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PREFACE

The purpose of the *Annual Scientific Report* is to make available, to the research community at-large, the individual summaries of the scientific work carried out by ILRAD staff and their collaborators during 1990. It also lists the scientific publications and the papers presented by staff at international meetings as well as the names, affiliations and research topics of visiting scientists who worked at ILRAD during the year.

The research summaries are grouped under each project area, and the project areas and their objectives are given below:

THEILERIOSIS

The theileriosis research programme is divided into three project areas with the following objectives.

TH1. Epidemiology of theileriosis

To increase knowledge of the epidemiology of bovine theileriosis and to introduce and evaluate improved immunological control methods in collaboration with national and international organisations.

TH2. Immunisation with sporozoite antigens

To identify, characterise and produce antigens from the sporozoite forms of *T. p. parva* and *T. p. lawrencei* which, when suitably delivered, will induce protective immunity.

TH3. Immunisation with schizont antigens

To identify, characterise and produce antigens from the intracellular forms of *T. p. parva* and *T. p. lawrencei* which, when suitably delivered, will induce protective immunity.

TH4. Vaccination

To develop novel vaccines against *Theileria* based on characterised parasite antigens and effective delivery systems.

TRYPANOSOMIASIS

The trypanosomiasis research programme is divided into four project areas with the following objectives.

TR1. Epidemiology of trypanosomiasis

To develop a more precise understanding of the epidemiology of tsetse-transmitted and non-tsetse-transmitted trypanosomiasis so that national organisations can better apply currently available control measures such as chemoprophylaxis and chemotherapy, use of trypanotolerant livestock and tsetse control.

TR2. Trypanosome biology and biochemistry

To obtain knowledge of important facets of the biology and biochemistry of the major pathogenic trypanosome species relevant to developing improved control measures for trypanosomiasis.

TR3. Immunology of trypanosomiasis

To determine the immunological and related mechanisms involved in the acquisition or expression of resistance to trypanosomiasis and investigate if such mechanisms can be manipulated in order to improve resistance to trypanosomiasis.

TR4. Resistance to pathogenic effects of trypanosomes

To determine the mechanisms which result in the resistance or susceptibility of livestock to the pathogenic effects of trypanosomiasis such as haemopoietic and reproductive dysfunction.

EPIDEMIOLOGY AND SOCIOECONOMICS

The epidemiology and socioeconomics programme has the following objectives.

To identify the factors at both national and farm levels which are essential for the successful delivery and acceptance of improved control measures for livestock disease.

To develop and verify predictive models for theileriosis which can be used in different locations and production systems to determine the probable epidemiological, economic, social and environmental consequences of the application of improved disease control measures, including immunisation.

To develop predictive models for trypanosomiasis which can be used in different locations and production systems to determine the probable epidemiological, economic, social and environmental consequences of the application of improved disease control measures, including tsetse control, chemotherapy, the use of trypanotolerant livestock and potential immunisation.

We continue to hope that this report will be of value to the scientific community, particularly to those scientists in both the developing and the developed world who also undertake research leading to improved control of livestock diseases.

J.J. Doyle
Director of Research

THEILERIOSIS

TH1.1 The *In vivo* characterisation of *Theileria parva* (Muguga) stabilate 3087 and the definition of an appropriate challenge for assessment of immunity

Scientists: T.T. Dolan, S.P. Morzaria, A.J. Musoke, G. Gettinby*
Research Associates: F. Mwakima, J. Scott, P.R. Spooner
Technician: J. Klarie

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The *Theileria parva* (Muguga) bulk stabilate, 3087, provides most of the sporozoites being used in the search for sporozoite antigens in developing a sporozoite vaccine. It is an important component in the development of cloning, characterisation and serological techniques for *T. parva* and is the parasite being used in attempting to identify factors which influence the infection rate in *Rhipicephalus appendiculatus* ticks. Infectivity, parasitological, clinical and pathological data have been collected over three years from 123 Boran cattle infected with different doses of the stabilate. One ml of undiluted, 1:10 or 1:20 dilutions of the stabilate produces fatal infections in all cattle tested. The stabilate has an estimated LD_{50} at a dilution of 1:100. Cattle suffering more prolonged disease reactions develop anaemia which may be profound (PCV 14%). Small numbers of naturally recovered animals have been shown to be partially immune to challenge with the heterologous stocks, *T.p. parva* (Marikebuni), (Mariakani), (Uganda) and the subspecies to *T.p. lawrencei* (7014) and *T.p. bovis* (Boleni). Among the responses to challenge is a transient neutrophilia, although this response is less marked than reported in an earlier study.

Stabilate 3087 is also being used either directly or through ticks in the initial testing of the immunity of cattle immunised with novel vaccines. It is essential to have information on the severity of challenge being applied in assessing the protection provided by any candidate vaccine. Stabilates provide reproducible infected doses of mature sporozoite which are delivered, usually, as a single dose. Ticks transmit sporozoites over 6-7 days with a peak output at 4 days. A single tick can induce a lethal infection and the contents of less than one infected acinus can be lethal. Examination of tick infection rate data shows a remarkable variability in both infection rate and the number of infected acini among ticks. Using a computer programme based upon tick infection rate data with stabilate 3087, it is possible to define the number of ticks to apply from a batch with a known infection and the cattle group sizes to use to give statistically valid results.

TH1.2 Characterisation of *Theileria parva* using monoclonal antibodies

Student: P.M. Rwambo
Scientist: T.T. Dolan
Research Associate: P.R. Spooner
Technicians: R. Njamunggeh, L.K. Juma

Monoclonal antibodies (MAbs) have been used in an indirect fluorescent antibody test (IFAT) to characterise different stocks of *Theileria parva* for ten years. The addition of new MAbs has resulted in a panel of twenty for routine use. It was decided to determine if all 20 MAbs were essential, whether different MAbs could improve the panel, and if the IFAT could be made less laborious.

The requirements of the panel were considered to be:

1.To distinguish between the six *T. parva* stocks selected for extensive characterisation (*T. p. parva* (Mariakani), (Marikebuni), (Muguga), (Uganda), *T. p. bovis* (Boleni), *T. p. lawrencei* 7014).

2.To characterise *T. parva* stocks resulting from isolates taken within the region affected by East Coast fever (ECF).

3.To separate other theilerial species isolated from cattle, buffalo and other Bovidae in the region.

4.To permit reproducibility of MAb profiles by different operators.

The criteria used for inclusion of a particular MAb into the panel were based on these requirements. The present panel contains nine MAbs from the original panel, plus two new MAbs numbers 21 and 22. This panel can separate the six selected stocks of *T. parva* and retains the sensitivity to characterise isolates within the region affected by ECF. Although specific MAbs for the identification of other *Theileria* species are unavailable, typical reactions of *T. taurotragi* and a *Theileria* species from buffalo permit differentiation of these species from *T. parva* using this panel. The reproducibility of the test has been improved by the removal of MAbs which gave weak variable reactions. MAbs 15 and 20 have been retained since they give good reactions which assist separation of selected stocks. However, they give weak reactions with certain other stocks. The IFAT has been simplified by assessment of results on a +/- basis rather than by titration of each MAb, and the test is performed with only two dilutions of each MAb.

TH1.3 Characterisation of *Theileria parva* schizont antigens by two-dimensional Western blotting

Scientists: M. Kishima, T.T. Dolan
Research Associates: P.R. Spooner, C. Nkonge
Technician: R. Njamunggeh

Previous studies demonstrated some differences in two-dimensional (2-D) gel electrophoresis patterns of schizont proteins among *Theileria parva* stocks.

In this study, schizont proteins of *T. parva* (Muguga), (Mariakani), (Marikebuni), (Uganda), *T .p. bovis* (Boleni) and *T. p. lawrencei* 7014 were compared by 2-D Western blotting using sera from cattle which were immunised or challenged with each stock and monoclonal antibodies raised against *T. parva*. Isoelectric points of the polymorphic immunodominant molecule (PIM) of schizonts which produced major antibody responses in cattle, ranged from acidic to basic. Molecular masses (Mr) of PIM of each stock were as follows: Muguga, 86 kDa; Mariakani, 83 kDa; Marikebuni, 83 kDa; Uganda, 83 kDa; Boleni, 83 kDa; 7014, 100 kDa. Two-D Western blotting patterns of *T. p. parva* Mariakani and Uganda were very similar. PIMs of *T. parva* subspecies were cross-reactive. It was very difficult to identify unique schizont proteins in *T. parva* for strain characterisation. However, as all animals responded to the PIM of the schizont, it may be a useful antigen for diagnosis.

TH1.4 Characterisation of *Theileria parva* using DNA technology

Scientists: R. Bishop, S.P. Morzaria, B. Allsopp*, T.T. Dolan
Research Associate: P. Spooner
Technician: B. Sohanpal

*Department of Biochemistry, University of Cambridge, UK

Primers derived from the *TpR* repetitive region of the *Theileria parva* genome have been successfully applied to detect parasites from several *T. parva* stocks in the blood of carrier cattle, using the polymerase chain reaction (PCR). Parasite DNA was detected in whole blood DNA made from *T. p. bovis* Boleni and *T. p. parva* (Marikebuni) but not *T. p. parva* (Muguga) cattle, which correlates with the fact that *T. parva* Muguga has never been observed to induce carrier states. In view of the importance of the carrier state in the epidemiology of East Coast fever, the ability to detect carrier animals without performing tick transmission experiments should prove valuable for future epidemiological work.

By cloning and sequencing PCR products from the *TpR* repetitive region, two stock specific oligonucleotides have been identified one for *T. p. parva* (Uganda) and one for *T. p. parva* (Marikebuni). The *T. p. parva* (Uganda)-specific oligonucleotide is being used in conjunction with a *T. p. parva* (Muguga)-specific oligonucleotide (developed by Dr. B. Allsopp) in an experiment to look for possible genetic recombination between these two stocks.

Three probes - (1) a *T. p. parva TpR* repetitive region probe, (2) a *T. parva* small subunit ribosomal DNA probe, (3) *T. p. parva* telomeric probe - have been used to examine restriction fragment length polymorphisms among a wide range of *T. p. parva* and *T. p. lawrencei* stocks. Parasite groupings defined by these methods and also by *Sfi* I restriction polymorphisms and reactivity with anti-schizont monoclonal antibodies (MAbs) will be examined for possible correlation with data from *in vivo* cross protection experiments carried out using cloned *T. p. parva* parasites.

A 1.6 kilobase (kb) *T. p. parva* telomeric clone isolated using a synthetic oligonucleotide derived from a published *Plasmodium berghei* telomeric sequence is being characterised and has been almost completely sequenced on one strand.

TH1.5 *Theileria* species specific DNA probes

Scientists: R. Bishop, B. Allsopp*, H. Baylis*, S.P. Morzaria, T.T. Dolan
Research Associates: P.R. Spooner, E. Gobright
Technicians: B. Sohanpal, R. Njamunggeh

*Department of Biochemistry, University of Cambridge, UK

In *Plasmodium* it has been demonstrated that species-specific synthetic oligonucleotides designed to probe parasite small subunit RNA can be sensitive epidemiological tools. In collaboration with Dr. B. Allsopp species-specific regions have been identified in the small subunit ribosomal DNA of five *Theileria* species. In Cambridge, small ribosomal subunit oligonucleotide probes specific for *Theileria parva*, *T. annulata* and a buffalo parasite (*?T.*

lawrencei) were synthesised and at ILRAD specific probes were synthesised for *T. taurotragi* and *T. mutans*.

Preliminary experiments in which these oligonucleotides were probed to acetic acid lysed RNA slot blots from infected lymphocyte cultures of different *Theileria* species suggest that they will be valuable for epidemiological studies. All four probes which were tested were species specific. The *T. parva* and *T. annulata* probes were capable of detecting as few as four hundred *Theileria*-infected cells, although the *T. taurotragi* and ? *T. lawerencei* specific oligonucleotides were less sensitive and conditions for their use require further optimization. A future priority will be to adapt these oligonucleotide probes for parasite detection in infected blood and *Theileria*-infected tick salivary glands.

