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ILRAD

ANNUAL REPORT 1980

INTERNATIONAL LABORATORY FOR RESEARCH ON ANIMAL DISEASES

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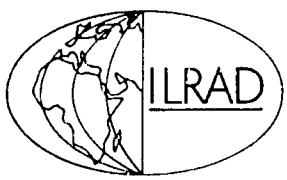

ANNUAL REPORT
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Naivasha Road, Kabete
P.O. Box 30709
Nairobi, Kenya

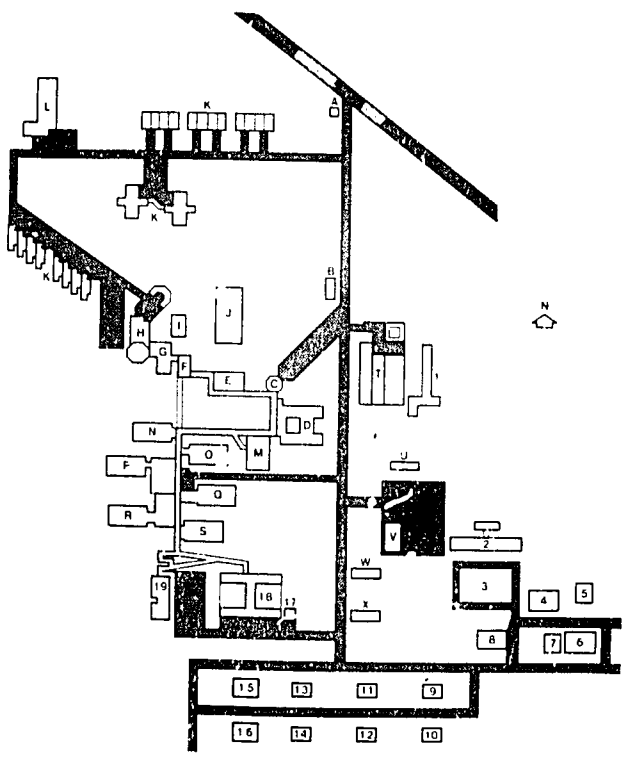
Telephone 592311
Telex 22040 ILRAD

ILRAD, the International Laboratory for Research on Animal Diseases, is supported by the Consultative Group on International Agricultural Research (CGIAR), which is in turn sponsored by the Food and Agricultural Organization of the United Nations, the World Bank and the United Nations Development Programme. The purpose of CGIAR is to improve the production of food crops and animals in the tropics and sub-tropics. Fourteen donors sponsor ILRAD directly and they are listed on page 29.

ILRAD, situated on 69 hectares near Nairobi, Kenya, was established in 1973 under an agreement with the Government of Kenya. Two diseases with far reaching deleterious effects on livestock production in Africa—trypanosomiasis and theileriosis—were selected for initial attack.

This report summarizes the research carried out at ILRAD during 1980. It also describes various training activities as well as those of the essential support services over the same period. It should be noted that ILRAD research projects and results are summarized here in a brief and simplified way. Scientists interested in the detailed findings of the reported research should refer to the list of publications on page 30.

ILRAD EXPERIMENTAL AND GENERAL FACILITIES



A Main gate
B Services office
C Main entrance
D Administration
E Conference hall
F Reception
G Social facilities
H Kitchen
I Swimming pool
J Tennis court
K Staff houses
L Tsetse unit
M Library
N Lab 1
O Lab 2
P Lab 3
Q Lab 4
R Lab 5
S Lab 6
T Main stores
U Store
V Transport yard
W Store
X Changing room

1 Clinical medicine/ diagnostic laboratory
2 Calf rearing building (production)
3 Weaners yard (production)
4 Old dip (outside holding pens)
5 Old calf rearing unit (production)
6 Experimental cattle building
7 Spray race (experimental pens)
8 Feed barn/farm office
9 Isolation unit 1 (experimental pens)
10 Isolation unit 2 (experimental pens)
11 Isolation unit 3 (experimental pens)
12 Isolation unit 4 (large animal surgery)
13 Isolation unit 5 (experimental pens)
14 Isolation unit 6 (experimental pens)
15 Tick facility
16 *T. vivax* building (experimental pens)
17 Radiation source
18 Laboratory animal unit
19 Pathology — post-mortem room

CONTENTS

The goat, an important animal in the African economy, is one of the domestic species susceptible to trypanosomiasis.

The mandate of ILRAD as established by the Consultative Group for International Agricultural Research (CGIAR) and the Board of Directors is to develop effective control methods for trypanosomiasis and theileriosis. After five years of operation ILRAD is on its way to accomplishing this mandate.

There have been considerable changes in the membership of the ILRAD Board of Directors over the last year. Five members who had been on the Board since its foundation in 1973 retired in 1980. These were Dr J. Pino, Chairman of the Board since its inception, Dr M. Cohen, Dr H. Goodman, Dr S. Toure, and Dr E. Weiss.

In addition, Dr A.C. Allison, the Director of ILRAD, resigned with effect from 30 November 1980. Another Board member, Dr K.F. Wells, was the Acting Director for the rest of the year.

All these people have contributed greatly to ILRAD's development and achievements, and the ILRAD staff and I wish to express sincere appreciation for their efforts.

The new Board members are: Dr L.L. Callow, Dr K. Eichmann, Dr P. Atang, Dr K.S. Warren, and Dr G.L. Kazyumba.

During 1980 ILRAD had its first Quinquennial Review by a committee of the CGIAR. The committee reviewed all aspects of ILRAD development and performance. The scientific staff at ILRAD is proud of the fact that the committee was well satisfied with the high standard of ILRAD's research work. The committee also supported the concept of mission-orientated research as the best means for tackling two of the most difficult disease problems in veterinary medicine.

ILRAD is in a unique position in that, at least so far as trypanosomiasis is concerned, it can test in sheep, goats and cattle some of the research findings obtained in laboratory animal models. The tendency, therefore, to use more ruminants for its research has been extended in 1980.

The progress ILRAD has made in 1980 in research is summarized in the main body of this report. I would, however, like to briefly mention some of the significant research achievements of 1980.

Our basic understanding of the mechanisms involved in an animal's ability to resist trypanosome infections has been extended. Susceptibility has been shown to be unrelated to major histocompatibility-associated genes which are involved in the control of immune responses. The ability of an animal to respond to *Trypanosoma brucei* infections is now known to depend on transformation of the parasite from its long slender bloodstream form to its 'stumpy' or tsetse-infective bloodstream form. The rate of this transformation is largely host dependent.

The *in vitro* cultivation of pathogenic trypanosomes has now been extended, following the early success with *T. brucei,* to the cultivation of animal-infective bloodstream forms of *T. congolense.*

The isolation and purification of genes of trypanosomes that code for variable antigens of *T. brucei* and the subsequent insertion of these genes into bacteria is a major step in the use of recombinant DNA technology in the study of trypanosomiasis.

For theileriosis, significant progress has been made in the laboratory identification of strains of *Theileria parva.* Two techniques, one involving recognition of parasite antigens by monoclonal antibodies, and another by induction of specific cytotoxic cells, promise to be useful *in vitro* systems for identification of parasite strains. Until now this has been possible only by cross immunity studies in cattle.

Epidemiological studies in theileriosis began in 1980 in collaboration with the Kenya Government Veterinary Department. Basic information on this disease is necessary for the development of vaccination programmes.

The ability to infect bovine cells *in vitro* with different strains of *T. parva* has further elucidated the immune responses of infected cattle. It is now evident that the host responds to the surface of parasitized cells and that recognition occurs in combination with its own (self) antigens. This is reflected by the fact that it is possible to infect and immunize animals successfully with low numbers of their own cells which have been infected by the parasite, but much greater numbers of cells are required to infect animals if the cells used are not their own and immunization is much more uncertain. The demonstration that during the course of infection large numbers of immune cells are generated which non-specifically kill other cells possibly explains the large-scale destruction of lymphoid cells in the terminal stages of this disease.

Research and facilities have now reached a point where ILRAD can offer new technologies and expertise for investigating the epidemiology of theileriosis and trypanosomiasis. Such studies will be carried out as an integral part of programmes established by national and international organizations concerned with the control of these diseases. These activities will also provide an important feed-back of information to keep ILRAD's laboratory programmes based on problems relevant to the control of disease under field conditions.

None of the measures used at present to combat trypanosomiasis has controlled or eradicated the parasite. Measures such as tsetse control, chemoprophylaxis, the use of trypanotolerant cattle, all have their individual uses and limitations. ILRAD is now in a position to contribute towards a better understanding of the interaction of these control measures and in collaboration with, for example, ILCA, to measure their effect on animal productivity. The role of strategic drug therapy in acquisition of resistance, the measurement of trypanotolerance exhibited by West African cattle, and the identification of genetic markers for trypanotolerance, are all areas where ILRAD can contribute to the efficient utilization of the control measures presently available. At the same time, such studies provide essential information for the development and successful utilization of any vaccine procedure in field situations.

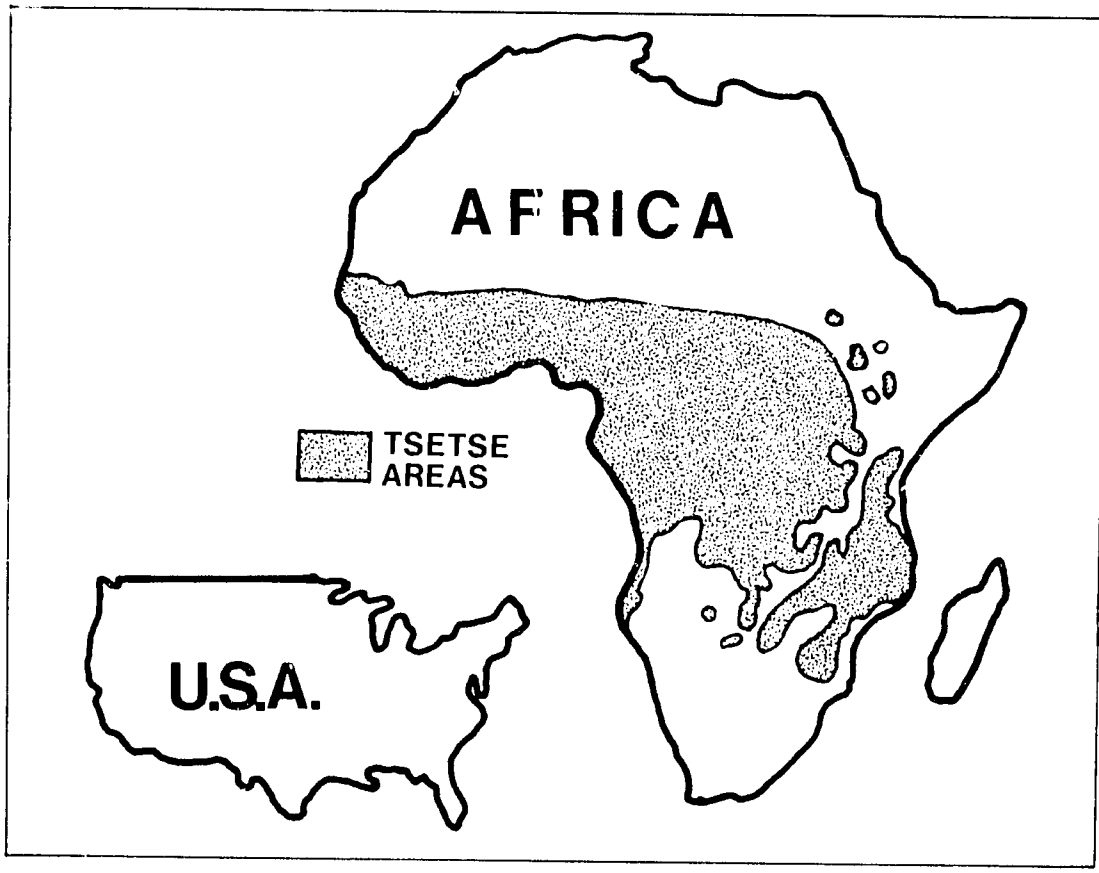
The ultimate success of any control measure depends upon effective implementation by trained personnel in the countries where these diseases occur. ILRAD has therefore placed a high priority on the identification and training of personnel at all levels and will attempt to develop stronger associations with national bodies concerned with disease control and livestock production

In conclusion it can be said that 1980 was a year of many changes in the management of ILRAD. The research, however, has continued to make steady progress. This was mainly due to the strenuous efforts of the ILRAD staff, for which we thank them.

