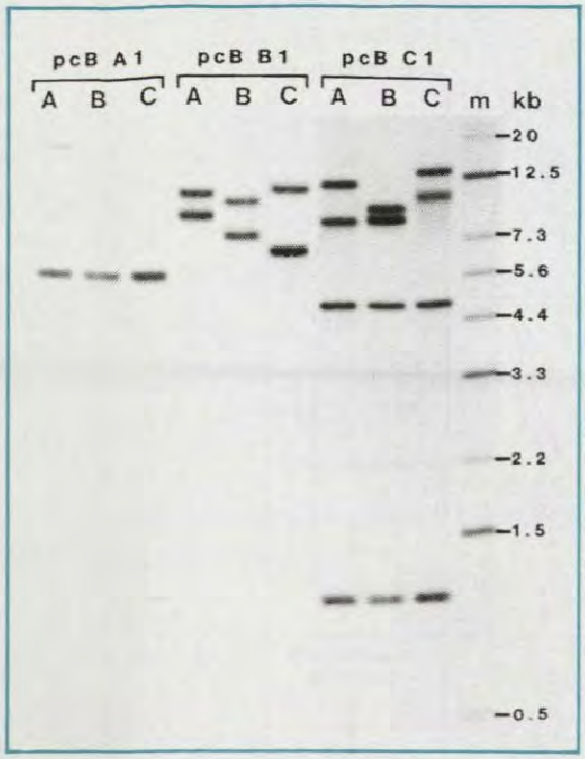


Figure 14. Comparison of rearrangements affecting the genes for VSGs A (ILTat 1.1), B (ILTat 1.2) and C (ILTat 1.3). DNA from trypanosome clones expressing each of these VSGs was digested with Hinc II (for hybridization to pcB A1 and pcB C1) and Sal 1 (for hybridization to pcB B1). These enzymes do not have sites in the cDNAs to which their digests were hybridized. Fragments were separated by electrophoresis on a single gel and transferred to nitrocellulose in 20 x SSC. Cut sections of the filter were hybridized to plasmids pcB A1 (first three tracks), pcB B1 (centre three tracks) and 1100 bp 5' fragment of pcB C1 (last three tracks), which had been labeled with 32p. The markers are fragments of known size which hybridize to pBR 322 sequences in the pcB C1 probe. The filter sections were washed twice in 0.1 x SSC, 0.1% SDS at 65° for 1 hour, dried, and realigned for exposure to X-ray film.

Because antigenic variation plays such an important role in the evasion of an animal's immune responses, it is important to understand the genetic mechanisms underlying this phenomenon. One serodeme of *T b brucei* was isolated at ILRAD and characterized genetically, and four genes were identified which code for four different variable antigens. In 1981, continuing study of the genetic mechanisms which control antigenic variation revealed that at least two complex types of gene rearrangement occur when different surface antigens are expressed (see Figure 14). Both of these rearrangements are unusual: the way in which they occur is probably related to the large number of variable antigens involved and the mechanism of variation.

Scientists have also investigated whether these four genes are present and how they are organized in other serodemes of the same trypanosome subspecies and in other species. Each of the four genes has been found in field populations of *T b rhodesiense*, but not in populations of *T b gambiense*, the two trypanosome subspecies which infect humans. Differences between two *T b brucei* serodemes have also been detected in the region of genetic material adjacent to the variable antigen genes. Besides providing evidence on evolutionary relationships, this work may lead to the development of simple tests to differentiate trypanosome subspecies and populations found in the field which will be an improvement over the cumbersome and expensive techniques currently available. Collaborative studies on gene rearrangements and the presence or absence of *T b brucei* genes in other *T brucei* subspecies are being carried out with the Université Libre de Bruxelles and the Vrije Universiteit Brussel.

Research has also continued on ways in which variable antigens are synthesized and maintained on the surface of trypanosomes, focusing on *T b brucei* and *T congolense*. Though the variable antigens are

proteins, previous work showed the importance of carbohydrate (CHO) side chains for the development and stability of the antigen molecules. Two types of CHO side chain are found in the final form of the variable antigen. The first is constructed and attached to the protein by a linkage found in mammalian and other systems. If this linkage is blocked by drugs, the antigen is still assembled but it is unstable and susceptible to breakdown. The second CHO side chain is linked to the variable antigen at a site close to the trypanosome surface membrane by a mechanism which has not yet been described in any system. The importance of this side chain is suggested by the fact that it remains constant on all the variable antigens of *T b brucei* and *T congolense* studied so far (see Figure 15). Its presence is probably required for the association of variable antigen molecules on the surface of the trypanosome to form an intact surface coat. The enzymes which are probably involved in its synthesis and attachment have been partially characterized. Interference with this process could disrupt the stability of the surface coat and render the trypanosome susceptible to the host's defences. Studies on the structure and antigenicity of *T b brucei* variable antigens are also being carried out in collaboration with the University of Cambridge's Molteno Institute.

Earlier observations indicated that trypanosomes in the metacyclic stage, transmitted by tsetse flies, display many fewer VATs than they do later in their bloodstream forms. More information is being sought on this stage of trypanosome development because it may be more feasible to immunize against metacyclic trypanosomes than against the bloodstream forms with their much larger repertoire of VATs. It is important to know whether an animal can develop effective immune responses to metacyclic VATs and to know how many different VATs exist in the metacyclic stage of a given serodeme. The VAT repertoires of *T b brucei*, *T b rhodesiense* and *T congolense* isolates are being characterized in a collaborative project with the Prince Leopold Institute for Tropical Medicine in Belgium.

Work which will be described in the section on parasite-host interactions showed that cattle exposed to metacyclic trypanosomes develop immune responses to metacyclic VATs of *T b brucei* and *T congolense*, but not *T vivax*. This may indicate that the metacyclic VATs of *T vivax* are not stable even within one serodeme or that the number of metacyclic trypanosomes extruded by the fly is too small to induce an immune response. By contrast, animals infected with an injection of bloodstream trypanosomes—whether *T b brucei*, *T congolense* or *T vivax*—all resisted challenge about 6 months later by metacyclics of the same serodemes. In the case of bloodstream infections with *T b brucei*, antibodies were observed which protected against metacyclic VATs of the same serodeme. This was probably due to the appearance of bloodstream VATs which are the same as or very similar to metacyclic VATs. Studies are now in progress to see if antibodies to metacyclic VATs arise in the same way in the case of *T congolense* and *T vivax* infections.

Highly specific monoclonal antibodies have been developed to identify individual VAT components. For one serodeme of *T b brucei*, 80 to 90% of a metacyclic population displayed only six different VATs. For one serodeme of *T congolense*, only three different VATs were displayed by approximately 60% of a metacyclic population.

Both sets of experiments confirm earlier observations that some, and perhaps all, of the metacyclic VATs are constant for a given serodeme of *T b brucei* or *T congolense*, no matter which bloodstream VAT is ingested by the tsetse fly which subsequently transmits the infection. The monoclonal antibody analyses further show that the number of metacyclic