Work has also been carried out on developing repetitive DNA probes specific for *T. mutans* and *T. taurotragi*. *Theileria taurotragi*, which is indistinguishable from *T. parva* in the *Rhipicephalus appendiculatus* tick vector by current methods, is a major source of confusion in the epidemiology of East Coast fever. To attempt to obtain a *T. taurotragi*-specific repetitive DNA probe which can specifically identify *T. taurotragi* in the tick vector a sheared DNA library in λ*gt*11 has been constructed using DNA prepared from schizonts purified from a *T. taurotragi*-infected sheep cell line.

A previously isolated *T. mutans*-specific repetitive DNA probe has been partially sequenced and primers derived from this sequence have been used to specifically amplify a 170-base pair region of the *T. mutans* genome using the polymerase chain reaction. It is intended to use these primers to detect *T. mutans* parasites in carrier cattle.

TH1.6 Molecular characterisation of the *Theileria parva TpR* gene family

Scientists: R. Bishop, H. Baylis*, B. Allsopp*, S.P. Morzaria, A.J. Musoke, T.T. Dolan
Research Associates: E. Gobright, C. Nkonge
Technician: B. Sohanpal

*Department of Biochemistry, University of Cambridge, UK

The *TpR* genomic region appears to be the major repetitive component of the *Theileria parva* genome and clones from this region have been extensively used for characterisation of *T. parva* stocks and strains, both in Cambridge and at ILRAD.

In view of the value of *TpR* region clones as characterisation reagents, it is desirable to know more about the evolution and possible functions of the *TpR* sequences.

In Cambridge sequencing of an 8.5 kilobase (kb) fragment of DNA from the *TpR* region of *T. parva* (Muguga) has revealed the presence of a series of tandemly arranged, partially repeated, long open reading frames.

At ILRAD, *TpR* region clones from three different *T. parva* stocks - *T. p. parva* (Muguga), *T. p. parva* (Uganda) and *T. p. bovis* (Boleni) - have been sequenced. These sequences also reveal the presence of large open reading frames, the 5' portions of which exhibit little homology between the stocks, although the 3' ends do exhibit some homology. Northern blot analysis reveals the presence of a 3.5 kb transcript in piroplasm RNA, but not

in sporozoite or schizont RNA, which hybridises to *TpR* genomic probes. *TpR* homologous clones have been isolated from a piroplasm cDNA library and sequencing of the 3' ends reveals the presence of three different 3' non-translated sequences among five clones. None of the cDNA clones were of full length and obtaining full length cDNA clones will be a future priority. Portions of two of the open reading frames present in the DNA sequences - one from *T. p. bovis* (Boleni) and one from *T. p. parva* (Muguga) - have been expressed in the pGEX plasmid vector and rats have been immunised with the fusion proteins. It is intended to use the rat antisera for Western blot analysis and electron microscopic work on the intracellular localisation of the putative proteins encoded by the open reading frames observed in the genomic sequences.

TH1.7 Attempts to complete the genomic map of *Theileria parva*

Scientists: S.P. Morzaria, J.R. Young*
Technicians: T. Batavia, T. Ndolo

*AFRC, Compton, UK

The objective and the strategies for the construction of a physical map of *Theileria parva* were described in the last year's annual scientific report. Using these strategies, an incomplete *Sfi* I restriction map was constructed which showed that there are four chromosomes in the genome of *T. parva*. Of the 30 *Sfi* I genomic fragments, 24 are allocated on various *T. parva* Muguga chromosomes using *Sfi* I linking clones. Putative location of all the *T. parva* genes isolated so far have been determined on the map.

One of the major inhibiting factors in completing the map was our inability to separate *Sfi* I and *Not* I DNA fragments greater than 1000 kb. We have now successfully separated these fragments using more sophisticated pulsed-field gel electrophoresis methods. The position of the ambiguous *Sfi* I fragments were identified by hybridisation of these fragments with chromosomes 1 and 3, and the larger *Not* I fragments above 1600 kb. Using the Smith/Bernsteil *Sfi* I partial digest analysis, the 3 *Sfi* I fragments were accurately located on the map.

At present, there is only one *Sfi* I fragment, fragment 12, which has been unassigned. The screening of *T. parva* (Muguga) *Sau 3A* partial library with nick-translated DNA eluted from the discrete *Sfi* I fragment 12 revealed several homologous clones. Of these, three were found to have an *Sfi* I restriction site. These clones are currently being tested on the Southern blots of the *Sfi* I digested genomic DNA. The map will be used for studies on possible sexual recombination in *T. parva*.

TH1.8 Development and evaluation of an ELISA for detection of bovine antibodies against *Theileria parva* schizont antigens

Scientists: P.M. Rwambo, T.T. Dolan, A.J. Musoke
Research Associates: C. Nkonge, J. Katende, S. Minja, P.R. Spooner
Technicians: R. Njamunggeh, J. Kiarie

The indirect fluorescent antibody test (IFAT) is currently the routine serological test for diagnosis of *Theileria parva* infections in cattle. For extensive epidemiological studies, the IFAT is not only expensive but also unreliable because of its subjective nature. There has therefore been a need to develop other more versatile, reliable, easy to interpret and less expensive immunoassays for the diagnosis of *T. parva* infections. The aim of this study was to identify *T. parva* schizont antigens for the development and evaluation of a micro ELISA for detection of anti-schizont antibodies in cattle infected with *T. parva*.

Serum samples were obtained from ILRAD cattle infected with either cloned or bulk *T. parva* (Muguga 3087), *T. parva* (Mariakani 3319), *T. parva* (Marikebuni 3014), *T. parva* (Uganda 3066), *T. p. lawrencei* 7014 (3081) and *T. p. bovis* (Boleni 3039) stabilates, respectively. The sera (n=134) were initially screened for *T. parva* anti-schizont antibodies by IFAT, which was used as the standard test for evaluating the ELISA. Western blot analysis of the sera using schizont antigens revealed that: 1) the antibody response was initially directed against the polymorphic immunodominant molecule (PIM) of the respective *T. parva* stocks; 2) antibodies to other schizont proteins appeared later and disappeared at a faster rate than those reactive with PIM; 3) no parasite stock or *T. parva* subspecies- and species-specific schizont antigens were identified; 4) nearly all cattle infected with *T. parva* produced antibodies against PIM of the respective parasite stock; and 5) PIM of one parasite stock had cross-reactive determinants that were recognised by antibodies from cattle infected with different stocks of *T. parva*. Based on these data, a *T. parva* schizont ELISA was developed.

Schizonts purified from a *T. parva* Muguga-infected bovine lymphoblastoid cell line were solubilised by sonication and used to coat ELISA plates. The optimum schizont protein concentration for the assay was determined in a checkerboard titration using reference *T. parva* positive and negative bovine sera as determined by IFAT. The results indicate that 70% of the 134 serum samples were positive by IFAT, while 71.6% of the same sera were positive by ELISA. An analysis of the serum samples that were positive by IFAT showed that 96.7% of these samples were positive by ELISA. A comparison of IFAT, ELISA and Western blotting showed that a few samples were positive or negative by one and not the other of the immunoassays. The correlation between the Western blot seropositivity to PIM and the ELISA was remarkably high. However, further evaluation and improvement of the ELISA is needed to improve on the sensitivity and specificity for *T. parva* infections in cattle.

TH1.9 Studies on the handling and storage of *Theileria parva* sporozoite stabilates

Student: E.N. Kimbita
Supervisor: T.T. Dolan

The standard method for the cryopreservation of *Theileria parva* sporozoite stabilates uses 7.5% glycerol and was adapted from a technique for preservation of trypanosomes. Other cryoprotectants, freezing rates and preparation procedures have not been reported for *Theileria* parasites. Attempts have been made to lyophilise *Theileria* sporozoites with a view to easing problems related to storage, transportation and delivery of stabilates.

Theileria p. parva (Muguga 3087) sporozoites were preserved in various concentrations of a range of cryoprotectants. The optimal concentration of each cryoprotectant was determined by *in vitro* titration of infectivity of the thawed stabilates. The optimal concentrations were found to be 5% dimethyl-sulphoxide, 5% polyvinyl pyrrolidone, 5% polyethylene glycol and 2.5% hydroxy ethyl starch.

Both acidity (pH 5-6) and alkalinity (pH 9) had adverse effects on the infectivity of sporozoites. The sporozoite infectivity was stable between the range pH 7-8. The loss of infectivity due to acidity (pH 5-6) was partially reversible; but beyond pH=9, infectivity was lost.

Sporozoite preparations were stabilized with polyvinyl-pyrrolidone, polyethylene glycol, hydroxyethyl starch, foetal bovine serum, MEM, sucrose peptone water, Leibovitz-15 and saline skim milk, then lyophilised. Most preparations were visibly dry after 4 hr. Samples were tested for infectivity after 4, 6, 12 and 24 hr drying. Some stabilate vials were sealed under vacuum while others were sealed under atmospheric conditions. The lyophilized material was resuscitated with distilled water, L-15 or a mixture of the two. None of the above combinations produced infective sporozoites.

TH1.10 Immunisation against *Theileria mutans*

Scientists: S.P. Morzaria, A.J. Musoke, V. Nene, T.T. Dolan
Research Associates: J. Katende, F. Mwakima, C. Nkonge
Technician: J. Kiarie

East Coast fever-immunised cattle undergo a disease characterised by anaemia and loss in productivity when exposed to natural tick challenge. This disease is associated with the appearance of parasites in the blood which have been isolated and identified as *Theileria mutans*. The cattle that recover from this parasite are immune to subsequent tick challenges. The objective of this study was to determine if cattle immunised with lysates of *T. mutans* mount a response similar to that induced by live organisms and to test if such animals can withstand severe *T. mutans* piroplasm challenge.

Five *T. mutans*-susceptible Kapiti Boran calves were divided into two groups. Group I consisted of three calves which were immunised with the Zanzibar stock while two calves in Group II were immunised with the Intona stock. The immunisation was performed using lysates of semi-purified *T. mutans* piroplasms. The primary immunisation was carried out with 300 µg of the lysate in complete Freund's adjuvant followed by five booster immunisa-

tions at approximately monthly intervals using the same amount of the lysate in incomplete Freund's adjuvant.

A week after the last boost, two animals in Group I and one animal in Group II were splenectomised. Additionally, two more *T. mutans* susceptible Kapiti Boran were splenectomised. Twelve days after splenectomy, all calves in Group I and II and the two splenectomised susceptible calves were challenged with *T. mutans* Zanzibar piroplasm stabilate. At the time of the challenge, the antibody titres of the immunised animals varied between 1/12500 and 1/62500 in the ELISA test using total piroplasm lysate as the antigen.

None of the immunised animals became infected while both the susceptible controls acquired *T. mutans* infection. One animal had a maximum *T. mutans* piroplasm parasitaemia of 36% on day 39 after challenge, and its PCV dropped from 29% at the time of infection to 9% on day 41. The other animal showed a milder reaction, with a maximum piroplasm parasitaemia of 3.6% on day 59 and a drop in PCV from the pre-infection level of 31% to 16% on day 64. The sera from all animals showed reaction to the 32 kDa and 72 kDa piroplasm antigens on Western blots. The reactivity of the sera to the 32 kDa was the stronger.

This is the first time protection against any tick-borne protozoan diseases has been demonstrated following immunisation with the whole lysates of piroplasms. In view of the fact that the predominant antibody response is to the 32 kDa piroplasm antigen, we are currently immunising cattle against this antigen.

TH2.1 The entry of *Theileria parva* sporozoites into bovine lymphocytes: MHC class I is involved

Scientists: M.K. Shaw, L.G. Tilney*, A.J. Teale, A.J. Musoke
Technician: P.M. Theuri

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We have re-examined the process of *Theileria parva* sporozoite entry into susceptible bovine lymphocytes and have begun to identify the molecular interactions involved in the process. The entry process involves a defined series of events and we have used a number of experimental procedures in combination with a method of quantitation to examine various aspects of this process.