Thanks are also particularly due to the donors who have given ILRAD continued support and encouragement. The research basis which has been laid during the previous five years encourages us to look at the future with confidence.

D. Zwart

Map 1: Tsetse distribution in Africa



RESEARCH ACTIVITIES

TRYPANOSOMIASIS

BACKGROUND

The African trypanosomiases represent a disease complex which affects man and his livestock. The disease in livestock is caused by three species of trypanosome, *Trypanosoma congolense, T. vivax* and *T. brucei,* and these can be transmitted by several species of tsetse (*Glossina*). Tsetse infect 36 countries in Africa, all south of the Sahara; this represents some 10 million km², a land mass larger than that of the United States of America (Map 1). At present this area is largely devoid of cattle, sheep and goats despite the fact that approximately 7 million km² could be used for livestock. It is estimated that exploitation of this region would double livestock production of the African continent. Currently the cattle population of Africa is thought to be around 160 million head. It should also be noted that there are large areas of South America where *T. vivax* is known to occur despite the absence of tsetse.

While each of the three trypanosome species which infect domestic livestock is pathogenic in its own right, mixed infections of all three commonly occur under natural field conditions. The pathogenesis of the disease is dominated by the development of anaemia which acts as a reliable indicator of the progress of the disease. The outcome of infection may be spontaneous recovery, death after an acute or chronic disease, or a chronic syndrome with animals living for years at a very low level of productivity.

The socio economic consequences of tsetse and trypanosomiasis are enormous for Africa. They are listed as follows:

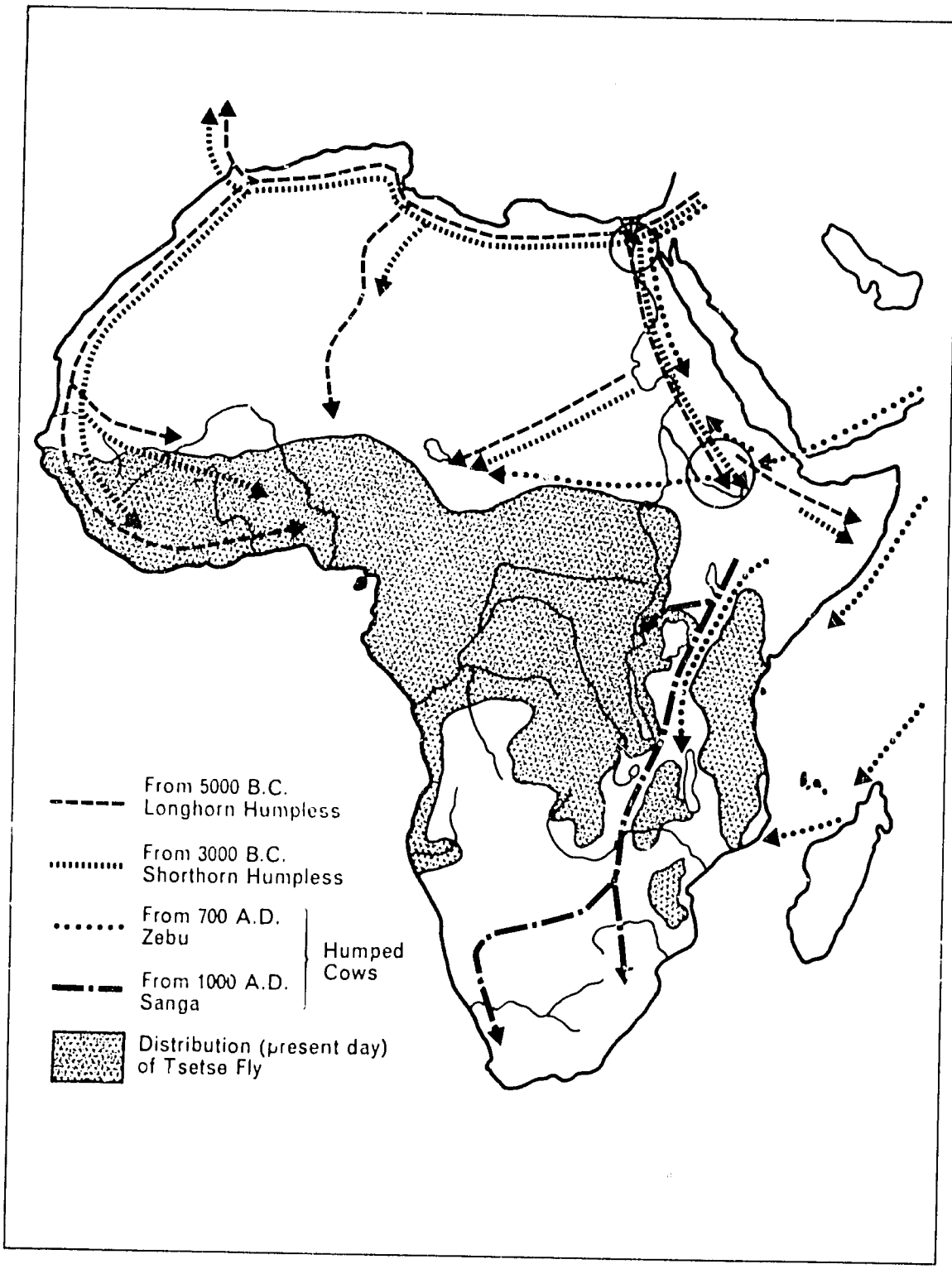
1. There are vast areas where livestock hardly exists because of the presence of tsetse.
2. In areas where it is possible to raise livestock there are huge economic losses due to the disease and the cost of controlling it. These losses are through:
   (a) mortality
   (b) retarded growth, weight loss, abortion, etc.
   (c) the cost of diagnosis and drug treatment
   (d) the cost of tsetse control.
3. Other major economic losses are due to the indirect consequences of tsetse and trypanosomiasis. These include:

   (a) The effect on human health caused by shortage of meat and milk. At present it is estimated that the production of animal protein from livestock farming per 1000 hectares is as follows:
   Africa 542 kg
   Latin America 4,113 kg
   Europe 38,085 kg.

   (b) The impact on agriculture because of the lack of draught animals. It is estimated that the availability of a draught ox to a family unit can increase agricultural output sixfold.

   (c) The effect on livestock production, e.g. improved breeds which are usually susceptible to trypanosomiasis cannot be developed and overpopulation of tsetse-free grazing areas may occur. (See Map 2.)

Tsetse fly feeding on ear of a half-lop rabbit.



Map 2: Origins of cattle in Africa with superimposed pattern of present-day distribution of the tsetse fly.

(d) Damage to national economies by compelling the importation of meat and milk in countries potentially capable not only of being self-sufficient in these commodities but also of their exportation.

4. Human trypanosomiasis, by itself, constitutes one of the major constraints to African rural development with an estimated 35 million people at risk. It has been a major factor in the depopulation of large areas of Africa with the consequent disruption of community structure and the resultant depletion of human resources upon which viable agricultural communities depend. The disease is on the increase and recently there have been several major outbreaks.

Effective methods for control of both animal and human trypanosomiasis are available and in some areas they have been successfully implemented. These methods include diagnosis and treatment, chemoprophylaxis and tsetse control or eradication with insecticides. Unfortunately, 50 years of such control strategies have had little net effect. If anything, the situation is deteriorating. A number of factors are responsible for this.

1. There are 22 species of tsetse capable of transmitting infection. Their adaptation to a wide range of habitats contributes to the widespread nature of the disease.
2. The three trypanosome species pathogenic for cattle exhibit a wide host range for both domestic and wild animals.
3 The phenomenon of antigenic variation with persistent parasitaemia provides an excellent opportunity for transmission of infection by tsetse.

At the same time, the control strategies available present several problems in implementation. The use of drugs, both therapeutically and prophylactically, can be costly because (a) repeated treatments are required, and (b) diagnostic facilities are necessary. Also, repeated use can lead to the development of chemoresistance to the extremely limited available drugs. Tsetse control followed in some cases by eradication has been successful in certain regions, e.g. in northern Nigeria. However, as with drug strategies, the cost is high and it is essential that the control area is kept under rigorous surveillance for several years and protected by natural or man-made tsetse barriers to prevent reinvasion. Furthermore, there is the question of possible environmental hazards from the widespread use of insecticides.

Our current state of knowledge about trypanosomiasis suggests that there are two main lines of investigation which could result in control of the disease in the field. The first is the possibility of the development of a field vaccine. Hope for such a solution lies with the fact that it is now known that resistance can be acquired in the field. The second approach is by the exploitation in endemic tsetse-infested areas of livestock selected for their ability to resist disease. This trait is known to be an innate characteristic and is termed trypanotolerance.

Thus there is a real possibility that basic research combined with an understanding of the field situation will permit the development of an effective means for controlling animal African trypanosomiasis.

Indigenous East African Zebu cattle.

Indigenous East African Boran cattle

RESEARCH RESULTS

ILRAD research into trypanosomiasis during 1980 focused on the following major but overlapping areas: the parasite, vector-parasite interactions and host-vector-parasite interactions. The overall objective of the research programme is to control trypanosomiasis by immunological, genetic or chemical means, either singly or in combination.

The parasite

Studies on the parasite itself can be subdivided into three overlapping areas. The first is research into the phenomenon of antigenic variation, its genetic basis and the structure and synthesis of the antigens involved. The second is the study of parasite antigens other than those responsible for antigenic variation and their possible role in protection, diagnosis and disease. The third is the development and utilization of *in vitro* cultivation techniques for both animal and insect forms of the trypanosome.

Antigenic variation

An understanding of the genetic basis and possible induction mechanisms underlying this phenomenon is important because this process represents a major avenue by which the trypanosomes establish a chronic infection in the face of protective immune responses by their mammalian host. This phenomenon can be simply described by saying that the trypanosomes present in a mammalian host have the ability to change the molecules (variable antigens) which constitute their surface coat in a diverse and rapid manner. Each new coat is different immunologically from the previous one to which the host has mounted a protective immune response. The size of this repertoire of surface variable antigen types (VATs) available to a single trypanosome is therefore of great interest and importance. Preliminary work suggests that this repertoire is large. There appears, however, to be considerable restriction in the number of surface coat types which can be displayed by the animal-infective (metacyclic) trypanosomes in the tsetse after cyclical transmission of trypanosomes possessing the same genetic background, i.e. belonging to the same 'serodeme'. It is therefore highly important to know if this restriction in the number of metacyclic VATs is a factor which contributes to the acquisition of immunity to trypanosomiasis in animals. The development of a vaccine based on the metacyclic VATs circulating in an area may be possible if the number of serodemes present in the trypanosome population of any area is sufficiently low.