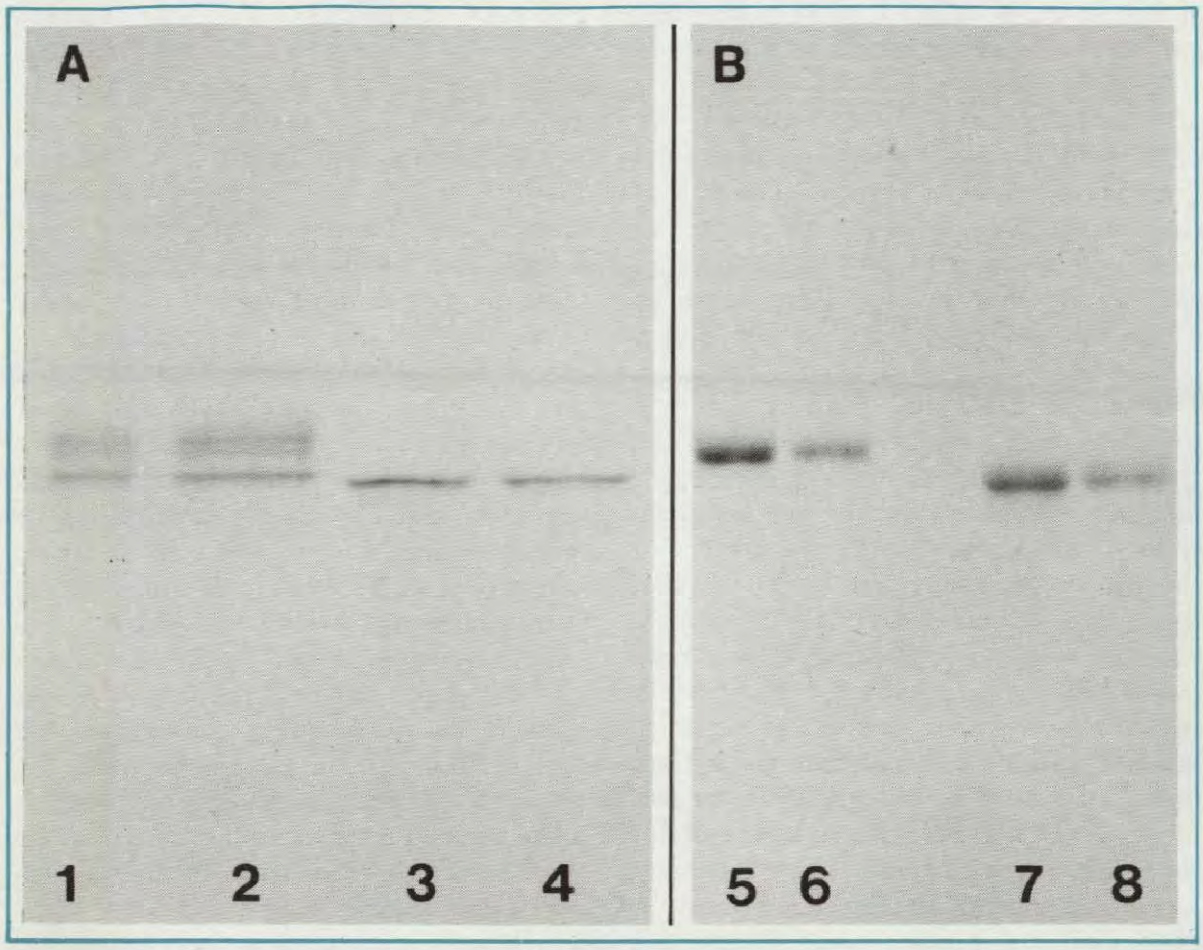


Figure 15. Complexity of the carbohydrate side chains of T b brucei *variable surface glycoproteins. In this experiment, two* T b brucei *clones were metabolically labeled in vitro in the absence or presence of tunicamycin, an inhibitor of N-glycosylation. The variable surface glycoproteins (VSG) were immunoprecipitated from radioactive cell lysates with the antiserum raised against the VSG which each clone expressed (homologous antiserum), and with an antiserum raised against an unrelated VSG, which recognized common determinant(s) associated with a carbohydrate side chain (heterologous antiserum). The figure is an autoradiograph of radioactive precipitates obtained in this way and analysed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. A. VSG from clone MITat 1.7 precipitated by homologous (track 1) and heterologous (track 2) antisera. The multiple bands suggest multiple glycosylation sites and/or microheterogeneity in carbohydrate side chains. The heterogeneity is abolished by synthesizing the VSG in the presence of tunicamycin, as shown by the pattern obtained after immunoprecipitation with the homologous antiserum (track 3). However, the carbohydrate-dependent cross reactivity revealed by immunoprecipitation with the heterologous antiserum is still detectable (track 4), suggesting that this carbohydrate side chain is not N-glycoside linked. B. VSG from clone MITat 1.2 precipitated by homologous (track 5) and heterologous (track 6) antisera. The VSG appears homogeneously glycosylated. The treatment with tunicamycin reduces the apparent molecular weight of the antigen immunoprecipitated by the homologous antiserum (track 7), showing the presence of N-glycoside-linked sugars. However, also in the case of this antigen, tunicamycin treatment does not interfere with the cross reactivity detected by immunoprecipitation with the heterologous antiserum (track 8).*

VATs present in any serodeme is likely to be limited, possibly more so in *T congolense* than in *T b brucei.*

A collaborative project was also carried out with the University of Zaire and the Program for Research and Training in Tropical Diseases of UNDP, the World Bank and WHO. Bloodstream trypanosomes isolated from human *T b gambiense* patients were cloned in laboratory rodents and individual VATs were identified using the immunofluorescent techniques developed for *T b brucei.* Parasites isolated from widely separated geographic areas gave rise to similar VATs, in contrast to the situation commonly found with *T b brucei* (see Figures 16 and 17). These findings suggest that the number of *T b gambiense* serodemes in Africa may be sufficiently limited to make immunological control feasible. The enumeration of serodemes by analysis of metacyclic VATs will be carried out in a further collaborative project. The possibility that domestic ruminants are potential carriers of human sleeping sickness will also be investigated, as this has significant implications for the introduction of livestock into areas where the human disease occurs.

OTHER ANTIGENS

Trypanosomes contain a number of antigens which are more stable than the variable antigen types found on their surface coats. Some of these antigens are common to all trypanosomes, some are found only in particular trypanosome species, and some are observed only at specific stages in the trypanosome life cycle. Work has been under way for some time at ILRAD and elsewhere aimed at characterizing these antigens, assessing their role in the course of disease and identifying how they might be used to develop immunological controls.

Studies continued in 1981 on the identification and characterization of parasite antigens. *T b brucei* and *T congolense* antigens are being characterized based on their recognition by antibodies present in bovine sera. Sera from resistant N'Dama cattle, susceptible Zebu and N'Dama x Zebu crossbreds have been provided for this work through a collaborative project with the Centre de Recherche et d'Elevage in Togo and the German (Federal Republic) aid agency (GTZ).

Antigens have been partly purified which are specific for *T b brucei, T congolense* and *T vivax* parasites, and monoclonal antibodies have been raised which will be used to purify them further. These antigens will be used in assays to determine the trypanosome species infecting animals in the field on the basis of the animals' serological responses. Such tests are essential for any detailed epidemiology investigation.

Further evidence of antigen differentiation among trypanosome species was obtained when antibodies were produced in goats immunized with uncoated forms of *T congolense* grown in the laboratory. When these antibodies were ingested by flies infected with *T congolense*, they interfered with the development of the parasites (Figure 18), but they had no significant effect on trypanosome development when ingested by flies infected with *T b brucei* or *T vivax.*

For *T b brucei*, antigens have been identified which are characteristic of specific stages in the parasite life cycle. Antigens have been found which differ between forms of the parasite found in the vector and in the mammalian bloodstream, and even between the long, slender and the short, stumpy bloodstream forms. These antigens may play a role in the transition of the parasites from one stage to the next: they are currently being isolated and their functions studied in more detail.

The role of trypanosomes and their breakdown products in the disease process is also being investigated. It was found

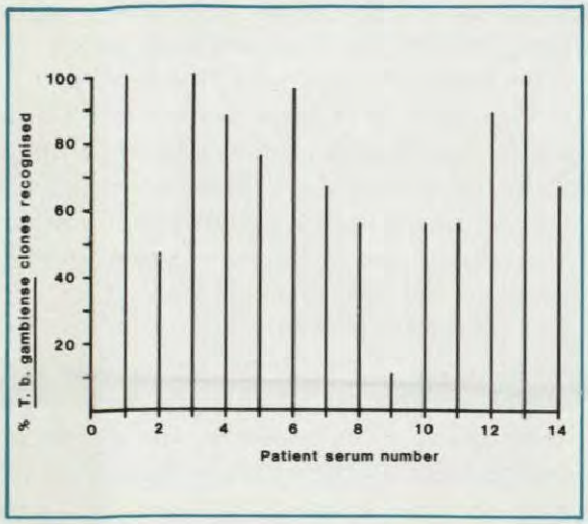


Figure 16. Percentage of 14 sera from human trypanosomiasis patients in Zaire recognizing the VATs carried by 17 clones of T b gambiense *derived from 4 isolates from different patients in Zaire.*

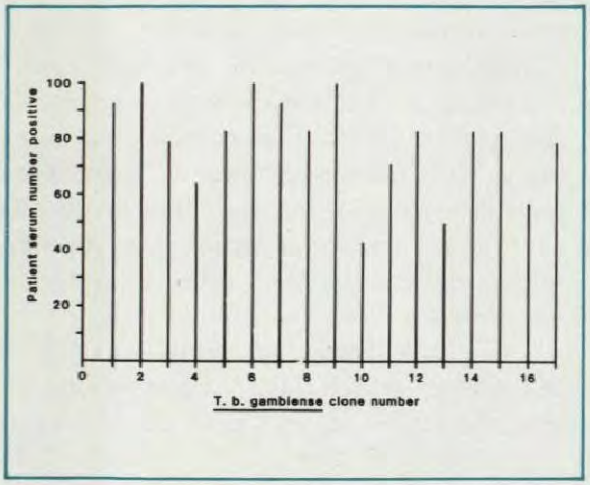


Figure 17. Percentage of 17 clones of T b gambiense *derived from 4 isolates from patients in Zaire recognized by individual sera from 14 different trypanosomiasis patients in Zaire.*

that all pathogenic species of African trypanosome can induce aggregation of platelets in the bloodstream of domestic ruminants and laboratory rodents (Figure 19). Platelet aggregation is likely to be the first stage of the coagulation disorders and tissue damage that are significant pathological effects of trypanosomiasis. In 1981, this work was extended to cover a limited number of wildlife species in a collaborative project with the Kenya Government, the Canadian International Development Association (CIDA) and the Netherlands Government. Work is now under way to isolate and characterize the specific parasite components which induce platelet aggregation, with a view perhaps to blocking their activity by the stimulation of immune responses. This work is being carried out in collaboration with the University of California at San Diego in the USA, supported by the UNDP/World Bank/WHO Program for Research and Training in Tropical Diseases.