Theileria parva sporozoites are non-motile organisms and the initial sporozoite-lymphocyte interaction is a chance event which can occur at 0-2°C. All subsequent stages in the process are temperature-dependent, require the participation of live intact sporozoites and host cells, and involve some cytochalasin-inhibitable rearrangement of the host cell surface membrane or cytoskeleton. Once internalized within the lymphocyte, a process that takes less than 3 min at 37°C, the sporozoite rapidly escapes from the encapsulating host cell membrane, a process which occurs concurrently with the discharge of the contents of the sporozoite rhoptries and microspheres.

Sporozoite entry can be inhibited by antibodies (MAbs) reactive with MHC class I molecules and with beta-2-microglobulin, whereas MAbs reactive with class II molecules, a common panleucocyte surface antigen (a bovine equivalent of CD11a) and a series of MAbs reactive with bovine lymphocyte surface molecules associated with cell activation had no effect on the process of sporozoite binding and entry.

Further evidence that MHC class I molecules are involved in the sporozoite entry process has been obtained from infection studies using a cloned lymphoblastoid cell line and a series of gamma-irradiation induced MHC deletion mutant subclones which clearly demonstrated that the level of sporozoite binding and entry was related to the level of class I expression.

While the present studies indicate a clear role for MHC class I molecules in the *T. parva* sporozoite entry process, further studies are in progress to try to identify other PBL surface molecules involved in determining sporozoite tropism.

TH2.2 Infection of bovine afferent lymph veiled cells with *Theileria parva* sporozoites

Scientists: D.J. McKeever, A.J. Musoke, M.K. Shaw, L.G. Tilney*
Technician: J. Gachanja

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As part of an effort directed at the elucidation of the mechanisms involved in sporozoite entry into bovine lymphocytes, the diversity of host cells which are capable of accommodating the parasite was investigated. During these experiments, it was observed that the

parasite appears to invade afferent lymph veiled cells (ALVC) more efficiently than lymphocytes.

Because the bite site of *Rhipicephalus appendiculatus* in cattle is characterised predominantly by the presence of mononuclear cells, most of which are of the macrophage type, we are interested in defining the significance of the interaction between ALVC and *T. parva* sporozoites.

Analysis by electron microscopy of ALVC after culture with sporozoites at 37°C for thirty minutes reveals that ALVC appear to take up the parasite more efficiently than the lymphocytes which commonly contaminate ALVC preparations. After seven days of culture, infected ALVC can be seen to contain early schizonts. Examination of similar cultures by Giemsa staining reveals that the parasite appears capable of development to the schizont stage within ALVC, although it has not been possible to demonstrate its ability to transform them. Because ALVC are believed to travel from the periphery to the T-cell areas of draining lymph nodes, and are likely to be derived in part from dendritic or macrophage-like cells within the dermis, we sought to determine whether the parasite could be transferred from infected ALVC to lymphocytes. ALVC were irradiated and incubated with sporozoites at 37°C for 30 minutes, and washed extensively to remove free sporozoites. Infected ALVC were then added to cultures of autologous and allogeneic lymphocytes, and the cultures were monitored for the development of proliferative infection. Transformation of both autologous and allogeneic PBM was observed after six days of culture, and schizont parasitosis was confirmed by Giemsa staining.

The results of these experiments suggest that ALVC constitute more permissive target cells than lymphocytes for sporozoite invasion, and provide preliminary evidence that infection can transfer between parasitised ALVC and uninfected lymphocytes. Future experiments will attempt to consolidate these results and investigate the significance of these observations to the development of infection *in vitro*.

TH2.3 A single exon codes for a cysteine protease of *Theileria parva*

Scientists: V. Nene, A.J. Musoke, J.D. Lonsdale-Eccles
Research Associates: E. Gobright, G.W.N. Mpimbaza
Technicians: D. Mwaengo, J. Ngugi

We have previously reported the identification of a cathepsin L-like protease activity in extracts of piroplasms of *Theileria parva*. The activity is optimal at pH 6.0 and is inhibited by E-64, a specific inhibitor of cysteine proteases. Sporozoites also contain protease activity and activity in schizonts has been inferred by the isolation of cDNA from schizont-infected cells. Hence, all stages of the parasite tested contain protease activity, although we cannot unequivocally equate the enzyme activities to each other nor can we assign the activity to the gene we have identified.

The complete gene and cDNA coding for a *T. parva* cysteine protease has been sequenced. The gene is divided into two exons by an intron which is 32 bp long. The first exon codes for a signal sequence and for part of the *pro* region. The second exon codes for the remainder of the *pro* region, including regions thought to be involved in zymogen processing, and for the entire enzyme domain. This is, to our knowledge, the first description

of a gene coding for a eukaryotic cysteine protease which lacks an intron in DNA coding for the enzyme domain. The mature parasite protease is predicted to consist of 212 amino acid residues with a calculated molecular mass of 23,500 Daltons. There is an overall homology of 44% with papain.

The second exon coding for the *T. parva* protease domain cross-hybridises with *Plasmodium falciparum* DNA at high stringency, indicating a significant DNA sequence homology between the two parasites. Indeed, an enzyme similar to the *T. parva* cysteine protease has been described.

Our interest in the *T. parva* enzyme was stimulated by reports that cysteine protease inhibitors are toxic for certain bacterial infections. Preliminary experiments with Z-Leu-Val-Gly-CHN2, a diazomethyl ketone inhibitor of cysteine proteases, shows that the growth of schizont infected cells is impaired.

Similar toxic effects on *P. falciparum* and *Trypanosoma brucei brucei* by the use of cysteine protease inhibitors have been reported. The availability of the *T. parva* protease sequence together with the possibility of producing active recombinant enzyme may allow the design of new drugs for chemotherapy

TH2.4 Expression of the *Theileria parva* p67 sporozoite surface antigen using the pAS expression vector system in *Escherichia coli*

Scientists: V. Nene, A.J. Musoke
Research Associates: C. Nkonge, S. Minja
Technicians: D. Mwaengo, J. Ngugi

This work was carried out in collaboration with SmithKline Beecham, Philadelphia, USA

The 2.3 kbp *BamH* I fragment containing the p67 gene was repaired and blunt end cloned into the *Hpa* I site *pMG* I, a derivative of the pAS vector system. This expression vector is derived from the *pAS* series of vectors which allows the construction of C-terminal fusions with the NSI non-structural protein of influenza virus. Expression is controlled by the PL promoter of bacteriophage λ and expression is induced by heat shock. The fusion protein, which is insoluble, contains 87 amino acid residues at the N-terminal end; 85 residues are derived from the N-terminal region of NS1, and all 709 residues of p67.

SmithKlineBeecham carried out a 20-litre fermentation to provide about 880 g wet weight cells. Antigen for cattle immunisations were prepared from this cell paste. One gram of cells was disrupted in 10 ml buffer A (50 mM glycine, 2 mM EDTA pH 8.0) using a bead beater. After a centrifugation step (13K, 30 min) to clear the lysate, the pellet, which contains the majority of the fusion protein, was washed once and the pellet was extracted with 10 ml buffer A containing 0.1% NP40 at 4°C for 1 hr. The supernatant recovered after centrifugation was aliquoted and stored frozen at -80°C until use for immunisation.

TH2.5 Expression of the *Theileria parva* p67 sporozoite antigen in insect cells using a recombinant baculovirus

Scientists: V. Nene, A.J. Musoke, M.K. Shaw, S. Inumaru*
Technician: D. Mwaengo

*National Institute of Animal Health, Tsukuba, Japan

We have previously reported the expression in *Escherichia coli* of the major sporozoite surface antigen, p67, as a C-terminal fusion protein of Sj-26, a glutathione-S-transferase of *Schistosoma japonicum*. However, the bacterial recombinant p67 protein is not recognised by murine monoclonal antibodies (MAbs) which bind to peptide epitopes on the native p67 on Western blots.

We decided to exploit the baculovirus expression system since the system is reported to yield high levels of authentic recombinant eukaryotic protein. Recombinant *Autographa californica* nuclear polyhedrosis virus (AcNPV) was used to infect *Spodoptera frugiperda* cell cultures, SF21AE, to produce non-fused p67.

The gene coding for p67 was cloned into a transfer vector, *pACYM* 1, and placed under the control of the polyhedrin promoter. Recombinant plasmid was co-transfected with AcNPV DNA into the insect cells using lipofectin. Extracellular virus particles were harvested and screened for an occlusion negative phenotype. Occ⁻ virus clones were analysed for p67 production in infected insect cells.

Western blot analysis with TpM 12.18.15.6, a MAb that neutralises sporozoite infectivity *in vitro*, showed that the MAb recognised a 67-kDa antigen in infected cells. However, pulse-labelling studies indicate that the majority of the label is incorporated into a molecule that migrates at about 110 kDa. This large species is recognised by a rat polyclonal anti-p67 antiserum and by TpM 12.18.15.6. The *E. coli* recombinant antigen also migrates with a higher apparent molecular mass than expected.

Live infected cells show positive immunofluorescence with TpM 12.18.15.6. Hence, it appears that the sporozoite antigen is processed to the surface membrane as it is in sporozoites. Immunocytochemistry has also localised the antigen to the surface of infected cells, but the antigen is also present inside the cells. Hence, the processing appears to be inefficient and in any case it does not occur exactly as in sporozoites since another neutralising MAb, 23F, does not recognise the insect recombinant antigen. These processing events are under further investigation.

TH2.6 Further characterisation of the gene coding for the *Theileria parva* sporozoite antigen, p67

Scientists: V. Nene, A.F. Barbet*, A.J. Musoke
Technician: D. Mwaengo

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Expression of the p67 sporozoite antigen appears to be stage specific. We have not been able to detect either the p67 antigen or the transcript in the schizont stage of the parasite. To test this more rigorously we have used the polymerase chain reaction on oligo-dT primed first strand cDNA made from mRNA isolated from infected lymphocytes. Preliminary results, with synthetic primers to N- and C-terminal regions of the gene, suggest that the p67 gene is not expressed in schizonts.

Amino acid sequence homology searches with p67 to proteins in the NBRF database have identified a significant sequence homology to the *Theileria annulata* sporozoite surface antigen. Comparison of the available 110 residues of the *T. annulata* antigen with the *T. parva* p67 antigen shows that there is a 55% homology between the two sequences. Southern blot analyses show that the p67 gene cross-hybridises with DNA from *T. annulata* at low stringency.

It appears that the major sporozoite surface antigens of *T. annulata* and *T. parva* are encoded by similar genes that have diverged during evolution. This degree of sequence homology raises questions on whether it may be possible to develop vaccines against both parasite infections using recombinant p67. It will also be of interest to determine whether the biological differences between the parasites, e.g., the host cell specificity, reside in the amino acid sequence differences between these two related genes.

TH2.7 Mapping of B-cell epitopes on the *Theileria parva* sporozoite surface antigen, p67

Scientists: V. Nene, A.J. Musoke
Research Associates: C. Nkonge, S. Minja
Techicians: D. Mwaengo, J. Ngugi

Monoclonal antibodies to the native p67 antigen do not recognise the *Escherichia coli* recombinant antigen although the MAbs do not bind to carbohydrate epitopes. To map B-cell epitopes, we have isolated several MAbs against the NS1-p67 fusion protein. Fifteen MAbs bind to native p67 on Western blots and 2 of these MAbs neutralise sporozoite infectivity *in vitro*.

To map the position of the B-cell epitopes, we have constructed NS1-p67 deletions by exploiting the presence of restriction enzyme sites within the p67 gene. These deletions remove coding DNA from the C-terminal end. Six deletions have been made which express residues 1-96, 1-175, 1-271, 1-397, 1-522 and 1-644 of p67. All the deletions contain the same N-terminal 87 residues derived from the expression vector. A construct which removed the NS1 portion, resulting in the synthesis of non-fused p67, was also made. This

antigen is, however, unstable and is rapidly degraded within the cell after inducing gene expression.

All the MAbs have been tested for recognition of the NS1-p67 deletion constructs and surprisingly the MAbs fall into two groups. Group A recognise epitopes that map between residues 522-644 and group B MAb epitopes map to residues 175-271. Hence, these peptide sequences also contain epitopes recognised by the neutralising MAbs.

The specificity of these MAbs with respect to each other and to the core amino acid sequence recognised remains to be determined. The NS1-p67 deletion derivatives will also be useful for mapping the epitopes. Such studies may help in defining functional immune responses to the recombinant antigen in cattle.