In studies of the genetic mechanisms which control this complex phenomenon, the molecular biology group at ILRAD have now been able to identify and isolate, from *Trypanosoma brucei* bloodstream parasites, four genes which code for four different variable surface coat antigens. Using these and related materials, it has been shown that the variability of the surface coat is associated with complex rearrangements of gene structure. This is also consistent with the probability that the trypanosome possesses two copies of each gene and indicates the likelihood that trypanosomes are diploid organisms. It is likely, therefore, that the metacyclic variable antigen composition of any serodeme may be altered in time as a result of sexual-like recombination events.

The isolation and characterization of these four genes allows very rapid and easy analysis of their presence and organization in different trypanosome populations. Evidence has already been obtained, for the one gene studied in detail, that part or all of the information coded in this gene is also present in trypanosomes of other *T. brucei* serodemes, and even populations of another trypanosome species, *T. vivax*.

Investigation of the structure of these genes and their adjoining regions is well advanced. The information obtained from these studies is important for understanding the mechanism of antigenic variation. It may also prove useful in offering alternatives to the present cumbersome and expensive techniques used to assess the number of genetic lines of trypanosome populations and their metacyclic populations present in any area.

Another significant step in the utilization of modern molecular biology techniques was achieved in 1980 when genetic information from *T. brucei* was successfully inserted into bacteria with the resultant production by the bacteria of trypanosome proteins. This could provide a means of obtaining sufficient metacyclic coat material for immunization studies. The conventional tsetse infection techniques produce low numbers of metacyclic trypanosomes which are insufficient for isolation of significant amounts of surface antigens.

Research has continued into the nature and pathway of formation of these variable surface coat antigens in an effort to identify differences in metabolic pathways between the parasite and its host which could be possible sites for drug action. It has been shown that the addition of carbohydrate molecules to the protein of the variable surface antigen is necessary for the formation and stability of these antigens. The sites of attachment, the enzyme pathways used and the nature of the carbohydrates involved have now been elucidated, and some differences in these pathways in the parasite and those in the mammal have been detected.

Studies continue on the number of determinants present in the variable surface antigen which are responsible for the induction of protective immune responses and the occurrence of similar determinants in different variable surface antigens.

The development of *in vivo* technology suitable for the analysis of the VAT composition of tsetse-

transmitted metacyclic trypanosomes has been approached in several ways. The first concerns the appearance of an indurated swelling (the chancre), at the site of an infected fly bite in the skin of a susceptible animal. Experimental evidence now shows, for the trypanosome populations tested, that such a chancre will not appear if the animal has been first rendered immune to the particular gene line (serodeme) of trypanosomes carried by the fly. However, if flies carrying trypanosomes of different serodemes feed on this animal, chancres will develop. This will be a very useful tool to identify the number of metacyclic populations, or different serodemes, in a given area. Another approach to the same question has been to produce antisera both by conventional and by monoclonal methods to the VATs present in metacyclic populations. These can then be used to type the metacyclic variable antigens produced by different serodemes of trypanosomes. Both types of experiments confirm earlier observations that, for a given genetic background, the variable antigen composition of the metacyclic population which develops in the tsetse flies is constant and characteristic for each serodeme but differs between serodemes and is independent of the variable antigen types carried by the trypanosomes ingested by the fly.

While cyclical development of the trypanosome through the tsetse fly produces only a limited number of variable antigen types, infections in the mammalian host are followed by the sequential appearance of a large and undefined number of variable antigen types. It had been thought previously that these types occurred in predictable sequence, but experimental results have shown that, even when infections are initiated with a single trypanosome the order of appearance of new variable antigen types is not constant. New variable antigen types appear to be generated with high frequency. Their sequence of appearance is only somewhat directional, and the number of variable antigen types present in each successive parasite population in a mammalian host is high. There is also some indication, at least in the case of *T. brucei* infections, that the same or very closely related variable antigen types can appear more than once in the course of an infection in a given host. The composition of the variable antigen types of the first detectable trypanosome population in animals infected by flies carrying trypanosomes of the same or different serodemes is not sufficiently constant or characteristic to allow identification of different serodemes of the same species of trypanosome.

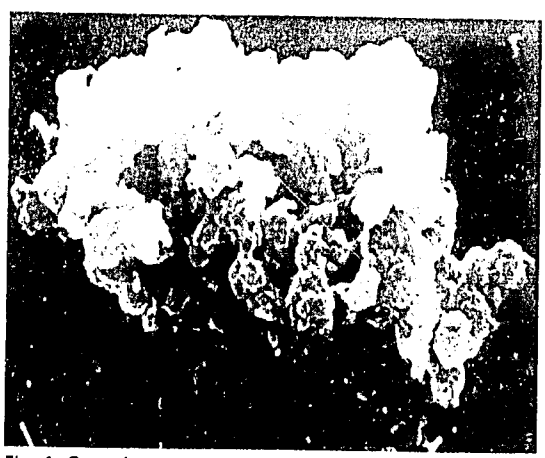


Fig. 1: Scanning electron micrograph of a platelet aggregate influenced by a factor derived from the trypanosome.

Parasite antigens other than variable antigens

Research has also been carried out on the properties of other parasite antigens which are common to all trypanosomes of a given species or are common to all species of trypanosomes with special relevance to their possible role in protection, diagnosis, and induction of disease processes.

Two different types of antigens have been prepared from *T. brucei* to assess their potential as vaccines. The first is a protein common to *T. brucei, T. congolense* and *T. vivax,* and the second is a preparation of the outer cell wall of *T. brucei* without its variable antigen. Both antigens were used to immunize goats and rabbits which were then challenged by infected tsetse flies. No significant protection has been achieved. Another three parasite antigens have been identified using antibodies present in the serum of N'Dama cattle which had recovered from trypanosome infections but which were not detectable in the sera of cattle which died from the disease. These antigens will now be isolated and their role in protection investigated.

Work also continues on the identification of parasite components specific for each major pathogenic trypanosome species. This will allow the development of tests which can be used to characterize the trypanosome species specificity of the antibodies in infected animals. Such an assay is fundamental for detailed investigation of trypanosomiasis as it occurs in the field.

Parasite components also play a role in the disease process. It has been demonstrated that trypanosomes can produce factors which destroy the host's red blood cells, aggregate platelets (Fig. 1) and consume certain proteins necessary for blood clotting. The resulting anaemia and coagulation defects are significant clinical/pathological manifestations of this disease in infected animals.

Studies utilizing in vitro *culture systems*

The development of *in vitro* systems for cultivation of both 'animal infective' and 'insect' forms of trypanosomes has continued. The ability to grow animal-infective *T. brucei in vitro* has been exploited to provide systems for assay of levels of parasite enzymes, activities of drugs, and other biological

factors. For example, the serum of ruminants has been shown to contain an enzyme (polyamine oxidase) which, in the presence of another biological reagent (spermidine), lyses trypanosomes. This enzyme is not present in non-ruminant sera.

A significant achievement has been the recent development of an *in vitro* culture system for *T. congolense* which has supported growth of animal infective *T. congolense* for up to 35 days. It is hoped that, with some modifications of the culture system, long-term cultivation of *T. congolense* will be achieved comparable to that already developed for *T. brucei*. Systems for the growth *in vitro* of metacyclic trypanosomes of *T. brucei* and *T. congolense* are being developed.

The vector

The tsetse is an obligatory haematophagous insect, hence the vector/trypanosome/host interaction maintains endemic and/or enzootic trypanosomiasis in a variety of ecological zones over an enormous area of tropical Africa. The objective of research is to understand the various interacting factors involved in the epidemiology of trypanosomiasis.

Three *Glossina* species have been successfully colonized; other species will be colonized in the near future. These different species will be used, among other things, to compare their vectorial capacity for *Trypanosoma brucei, T. congolense* and *T. vivax*. It has been demonstrated that *Glossina morsitans morsitans* and *G. m. centralis* are equally efficient in transmitting the three trypanosome species. *G. morsitans* can be simultaneously infected with different trypanosome species and can transmit the mixed infections to susceptible hosts. Tsetse have also been shown to transmit all three species of trypanosomes non-cyclically, i.e. mechanically. The possibility of mechanical transmission of all three species in a field situation is important when considering the practicability of immunization against metacyclic forms of trypanosomes since the mechanically transmitted trypanosomes will be different antigenically from the metacyclics.

Another important finding is that all three pathogenic species of trypanosomes can develop normally in sterile male tsetse, and the latter can transmit the infections to livestock as efficiently as do sexually fertile tsetse. The sterile insect release (SIR) method has been identified as one of the methods for tsetse eradication. But, if this SIR method is used without initial reduction of the tsetse population in a selected field site, there will be an increase in the disease transmission rate to man and/or his associated livestock, at least in the early phase of the programme.

It has also been shown that when tsetse carrying developing but not yet mature trypanosome infections feed on goats treated with drugs active against trypanosomes, i.e. Berenil or Samorin, the infection in the vector is frequently suppressed. The effect of such feeding on mature trypanosome infections in tsetse is under investigation. This finding is again relevant in terms of trypanosomiasis challenge to livestock in the field when such drugs are in use to control trypanosomiasis.

Tsetse feed mainly on wildlife and they favour a few species, though some tsetse species are catholic in their choice of hosts. Studies have shown that the performance, i.e. survival, fecundity and mean pupal weights, of *G. m. morsitans* did not differ significantly when maintained by artificial feeding through silicone membrane upon the fresh defibrinated blood of buffalo, bushbuck, cow, eland, oryx, warthog, waterbuck or wildebeest. Hence the host preference shown by *Glossina* species is probably based on the behavioural patterns of the tsetse and their hosts, and not on the nutritional value of the different hosts' blood.

The host

An understanding of the complex interactions between the parasite, its vector and the host which result in death or survival of that host is fundamental to the possible development of means to control

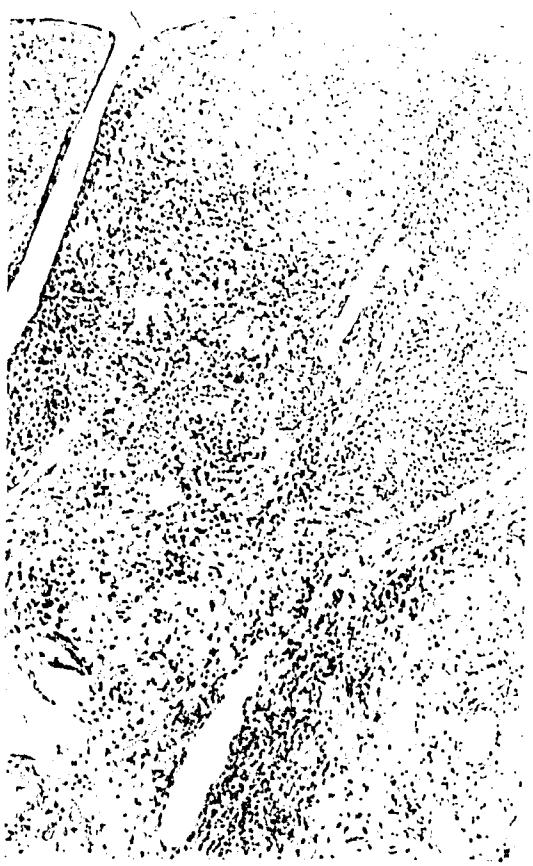
Fig. 2: Raised indurated plaques (chancres) several centimetres in diameter on the flank of susceptible bovine ten days after being bitten by a tsetse infected with *T. congolense*. The lesion is first detectable after five days and disappears during the fourth week.

trypanosomiasis in the field. Research focuses on the mechanisms which allow or prevent development of a protective immune response in the host, and the genetic factors that permit the host to survive trypanosome infections.