THE DEVELOPMENT OF IN VITRO CULTURE SYSTEMS

Significant progress continues in the development of techniques to cultivate and maintain trypanosomes in the laboratory. A major achievement in 1981 was the growth in culture of a population of *T vivax* which retains the morphology and animal infectivity of the parasite bloodstream form. This work was carried out in collaboration with scientists from the Swiss Tropical Institute. The culture system for parasites which resemble the *T congolense* bloodstream form has also been improved. With the addition of new technology developed at the University of Edinburgh's Centre for Tropical Veterinary Medicine, ILRAD now maintains forms similar to all stages of the *T congolense* life cycle in vitro (see Figure 20). The development of a system to maintain forms of *T b brucei* similar to all stages of

the life cycle was a major achievement in earlier years. These in vitro culture systems have greatly facilitated parasitological, immunological and biochemical investigations which could not be carried out until the parasite could be maintained outside its mammalian host.

PARASITE-VECTOR INTERACTIONS

Colonies of five tsetse species were maintained at ILRAD in 1981, as described later in this report. Research focuses on the interactions between the parasites, the tsetse vectors and the mammalian hosts. The goal is to develop a fuller understanding of the role of the vector in the epidemiology of trypanosomiasis in different environments and ultimately more effective methods of disease control.

An extensive study was carried out in 1981 on the feeding behaviour of tsetse flies infected with *T vivax*, *T congolense* and *T b brucei*, compared with the behaviour of non-infected flies. It was shown that trypanosome infections do not significantly impair the tsetse's feeding ability nor increase the frequency of probing before feeding, as had been proposed by other researchers.

In a collaborative study with the Swiss Tropical Institute, it was shown that *T b brucei* infections do not alter the ultrastructure or function of midgut cells in tsetse flies, though trypanosome infections are often accompanied by secondary bacterial infections in the tsetse gut. Thus it appears, at least in the areas studied, that trypanosome infection does not exert a significant pathogenic effect on tsetse flies.

While tsetse flies normally show feeding preferences for certain hosts in the field, work at ILRAD with *Glossina morsitans morsitans* demonstrated that survival, fecundity and mean pupal weight did not differ significantly when flies were fed artificially on defibrinated blood from

	Infection rates in Tsetse	
	Immunized Goats	Non-immunized Goats
Site		
midgut	16 (9%)[a]	47 (28%)
proboscis	5 (3%)[a]	41 (24%)
Number of tsetse examined	171	168

[a] Significantly different from controls $\chi^2 = 20.67, P>0.001$.

Figure 18. The effect on infection rates of feeding G m centralis *infected with* T congolense *on goats immunized with uncoated forms of* T congolense *grown in vitro.*

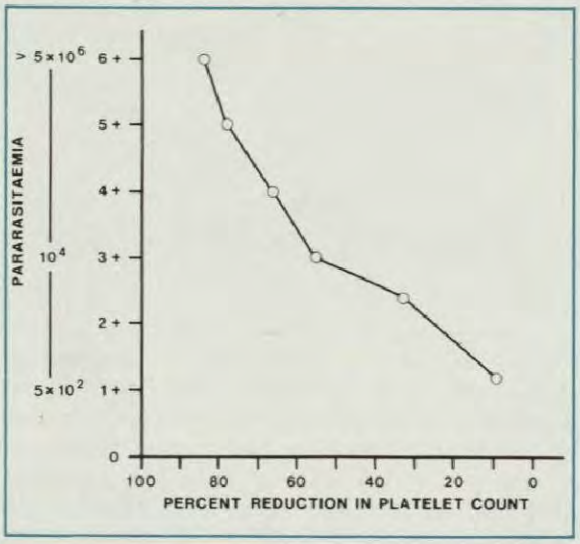


Figure 19. Thrombocytopaenia, induoed by platelet aggregation, develops with the onset of parasitaemia in trypanosome-infected animals. The degree of thrombocytopaenia is associated with the level of parasitaemia, as shown in this Boran steer which was infected with T congolense.

several mammalian species. In 1981, it was shown that proteins found in tsetse haemolymphs after digesting blood meals from different animals were remarkably consistent in terms of size and electrophoretic mobility, though the blood proteins of the species on which the tsetse had been fed differed markedly in these characteristics. These tsetse are thus able to maintain a physiological balance in terms of protein metabolism, even when the proteins they ingest come from a wide variety of animals on which they do not normally feed.

Investigations were also conducted on the infection rates and transmission characteristics of sterile male *G m morsitans* infected with *T vivax*, *T congolense* and *T b brucei*. No significant differences were observed between the gamma-irradiated sterile males and the non-irradiated controls. These results indicate that the release of sterile males as part of a tsetse control program could increase the immediate risk of trypanosomiasis in the affected area unless appropriate measures are taken, such as the strategic use of trypanocidal drugs. This study continues in 1982, with support from IAEA.

Figure 20. Animal infective T congolense *(Stock 687) propagated in vitro. The cultures were initiated by adding infected proboscides of tsetse vectors (*G m centralis*) in the presence of bovine dermal collagen explants and maintained over 1 year in HEPES (25 mM)-buffered Eagle's MEM with Earle's BSS and 20% (v/v) heat-inactivated fetal bovine serum at 28°C. Animal-infective forms were harvested 2 to 3 times a week from day 30 onwards by passing the cultured trypanosome populations, which consisted of non-infective trypomastigotes, epimastigotes and metacyclic forms, through a DEAE cellulose (DE52) column. The majority of the harvested parasites resembled metacyclic forms. Intradermal inoculation produced local skin reactions in cattle 7 days after inoculation. The trypanosomes shown here were harvested from the cultures on day 398 and stained with Giemsa solution.*

PARASITE-HOST INTERACTIONS

An understanding of the complex interactions between trypanosomes and their mammalian hosts is fundamental to the development of measures to control the disease. Research in 1981 concentrated on factors which affect the host's immune response, critical stages in the pathogenesis of the disease and genetic differences which enable some species and breeds to survive trypanosome infections better than others.

When cattle or goats are exposed to metacyclic VATs through the bite of infected tsetse flies and treated 15 days later with trypanocidal drugs, they are immune

when bitten later by flies infected with the same serodemes of *T congolense* or *T b brucei* (see Figure 21). However, to date metacyclic infection and treatment has not conferred immunity to *T vivax*. The onset and duration of immunity induced in this way was studied in more detail in 1981 for *T congolense* infections. Only animals treated 15 days or more after infection were subsequently resistant to challenge with the same serodeme: animals treated sooner remained fully or partly susceptible to reinfection. Successful immunity was shown to last as long as 6 months after initial infection and treatment. This immunity was shown to be directed against metacyclic VATs by neutralization assays, failure of chancre appearance on rechallenge and the absence of detectable trypanosomes in the lymph channels draining the site of the rechallenge bite.

It has been known for some time that cattle which undergo chronic trypanosome infections and recover without treatment are subsequently resistant to reinfection by the same serodeme. These animals would have been exposed and responded to the full range of bloodstream VATs. A similar situation has been induced experimentally by infecting animals directly with bloodstream trypanosomes. It was shown earlier that animals infected in this way with serodemes of *T b brucei* resist challenge by the metacyclic form of the same serodemes when bitten later by infected flies. In 1981, it was shown that cattle infected with bloodstream forms of *T congolense* and *T vivax* also resist subsequent challenge by the metacyclics of the same serodemes. The ability to render animals immune in this way to the metacyclic form of *T vivax* is particularly encouraging in view of the failure of previous attempts based on metacyclic challenge and treatment.