TH3.1 Direct evidence of a role for parasite-specific cytotoxic lymphocytes in clearance of *Theileria parva* infection

Student: E.L.N. Taracha
Scientists: D.J. McKeever, E.A. Innes
Research Associate: N.D. MacHugh
Technician: E. Awino

There is strong evidence that protection of immune cattle against challenge with *Theileria parva* is mediated by cellular mechanisms directed at the schizont-infected cell. The appearance of class I MHC-restricted $CD8^+$ cytotoxic T cells (CTL) in the blood of immune animals around the time of remission has led to the belief that parasite-specific CTLs are responsible for clearance of the parasite in immune animals. Direct evidence for this conclusion has, however, been lacking, and although a previous experiment at ILRAD demonstrated that immunity could be transferred between immune and naive chimaeric twins in the cellular fraction of thoracic duct lymph, a role for non-CTL cellular mechanisms in this effect could not be ruled out.

We have prepared $CD8^+$ CTLs from efferent lymph draining a responding immune lymph node during the period of maximal parasite-specific CTL activity, and transferred them to a naive twin lethally infected with the parasite at the time of the emergence of schizont parasitosis. Cells were prepared for transfer by complement lysis of $CD8^-$ lymphocyte populations using lineage-specific monoclonal antibodies. Transferred cells were capable of high levels of parasite-specific killing, and limiting dilution analysis revealed that the frequency of precursor CTLs in this population was 1:31. Complement lysis of $CD8^+$ cells abrogated direct parasite-specific killing and rendered CTL precursors undetectable. A total of 1×10^{10} cells were transferred to the recipient over a period of three days. Direct parasite-specific cytotoxicity was detectable in the blood of this animal one day after the completion of transfer, and subsequently this activity reached higher levels than were detected in the donor animal at equivalent times of infection. Schizonts were first detected in lymph node smears prepared from recipient and control animals on day seven following challenge, the third day of transfer. In the recipient, parasitosis reached peak levels of two per cent on day nine after challenge, and had dwindled to undetectable levels by day thirteen. In contrast, parasitosis increased steadily in the control animal reaching levels of 44% by day fourteen, at which time treatment was initiated.

These results suggest that the transfer of large numbers of parasite-specific CTLs derived from an immune twin can confer protection against patent infection following lethal challenge with *T. parva*. The kinetics of this effect suggest that these cells are capable of rapidly clearing the parasite from the animal. We believe that this observation provides strong evidence that parasite-specific CTLs constitute the major immune mechanism behind protective immunity to *T. parva* in cattle.

TH3.2 Analysis of CTLp frequency and MHC bias in an animal immunised with *Theileria parva*

Scientists: P.G. Toye, A.J. Teale
Research Associate: G.M. Lamb
Technicians: D. Ngugi, J. Mwakaya

We have analysed the CTLp frequency and MHC restriction bias in an animal undergoing immunization with *Theileria parva* (Muguga). The MHC type of the animal is W1/KN104. CTLp frequency analysis was performed on autologous infected cells (W1/KN104), semi-matched infected cells (W1/W1 and KN104/-) and mismatched infected cells (W25/W25). Analyses were performed on days 10, 12, 14 and 21 after infection.

The peak CTLp response to the autologous infected cell occurred on day 14 of infection, with a CTLp frequency of 1/218. A similar frequency was observed using target cells of the W1/W1 BoLA type, and a lower frequency for the KN104/- cells (1/1106).

The above results were obtained using a cut-off point of the mean, plus three standard deviations of the mean, of release of label from control wells. Using a higher stringency cut-off point (10% specific release of label), the frequency of CTLp specific for the W1/KN104 and W1/W1 cells was 1/580 and 1/473, respectively. However, the CTLp frequency against the KN104/- cells was less than 1/20,000.

A CTL line obtained from the animal and stimulated with autologous infected cells *in vitro* showed potent killing against the W1/KN104 and W1/W1 target cells, but lysed the KN104/- target cells very poorly.

The results indicate that the bias in the MHC restriction of cell lines stimulated *in vitro* is reflected in the frequency of CTLp of higher potency.

TH3.3 Development of technology to enable screening of a *Theileria parva* schizont gene library with CTL

Scientist: P.G. Toye
Research Associate: G.M. Lamb
Technician: D. Ngugi

We are attempting to develop methods to enable us to use CTL from cattle immune to *Theileria parva* to identify their corresponding antigens in a library of schizont genes expressed in eukaryotic cells. One possibility is to identify the "positive" transfectant by its ability to restimulate cloned, or bulk, CTL lines.

To test this possibility, we have examined the ability of *T. parva*-infected cells, when mixed with uninfected cells (representing "negative" transfectants), to restimulate a CTL clone. In each of twelve wells containing 10^2 infected and 10^5 uninfected cells, positive responses were observed when compared to wells containing only 10^5 uninfected cells.

As the initial attempts to establish a eukaryotic schizont gene library will employ mouse L fibroblasts, we have tested this system using L-cells transfected with the KN104

MHC class I molecule (IL-X2) and an alloreactive CTL clone specific for this molecule. At a level of 10^3 IL-X2/10^5 parent Ltk$^-$ cells, ten out of twelve wells were positive, whereas at 10^2 IL-X2/10^5 Ltk$^-$ cells, no positive responses were generated.

The results suggest that this approach may prove successful in identifying antigens recognized by immune CTL, although improvements in the sensitivity of the system employing mouse L-fibroblasts may be required.

TH3.4 Evaluation of bovine papilloma virus-based vectors for expression of *Theileria parva* genes in mammalian cells

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In order to identify parasite antigens recognized by CTL from immune cattle, it is desirable to express parasite genes in mammalian cells. Bovine papilloma virus (BPV)-based vectors have been used to obtain expression of foreign genes in mammalian cells. These vectors replicate episomally to high copy number, thus inducing strong and stable expression of foreign genes and allowing straightforward rescue of the vector from the host cell. To evaluate the usefulness of this system, a reporter gene, secreted placental alkaline phosphatase (SEAP), was subcloned into the BPV-based vector, *pBMG-neo* [Karasuyama and Melchers (1988), *Eur. J. Immunol.*, 18:97-104]. This vector contains 69% of the BPV genome and induces expression of foreign genes from a metallothionein promoter.

The recombinant plasmid was transfected into L929 fibroblasts by the calcium phosphate precipitation method. About 800 stable transfectants per microgram of plasmid were obtained. The SEAP gene was strongly expressed in some, but not all, of the cloned transfected cells. However, it was evident that subcloning of genes into the *pBMG-neo* plasmid is not straightforward, which would detract from its usefulness in the expression of a *T. parva* schizont gene library. Thus, other BPV based vectors are being sought.

TH3.5 Eukaryotic expression cloning of genes encoding antigens from a *Theileria parva*-infected lymphocyte cell line

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Initial efforts are aimed at the generation of cDNA libraries in the COS-cell expression vector *pCDM8*. mRNA was obtained from the *Theileria parva* (Muguga)-infected "null" cell line D409-N2 (BoLA type: w7/w10, KN104; CD2+; CD8 (a)+; IL-A29+) and from a human megakaryocyte cell line. As judged by the number of primary clones, insert size and screening with a KN104 cDNA probe, the libraries are of good quality. Full length MHC class I clones should be identified in the near future.

A novel technique was established for the identification of intracellular antigens. In this technique, transfected COS cells are grown on filters, fixed/permeabilized and probed with radiolabelled antibody specific for the molecule of interest. Positive cells can be visualized by autoradiography of the filters. Plasmids can be directly extracted from the cells fixed on the filters. This method has been successfully employed to clone the gene encoding the human lysosomal antigen CD63 and may prove crucial to the isolation of parasite antigens. However, attempts to clone the gene encoding the *T. parva* polymorphic, immunodominant molecule have been unsuccessful so far.

TH3.6 Transfection of *Theileria parva* genomic DNA

Scientists: P.G. Toye, S.P. Morzaria
Research Associate: N.D. MacHugh

In order to produce a library of *Theileria parva* genes expressed in eukaryotic cells, we attempted to transfect genomic DNA of *T. parva* into mouse L cells. The DNA was obtained from piroplasms of *T. parva* (Muguga) and transfected into mouse L cell transfectant IL.X2, which expresses the bovine class I MHC molecule, KN104. The transfection was performed using the calcium phosphate precipitation method, which has been used routinely in the laboratory to transfect genes encoding bovine cell surface antigens.

The transfected cell population was sorted on the FACS using monoclonal antibodies recognizing the 67 kD and PIM antigens and also with bovine sera from cattle that had recovered from *T. parva* infection. The sorted cell population was expanded and re-screened for both cytoplasmic and cell surface parasite gene products with the same panel of antibodies. There was no evidence of either cell surface or cytoplasmic reaction with any of the antibodies used. It was, therefore, concluded that the *T. parva* genomic DNA had either not been transfected, i.e., incorporated into the host DNA, or that it was not being efficiently expressed. Northern and/or Southern blot analysis may help demonstrate whether or not genomic DNA transfection is a viable method for the establishment of stable parasite gene transfectants.

TH3.7 Further characterisation and manipulation of a soluble *Theileria parva* schizont antigen

Scientists: D.J. Grab, E.A. Innes, W. Brown, C. Baldwin, K.P. Iams
Technicians: F. Mbwika, Y. Verjee

The aim of this project is to further characterise and manipulate an antigen contained in a high speed supernatant (HSS) fraction obtained from lysed *Theileria parva*-infected cells. This antigen was recognised by T lymphocytes from *Theileria* immune but not naive cattle.

A protocol has been devised to produce material enriched for the antigen of interest. The HSS is collected and subjected to DEAE 53 column chromatography and hydroxyl apatite chromatography. The resultant eluant is concentrated and further fractionated by HPLC. The protein fractions obtained from the HPLC were then tested for their ability to stimulate T-cell clones known to respond to the crude unfractionated HSS antigen. Using this method,

appropriate protein fractions were thus identified. The molecular weight of the purified antigen was thought to be around 10,000 daltons as determined by autoradiography of labelled proteins on an SDS PAGE gel.

In order to generate larger amounts of the purified antigen for further analysis, 10^{10} *T. parva*-infected cells were generated in tissue culture and snap frozen at -80°C.

To establish whether any of the protein bands visualised on the SDS PAGE gel are a result of contaminating serum proteins, a sample of bovine serum radiolabelled with ^{125}I will be added to an aliquot of the crude unfractionated supernatant. This supernatant will then be processed as described above and the fractions from the HPLC known to contain the antigen(s) of interest would be collected, concentrated and radiolabelled using ^{3}H succinimidyl propionate. Using this method, it should be possible to distinguish bands on a gel with ^{3}H activity (presumably only of *T. parva*-infected cell origin) from bands with ^{125}I activity (contaminating serum proteins).

The remainder of unlabelled sample from the 10^{10} *T. parva*-infected cells would be processed and fractionated with hopefully enough of the protein antigen to allow transfer from a gel to a nitrocellulose filter.

The nitrocellulose-bound protein could then be used to generate immune sera to the purified antigen to facilitate the identification of the appropriate gene. Another approach could involve obtaining an N-terminal sequence of the protein, which again may provide enough information to obtain the relevant gene.

TH3.8 Identification of schizont antigens recognised by *Theileria*-specific T cells

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Research Associate: G.M. Lamb
Technicians: F. Mbwika, J. Nyanjui

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The antigenic changes on the surface of a *Theileria parva*-infected cell can be recognized by immune T cells but not by antibody. Therefore, it seemed appropriate to identify relevant schizont antigens directly using immune T cells as primary probes. This study continues previous work reporting on the potential use of nitrocellulose-bound antigen to analyse immune T-cell specificities. The main emphasis of this study has been to identify and manipulate appropriate T-cell reagents to use for antigen screening.

It was possible to use PBM from immune animals to screen for reactivity to nitrocellulose-bound fractions of a purified schizont antigen. Certain fractions appeared to be immunodominant but the proliferative responses did not tend to be very strong, perhaps due to the low frequency of antigen-specific T cells present in PBM.