It has been demonstrated that mice, cattle and goats which have been treated with trypanocidal drugs after cyclical infection are resistant to cyclical challenge by trypanosomes of the same serodeme, but they are not resistant to heterologous cyclical challenge. In susceptible cattle and goats, the site in the skin of the bite of an infected tsetse fly becomes indurated and a chancre develops, whereas such a chancre does not develop on challenge of an immune animal.

Chancres are readily induced in susceptible cattle and goats by tsetse infected with *T. congolense, T. vivax* and *T. brucei* (Fig. 2). They have also been elicited in certain species of wild animals by tsetse infected with *T. congolense* and *T. brucei*. The chancre represents the first response of the host to the parasite. It is the location at which the trypanosome first becomes established and then proliferates prior to dissemination to the bloodstream via the lymph. The initial reaction is an intense inflammatory response accompanied by infiltration of neutrophils (Fig. 3); trypanosomes are readily recognized (Fig. 4). Soon after this, the cellular population becomes dominated by lymphocytes (Fig. 5) and plasma cells, the presence of which is indicative of a local immunological reaction. It is important to understand the immunological and cellular mechanisms of the host's immune response at this site. It is now important to evaluate in a field situation the role of acquired immunity to metacyclic VATs in animals developing resistance to trypanosome infections and whether the more logical use of chemotherapeutic agents can improve this situation.

Fig. 3: The intense inflammatory reaction in a chancre seven days after infection. There is marked congestion, oedema and neutrophil infiltration.



Recently it has also been demonstrated that on self-cure from a needle challenge, cattle become resistant to a subsequent cyclical rechallenge by trypanosomes of the same serodeme, but they are not resistant to a heterologous cyclical or needle challenge. It is possible that such animals go through and acquire immunity to the entire antigenic repertoire of the serodeme thus developing resistance to trypanosome challenge under field conditions, in the absence of chemotherapy.

It is also important to understand the factors which control the generation of immune responses against the parasite after an infection is established in the mammalian host. It has been demonstrated for *T. brucei* infections that, in the course of each parasitaemic wave, the immune response against the surface variable antigens which eventually destroys the parasites are not initiated until the parasite differentiates into its tsetse-infective, or 'stumpy', form. This lag in production of lytic antibody probably allows the appearance of new variable antigen types before the destruction of the original population. The rate at which bloodstream forms of *T. brucei* differentiate into stumpy forms is, in some way, influenced by the host itself in that parasite clones which do not differentiate in mice readily do so in cattle; differentiation also varies between strains of mice which have different susceptibilities to trypanosomiasis. Differentiation rates can also be altered after cyclical transmission in that parasite populations which transformed only a very low percentage of their populations into stumpy forms may increase the rate of this differentiation after cyclical transmission.

The magnitude of the immune response to the variable antigen of the trypanosome appears to be amplified and accelerated by factors (mitogens) released by the dying trypanosomes. Analyses and characterizations of the antibody responses to the VATs of *T. brucei* show that antibodies of the IgM and IgG classes are formed in a normal fashion and that secondary immune responses occur on the reappearance of the same or closely related VATs during the course of infection. This study is now being extended to *T. congolense* and *T. vivax* infections.

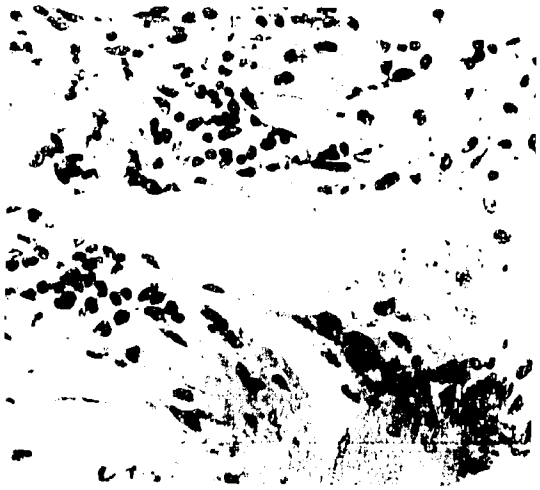


Fig. 4: A lymphatic vessel in the chancre. It is dilated with fluid and contains trypanosomes (*T. congolense*).

It has been recognized for some time that trypanosome infections in mice greatly alter and suppress their ability to respond to other antigens, due to marked alteration of the cellular organization necessary for these responses. This was thought to be one reason why animals could not efficiently control trypanosome infections. It has now been shown that even in mice this alteration in cellular organization is confined mainly to the spleen, and that cells in other sites, e.g. the lymph nodes, can respond normally during trypanosome infections.

Similar work in cattle has shown little evidence of structural disorganization of lymphoid tissue or functional impairment of immune responses as judged by their ability to respond to a variety of antigenic stimuli. This does not mean, however, that infected cattle respond normally to all antigens. There can be competition between the immune responses to trypanosome variable antigens or other antigens if they are presented simultaneously to the host with suppression of the response to one or another antigen. The delayed response to new variable antigen types as they arise in the host may be another route for parasite survival.

The different trypanosome populations present at any one time in the host may also interact between themselves, one suppressing the growth of another. This has been demonstrated in cattle subjected to challenge with two unrelated populations of *T. congolense* six weeks apart. Thus establishment of the second infection is inhibited whether the challenge is delivered as bloodstream forms or by infected tsetse flies despite the absence of specific antibody.

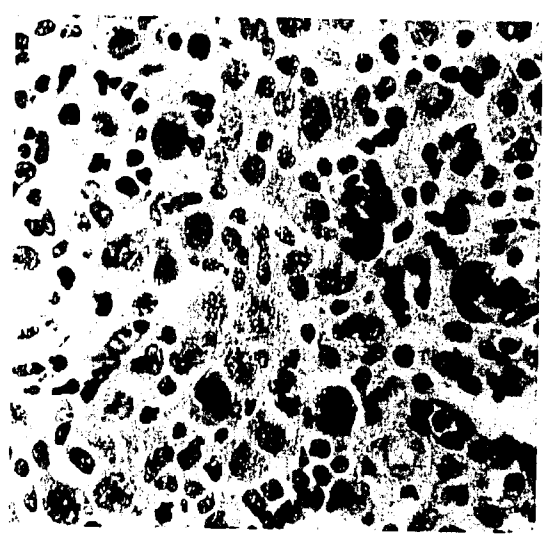
An understanding of the mechanisms involved in such suppression where one infection inhibits the establishment of a second infection may provide a new approach to stimulating resistance to trypanosomiasis.

The use of genetically defined inbred strains of mice has shown that the resistance of such mice to trypanosome infections is under genetic control but not solely under the control of genes which have been identified as controlling immune responses. Other genes therefore influence the degree of resistance exhibited by different inbred mouse strains. Nevertheless, in mice infected with *T. congolense,* the ability to control parasitaemia is correlated with the production of specific antibody. Despite the absence of specific antibody in those animals which fail to control parasitaemia, there is a marked increase in immunoglobulin production, particularly of the IgG class. In the case of *T. brucei* infections, the genes controlling susceptibility may exert their effect by influencing the differentiation process of this parasite, an event related to the induction and expression of the immune response against these trypanosomes.

Genetic resistance to trypanosomiasis, or trypanotolerance, has been confirmed in the West African N'Dama. It has also been shown to occur in certain breeds of cattle, sheep, goats and wild animals in East Africa; the latter include waterbuck, eland and buffalo. These results were achieved using animals which had not been previously exposed to trypanosomiasis. The trypanotolerant trait would appear to be related to the capacity to control parasitaemia and as a result develop less severe pathogenic changes such as anaemia. Trypanotolerance can be supplemented by previous exposure but decreases with age, stress or intensity of challenge.

It has now been shown that cattle which recover from trypanosome infections produce, during the course of infection, antibodies to a wider range of parasite antigens than cattle which ultimately die of the disease. The elucidation of marker systems by which cattle could be assessed for their innate ability to resist trypanosome infections would be a significant step in the development of herds with trypanotolerance.

Fig. 5: The striking large lymphocyte reaction in the chancre at day 18.



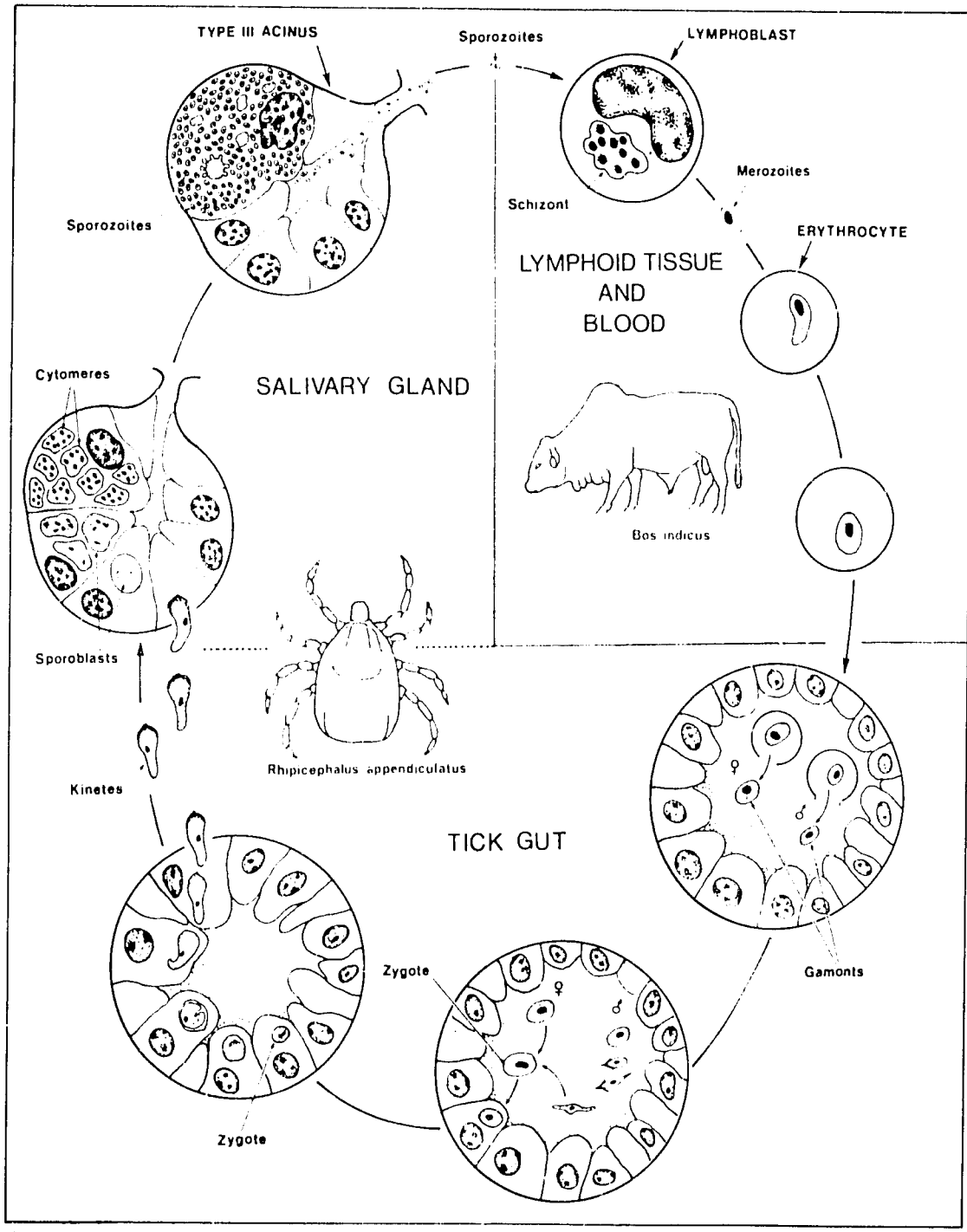


Fig. 6: Schematic representation of the life cycle of *Theileria parva*.