Studies were initiated in 1981 on the use of irradiated trypanosomes and isolated variable antigens to induce an immune

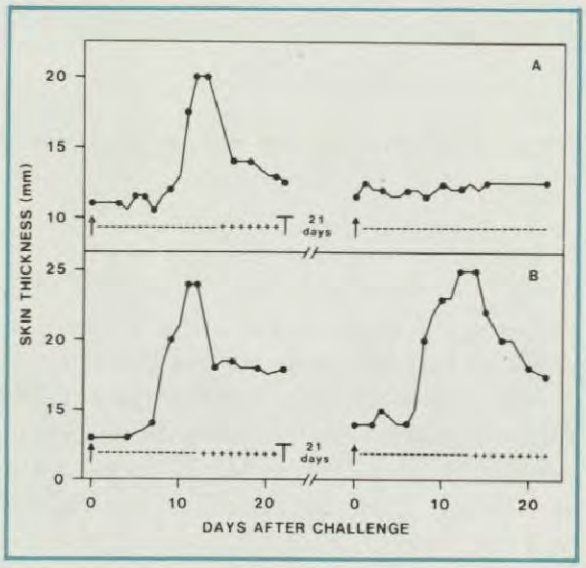


Figure 21. Cattle infected through the bite of tsetse infected with T congolense *and treated with the trypanocidal drug Berenil (Hoechst) 21 days later were subsequently immune to homologous cyclical rechallenge with the same serodeme (A). This was assessed by the failure to develop chancres or parasitaemia and by the development of antibodies which completely neutralized the metacyclic population. However, these animals were completely susceptible to rechallenge with tsetse infected with a different serodeme (B). Chancres and parasitaemia developed in these animals as rapidly as in susceptible controls.*

Dose of Irradiated Trypanosomes	Peak Antibody Titres				Protection Against Challenge with Homologous Trypanosomes
	Neutralization	Farr Assay	Immunofluorescence IgM	IgG	
10^9	10^4	1.78	128	128	complete
10^8	10^2	0.75	16	16	complete
10^7	10^2	0.92	16	16	complete
10^6	10^0	0.18	0	0	partial
10^5	0	nd	0	0	none

Figure 22. Antibody responses and protective immunity elicited in pairs of cattle inoculated intravenously with different doses of irradiated T b brucei *IL Tat 1.3.*

response. Both irradiated *T b brucei* and isolated variable antigens derived from *T b brucei* were shown to induce protection in cattle against challenge by the same trypanosome serodeme. Work focused on the number of irradiated trypanosomes and the type of adjuvant required for use with isolated antigens. It was found that 10 million or more irradiated organisms stimulate a good antibody response and confer protective immunity against rechallenge with the same clone (Figure 22), while a 200 μg injection of purified surface antigen (equivalent to about 500 million trypanosomes) requires the addition of an adjuvant to stimulate protective immunity. Adjuvants such as saponin, which promote mainly humoral immunity, were the most effective.

Immune responses to trypanosomes of more than one serodeme or species are important under field conditions where animals are exposed to mixed parasite populations. It was reported previously that when cattle are infected with a serodeme of *T congolense* and then infected with another serodeme of the same species 5 or 6 weeks later, the establishment of the second infection is significantly impaired. In 1981, this phenomenon was shown to operate in sequences of infection involving different trypanosome species as well as different serodemes within the same species. This finding suggests that levels of parasitaemia may be influenced by factors other than host immune response. Current investigations concentrate on the mechanisms involved and the implications for trypanosomiasis epidemiology.

Another research approach focuses on responses of the host which may limit the expansion of the trypanosome population in the bloodstream. Normally after infection with *T b brucei*, the number of parasites increases rapidly in the bloodstream, declines and increases again in a series of waves. In many cases, the severity of the disease appears to be related to the level and duration of these parasitaemic waves. In some cases with laboratory animals,

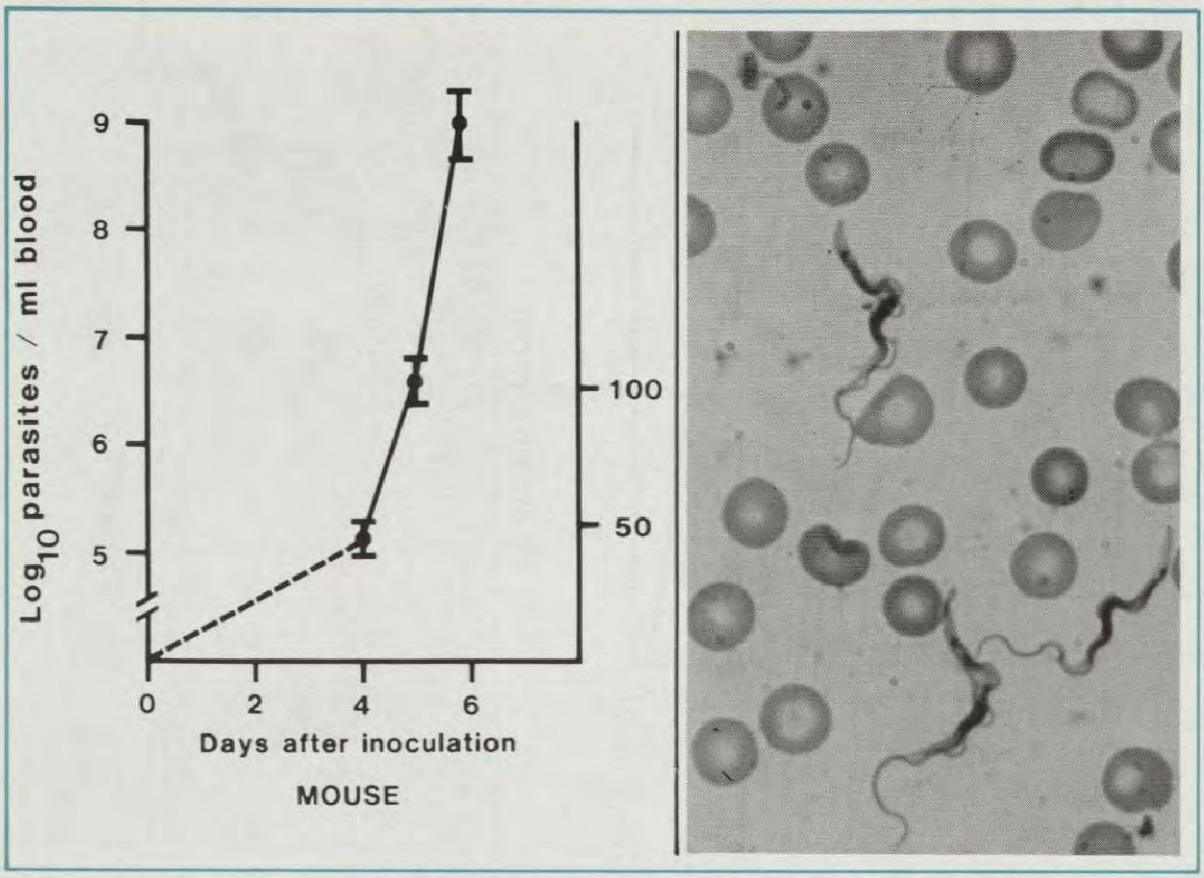


Figure 23. Balb/c mice were inoculated with 10^2 T b brucei *ILTat 1.4 (Lump 227, UHEMBO/64/EATRO/795). Parasitaemia was assessed daily and smears of tail blood were prepared, air-dried, methanol-fixed and Giemsa-stained for analyses of slender and stumpy morphological types, using the method of Wijers (in* Annals of Tropical Medicine and Parasitology, *1959, 53: 59-68). No stumpy forms and fewer than 1% intermediate forms were detected.*

the parasites simply increase until the animal dies.

It has been shown that control of *T b brucei* parasitaemia is dependent on the parasite growth rate and the induction of immune responses in the host. Declines in the parasite population are associated with a change in the parasites from the rapidly dividing long, slender form to the nondividing short, stumpy form and with host production of antibodies specific to the parasite surface antigens. Actively dividing parasites do not stimulate the production of antibodies, but the short, stumpy parasites are senescent and as they die the presence of dead parasites appears to stimulate antibody production. Thus, if the transition to the short, stumpy form could be accelerated, the result would be a quicker immune response and a less severe infection.