Problems were encountered when using polyclonal T-cell lines, generated by *in vitro* stimulation of immune PBM with autologous infected cells, due to consistently high background proliferative responses to the negative control nitrocellulose fraction which did not contain any bound parasite antigens.

Attention is currently focused on the use of $CD4^+$ T-cell clones as screening reagents. A more efficient method for obtaining parasite-specific-T-cell clones was released by a procedure of direct cloning of $CD4^+$ T cells from lymph draining a node responding to parasite challenge.

T-cell clones that responded to the whole unfractionated schizont antigen also tended to respond to certain nitrocellulose bound antigen fractions, although this was not always the case. Differing reaction patterns were observed with some of the clones tested against the nitrocellulose antigen fractions.

The preliminary results gained so far using the T-cell clones have been promising. The advantages of using T-cell clones is their consistent antigenic specificity which is important if they are to be used as screening reagents to identify relevant antigens. The main disadvantage is that they represent only a limited component of the potential T-cell repertoire.

TH3.9 Direct screening of *Theileria parva* DNA expression library using immune $CD4^+$ T-cell clones

Scientists: E.A. Innes, K P. Iams
Research Associate: G.M. Lamb

The possibility of using *Theileria parva*-specific $CD4^+$ T-cell clones as primary probes to screen an expression library of *T. parva* genomic DNA is currently being investigated.

Recombinant fusion proteins have been produced fused to B-galactosidase in the expression vector *pUR*. It is initially planned to screen twenty pooled mixtures, each comprising approximately 200 fusion proteins, for their ability to stimulate a panel of $CD4^+$ T-cell clones.

The T-cell clones were derived from a direct cloning of $CD4^+$ T cells from lymph draining a node responding to parasite challenge. The T-cell clones had been screened for antigen specificity and were also found not to respond to B-galactosidase or a lysate of *E. coli*.

By using immune T-cell clones, the problem of background reactivity to B-galactosidase, which is apparent when using PBM or polyclonal T-cell lines derived from immune animals, is circumvented.

TH3.10 IL-A29$^+$ cells: Preliminary characterisation of the *in vitro* culture conditions and functional activity.

Scientists: E.A. Innes, A. Diaite
Research Associates: N.D. MacHugh, G.M. Lamb

The monoclonal antibody IL-A29 defines a unique population of CD4$^-$ CD8$^-$ γ/δ bovine T-cells. The precise function of this cell population is not yet known although studies in mice and humans indicate that this cell population may exhibit some cytotoxic activity which is usually of a non-MHC restricted nature. Previous work on the functional properties of IL-A29$^+$ cells in cattle indicated that these cells could be stimulated in autologous mixed lymphocyte cultures, would not respond to pokeweed mitogen and proliferated very weakly to either Con A or rIL2. However, if rIL-2 was subsequently added to the Con A stimulated cultures, a marked increase in the proliferative response was observed. The conclusions from the early work suggested that the ILA-29$^+$ cells were reacting to autologous cell surface determinants in the presence of various cytokines.

The bovine autologous MLR response appears to be a proliferative response of null cells to MHC class II antigens. As *T. parva*-infected cells express high levels of class II molecules, they may also be capable of inducing an autologous MLR response. Indeed, it has been observed that the majority of cells proliferating in the autologous *Theileria* MLR are cells lacking B- and T-cell markers.

In this study we attempted to exploit the autologous *Theileria* MLR response to culture IL-A29$^+$ cells *in vitro* in order to examine their functional activity.

IL-A29$^+$ cells were sorted using a fluorescence-activated cell sorter from PBM of a pair of twin cattle, one of which was immune to *T. parva*. The sorted cells were then cloned out in limiting dilution and stimulated with a mixture of autologous *Theileria*-infected cells and 15% final concentration of bovine TCGF. A small amount of proliferation was observed when the cells were stimulated only with TCGF but marked proliferation was achieved with cells cultured in the presence of both TCGF and autologous infected cells. There was no obvious difference between the immune and the naive animal in the ability of their respective IL-A29$^+$ cells to proliferate.

Phenotypic analysis showed that the proliferating cells did express the γ/δ T-cell receptor and the molecule recognised by IL-A29. They did not express CD8 but there did appear to be a small contaminant CD4$^+$ population.

Cytotoxic function of the proliferating cells was measured using a four-hour ^{111}Indium -release assay. Low levels of cytotoxicity varying from 7 to 20% was observed directly, mainly against the BoLA mismatched infected target. Therefore, the cytotoxicity did not appear to be MHC restricted and again there was no obvious difference between the response of the immune and naive animal.

These preliminary data suggest that the autologous *Theileria* MLR can be exploited to culture populations of γ/δ T-cells *in vitro*. When larger numbers of cells are available, more extensive study on phenotype and function of this cell population will be possible.

TH3.11 Casein kinase II in *Theileria parva*-infected lymphocytes

Scientists: O.K. ole-MoiYoi, K.P. Iams
Research Associate: M.D. Macklin

Functional characterisation of protein kinase activity dominant in *Theileria parva*-infected lymphocytes had revealed, in previous years, that the activity was that of a siren/threonine kinase which was cyclic nucleotide independent, insensitive to Ca^{++}/calmodulin and was capable of hydrolysing γ GTP as well as γ ATP as sources of Pi. The enzyme phosphorylation of endogenous substrates as well as casein, phosritin and other exogenous substrates was inhibited by heparin and activated by polycations. The enzyme was thus considered to be casein kinase II-like. An anti-bovine casein kinase II IgG was used both on Western blots and in the preparation of immunoprecipitates from both uninfected, TCGF-dependent cloned cell lines and from *T. parva*-infected lymphocytes. These studies revealed increased (11- to 18-fold) functional casein kinase II-like activity in immunoprecipitates prepared from *T. parva*-infected cells compared with uninfected TCGF-dependent lymphocyte controls. Using this antibody on western blots showed a marked increase in CK II antigen bands corresponding to the typical α/α' and β subunits of bovine CK II. In addition to these bands, other related antigens with approximate Mr of 130, 69 and 14-19 also reacted with this antibody. The latter were presumed to be of parasite origin.

A *Drosophila* CK-II α cDNA hybridised strongly to *T. parva* DNA but gave no hybridisation signals to bovine DNA. This cDNA was used to isolate a CK II gene from a library of *T. parva* genomic DNA cloned in λgt 11. This clone was sequenced and the derived amino acid sequence was found to be highly homologous to other α CK II sequences. At the DNA level the *T. parva* CK II sequence has, within its non-translated 3' region, three of the mRNA lability-conferring sequence motifs characteristic of transcripts for short-lived, tightly-controlled growth-regulating molecules such as lymphokines and a variety of oncogenes. The N-terminal amino acid sequence, deduced from the *T. parva* CK II-α sequence and which appears to be unique to *T. parva*, has now been confirmed by sequences obtained from putative full length cDNA clones of *T. parva*. This sequence (78 aa residues) has similarity with transmembrane domains of other proteins.

TH4.1 The role of afferent lymph veiled cells in the induction of bovine immune responses to soluble antigens

Scientist: D.J. McKeever
Technician: E. Awino

Previous reports have described investigations of the accessory function of afferent lymph veiled cells (ALVC) in bovine T lymphocyte responses to soluble antigens. It has been shown that ALVC are considerably more potent than blood monocytes in the presentation of trypanosomal VSG for recognition by antigen-specific T-cell clones, and that this function is the result of a chloroquine-sensitive processing step. These results suggest that ALVC are important in the induction of bovine immune responses to soluble antigens, and have prompted an investigation of their function *in vivo*.

Experiments were carried out in order to determine whether ALVC are involved in the carriage of soluble antigens from the inoculation site to the draining lymph node. These experiments made use of identical *Bos taurus* twins, one of which was immunised with IITat 1.3 VSG. Afferent lymph was collected from the immune twin for various periods following inoculation with 500 µg of the antigen in PBS, and ALVC purified from this lymph were included in cultures of peripheral blood mononuclear cells prepared from both the immune and naive twins. Results from two such experiments have revealed that as early as 30 minutes following inoculation, ALVC draining from the area are capable of inducing maximal responses in primed but not in unprimed lymphocytes, and that antigen carriage persists for up to 48 hours. Results from an additional experiment suggest that inoculation of antigen in complete Freund's adjuvant delays the uptake of antigen by ALVC. These observations provide strong evidence that soluble antigens in the periphery are rapidly taken up by ALVC and transported to the draining lymph node.

In an attempt to determine if ALVC which have taken up antigen are capable of inducing T-cell responses in the draining lymph node, antigen-pulsed ALVC were transferred from the immune animal to its naive twin and to an unrelated animal. Proliferative responses of these animals to VSG were monitored over a nine-week period. Significant antigen-specific T-cell proliferative responses were detected in the infected twin one week following transfer, and responsiveness was maintained during the monitoring period.

These results provide evidence that ALVC play a major role in the induction of primary T-cell responses to soluble antigens in cattle, and identify ALVC as an important target for antigen delivery strategies. It is anticipated that similar experiments might be used to assess the efficacy of antigen delivery systems.

TH4.2 Monoclonal antibodies recognising differentiation antigens on bovine peripheral blood monocytes and afferent lymph veiled cells (ALVC)

Research Associate: N.D. MacHugh
Scientists: D.J. McKeever, B.M. Goddeeris
Technicians: D. Ngugi, J. Kamau

Bovine monocytes and afferent lymph veiled cells have been shown to be extremely efficient at processing and presenting antigen to cells of the bovine immune system. Monoclonal antibodies have been described which identify these cells *in vivo* but the antigens recognized lack sufficient restriction to differentiate between monocytes and ALVC; nor have they been shown to be involved in the processing or presenting mechanisms. To further characterise these cell types, and the mechanisms important for the immune responses which they elicit, attempts have been made to generate new MAbs which would clearly differentiate monocytes from veiled cells and discriminate between these cells and other cells of myeloid origin.

Balb/c mice were immunised with monocytes cultured from a bovine bone marrow biopsy. Spleenocytes from these mice were fused with the mouse myeloma cell-line X63 and the following monoclonal antibodies were selected.

MAb IL-A96 reacts with peripheral blood monocytes, granulocytes and ALVC and its ligand has a relative molecular mass (Mr) of between 20 and 25 kDa. MAb IL-A106 reacts with monocytes and ALVC but does not react with granulocytes. The antigens recognised by MAbs IL-A100 and IL-A109 are restricted to ALVC and monocytes, respectively.

In addition to the above reactions, MAb IL-A96 reacts with a *Theileria parva*-infected clone derived from the non-MHC-restricted cytotoxic cell line T36.78. Cells with similar unrestricted killing properties have recently been identified in cattle undergoing lethal infection with *T. parva*. This MAb may therefore be of use in the investigation of this NK-like bovine cell population. MAb IL-A100 recognises an antigen restricted to ALVC and may thus contribute to the determination of the origin and distribution of these cells in cattle. The antigen recognised by MAb IL-A109 has a similar distribution to that of CD64 in man. CD64 defines the high affinity Fc receptor for IgG (FcRI); this receptor can be up-regulated by interferon γ and is involved in the pinocytosis of immune complexes, phagocytosis of opsonised particles and antibody-dependant cell-mediated cytotoxicity (ADCC). Confirmation that MAb IL-A109 recognises the bovine equivalent of CD64 will require determination of the Mr of its ligand.