THEILERIOSIS

BACKGROUND

Part of ILRAD's mandate is to develop effective control measures against bovine theileriosis. Emphasis is being placed on East Coast fever (ECF), caused principally by *Theileria parva* (see Fig. 6). This disease affects large numbers of cattle in East and Central Africa, and in many areas is the main constraint to expansion or upgrading of the livestock industry.

It has been known for many years that animals which recover from ECF develop a long-lasting immunity to homologous challenge. It is also well established that the degree of immunity is not dependent on the severity of the initial reaction: animals may undergo a mild or inapparent reaction and subsequently be fully resistant to a lethal challenge with a homologous strain. Previous investigators have been able to exploit these findings and have shown that animals can be effectively immunized against ECF by concurrently injecting them with sporozoites of *T. parva* and oxytetracycline drugs which limit the ensuing infection.

A number of points have emerged from this work:

1. In order to render cattle immune, living parasites must infect the animals. This can place their lives at risk.

2. Animals immune to one strain may be only partially immune, or even fully susceptible, to challenge with a heterologous strain; this seems to be particularly true of strains derived from buffalo (*Syncerus caffer*). This finding suggests that, for effective field immunization to be achieved, cattle should be protected against several strains.

3. Oxytetracycline cannot control infection induced by some strains of *T. parva*.

These problems raise a number of secondary questions, for example: what is the mechanism of immunity, how can it be induced safely, how many strains should be used to provide effective coverage against field challenge, can strains be identified *in vitro*?

Considerable progress has been made towards answering some of these questions at ILRAD during 1980, particularly in relation to understanding the mechanism and nature of immunity in ECF.

RESEARCH RESULTS

The immune response

To formulate rational strategies for vaccination against East Coast fever it is necessary to understand the mechanisms by which immunity is induced in cattle and which immune responses confer protection against reinfection. The duration of acquired immunity against *T. parva* strongly suggested the participation of cell-mediated immunity. This premise was substantiated by studies at ILRAD where protection against infection was adoptively transferred to susceptible cattle by leucocytes from immunized animals. In addition, macroschizont infected cells from culture or from infected cattle were shown to initiate strong proliferative responses in autologous (same animal) peripheral blood leucocytes in a reaction of mixed-lymphocyte type (termed the autologous theileria-lymphocyte culture AuTLC since the reaction does not accord completely with conventional mixed lymphocyte responses).

A major advance towards delineation of the protective immune response in theileriosis arose from experiments conducted at ILRAD during 1980. Leucocytes taken from lymph nodes, lymph and blood of cattle terminally infected with *T. parva* were found to destroy a range of infected and non-infected target cells from cattle and a murine tumour cell line. These natural killer' cells may be responsible for the cytolysis seen in lethal infections with *T. parva*. In contrast, cattle being immunized or rechallenged (immune) with *T. parva* mount cytotoxic responses which lyse parasitized

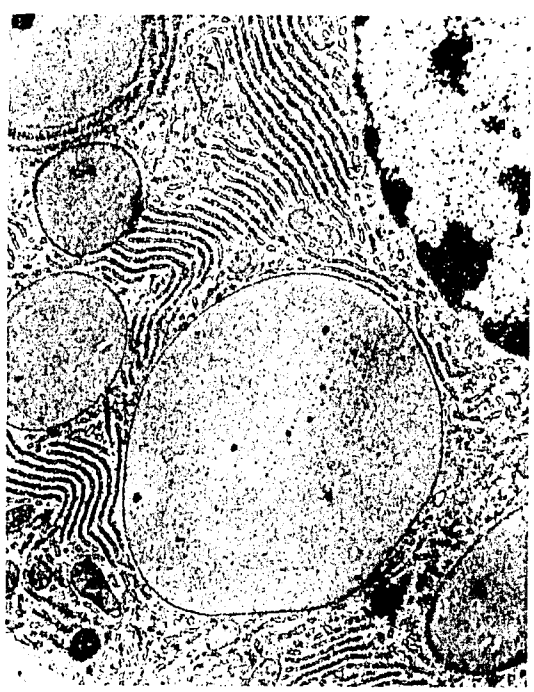
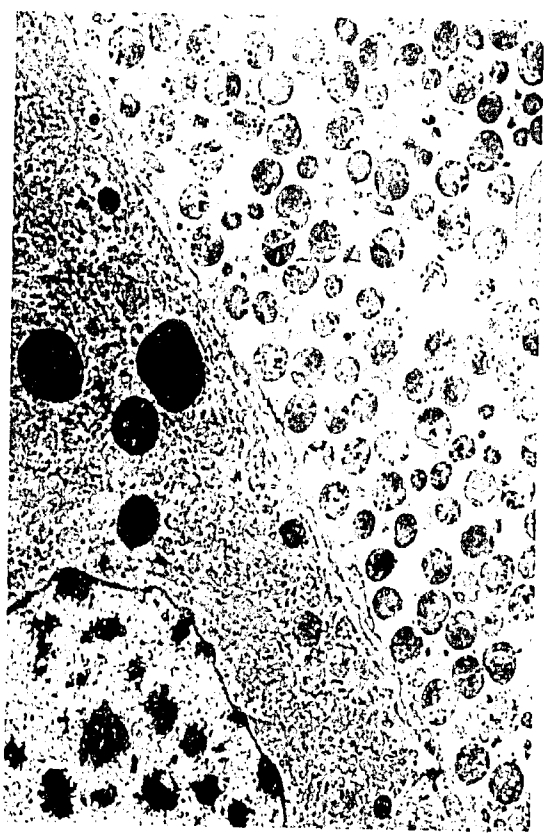


Fig. 7: An electron micrograph of a normal E-cell of a type III acinus showing the nucleus (upper right), the protein synthesizing endoplasmic reticulum and the characteristic large secretory granules. This is the cell type preferentially invaded by the parasite and the site of development of the sporozoites, the stage of the life cycle infectious to cattle.

autologous (but not allogeneic) target cells after the parasite has been eliminated. When cytotoxic lymphocytes from cattle immune to one isolate of *T. parva* are confronted with autologous cells infected with different isolates of the parasite, variable lysis ensues. Thus the cytotoxic response is genetically restricted by virtue of the fact that it is only operative against autologous parasitized cells. The response also shows some specificity for the strain of the parasite. The possibility that the magnitude of lysis *in vitro* of heterologous isolates of *T. parva* correlates with cross-protection between the respective isolates *in vivo* is being studied.

Given the participation of the AuTLC and cytotoxicity in theileriosis (and possibly other cell-mediated responses) then a comprehensive knowledge of the impact of *T. parva* on the bovine lymphoid system becomes essential. Since little was known of the functional nature of cell-mediated immunity in cattle a major avenue of research at ILRAD is analysis of the normal bovine immune response. Initial studies at ILRAD indicate that the induction of cellular reactions in cattle is essentially similar to those in other species. Monoclonal antibodies raised against the membrane determinants of bovine leucocytes were used as markers for leucocyte subpopulations. In essence,

Fig. 8: Uninfected E-cell of tick salivary gland at left and at right the same cell type filled with sporozoites of *Theileria parva*.



determinants on leucocytes and other cells concernad with regulatory phenomena and the discrimination of 'self' and 'non-self' are coded by the major histocompatibility complex (MHC). Differences between one set of determinants, the 'lymphocyte-defined' (LD) antigens on viable 'stimulator' cells of haematopoietic origin, are involved in the initiation of mixed-lymphocyte reactions from which cytotoxic cells emerge. Differences in the 'serologically defined' (SD) antigens are recognized for lysis by cytotoxic cells. Thus following the inoculation of foreign cells (e.g. allogeneic lymphocytes), the induction of antibody and cytotoxic responses resulting in the elimination of these cells is determined principally by differences in the MHC antigens. However, modifications of autologous MHC antigens (e.g. during viral infections) also elicit the development of cytotoxicity. This is probably the means by which *T. parva* induces immunity and through which parasitized cells are recognized and eliminated by cytotoxic cells. Consequently, effort has been devoted to the serological and biochemical analysis of the membranes of parasitized lymphoblasts to identify parasite-induced alterations. Since the analysis of both cytotoxicity and cell membranes is complicated by the antigenic complexity of cell lines established with parasite isolates, cultures of cloned parasitized cells are being generated to provide defined homogeneous experimental material from which the total antigenic complement of *T. parva* could be compiled. Those cells most important for immunity will then be defined.

Studies *in vitro* have provided important information concerning the types of cellular interactions likely to occur during infection. Analyses *in vivo* are concerned with identification of the cell type(s) parasitized by *T. parva* and the subsequent impact of parasitism of host lymphocytes on the normal immune response; the differences in cytotoxicity manifested between lethal and sublethal infections exemplify these diverse effects. For this purpose monoclonal antibodies are being used to probe for markers characteristic of subpopulations of bovine lymphocytes.

Work at ILRAD has confirmed that the induction of immunity to *T. parva in vivo* requires the use of viable parasites, an observation consistent with protection mediated by cytotoxic cells which require infection of host cells for their generation and restricted response. If cross-protection between different strains of the parasite *in vivo* is accurately reflected in cytotoxic responses *in vitro*, then the number of antigenic types of *T. parva* can readily be identified. Current research at ILRAD, and other institutes, is directed towards the induction of primary cytotoxic responses using defined antigens or non-viable material with or without adjuvants; such a breakthrough could be directly applicable for field use.

Immunization with macroschizonts

Earlier workers showed that it was possible to cause

infection of cattle by injecting macroschizont infected bovine lymphoid cells. Such cells could be derived by adding sporozoites to bovine peripheral blood leucocytes *in vitro:* sporozoites invaded the cells and induced their transformation to lymphoblastoid cells infected with macroschizonts of *T. parva.*

Workers at ILRAD showed that when macroschizont-infected cells were inoculated back into an autologous donor animal, 10^2 to 10^5 infected and immunized cattle, whereas 10^7 or more cells were needed to infect or immunize allogeneic (unrelated) cattle. This difference was attributed to the fact that when allogeneic infected cells were inoculated into cattle the allogeneic cells were ultimately rejected, so that the establishment of infection required macroschizonts to transfer from donor into host lymphocytes. It is estimated that this event occurs with a frequency of around 10^{-5}. It has also been shown that prior induction in the host of antibody to the macroschizont can interfere with the transfer of macroschizonts and diminish the infectivity and immunogenicity of parasitized allogeneic cells.

Since the generation of autologous cell lines is not practical, and production of 10^7 allogeneic cells is cost prohibitive, methods need to be developed to facilitate the transfer of macroschizonts to host cells *in vivo* before macroschizonts can be used for practical immunization.