Some factor in the host appears to play a role in the timing of parasite transition, because when identical parasites are introduced into mice and then cattle the timing of the transition—and thus the level of parasitaemia observed—differ dramatically in the different host species (see Figures 23 and 24). When the parasites are isolated from cattle and cloned back into mice, their transition rate, and the parasitaemia level, resemble the original situation when they were maintained in mice (see Figure 25). As the trypano-

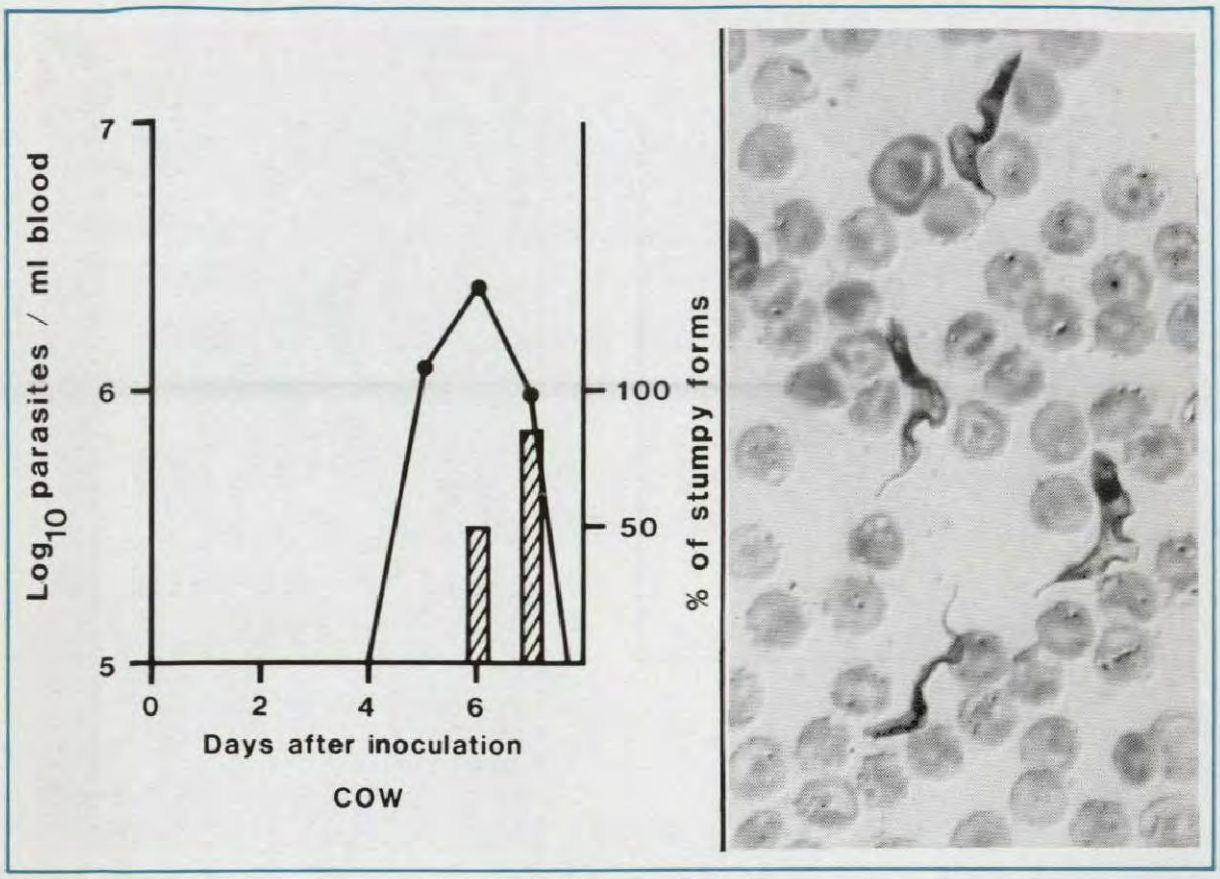


Figure 24. A 6-month-old Hereford heifer was inoculated with 10^5 T b brucei ILTat 1.4 harvested from the mice described in Figure 23. Several waves of parasitaemia were observed and a predominance of stumpy forms was noted prior to remission of each wave. T b brucei *ILTat 1.4 organisms also give rise to stumpy forms in sheep, eland, waterbuck and African buffalo.*

somes used in the experiment all have the same genetic background, the results clearly indicate the role of the host in the transition process.

Studies on pathogenesis continue, with an emphasis on chancre development, platelet aggregation and thrombocytopaenia. The trypanosomes first become established and multiply in the skin at the site of an infected tsetse bite prior to dissemination to the bloodstream via the lymph. The chancre which develops at this site represents the first response of the host to the parasite. Investigations continue on the pathological events which result in chancre formation and the role of immune responses in the skin.

Recently, clinical manifestations of central nervous system involvement have been observed in cattle at later stages of *T b brucei* infections. The basic lesion is a severe meningoencephalitis (Figure 26),

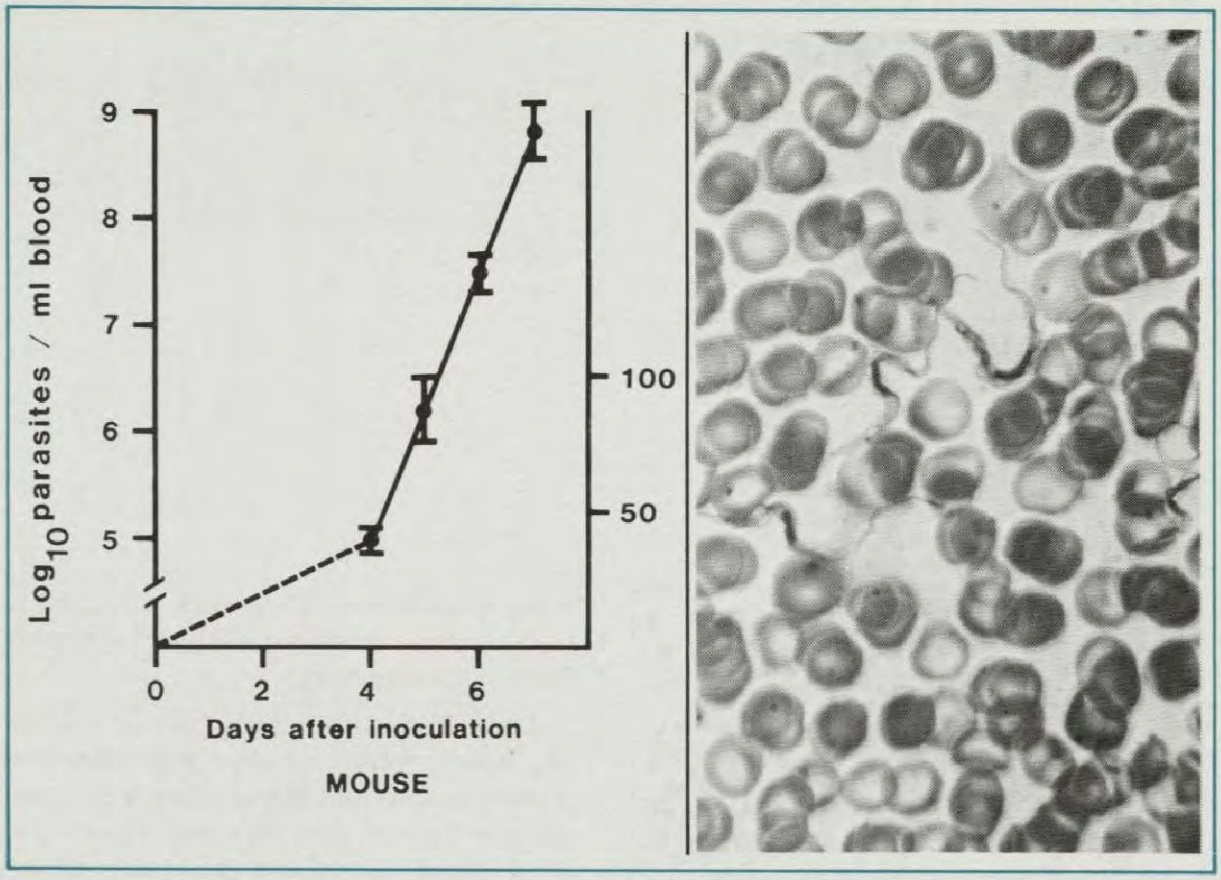


Figure 25. Several subclones of T b brucei *ILTat 1.4, all of the same surface antigenic type, were raised in Balb/c mice from trypanosomes collected 5 days after infection of the calf described in Figure 24. The figure shows the growth of a typical subclone in the mice: all of the subclones resembled the parent clone described in Figure 23 and did not give rise to stumpy forms. Similar results were obtained using subclones derived from the third parasitaemic wave in the calf. These subclones did not express the ILTat 1.4 variant surface glycoprotein.*

similar to that recorded in human sleeping sickness. Cases of central nervous system involvement have also been found in animals which had been treated with trypanocidal drugs: trypanosomes could not be detected in the bloodstream but were present in the brain. This could explain the occasional occurrence of 'relapsing' infections observed in the field.