TH4.3 Characterisation of bovine T-helper cell specificity for defined antigens

Student: H. Pereira
Supervisors: A.J. Teale, D.J. McKeever
Scientists: A.J. Musoke, V. Nene

Animals immunised with gp67, a 67-kDa surface protein of *Theileria parva* sporozoites, produce antibodies which neutralise the infectivity of the sporozoites. The antibody response is cross-protective and neutralises homologous *T. parva* sporozoites as well as those of other *T. parva* stocks. The gp67 molecule is a potential vaccine component and it

is therefore important to know the influence of the MHC on its recognition in the bovine system. Seven Boran (*Bos indicus*) animals homozygous for their MHC haplotypes were immunised using gp67 electro-eluted from SDS-PAGE gels. The MHC haplotypes encoded the workshop specificities w10, w25 and locally-defined specificities KN8, KN12 and KN103 at the A locus. They were shown to be homozygous for class II molecules using mixed lymphocyte reactions and isoelectric focusing techniques. All animals except the w25 animal produced *in vitro* proliferative responses to gp67. All animals respond to foot and mouth virus antigens used as positive control antigens but not to keyhole limpet haemocyanin used as a negative control. PBM from animals not immunised with gp67 do not proliferate to this antigen in these assays. It was difficult to demonstrate proliferative responses to any of the antigens in the w25 animal due to high background proliferation. Sera tested using Western blot analysis and ELISA indicated that all animals were producing anti-gp67 antibody. Responding cell populations were of the $BoT4^+$ phenotype and responses of PBM were blocked by anti-class II MAb. Thus, *in vitro* proliferative responses to gp67 are not apparently influenced by the MHC in the cattle populations studied. The fact that the gp67 is a large molecule which probably contains several T-helper epitopes to which the differing class II antigens might bind may explain the apparent lack of variation in immune responsiveness to this molecule. T-helper cell epitopes on gp67 have not yet been identified. However, T-helper cell epitopes have been identified on the variant surface glycoprotein (VSG) of *T. brucei* IITat 1.3, and eight homozygous animals (seven from the gp67 immunised group and 1 other w1 homozygote) have been immunised with this molecule. We are currently examining the responses of PBM from these animals to intact VSG and will shortly be assessing their responses to two peptides which represent T-cell epitopes of the antigen.

TH4.4 Mapping of T-cell epitopes on *Trypanosoma brucei* variable surface glycoprotein

Scientists: D.J. McKeever, V. Nene
Technicians: E. Awino, J. Nyanjui

A previous report described the development of a strategy for the mapping of bovine T-cell epitopes using recombinant antigens expressed in *Escherichia coli* and antigen specific T-cell clones. This method is based on the generation of 3' deletions in the gene using exonuclease III and mung bean nuclease, and the screening of their expressed products with T-cell clones. A set of deletions derived from the gene which encodes IITat 1.3 VSG had been used to resolve the epitope of a VSG-specific clone to a stretch of 80 amino acids, but problems relating to the removal of stop codons from the pGEX expression vector during the deletion reactions precluded the definition of the epitopes of three other clones to a satisfactory resolution. It was considered expedient to the success of the strategy to construct a modified expression vector more suited to the generation of exonuclease III/mung bean nuclease deletions.

A modified expression vector based on *pGEX* has been constructed which allows the generation of reliable 3' deletions in incorporated genes. This has been achieved by the insertion of stop codons at a site which is protected from exonuclease III digestion, ensuring that transcription of truncated gene sequences does not run on into vector sequences. Using this vector, an additional set of truncated IITat 1.3 genes has been made, and these have been screened with VSG-specific T-cell clones derived from two cattle. In conjunction with

the results of previous experiments with the original vector, these studies have allowed the identification of three areas (of 14, 18 and 21 amino acids, respectively) on the VSG molecule which contain bovine T-cell epitopes. Peptides corresponding to two of these areas are being synthesised in order to confirm these observations.

The results of these efforts suggest that the use of exonuclease III/mung bean nuclease deletions constitutes a useful method for the identification of T- or B-cell epitopes in antigens expressed in *E. coli*. A system has been developed for this purpose which can readily be applied to other antigens under study at ILRAD.

TH4.5 Immunising capacity of a recombinant vaccinia virus encoding the 67-kDa surface antigen of *Theileria parva*

Scientists: D.J. McKeever, A.J. Musoke
Technicians: C. Nkonge, J. Gachanja

A previous report described the construction of a recombinant vaccinia virus (VVp67) incorporating the gene which encodes the 67-kDa surface antigen of *Theileria parva*. A preliminary experiment involving the infection of a rabbit with this virus revealed that it gave rise to the production in this species of antibody capable of neutralising the infection of bovine lymphocytes *in vitro*. Further characterisation of the immunising capacity of this virus has been carried out in guinea pigs and cattle.

Two pairs of p67-seronegative guinea pigs were infected by intradermal inoculation with 2.5×10^7 and 5×10^7 plaque forming units (pfu) of VVp67 respectively. Serum was prepared from the animals three weeks after infection and assayed by Western blotting and serum neutralising tests for the presence of p67-specific antibodies. One animal which received the lower rate of inoculation failed to produce antibodies detectable by either assay. The remaining animals produced low titres of antibody as detectable by Western blotting and neutralisation assays.

In a separate experiment, two *Bos indicus* calves were infected with 10^8 pfu of the virus by intradermal inoculation on the lateral aspect of the neck. The calves were bled at weekly intervals for the assessment of serological and cellular immune parameters. Sera were assayed for reactivity by ELISA, Western blotting and a serum neutralising test. Peripheral blood mononuclear cells were also prepared from weekly samples and cultured in the presence of p67 derived by preparative SDS-PAGE from sporozoites. Low titres of specific antibody were detectable by all of these assays in both animals, and in one animal a transient but weak proliferative response to p67 was detected in PBM.

These results provide evidence that inoculation with the VVp67 recombinant virus gives rise to the generation of p67-specific antibodies in cattle and experimental animals. In all cases, however, titres were low and unlikely to be protective. Future studies will entail an assessment of the effects of inoculum dose and secondary boosting inoculations on the antibody responses induced by the virus.

TRYPANOSOMIAIS

TR1.1 African Trypanotolerant Livestock Network: Tsetse component

Research Associate: S.G.A. Leak
Collaborating Scientists: ILCA Scientists and ATLN field-site personnel

The project has been examining the relationships between estimates of tsetse challenge or trypanosomiasis risk and trypanosome prevalence in cattle at sites of the African Trypanotolerant Livestock Network. Tsetse control interventions have taken place at Ghibe and Tolley in south-west Ethiopia and in the Boundiali area of northern Côte d'Ivoire.

In May 1990, 500 deltamethrin impregnated cloth targets were sited at roughly 200-metre intervals in suitable tsetse habitat in 125 km^2 of the Ghibe Valley in order to reduce the populations of *Glossina pallidipes* and *G. fuscipes*. It is hoped that this will ameliorate a situation in which an increasing level of trypanosome prevalence is being detected in cattle and a serious problem of resistance to isometamidium, diminazene and homidium has been identified. Initial results of catches in odour-baited biconical tsetse traps have shown reductions of 85% and 77% in the populations of *Glossina pallidipes* and *G. fuscipes*, respectively. As tsetse relative density at this site normally shows a decline during the rainy season, a better idea of the effectiveness of the control will come during the last quarter of 1990 when relative density normally rises. Simultaneously with the tsetse control intervention, trials are being conducted to determine the most effective chemotherapeutic drug regime for the area.

In October 1990, a tsetse control intervention using Ectopor™ pour-on insecticide will start at Tolley, about 25 km upstream from Ghibe. All of the estimated 2000 cattle in the area will be treated at three-week intervals with Ectopor™. The effectiveness of the tsetse control interventions at Ghibe and Tolley will be assessed by monthly trapping and from monthly estimates of trypanosome prevalence in two cattle herds.

TR1.2 ILRAD contribution to the African Trypanotolerant Livestock Network (ATLN): health aspects

Scientist: E. Authié

Trypanotolerance is a genetic characteristic which involves an ability to limit both the parasitaemia and the development of anaemia. Further analysis of data from ATLN sites in Gabon and Zaire showed that the ability to control the development of anaemia was more closely correlated with productivity of trypanotolerant N'Dama cattle under natural challenge than the ability to control parasitaemia. Heritabilities of packed cell volume (PCV) and its genetic correlations with growth parameters indicated that selection on the ability to control anaemia might be possible. However, the evaluation of the ability to control parasite growth is impaired by the lack of sensitivity of current diagnostic techniques. The antigen detection ELISA developed at ILRAD has been used recently for more precise analysis of data from the Network site in Gabon. Preliminary results indicate that about 40% of parasitaemic N'Dama cattle have circulating trypanosome antigens, with no significant effect on PCV and growth rate. Investigations are continuing on the bovine major histocompatibility complex and common leucocyte antigens, with a view to identify markers of trypanotolerance. Other collaborations with ILCA include studies on the use of trypanocides. The occurrence of

drug-resistant *Trypanosoma congolense* at the Ghibe site (Ethiopia) was confirmed by analysis of field isolates at ILRAD.

Alternative strategies for trypanosomiasis control in cases of multiple drug resistance are now being studied. Larger scale studies on drug resistance have also been implemented in Côte d'Ivoire. A one-month anglophone training course, involving six participants, was held in 1990 on diagnosis and control of bovine trypanosomiasis.

TR1.3 Analysis of *Trypanosoma congolense* isolates from East Africa

Scientists: R.A. Masake, V.M. Nantulya, S.K. Moloo, L.H. Otieno*
Technician: J.M. Makau

*International Centre of Insect Physiology and Ecology, Nairobi, Kenya

In 1982 and 1983 *Trypanosoma congolense* parasites were isolated from a fairly secluded farm in Kilifi. One hundred and seventeen clones were derived from these isolates. Chromosome-sized DNA molecules of the cloned trypanosomes were studied and were categorised into 18 chromosome profiles. Furthermore, it was observed that infectivity of clones displaying similar chromosome profiles could be neutralised by sera obtained following chronic infection by any of them. This clearly implied that the clones in question belonged to the same serodeme. It soon became clear that chromosome profiles could be used in serodeme identification.

Despite the fact that analysis of chromosome profiles would offer a faster and quicker methodology for serodeme identification, there was still uncertainty as to how stable the chromosome-sized DNA molecules are. In an attempt to answer this question, goats were infected with ten clones from ten *T. congolense* serodemes and left to run a chronic infection for four years without chemotherapeutic intervention. Parasites were recovered from the goats after 1, 6, 12, 24, 36 and 40 months of infection and subjected to chromosome profile analysis. The chromosome sized DNA molecules of all the ten serodemes remained stable. Soon after this, trypanosomes isolated from Serengetti, Nguruman and Lambwe Valley were cloned and their chromosome profiles were studied. Surprisingly, there was great variability in the chromosome pattern. We are presently trying to verify whether or not each of these profiles represent a different serodeme.

TR1.4 Identification, cloning and characterisation of the diagnostic antigen for *Trypanosoma congolense* infections

Student: A. Jaye
Supervisor: V.M. Nantulya

Recently, new assays have been developed for detection of species-specific circulating antigens in sera from *Trypanosoma congolense*, *T. vivax* or *T. brucei*-infected animals as a means of diagnosis. These assays are based on monoclonal antibodies with specificity for trypanosome species-specific invariant antigens. The species-specific diagnostic monoclonal antibody (Tc39, an IgM isotype) recognises multiple bands of proteins (Mr of between

69 and 38 kDa) in Western blots using freshly prepared *T. congolense* lysates. The electroeluate of these bands have been used to raise IgG-class monoclonal antibodies. One of the monoclonal antibodies produced, C2 (an IgG1 isotype), was found to react strongly with *T. congolense* lysates by Western blotting. The monoclonal antibody recognized 38 kDa and 40 kDa, and was found to be specific for *T. congolense* both by Western blotting and by sandwich ELISA. C2 antibody has been compared with Tc39 in the sandwich antigen capture ELISA using both *T. congolense* lysates and infection sera. Their pattern of capture of the antigen was similar and one conjugated antibody was found to reveal the antigen that is captured by the other, suggesting that the epitopes recognised by the two monoclonal antibodies were present on the same antigen molecule. Rabbits have been immunised with the 38-kDa and 40-kDa protein eluates recognised by C2 to produce polyvalent antisera (R-AT99 and R-BT9). The rabbits responded strongly to the eluates, antisera reacting with their respective protein bands and were specific for *T. congolense*. The rabbit sera are currently being used for the screening of a cDNA library to identify and clone the gene fragment encoding the diagnostic antigen.