Immunization with sporozoites

The other main candidate for use as an immunogen in ECF is the sporozoite. The Tick Laboratory provides a regular supply of infected *Rhipicephalus appendiculatus* ticks, the vector of ECF. From these ticks, sporozoites of *T. parva* can be isolated for use in both *in vitro* and *in vivo* experiments.

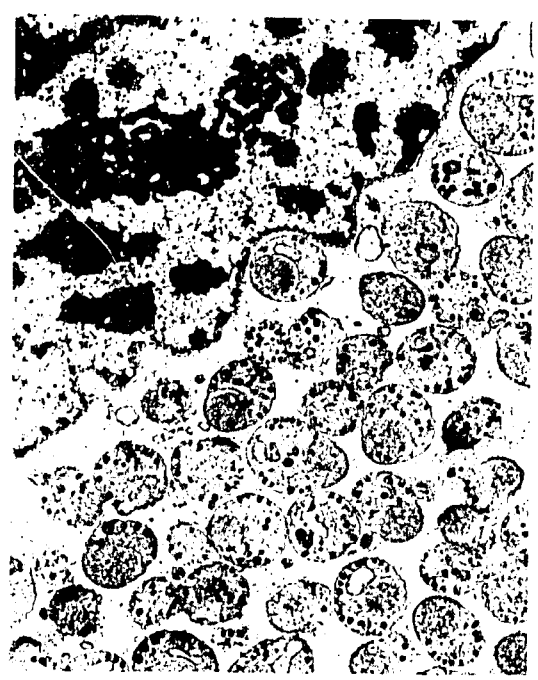
At ILRAD a series of ultrastructural studies of infected tick salivary glands has described the mode of parasite development within the E cell of the type III acinus of the vector tick, and also showed that one infected cell can contain 30,000 to 50,000 sporozoites. This is important information which will assist in quantifying sporozoite preparations. Another finding which may assist in quantification is that sporozoites will selectively incorporate radio-labelled hypoxanthine. Sporozoites are too small to count directly, so development of other assay systems is important if sporozoites are to be used for immunogenesis. For this reason the possibility of producing sporozoite specific monoclonal antibodies is being pursued. Such antibodies could be of value as sporozoite markers and they may also be used to detect strain differences.

A collaborative study with the Veterinary Research Department, Kenya Agricultural Research Institute, has yielded the first electron micrographs of the entry of sporozoites into peripheral blood lymphocytes *in vitro.* These observations shed new light on the mode of entry and suggest the mechanism by which the parasite escapes destruction by the phagolysusomal system of the host cell. The time course of the initial events was found to be very rapid, sporozoites being found in the lymphocytes within minutes rather than hours.

The initial interaction of sporozoites with host lymphocytes has also been studied by testing the infectivity for cattle of lymphocytes incubated with sporozoites for short periods of time *in vivo.* The rapid association of sporozoites with host cells was also evident from this study which showed that after one hour's exposure to parasites as few as 2,000 cells initiated infections in autologous hosts. Large numbers of such cells failed to infect allogeneic recipients. It is hoped to use this system to examine different subpopulations of lymphocytes as target cells for the infection.

At this Institute anti-sporozoite antibodies have recently been detected in the sera of ECF-recovered cattle but their role in neutralizing sporozoites *in vivo* remains to be determined. If such antibodies can neutralize sporozoites before they enter host cells, a potential means of immunizing cattle is indicated which could obviate the need to establish infection within the host to generate effective immunity.

Fig. 9: Sporozoites of *Theileria parva* in the cytoplasm of an E cell of acinus III. Host cell nucleus at upper left.



The other main approach to using sporozoites as immunogens is to use sufficient parasites to generate specific immunity, and then to control subsequent parasite development before the host becomes overwhelmed. As already described, certain drugs, notably oxytetracycline, can achieve appropriate control. There is also some evidence that similar parasite attenuation can be achieved by ionizing radiations. This aspect is currently being examined in further detail in conjunction with the Veterinary Research Department of the Kenya Agricultural Research Institute.

The possibility of using viable sporozoites as immunogens will depend very much on improving techniques for quantifying them and for controlling the ensuing infection. When macroschizont-infected cells are used, quantification is no problem, but there is still the question of controlling the infection and above all of overcoming or by-passing problems relating to histocompatibility restriction in allogeneic situations.

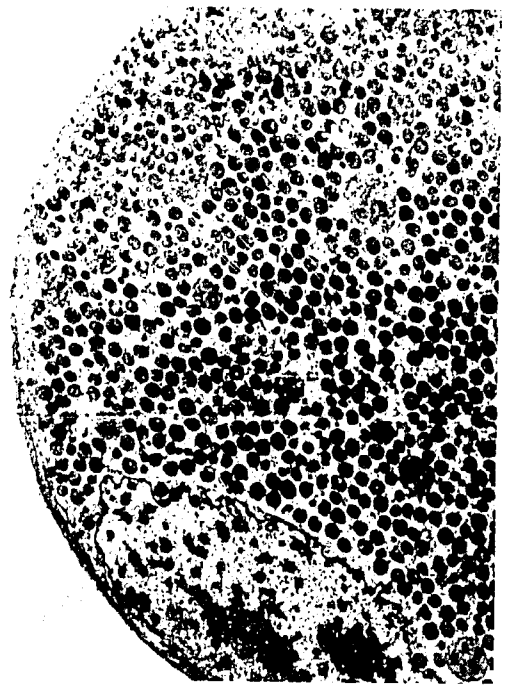
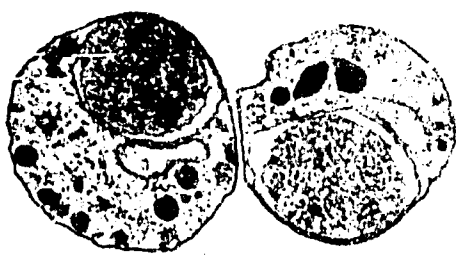


Fig. 11: A low-power micrograph of an infected cell in a tick salivary gland acinus, showing the great hypertrophy of the parasitized cell and the very large number of sporozoites resulting from intracellular proliferation of a single invading kinete of *Theileria parva*.

Table 1: Summary of theileria immunization work on cell mediated cytotoxicity

	Infection	*Cytotoxicity*
1. Lethal infection	Patent day 7, death day 18 - 20	Non-specific day 14 onwards
2. Infection and treatment	Patent day 14 - 18	Specific when parasites disappear
3. Allogeneic infected cell line (10^7)	May be transiently positive	Specific both to allogeneic cell and to autologous cell line
4. Autologous infected cell line	$>10^6$ lethal infection $<10^6$ infections and recovery	Non specific Specific when parasites disappear
5. Challenge of immune animals with stabilate	May be transiently positive	Specific 7 - 10 days

Fig. 10: High magnification image of two sporozoites showing their nuclei, mitochondria, rhoptries and micronomes.



Strain differentiation

An *in vitro* test to differentiate strains of *Theileria* has been needed for many years. Recent work at ILRAD has indicated three possible methods:

(a) the use of isoenzyme markers
(b) the use of monoclonal antibodies, and
(c) the use of specific cell cytotoxicity.

Preliminary tests with different strains of *T. parva*, indicate there may be correlation between the results of *in vitro* cross matching and cross immunity *in vivo*. At the moment emphasis has been placed on the use of anti-macroschizont monoclonal antibodies, a battery of which is being tested against different macroschizont-

infected cell lines derived from strains isolated from the field. For these studies cloned cell lines are being raised. Further work will show whether this, or other tests, provides reliable information on strain specificity which can be extrapolated to *in vivo* cross immunity.

Epidemiology

The initial phase of a field programme began in 1980. A project was started in conjunction with the Kenya Ministry of Livestock Development to determine the epidemiology of ECF in the Coast Province of Kenya. This area was chosen because the disease here is limited in distribution and incidence, and may therefore be more amenable to control than in areas where it is more widespread. The preliminary stage has been to collect sera and ticks and to attempt strain isolation from Kilifi District. The indirect fluorescent antibody test has been used to screen sera, and good correlation has been found between the presence of *R. appendiculatus* and animals serologically positive to *T. parva*. Several strains have been isolated and these are being characterized in the laboratory with a view to using them when an immunization strategy becomes feasible.

As the programme develops it is hoped that the International Centre for Insect Physiology and Ecology and the International Livestock Centre for Africa will also participate.

Studies on the tick vector

Studies of the tick vector are relevant to ILRAD's mission in that a better understanding of its physiology may contribute to the development of more effective acaricides. Similarly a thorough analysis of parasite-host relationships in the tick may identify steps in the complex life cycle potentially vulnerable to pharmacological or immunological attack.

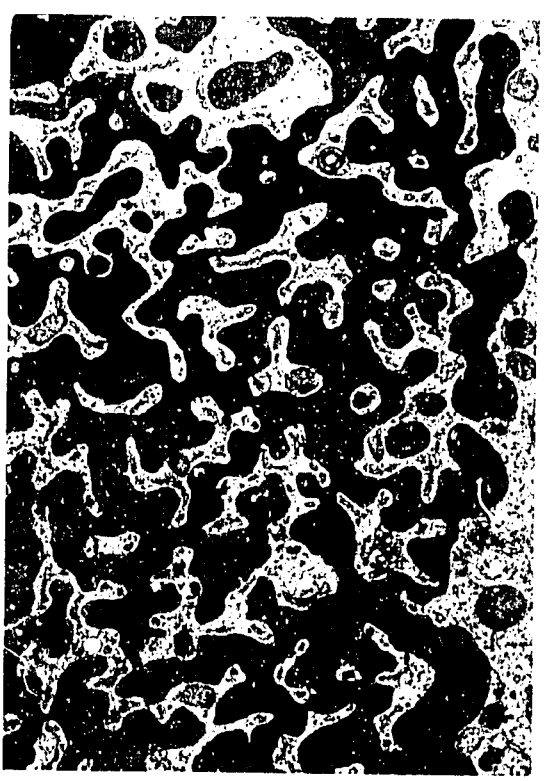
The infective stage of *Theileria parva* develops in the tick salivary gland and is introduced into the bovine host in the saliva (see Fig. 6, page 16). The tick salivary gland consists of about 300 secretory units called acini. These are of three structurally and functionally distinct types, each composed of several different cell types which are not easily distinguished with the light microscope. An electron microscopic study of the gland has now established unambiguous criteria for identification of acinus and cell types, thus providing basic information essential to correct interpretation of host parasite relations.

The parasite had previously been thought to proliferate in two of the three types of acini and to be relatively unselective as to the cell type entered. Using the more discriminating electron microscopic criteria for identifying cell types, it has been found that *Theileria parva* exhibits a high degree of selectivity for a single host-cell type – the E-cell of acinus III (see Figs. 7 – 10).

The greater resolving power of the electron microscope has also made it possible to analyse the changes in the host cell induced by the invading parasite. These include a striking cell hypertrophy (see Fig. 11); reorganization and progressive autophagic reduction in the organelles involved in protein synthesis; and changes in carbohydrate metabolism resulting in deposition of glycogen as an energy source for the parasite. In the previous literature on host-parasite relationships of *Theileria*, the alterations in appearance of the infected salivary gland cell had been interpreted as degenerative changes. The new observations suggest instead that the invading organism induces active adaptation of the host cell's internal structure and modification of its metabolism to create an intracellular environment favourable for its own rapid proliferation.