Certain indigenous taurine cattle breeds of West and Central Africa, the N'Dama and West African Shorthorn, are significantly more resistant to trypanosomiasis than *Bos indicus* breeds. This trait, termed trypanotolerance, has been shown to be an innate genetic characteristic. As such animals are not available in East Africa, a study was made of indigenous East African ruminants to determine if they possess any degree of genetic resistance compared with recently imported breeds and, if so, to investigate the under-

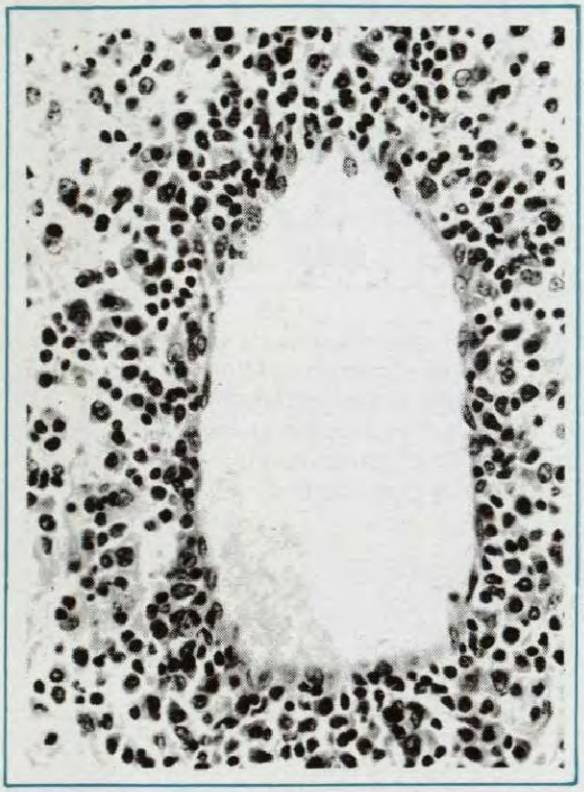


Figure 26. Severe meningoencephalitis in a cow infected with T b brucei. *The perivascular spaces throughout the brain are dilated with fluid and packed with lymphocytes, plasma cells and macrophages.*

lying mechanisms. This work was done in collaboration with the Kenya Veterinary Research Laboratory.

Local *Bos indicus* cattle breeds, including Boran, were compared with imported Ayrshires, and Red Masai sheep were compared with imported Merinos. The indigenous breeds proved less susceptible to trypanosomiasis, as judged by the severity of anaemia and rates of survival. With the indigenous cattle, reduced susceptibility was associated with a greater capacity to control parasitaemia, whereas no differences in parasitaemia were found between different breeds of sheep, though onset of anaemia was later in the indigenous than in the imported breed. These results are being pursued to determine how parasitaemia is controlled in local cattle and the reasons for the milder anaemia observed in Red Masai sheep. However, the degree of reduced susceptibility found in East African types appears to be much less than that of the recognized trypanotolerant breeds of West Africa.

Research in the laboratory has provided results which could be of great relevance to the study of trypanosomiasis epidemiology under different field conditions. Epidemiological investigations of the role of acquired immunity and the existence of the trypanotolerant trait will be empha-

sized in 1982. In collaboration with the International Livestock Centre for Africa (ILCA), these two aspects are being studied on a ranch in Kenya where detailed records have been kept for many years. Based on the number of drug treatments which have been required for trypanosomiasis, there is evidence of both acquired and a degree of genetic resistance in Ayrshire x Sahiwal crossbred cattle.

ILRAD scientists are also collaborating with ILCA in an ongoing study of trypanotolerant livestock in West and Central Africa. Beginning in Zaire, Gabon, Ivory Coast and Nigeria and expanding to Benin, Congo, The Gambia and Senegal, ILCA will assess the biological and economic productivity of trypanotolerant herds and flocks kept under various husbandry conditions. ILRAD will provide assistance in monitoring levels of disease challenge, including tsetse and trypanosomiasis risk■

RESEARCH SUPPORT

TSETSE LABORATORY

Thirty species and subspecies of tsetse are found in tropical Africa. All of these are potential vectors of trypanosomiasis, though the actual vectorial capacity of many species is not fully established.

In addition to research activities described in the trypanosomiasis section of this report, ILRAD's tsetse laboratory maintains breeding colonies of five tsetse species which are used in collaborative research with other ILRAD scientists. In the past, the main colony was of *G m morsitans* originating from the Kariba region of Zimbabwe. Because this species is available elsewhere, ILRAD's colony has been reduced to 1200 breeding females. The colony of *G m centralis*, originating from Singida in Tanzania, has been expanded to 10 000 breeding females and now provides material for all of ILRAD's trypanosomiasis work involving use of the vector. Colonies were established in 1981 of three additional tsetse species. These are *G austeni* originating from Zanzibar and *G tachinoides* from Chad, both of which have reached the target breeding population of 2000 females, plus a colony of *G fuscipes fuscipes* collected from the Lake Victoria area in Uganda which is still being expanded. Present colonies now provide representatives of two of the three main tsetse groups, *morsitans* and *palpalis*. Colonies of two additional species are being established in 1982 — *G pallidipes* and *G brevipalpis*. *G brevipalpis* is a representative of *fusca*, the third tsetse group.

These tsetse species are all being evaluated in terms of their capacity to transmit *T vivax*, *T congolense* and *T b brucei* to ruminants. Earlier work indicated that *G m morsitans* and *G m centralis* are equally efficient vectors of the three trypanosome species. Work in 1981 showed that *G austeni* has a much lower *T congolense* infection rate than the two subspecies of *G morsitans* when fed on the same infected goat.

In addition to tsetse produced for ILRAD's research, flies were supplied in 1981 to the Centre de Recherche et d'Elevage in Togo and to the Swiss Tropical Institute.

TICK LABORATORY

ILRAD's tick laboratory maintains breeding colonies of three strains of *Rhipicephalus appendiculatus* and one strain of *R evertsi*, all of which transmit *T p parva* and *T lawrencei*. A second tick species is also maintained, *Amblyomma variegatum*, the vector for *T mutans*. The tick laboratory

routinely provides ILRAD scientists with infected and uninfected ticks, tick organs and tick-derived *Theileria* stabilates.

When cattle are challenged with tick-derived stabilates, the course of infection is monitored and the infectivity of the stabilates is tested. Research is also carried out in collaboration with other scientists involved in ILRAD's theileriosis program, including a long-term study on the factors which affect *Theileria* infection levels in ticks.

FARM ANIMAL PRODUCTION

The ILRAD calf-rearing facility provided 200 calves for research in 1981, and 110 weaned calves were purchased from selected ranches in Kenya. A total of 380 cattle were used for research throughout the year, including animals which were available at the end of 1980. Of this total, roughly 60% was used for ECF experimentation and 40% for work on trypanosomiasis. This increase in the proportion of cattle used for ECF research over previous years partly reflects the implementation of ILRAD's ECF epidemiology program at the Kenya coast, with animals used for the isolation and characterization of ECF strains and for cross-immunity trials. ILRAD scientists also carried out collaborative research with the Kenya Veterinary Research Laboratory on trypanosomiasis in cattle. In 1981, 80 animals were used for this work, housed at the government laboratory.

A total of 337 sheep and goats were used throughout the year for trypanosomiasis research. The screened accommodation available at ILRAD for trypanosomiasis experiments can accommodate 300 small ruminants at one time. This has been sufficient in the past, but an increase in trypanosomiasis work with the development of an epidemiology program may require the construction of additional fly-proof facilities.