TR1.5 The sensitivity of antigen-ELISA in diagnosing *Trypanosoma congolense* infections in goats

Scientists: R.A. Masake, V.M. Nantulya
Technicians: J.M. Makau, J.T. Njuguna

The sensitivity of a monoclonal antibody-based antigen-detection ELISA for the diagnosis of *Trypanosoma congolense* was evaluated using sera from experimentally infected goats as well as field sera from goats in a trypanosomiasis endemic area. Ten goats (Galla × East African Maasai) were infected with different clones of *T. congolense* and left to run a chronic course for 46 months. During this period, monthly blood samples were collected and analysed for the presence of trypanosomes and antigens in peripheral blood. The total number of blood samples tested was 383. Out of these, 361 were positive for circulating antigens while only 42 had demonstrable trypanosomes as revealed by the microhaematocrit centrifugation technique. In an analysis of serum samples from goats in an area known to be endemic for trypanosomiasis, 106 out of 131 were positive for *T. congolense* antigens, while none of the corresponding blood samples had detectable trypanosomes. Control sera from 24 goats in a trypanosomiasis-free region were all antigen-ELISA negative. Hence, the antigen-ELISA was nine times more sensitive than the microhaematocrit centrifugation technique in monitoring *T. congolense* infections in goats.

TR1.6 Identification of a gene fragment encoding *Trypanosoma vivax* species-specific antigen

Scientists: R.A. Masake, V.M. Nantulya, O.K. ole-MoiYoi
Technician: J.M. Makau

A gene coding for *Trypanosoma vivax* species-specific antigen has been isolated from a λ*gt* 11.2 library. The fragment was subcloned into *pUR* 291 to facilitate production of fusion-protein in XL-1 cells. The fusion-protein produced reacts with monospecific antisera and monoclonal antibody raised against *T. vivax* species-specific antigen. The fusion-pro-

tein was subsequently electro-eluted from SDS-PAGE gels and used to immunise rabbits. The serum obtained has activity against both the fusion-protein and the *T. vivax* lysate. The DNA itself is currently being sequenced.

TR1.7 Development of monoclonal antibody-based assays for species identification of trypanosomes in the tsetse fly (*Glossina* spp.)

Student: K.M. Bosompem
Supervisors: V.M. Nantulya

Glossina species (tsetse) are the vectors of trypanosomiasis. The standard method for identification and differentiation of trypanosome infections in tsetse is by dissection. This method, however, presents several problems, especially that of differential diagnosis of trypanosome species and subspecies in the fly. There is, therefore, pressing need for a more reliable field test for trypanosome species identification in *Glossina*. In view of this, we are developing techniques based on monoclonal antibodies for differentiation of the trypanosome species (*Trypanosoma congolense*, *T. brucei* and *T. vivax*) and subspecies (*T. simiae* and *T. congolense*) infections in the tsetse vector.

So far we have produced several monoclonal antibodies against trypanosome insect stages (procyclics and epimastigotes) and utilised them in the Dot-enzyme-immunoassay (Dot-blot) for differentiating *in vitro* propagated trypanosome vector stages. This panel of monoclonal antibodies is capable of distinguishing *T. congolense*, *T. brucei* and *T. vivax*, and also *T. congolense* from *T. simiae*. Evaluation of the sensitivity of the antibodies in terms of the minimum number of trypanosomes that can be detected in the Dot-blot assay indicate that the *T. brucei* and *T. congolense* and Nannomonas specific antibodies can detect as low as ten organisms, whilst the *T. vivax* antibodies can detect only 1,000 to 10,000 organisms. Further experiments are under way to improve the sensitivity of the *T. vivax*-specific antibodies in the Dot-blot assay, and also to produce more *T. vivax*-specific monoclonal antibodies with greater specificity.

TR1.8 Pharmacokinetics of diminazene in Boran (*Bos indicus*) cattle

Scientists: Y.O Aliu*, A.S. Peregrine
Student: M. Mamman

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The disposition and absorption kinetics of diminazene (Berenil®, Veriben®) were studied in five normal, 11-14 month-old female Boran (*Bos indicus*) cattle. Single doses of diminazene (3.5 mg/kg^{-1}), as the diaceturate salt, were injected intravenously (IV) and intramuscularly (IM) in a single cross-over design with a 6-week wash-out period. Following paired-ion extraction, high-performance liquid chromatography was used to determine the concentration of intact diminazene in plasma, whole blood and urine samples collected prior to, and at various time intervals after, each drug administration. The disposition kinetics relating to both the IV and IM administration experiments, as analysed by non-linear least

squares regression, were best described by a three-compartment open model. Following IV bolus injection, plasma diminazene concentrations rapidly declined from 10.16 ± 2.02 $\mu g\ ml^{-1}$ at 5 minutes to 2.34 ± 0.40 $\mu g\ ml^{-1}$ at 1 hour and 0.47 ± 0.10 $\mu g\ ml^{-1}$ at 48 hours. In three animals, plasma levels of 0.05 ± 0.02 $\mu g\ ml^{-1}$ were detected at four weeks. However, the drug was not detected in plasma from any of the five animals at six weeks. One of the characteristics of the IM dose was rapid absorption; the absorption half-life was 8.6 ± 4.2 minutes. The maximum plasma drug concentration (C_{max}), 3.71 ± 0.56 $\mu g\ ml^{-1}$, was attained in 10 - 15 minutes and a systemic availability of 100.0 ± 2.0% was estimated. Although the plasma concentrations at 1 hour (2.21 ± 0.26 $\mu g\ ml^{-1}$) and at 48 hours (0.52 ± 0.06 $\mu g\ ml^{-1}$) were not significantly different from those present at the same time intervals after IV administration, 0.13 $\mu g\ ml^{-1}$ was detected up to, but not beyond, 10 weeks in three animals following the IM dose. About 8.3 ± 0.8% of the IM dose was excreted intact in the urine within the first 24 hours following administration of the drug.

TR1.9 Pharmacokinetics of diminazene (as Berenil®) in healthy cattle, and during acute and chronic infections with *Trypanosoma congolense*

Student: M. Mamman
Supervisor: A.S. Peregrine

In earlier work we have characterised the pharmacokinetics of diminazene in *Bos indicus* cattle following intravenous (IV) and intramuscular (IM) administration of the diaceturate salt. In the work described here, we have endeavoured to define the pharmacokinetics of diminazene (as Berenil®) in five cattle when treated in health, and during acute and chronic trypanosome infections, i.e., at first peak parasitaemia and when the packed red blood cell volume (PCV) had fallen below 17%, respectively. In each study, a single IM dose of 3.5 mg diminazene base kg^{-1} body weight was administered as a 7.0% w/v solution (in normal saline) of diminazene aceturate. For studies on the pharmacokinetics of diminazene in infected cattle, *T. congolense* clones IL1180 and IL2642 were used. The populations were expanded in mice and cattle were infected via IV inoculation of 1.0×10^7 trypanosomes. When the cattle were infected with IL1180, treatment was instituted at the first peak of parasitaemia (acute infection). In contrast, infection with IL2642 was allowed to progress until the PCV had fallen below 17% (chronic infection) before treatment was administered. In each of the studies, blood was collected at various time intervals, from 0 to 48 hours, following administration of the drug. Plasma samples were initially processed by paired-ion extraction and subsequently analysed for intact diminazene using reversed-phase high-performance liquid chromatography. Using least squares non-linear regression analysis, a three compartment open mammillary model was fitted to the concentration-time data from each study. A non-compartmental approach was also used to calculate the area under the concentration-time curve (AUC) from zero time to infinity, area under the (first) moment curve (AUMC), mean residence time (MRT), clearance (Cl) and the apparent volume of distribution at steady state (V_{ss}). The peak concentration (C_{max}) of plasma diminazene attained during the acute phase of infection, 8.63 ± 1.04 $\mu g\ ml^{-1}$, was significantly greater than that which occurred when animals were treated in the healthy state, 5.51 ± 1.02 $\mu g\ ml^{-1}$, or during chronic infection, 5.68 ± 0.46 $\mu g\ ml^{-1}$. The time corresponding to the C_{max} when treatment was conducted during the acute phase, 0.40 ± 0.33 hr, was significantly shorter than that in healthy cattle, 1.40 ± 0.54 hr, or subsequent to treating chronic infections, 1.25 ± 0.28 hr. In addition, the absorption half-life subsequent to treating acute infections, 0.13 ± 0.05 hr, was significantly greater than that in the healthy state, 0.06 ± 0.03

hr. However, despite these differences, non-compartmental analysis of the plasma diminazene concentration-time data did not indicate significant differences in the AUC, AUMC, MRT, Cl and V_{ss} between the three studies. Therefore, when cattle are treated in the healthy state, or during acute or chronic trypanosome infections, there appears to be no significant difference in the total amount of diminazene quantified in the plasma up to and beyond 24 hours following treatment.

TR1.10 Characterisation of Ethiopian field isolates for their sensitivity to trypanocides used in domestic livestock

Student: V. Codjia
Supervisor: A.S. Peregrine
Collaborating ILCA Scientists: W. Mulatu, J. Rowlands, G.D.M. d'Ieteren

The International Livestock Centre for Africa (ILCA) has, for the past four years, been involved in a field project at Ghibe, Ethiopia, to define the constraints to livestock productivity at that site. Since data obtained from the site indicated that there might be a significant problem with trypanocide resistance, trypanosome isolates have been obtained from cattle at the site and characterised at ILRAD for their drug-resistance phenotypes. Thirteen isolates have been chosen at random and all are *Trypanosoma congolense*. All 13 isolates have been characterised *in vivo* (calves, goats and mice) for their sensitivities to diminazene, isometamidium and homidium. Studies in cattle showed that of the 13 isolates, 12 were resistant to intramuscular (IM) treatment with 7.0 mg diminazene aceturate kg^{-1} body weight (bw), and 11 were resistant to IM treatment with 0.5 mg isometamidium chloride kg^{-1} bw. The same 11 isolates were also resistant to IM treatment with 1.0 mg homidium chloride kg^{-1} bw.

Five clones were derived from one isolate which expressed high levels of resistance to diminazene, isometamidium and homidium, and characterised in mice for their sensitivities to the same three drugs. All five clones expressed high levels of resistance to each of the three drugs. It therefore appears that there is a high prevalence of multi-drug resistance at Ghibe and that in at least one isolate this is due to clones expressing multi-drug resistance.

Electrophoretic variants of ten enzymes are being determined for ten of the isolates to provide information on the homogeneity of the trypanosome populations from Ghibe. Karyotyping of the same isolates will also be conducted.

TR1.11 Field studies in Côte d'Ivoire to determine the prevalence of resistance to trypanocides

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Technician: S. Kemei

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Since 1983 the International Livestock Centre for Africa (ILCA) and ILRAD have been collaborating with the Veterinary Department of Côte d'Ivoire (SODEPRA) in a project to determine the constraints to ruminant production in the northern part of the country. Subsequent to a request from the Director for Tsetse and Trypanosomiasis Control for Côte d'Ivoire, and since resistance to isometamidium and diminazene has been shown to occur in this region of West Africa, a three-year project has been designed to determine the prevalence and level of trypanocide resistance in northern and central Côte d'Ivoire. The project began in January, 1990, and experimental sites were established in Boundiali, Bouaké, Ferkessédougou and at Marahoué ranch. At each site groups of approximately 600 cattle are being monitored regularly for the presence of trypanosome infections. The efficacy of diminazene aceturate, isometamidium chloride and homidium bromide at eliminating infections from cattle at each of the sites is being determined. Various production parameters are also being evaluated. Data from the four project sites is being stored on computers at Boundiali and programs have been designed by ILCA personnel to enable measures of *new infection rates* and *rates of relapse to treatment* to be made. As an adjunct to the field work, isolates are being collected from cattle at the beginning, middle and end of the study. Their drug-resistance phenotypes will be determined at ILRAD. To date, 14 isolates have been obtained; seven from Boundiali, three from Marahoué ranch and four from Ferkessédougou. In mid-1991 the field and laboratory data will be used to design efficacious strategies which will be evaluated in the field for the final 18 months of the project.