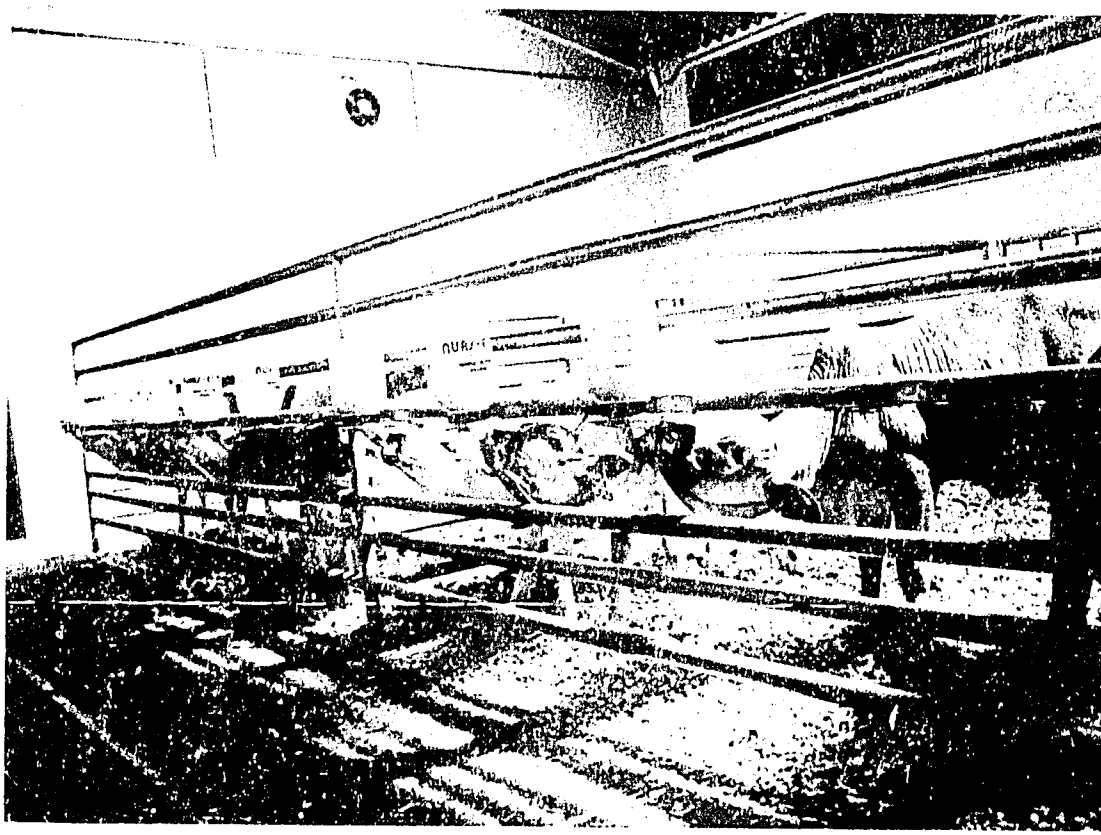
Traditional depictions of the developmental events leading to formation of infective sporozoites have been based upon inference from the number and distribution of stained parasite nuclei seen in sections or squash

Fig. 12: Electron microscopic studies have revealed a hitherto unexpected complexity in the relationship of the developing sporoblasts in the host cell of the tick salivary gland. In this micrograph the parasite has been opaqued to make more apparent its remarkable labyrinthine configuration which presents an enormous surface area for interchange of nutrients and metabolites with the host cell cytoplasm.



preparations of infected salivary glands examined with the light microscope. This instrument was not able to resolve the boundary between parasite and host cell cytoplasm. The actual configuration of the developing sporoblast was therefore conjectural. Electron micrographs of these stages of sporogony have disclosed a hitherto unsuspected complexity in the topographical relations between parasite and host cytoplasm. The proliferating nuclei of the parasite are situated in lobular processes at the periphery while its central region takes the form of a labyrinthine network of slender branching and anastomosing processes which present an enormous surface area for metabolic exchange with the host cytoplasm (see Fig. 12). Electron micrographs of later stages of sporogony reveal an elaborate process of segmentation and fission which culminates in production of 30,000 to 50,000 sporozoites in a single hypertrophied salivary-gland cell. These new observations on the genesis of the infective particles are at variance with the traditional diagrams depicting budding of sporozoites from the periphery of more or less spherical tertiary sporoblasts.

Calves feeding in the calf-rearing building.



RESEARCH SUPPORT

EXPERIMENTAL ANIMAL PRODUCTION AND UTILIZATION

LARGE ANIMALS

A total of 392 cattle were used in experiments during 1980; 5% of these animals were used for experiments related to theilerioses research and the balance for trypanosomiasis research. In addition 510 small ruminants were used for trypanosomiasis research. The ILRAD calf rearing facility provided 168 calves for research purposes, 104 weaned calves were obtained from outside sources, and the balance of the experimental cattle had been obtained in 1979.

The major constraint in the utilization of larger numbers of cattle for experimental purposes was the lack of sufficient animal accommodation on the ILRAD site. In order to partly alleviate this problem ILRAD made use of facilities offered by the Government of Kenya at its Central Veterinary Laboratory to house 25 cattle for collaborative research on trypanosomiasis. The problem of cattle accommodation on the ILRAD site will be resolved when construction of the new experimental cattle holding unit is completed in mid 1981. The funds for construction of this building were made available in the 1980 budget. The building will house 350-400 cattle, dependent on age, thus giving a total housing capacity of approximately 450 experimental cattle at any one time. As the average duration of experiments in trypanosomiasis and theileriosis is approximately six months, the potential capacity would be 900 experimental cattle per year. The fly proof accommodation available for small ruminant trypanosomiasis experiments has a total capacity of approximately 300 goats/sheep which is sufficient for the present. A substantial increase in the utilization of such animals by the trypanosomiasis epidemiology programme may however, put pressure on the screened facilities available for cattle experiments from 1984 onwards.

The ILRAD calf rearing facility has a total production capacity of 300 - 350 calves a year and the low numbers produced in 1980 reflect the lack of experimental accommodation causing a backlog in the rearing and holding facilities and also the failure of supply of suitable calves in the last quarter of 1980 due to foot and mouth quarantine restrictions on the farm supplying these calves. It is anticipated that the final purchase of an ILRAD farm will take place in 1981 and that this farm will then be capable of supplying all of ILRAD requirements for both calves and older animals (*Bos indicus*) from 1982 onwards with the exception of specific requests by scientists for cattle of European breeds (*Bos taurus*) which will be met from the already existing supplier.

Determining *in vitro* cytotoxic responses to theileria infected bovine lymphocytes.

Collection of blood samples from the jugular vein of a goat.

SMALL ANIMALS

Utilization of laboratory animals for experimental purposes in 1980 was as follows: 17,540 inbred mice, 12,400 rats and 503 rabbits. Production colonies of five inbred mice strains have been established with a maximum annual production of approximately 20,000 mice. This is sufficient to meet research needs. The rat breeding colony has a maximum capacity of 12 – 13,000 rats annually and this also is sufficient to meet experimental needs. A total of 136 of the 503 rabbits used in 1980 were bred at ILRAD and the remainder purchased locally. ILRAD will become self-sufficient in rabbit production in 1981/82 on completion in mid-1981 of the construction of the new rabbit breeding/holding facility approved and funded in 1980.

DIAGNOSTIC LABORATORY

The main functions of the Diagnostic Laboratory during 1980 were again to provide serological and bacteriological services to the research laboratories and the animal production facilities. A total of 10,774 samples were received, 10,351 for serological analyses and 432 for bacteriological analyses.

The laboratory provides serological screening procedures for *Trypanosoma brucei*, *T. congolense*, *T. vivax*, *Babesia bigemina*, *Theileria parva*, *T. mutans* and *Anaplasma marginale*. It provides routine bacteriology for the isolation and identification of common pathogens as well as diagnostic and preventive screening for helminths.

Apart from its services to ILRAD departments, the Diagnostic Laboratory provided assistance to other centres or projects. Tests against East Coast fever were performed for an FAO project in Zanzibar, and on the request of the Veterinary Laboratories of Malawi, one of their technicians was trained for three months. *Theileria parva* antigen, antiserum and conjugate were supplied to scientists in Nigeria, Uganda, Rwanda and Burundi.

The preparation of the antigens and the conjugate used in the indirect fluorescent antibody test are prepared by the Diagnostic Laboratory. The procedure of fixing the antigens in suspension and preservation by lyophilization or freezing has greatly improved the test as regards morphology and durability of the antigen. Preincubation of test serum with homogenized lymphocyte supernatant has brought non-specific staining of the infected lymphocyte in *Theileria parva* to a minimum. Significant progress has been made in preparing antigens and conjugates for the enzyme-linked immunospecific assay; the test is in use for trypanosomiasis and under study for East Coast fever.

In order to be able to include a serological test for screening small ruminants, the Diagnostic Laboratory is in the process of raising both rabbit and anti-sheep and anti-goat sera. These will be used to prepare conjugates for the indirect fluorescent antibody test and the enzyme linked immunospecific test.

The spray-race.

TRAINING ACTIVITIES

ILRAD undertakes to contribute to the training of personnel in developing nations who are engaged in research activities similar to those defined by ILRAD's mandate. ILRAD undertakes to contribute towards the training of these individuals on the understanding that such training activities will not be a major hindrance to its research effort. It is expected that the trainees will contribute towards the overall research effort of ILRAD. The training programmes and the numbers of trainees taken each year are determined by the funds available for the training budget.

INTERNATIONAL TRAINING PROGRAMMES

TECHNICAL TRAINING

Four individuals came to ILRAD this year under this programme. A technician from Malawi came in February for three months training in serology in the Diagnostic Laboratory. He learnt immunofluorescence and autoradiography, immunodiffusion and immunoassay techniques, together with conjugate and soluble antigen preparation. He was sponsored by DANIDA.

Two German researchers from the German Agency for Technical Co-operation (GTZ) also came in February. One, a veterinarian, came for six weeks training. He learnt methods to detect surface antigens of lymphocytes including immunofluorescence and autoradiography together with the preparation of specific reagents necessary to detect these antigens.

The other, a physician and veterinarian, came for three weeks' training. The techniques he learnt involved lymphocyte culture, incubation of bovine PBL with various mitogens and the establishment of optimal dose response kinetics for this reaction.

Both researchers went to work in Togo, West Africa, after leaving ILRAD.

A Senior Technologist from Nigeria arrived in October for four months' training in cell culture techniques. Her sponsorship was provided by the World Health Organization.

Course

One course was held at ILRAD this year. It was entitled 'Advanced Techniques in Immunological and Biochemical Approaches to Hemoparasitic Research' and was held from 14 to 28 September 1980. The course provided a very intensive laboratory and lecture programme on the techniques used in the study of

Routine examination of a goat.

blood-borne parasites. Fourteen participants took part from African countries of which five were from Kenya. One participant came from Cuba and another from the U.S.A. The sponsors were UNESCO ICRO, WHO, DSE and ILRAD.

POST GRADUATES

The number of post graduate trainees at ILRAD this year totalled twelve. Of these four were Kenyans, two Sudanese, two Germans, one Ugandan, one Rwandan, one Australian and one from Holland (Table 2). The graduate from Holland successfully defended his Ph. D. thesis at the University of Utrecht, Holland, in December. Seven of these individuals were funded by ILRAD and five were paid a salary by their sponsoring governments.