As in previous years, research was constrained to some extent in 1981 by a shortage of cattle, partly because of difficulties in purchasing suitable animals and partly due to a shortage of accommodation. Both of these problems were overcome at the end of the year with the completion of a new experimental cattle holding unit at ILRAD and the purchase of a 13 000 ha ranch on the Kapiti plains about 80 km from Nairobi. Over 2000 Boran (*Bos indicus*) cattle were purchased with the ranch, including a breeding herd of about 750 mature cows and 220 heifers (see Figure 27). Plans are to increase the breeding herd to 1200 animals. Offtake should be sufficient to meet ILRAD's

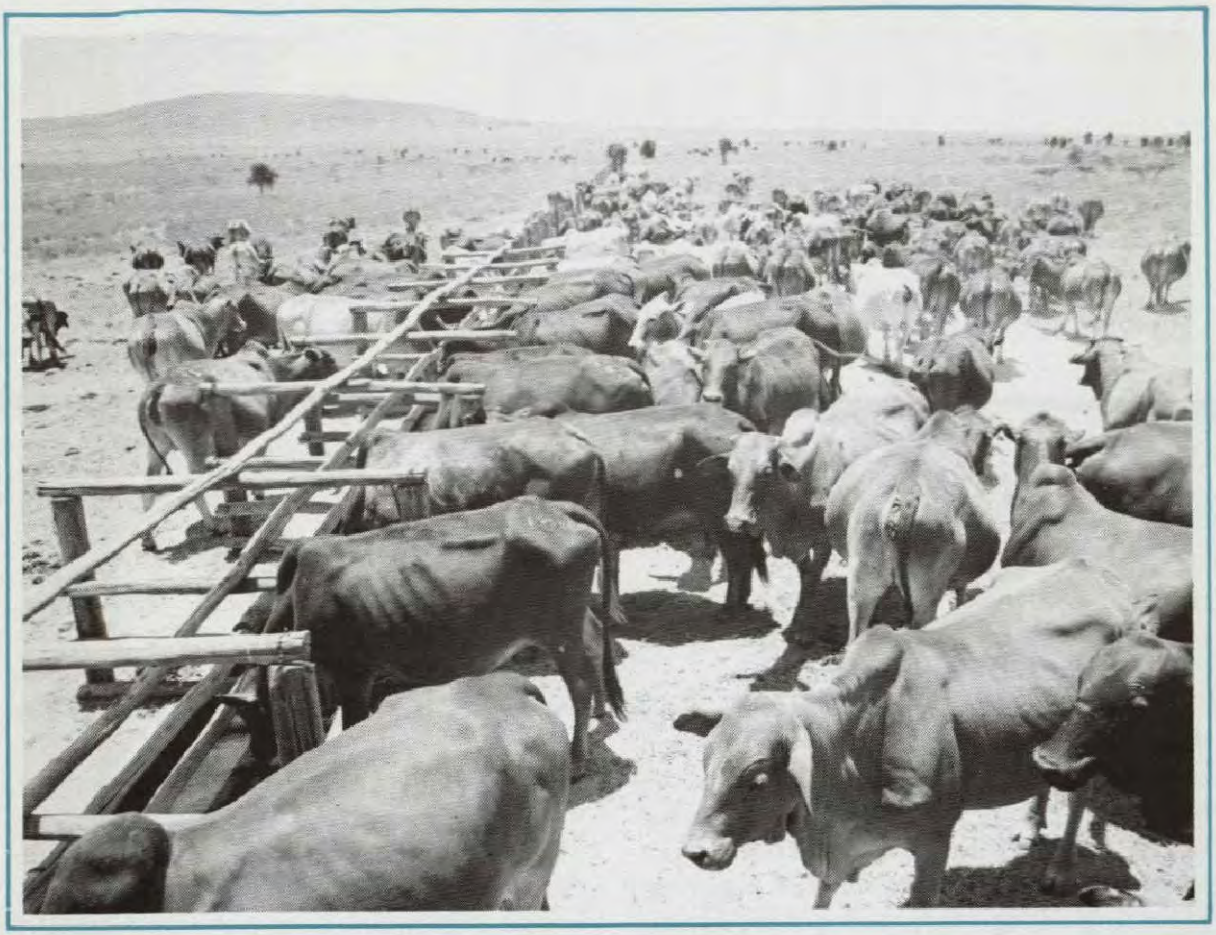


Figure 27. Boran herd at ILRAD ranch on the Kapiti Plains.

future research needs, but specific requests by scientists for cattle of European (*Bos taurus*) breeds will still be met by purchases from outside.

LABORATORY ANIMAL PRODUCTION

ILRAD's breeding unit supplied nearly all the small animals used in the laboratories in 1981 and supplied animals to several national and international research organizations working in Kenya. Five inbred mouse strains and one inbred rat strain are maintained. The mice are used to study the mechanisms of resistance to trypanosomiasis: important differences in resistance between different mouse strains have been observed. The rats are used primarily to produce the trypanosomes required by ILRAD scientists. Rabbits are bred to produce sera used for research and diagnostic purposes and to support the tsetse and tick colonies.

In 1981, ILRAD supplied laboratory animals as available to the International Centre of Insect Physiology and Ecology (ICIPE), the Kenya Trypanosomiasis Research Institute, UNESCO's (United Nations Educational, Scientific and Cultural Organization) Integrated Project on Arid Lands, the University of Nairobi and the National Museums of Kenya.

CLINICAL AND DIAGNOSTIC SERVICES

In 1981, ILRAD's diagnostic laboratory continued to provide routine analyses for the research laboratories and animal production facilities. During the year, a total of 11 645 samples were received: 10 161 for serological, 839 for haematological, 385 for bacteriological and 260 for helminthological analysis.

Serological screening and titration were provided for six bloodstream parasites found in cattle — *Trypanosoma brucei brucei, T congolense, T vivax, Theileria parva parva, T mutans* and *Anaplasma marginale*. Most routine screening was by the indirect fluorescent antibody test (IFA) and the enzyme-linked immunosorbent assay (ELISA). The ELISA test was used for the routine screening of bovine sera for the three trypanosome species. This technique will also be applied to detect antibodies to *T p parva* schizonts and *T mutans* piroplasms. The card agglutination test is used to screen sera for *A marginale* antibodies.

The capacity was also developed to screen sheep and goat sera for antibodies to the three trypanosome species. Requests for routine bacteriological screening of small laboratory animals were fewer than in previous years as ILRAD became increasingly self-sufficient in this area and very few animals were purchased from outside.

During 1981, 180 post-mortem examinations were carried out as part of the veterinary diagnostic services. Of these, 131 cattle were examined and 49 sheep and goats.

Apart from services provided to ILRAD scientists, the diagnostic laboratory assisted other centres and projects. Tests for *A marginale* antibodies were performed for a German (Federal Republic) technical aid project in Somalia, and *T p parva* schizonts and *T mutans* IFA antigens were provided to FAO projects in Zambia and Mozambique and a German (Federal Republic) aid program in Burundi.

LIBRARY

The library provides support for ILRAD's research and training activities and is used by scientists from other research institutes in the Nairobi area. The highly specialized collection included subscriptions to 202 scientific periodicals by the end of 1981, and over 2000 books. Journals are received in the fields of biochemistry, parasitology, immunology, pathology, cell culture and entomology, among others. The library also receives articles requested by ILRAD scientists through a regional interlibrary loan system and by purchase from the British Library Lending Division.

Conference papers and journal articles published by ILRAD scientists are held in the library, and copies distributed on request. Close cooperation is also maintained with the libraries of the University of Nairobi's Faculty of Veterinary Medicine, the Veterinary Research Laboratory of the Kenya Agricultural Research Institute, ICIPE and the Kenya Trypanosomiasis Research Institute.

TRAINING AND . . .

Recent science graduates and senior technicians participated in ILRAD's research program in 1981, receiving training in specialized scientific areas and laboratory techniques. These trainees were working within the scope of ILRAD's research mandate, and most were from African countries. The number of trainees who can be accomodated at any level depends on the availability of supervisory staff, laboratory space, equipment and accommodation. Within budgetary limits, a few individuals are also sponsored for training at other institutions.

Eight post-doctoral fellows worked at ILRAD in 1981—one from Belgium, one from India, two from Kenya, one from Japan, one from the Netherlands, one from the USA and one from Zaire. These scientists are recruited on an international basis and generally stay at ILRAD for 2 years. Their work falls directly within ILRAD's research program.