Conferences

In September, ILRAD hosted a conference entitled 'The Impact of Animal Disease Research and Control on Livestock Production in Africa'. The conference covered the important aspects of animal production, animal disease costs, strategies and research needs. The conference was organized by the Association of Institutes for Tropical Veterinary Medicine, and of ninety participants fifty were from African nations. This meeting brought together deans of veterinary faculties in African universities, heads and other personnel from ministries of livestock development and veterinary services in Africa, individuals from leading research centres in tropical veterinary medicine in Europe and America, and ILRAD researchers to discuss these issues.

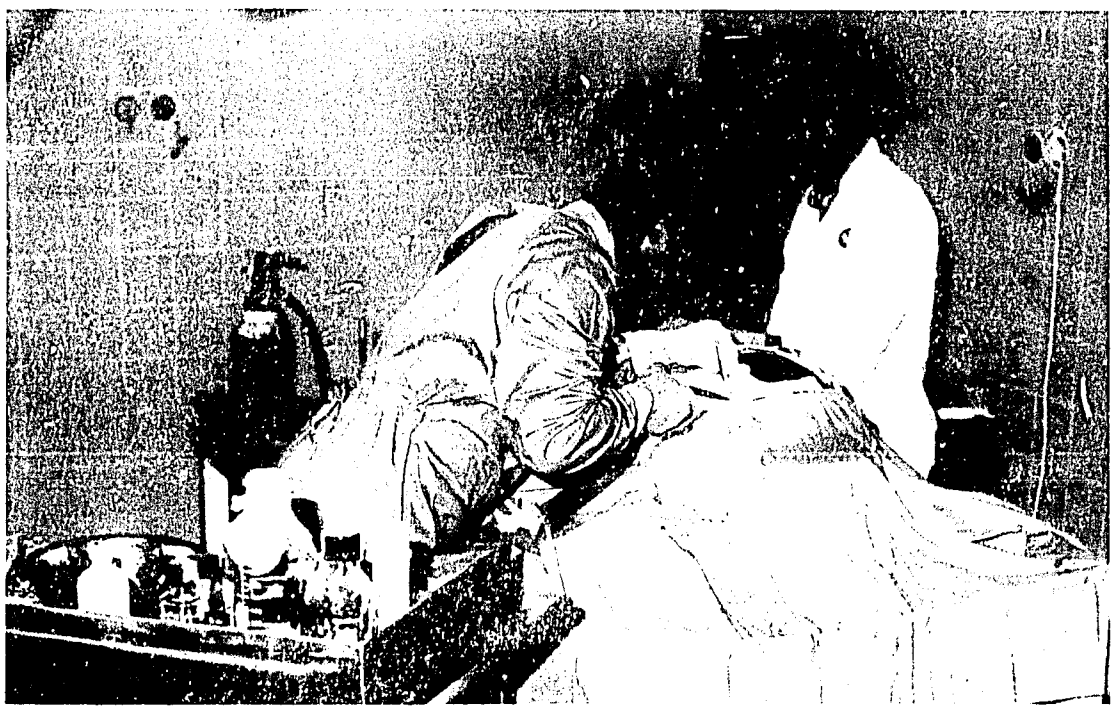
The co sponsors were OAU, ILCA, GTZ, ILRAD, FAO, Belgium, Switzerland and the EEC.

Workshops

In February, the UNDP World Bank WHO Special Programme for Research and Training in Tropical Diseases, held its second meeting of the Scientific Working Group in Immunology and Pathology of African Trypanosomiasis. Approximately 40 people attended the meeting, including five Kenyans from local organizations.

On 9 and 10 September, an IDRC sponsored workshop on 'Wildlife Disease Research' was held at ILRAD. The workshop focused on the practical importance of data generated by wildlife disease research. The proceedings which resulted from the conference should be a useful reference source for the development of land use policies in areas of Africa enriched by wildlife. Participants from Kenya and several other African countries included veterinarians, administrators in the ministries of livestock development and environment and natural resources, wildlife managers, and ecologists. The workshop was organized by the IDRC sponsored Wildlife Section at the Kabete Veterinary Laboratory.

Lymphatic cannulation of a calf.



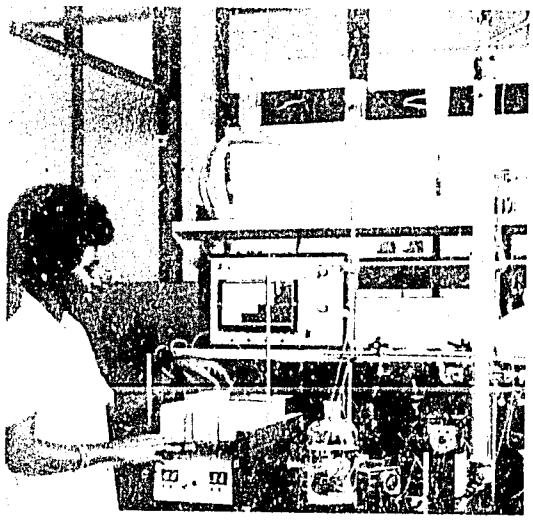
ILRAD STAFF PROGRAMMES

TECHNICIANS

ILRAD sponsors a number of its technicians to attend day-release courses at the local Kenya Polytechnic in Nairobi. Ten individuals were sponsored in 1980.

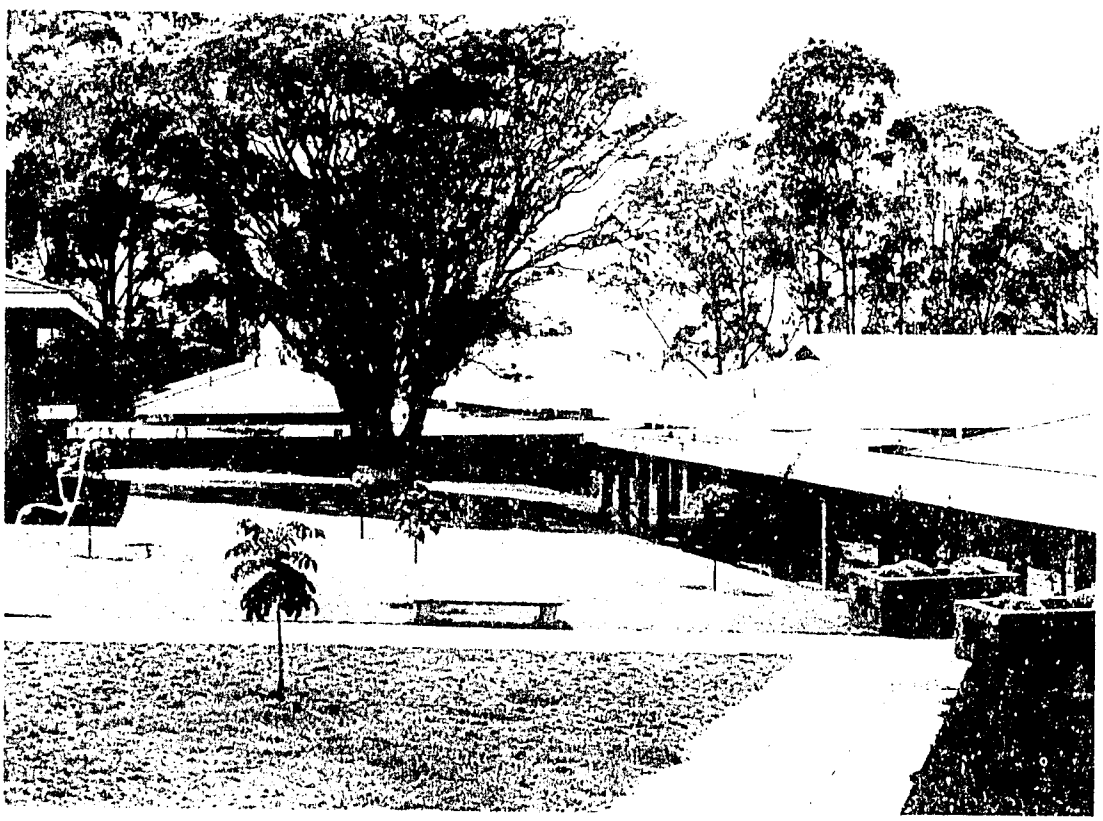
Table 2: Post-graduate research fellows in 1980

Name	*Country*	*Sponsor*
Akol, G.	Uganda	ILRAD
Borowy, N	Germany	ILRAD
Bwayo, J.	Kenya	Kenya
Chumo, R.	Kenya	Kenya
deGee, T.	Holland	Holland/ILRAD
Ismail, A.	Sudan	Sudan
Kratzer, R.	Germany	ILRAD
Lalor, P.	Australia	ILRAD
Mahan, S.	Kenya	ILRAD
Monirei, J.	Kenya	Kenya
Musa, M.	Sudan	Sudan
Sendashonga C.	Rwanda	ILRAD



Fractionating ascitic fluids containing monoclonal antibodies.

View of the ILRAD facility. At left, the conference hall; centre, the administration wing; right, the library



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Campus de Kinshasa
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STAFF

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Parasitology

J.J. Doyle	Co-ordinator
A.D. Irvin	Co-ordinator
S.Z. Shapiro	Scientist
J.D. Barry	Post-doctoral
D. Dobbelaere	FAO associate expert
G.L. Kazyumba (January, November & December)	Visiting scientist
T. Minami	Visiting scientist
K. Nguli	Research associate

Biochemistry/Molecular Biology

L. Rovis	Co-ordinator
R.O. Williams	Co-ordinator
A.F. Barbet	Scientist
N. Agabian (January-March)	Visiting scientist
S. Dube	Visiting scientist
J. Donelson (April-December)	Visiting scientist
P. Englund (June-August)	Visiting scientist
P. Majiwa	Research associate
G. Mpimbaza	Research associate
J.R. Young	Research associate

Cell Biology

H. Hirumi	Co-ordinator
D. Dube	Visiting scientist
A. Ferrante (April-July)	Visiting scientist
O. Nyormoi	Visiting scientist
K. Hirumi	Research associate
R.T. Nelson	Research associate

Immunobiology

S.J. Black	Co-ordinator
E. Eugui	Scientist
J. Ferluga	Scientist
R. Jack	Post-doctoral
R. Masake	Post-doctoral
R. Hewett	Research associate
L. Lundin	Research associate

J. Newson	Research associate
C. O'Brien	Research associate
C. Simmons	Research associate
C. Withey	Research associate

Immunology

G. Roelants	Co-ordinator
A.J. Musoke	Scientist
V.M. Nantulya	Scientist
M. Pinder	Scientist
P.C. Creemers	Post-doctoral
S.K. Kar	Post-doctoral

Pathology

M. Murray	Co-ordinator
D. Emery	Scientist
W.I. Morrison	Scientist
C. Davis	Visiting scientist
D. Whitelaw	Visiting scientist
D.L. Bovell	Research associate
C.A. Hinson	Research associate
J. Paris	Research associate

Electron Microscopy

D. Fawcett	Co-ordinator
S. Doxsey	Visiting scientist

Tick Laboratory

G. Buscher	Co-ordinator
B.J. Ellis	Research associate

Tsetse Laboratory

S.K. Moloo	Co-ordinator
H. Hecker (Jan-march)	Visiting scientist
S.B. Kutuza	Research associate

Clinical Medicine and Diagnostic Laboratory

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D.K. Mwangi	Post-doctoral
B. Goddeeris	FAO associate expert
J. Katende	Research associate
M. Sandiford	Research associate

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United Kingdom
United Nations Development Programme
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World Bank

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