At the post-graduate level, 14 trainees worked with ILRAD scientists in 1981, half of them from Kenya. Graduates undertake research projects which last from 1 to 3 years, and they generally submit work done at ILRAD for a higher degree at the University of Nairobi or at other universities overseas. In 1981, one graduate was working at ILRAD from Australia, two from Germany (Federal Republic), seven from Kenya, one from Rwanda, two from Sudan and one from Uganda.

Ten scientists and senior technicians from four African countries received training in 1981 in specialized laboratory techniques. Two Kenyans were trained in immunodiagnostic techniques, and two others in trypanosomiasis pathology. Four Ethiopians were trained in parasitology, one Togolese worked on tsetse flies as trypanosomiasis vectors and one Nigerian learned in vitro cultivation techniques. All were staff members of university laboratories or national research centres in their home countries.

ILRAD also provided support for three scientists to attend the Third Training Seminar on Trypanosomiasis, sponsored by the OAU, FAO and WHO in Arusha, Tanzania. Two of these participants were from Togo and one was from The Gambia. In addition, 18 ILRAD technicians were sponsored for training courses at the Kenya Polytechnic.

A regular seminar series was initiated in 1981. Seminars were held every 2 weeks, providing a forum for researchers from centres in Nairobi and visiting scientists to discuss their current research projects. These seminars were well attended by ILRAD staff and scientists from the

OUTREACH

University of Nairobi and other national and international institutes.

An international conference was held in February 1981 on Advances in the Control of Theileriosis, sponsored by ILRAD, FAO, UNDP, IAEA and the Danish International Development Agency (DANIDA). Altogether, 185 participants attended, including 85 from Kenya and 48 from other African countries. The remainder were from Australia, Brazil, India, Israel, Pakistan, the UK and the USA. Papers presented at the conference were published in a proceedings volume.

An exhibit of ILRAD's scientific work was prepared for the Nairobi International Show in September 1981. This show is sponsored by the Agricultural Society of Kenya. ILRAD's exhibit was visited by thousands of Kenyans, including farmers, businessmen, government officials and school children, and received a second prize from the show committee. A booklet on ILRAD's activities was distributed at the show and to visitors to the laboratory.

Publications by ILRAD scientists in 1981 included 59 journal articles, chapters in books and published conference papers. Reprints of journal articles and other publications are distributed to scientists and research centres around the world. ILRAD's 1980 annual report was also prepared in 1981 and widely distributed.

ILRAD staff members have been instrumental in setting up the Nairobi Cluster, an organization to promote collaboration between scientists in Kenya working on trypanosomiasis and ECF and their vectors. Current members of the cluster include ICIPE, the Kenya Veterinary Research Laboratory, the Veterinary Research Department of the Kenya Agricultural Research Institute, the Kenya Trypanosomiasis Research Institute, the OAU's Inter-African Bureau of Animal Resources, ILCA, UNESCO's Integrated Project in Arid Lands, the University of Nairobi's Faculties of Medicine and Veterinary Medicine and the Clinical Research Centre of the Kenya Medical Research Institute, as well as ILRAD. ILRAD scientists also serve on the WHO Steering Committee on Immunology and Pathology of African Trypanosomiasis, the FAO Panel of Experts on the Ecology and Technical Aspects of the Program for the Control of Animal Trypanosomiasis and Related Development, and the FAO Expert Panel on Ticks and Tick-borne Diseases.

In addition to the institutions in the Nairobi Cluster, research and technical collaboration was in progress in 1981 with the Kenya Ministry of Livestock Development, the Centre de Recherche et d'Elevage in Togo and the national veterinary laboratories of Botswana, Burundi, Malawi, Mozambique, Rwanda, Tanzania and Zimbabwe. Outside Africa, ILRAD scientists collaborated with colleagues at the Prince Leopold Institute for Tropical Medicine, the Vrije Universiteit Brussel and the Libre Université de Bruxelles in Belgium, the Swiss Tropical Institute, Cambridge, Glasgow and Edinburgh Universities in the UK and the Universities of California, Iowa, New York (Stony Brook), Washington and Washington State in the USA■

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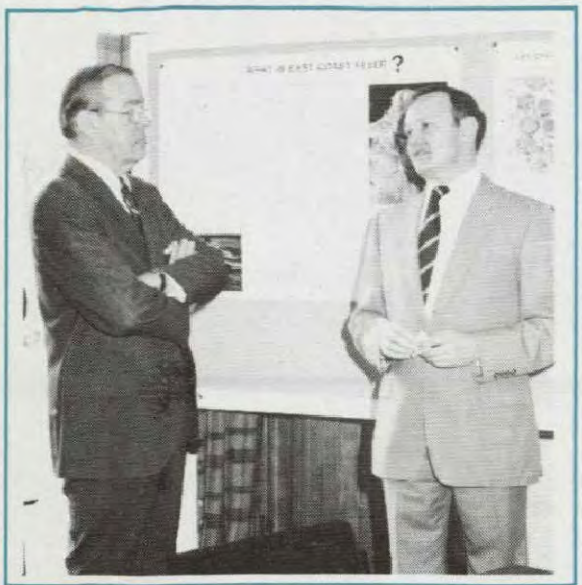

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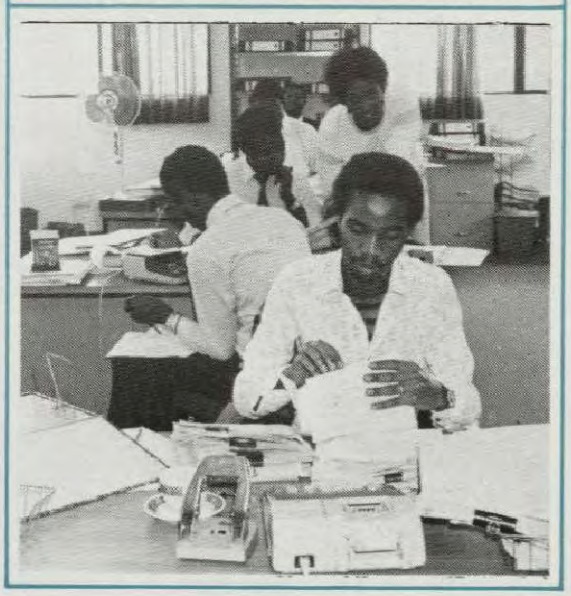

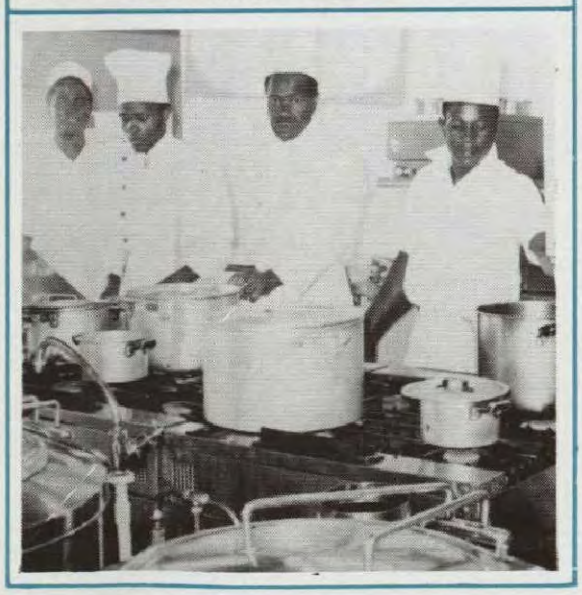

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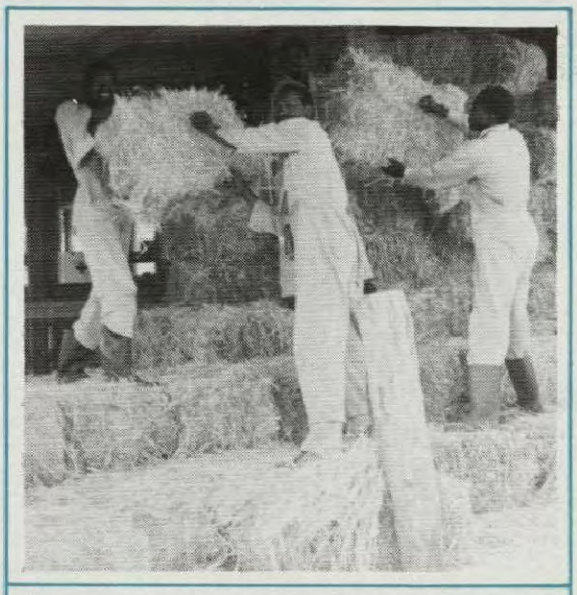

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