TR1.12 Biodynamics of drug-resistant *Trypanosoma congolense*

Scientists: A.S. Peregrine, M.A. Gray*, S.K. Moloo, N.B. Murphy
Research Associate: J. Scott
Student: G. Ndoutamia
Technician: S. Kemei

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Trypanosoma congolense IL1180 is a cloned population which expresses a high level of sensitivity to isometamidium chloride in mice (50% curative dose [CD_{50}] = 0.007 mg kg^{-1}). Populations have been derived from IL1180 which relapse in mice following treatment with 100X the CD_{50} (IL1180/100X) and 200X the CD_{50} (IL1180/200X). Studies *in vitro* have shown that transformation from bloodstream to metacyclic trypanosomes takes eight to nine days for IL1180 but 23-24 days for IL1180/100X and IL1180/200X. However, when the rate of transformation was studied in *Glossina morsitans centralis*, fed on infected goats, no significant difference was detected between the three populations.

Studies on stability of drug resistance have used two clones of *T. congolense*: IL1180 and IL3270. IL3270 has been shown to be approximately 1000-times more resistant to isometamidium than IL1180 and, over a period of seven months, has been passaged 37 times in mice. Five clones were derived from the population that arose during the 37th passage and characterised in mice for their sensitivity to isometamidium. Whilst the isometamidium chloride CD_{50} value for IL3270 was 5.1 mg kg^{-1}, the CD_{50} values for the five clones ranged from 8.1 to 12.1 mg kg^{-1}; four of the five clones were significantly more resistant than IL3270. Similarly, IL1180 has been passaged 79 times in mice over a 14-month period. Populations occurring during passage eight, passage 40 and passage 79 were characterised in mice for their sensitivity to isometamidium. Whilst the sensitivity of passage 8 did not differ significantly from IL1180, passage 40 was significantly more sensitive to isometamidium than IL1180 (by a factor of approximately two) and passage 79 was significantly more resistant than IL1180 (by a factor of approximately two).

In studies to determine the cross-resistance phenotype of trypanosomes made resistant to isometamidium, metacyclic forms of *T. congolense* IL1180 and IL1180/200X, derived *in vitro*, were characterised *in vitro* for their sensitivity to isometamidium, homidium and diminazene. These studies showed that IL1180/200X was 200-times more resistant to isometamidium than IL1180. It was also 200-times more resistant to homidium. No difference in sensitivity to diminazene was detected. In mice, the isometamidium chloride CD_{50} values for IL1180, IL1180/50X, IL1180/100X and IL1180/200X were 6.6 µg kg^{-1}, 470 µg kg^{-1}, 1,700 µg kg^{-1} and 2,100 µg kg^{-1}, respectively, and all four CD_{50} values were significantly different from each other. The diminazene aceturate CD_{50} values for the same four populations were 2.3 µg kg^{-1}, 5.7 µg kg^{-1}, 7.8 µg kg^{-1} and 6.9 µg kg^{-1}, respectively. The values for IL1180, IL1180/50X and IL1180/100X were significantly different from each other. However, that for IL1180/100X was not significantly different from IL1180/200X. Thus, the mouse characterisation system appears to be a more sensitive system than the *in vitro* cultivation system for quantifying resistance.

TR1.13 Variation in the sensitivity of *Trypanosoma congolense* to diminazene during the course of infection in goats

Student: M. Mamman
Supervisor: A.S. Peregrine

Earlier work has shown that if goats are infected with *Trypanosoma congolense* IL3274 via the bites of infected tsetse and animals are treated intramuscularly (IM) with diminazene aceturate (DA) at a dose of 7.0 mg kg^{-1} body weight, infections relapse in all animals. However, infections with IL3274 are eliminated in goats infected in a similar manner but treated IM with DA 24 hours following infection. We have repeated and extended this work to determine the interval following fly infection when IL3274 apparently alters its level of resistance. Groups of five goats each were infected with IL3274 via the bites of infected *Glossina morsitans centralis* and treated IM with 7.0 mg DA kg^{-1} body weight at intervals of 1, 4, 8, 12 or 19 days following infection. As before, treatment on day one eliminated infections in all goats and treatment on day 19, after animals had been detected parasitaemic, failed to eliminate infection in any goat. In groups treated 4, 8 or 12 days following challenge, two out of five animals in each group were cured. Therefore, IL3274 appears to alter its expression of resistance within the first 19 days of infection, most especially between day one and day four. Since the alteration in apparent resistance between day 1 and day

19 could be due to changes in resistance by the trypanosomes as the population expands, part of the experiment was repeated but with IL3274 parasites that relapsed in goats after IM treatment with 7.0 mg DA kg^{-1} body weight. Such parasites from five donor goats were transferred intravenously (IV) into five clean goats. The recipient goats were treated immediately IM with 7.0 mg DA kg^{-1} and monitored daily. Only three of the five goats became parasitaemic and the onset of parasitaemia in these animals appeared greatly delayed. It therefore appeared that only a proportion of the parasites occurring in the donor goats after treatment were resistant to the dosage of diminazene used. In a final study, tsetse flies were fed on each of the aforementioned donor goats on the day that parasites were transferred IV. Thereafter they were fed on clean rabbits until infective. The flies were then used to challenge clean goats. All the goats were treated IM with 7.0 mg DA kg^{-1} 24 hours following infection. In a similar manner to the parental IL3274 study, infections failed to develop in any animal. Thus, although tsetse were infected with parasites that had arisen in goats after IM treatment with 7.0 mg DA kg^{-1}, feeding of the flies on clean goats, when infective, failed to result in infection when the goats were treated 24 hours after infection with the same drug dosage. Further work is under way to determine the aetiology of this phenomenon.

TR1.14 Efficacy of diminazene repeat treatment regimes at eliminating diminazene-resistant *Trypanosoma congolense* infections in goats

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Workers conducting studies on the pharmacokinetics of diminazene in goats have suggested that repeat treatment regimens with this drug may enhance the drug's therapeutic activity. Since some countries are unable to obtain the full repertoire of trypanocides, such regimes would be of benefit in eliminating parasites which express resistance to the recommended intramuscular (IM) dose of 3.5 or 7.0 mg diminazene aceturate (DA) kg^{-1} body weight. A study was therefore conducted to investigate the efficacy of repeat, as opposed to single, treatment with DA at eliminating infections with *Trypanosoma congolense* IL3274. IL3274 is a cloned population which originates from Burkina Faso and expresses a high level of resistance to diminazene both *in vivo* and *in vitro*.Twenty-eight goats were divided into six groups: five groups of five animals each (Groups A, B, C, D and E) and one group of three animals (Group F). On day O, Groups A-E were infected with IL3274 via the bites of infected *Glossina morsitans centralis*. Animals in Group F were infected in a similar manner, but with *T. congolense* IL1180, a clone which expresses a high level of sensitivity to diminazene. All animals in Groups A, B, C and F were treated IM with 7.2 mg kg^{-1} DA three days after parasites were first detected. In contrast to Groups A and F, Groups B and C received a second treatment with the same drug dosage, eight or 24 hours, respectively, after the first treatment. Animals in Group D were also treated IM with 7.2 mg DA kg^{-1} body weight, but treatment was administered 24 hours after infection. Animals in Group E remained untreated as an infectivity control. Infections in all five animals in Group A, infected with IL3274 and treated with a single dose of DA after detection of parasites, relapsed within a period of seven to twelve days following treatment. Similarly, infections in seven of the ten animals in Groups B and C (treated twice with DA) relapsed

within a period of 10-15 days following treatment. By contrast, none of the animals in Group D (treated 24 hours after infection) were detected parasitaemic during the 120-day observation period. Similarly, relapse infections were not detected in any of the animals in the drug sensitivity control group (Group F) following treatment. These results suggest that, as compared to single treatment, repeat treatment with 7.2 mg DA kg^{-1} at either eight-hour or 24-hour intervals does not greatly enhance the therapeutic activity of the drug. However, and more importantly, IM treatment with 7.2 mg DA kg^{-1} eliminated infections when treatment was conducted 24 hours after infection but failed to do so if treatment with the same drug dosage was delayed until after onset of parasitaemia. This suggests that failure to respond to treatment may not have been due to drug resistance *per se*.

TR1.15 Diminazene aceturate susceptibility of *Trypanosoma congolense* bloodstream forms *in vitro*

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The susceptibility of six *Trypanosoma congolense* stocks or clones to diminazene aceturate (Berenil®) was determined *in vitro*. *T. congolense* bloodstream forms have been adapted to continuous growth in a feeder cell-free culture system. Samples from continuously growing cultures were diluted in medium to a density of 6-8 × 10^5 trypanosomes/ml and incubated with various drug concentrations for 24 hrs at 34°C. The number of generations which occurred in drug-treated cultures was calculated and the relative growth of trypanosome populations was determined by comparison with the number of generations occurring in control cultures. In this Growth-Inhibition-Assay diminazene aceturate produced sigmoidal inhibition curves on all six tested *T. congolense* populations. EC_{50}-values for IL1180 were two - ten-fold lower than those of *T. congolense* stocks or clones which were less sensitive to the drug in mice. Diminazene aceturate-induced growth inhibition of IL1180 was similar as in drug-sensitive *T. b. brucei* clones when tested under the same conditions. Different generation times in control cultures did not seem to influence the anti-trypanosomal effect of diminazene. However, results were inconsistent with two out of the six populations tested.

In the Long-Term-Viability-Assay (10 days), none of the four tested *T. congolense* populations were able to grow in the presence of 100 ng diminazene aceturate/ml. Concentrations up to 30ng drug/ml did not show anti-trypanosomal effects.

TR1.16 Further studies on the initiation of *Trypanosoma vivax* cultures from infected cattle

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A necessary condition for employing *in vitro* assays for assessment of isometamidium resistance in *Trypanosoma vivax* is the successful initiation of *in vitro* cultures with trypanosomes from infected cattle or tsetse. A medium based on Iscove's medium containing 2 mM L-glutamine, 2 mM sodium pyruvate, 4.3 mM proline, 1.1 mM cysteine, 0.1 mM hypoxanthine, 0.075 mM adenosine and 20% fetal bovine serum supported transformation and growth of *T. vivax* from West (IL3185, IL1392 from mouse blood) and East (IL2242, CP 2171, IL3067) Africa. Cultures were initiated with five ml medium in T-25 flasks at 27°C.

A seeding density of 2×10^6 bloodstream form trypanosomes/ml appeared to be necessary to secure successful initiation of feeder cell-free cultures of insect forms. Occasionally, transformation and formation of colonies of epimastigotes occurred in cultures seeded with fewer trypanosomes, in particular with *T. vivax* clone IL3185. Subpassage from established cultures was possible in all attempts, a necessary condition for setting *in vitro* drug sensitivity assays. Initiation of cultures was successfully performed with trypanosomes isolated from the first to the sixth wave of parasitaemia in cattle. Thus, it appears that the state (duration) of parasitaemia in cattle does not have a major influence for the initiation of cultures provided a sufficient number of trypanosomes can be isolated.

TR1.17 Studies on *In vitro* assessment of drug resistance in *Trypanosoma vivax*

Student: D.S. Kitosi
Scientist: R. Kaminsky

Six stocks of *Trypanosoma vivax* were investigated for their drug sensitivity to isometamidium chloride and diminazene aceturate. It was evaluated whether *in vitro* tests had the potential to distinguish between drug-sensitive and drug-resistant stocks of *T. vivax*. Four *in vitro* tests were employed in drug sensitivity assessment: (1) Long-Term Viability Assay (20 days) with insect forms at 27°C, (2) ^{3}H-Hypoxanthine Incorporation Test (48 hr) with bloodstream forms at 34°C, (3) Drug Incubation Infectivity Test (DIIT) with bloodstream forms at 37°C and (4) Drug Incubation Survival Test with bloodstream forms at 34°C. All tests were able to show an anti-trypanosomal effect of both isometamidium chloride and diminazene aceturate on *T. vivax in vitro*. The anti-trypanosomal effects were detected by rounding and death of epimastigotes, inhibition of ^{3}H-Hypoxanthine incorporation by bloodstream forms, loss of infectivity to mice and reduction in the number of survivors.

The Long Term Viability Assay and the Drug Incubation Survival Test showed potential to distinguish between isometamidium chloride-sensitive and isometamidium chloride-resistant stocks or clones of *T. vivax*. Results on drug sensitivity of *T. vivax* stocks obtained with these tests correlated with results in cattle and mice. A correlation between *in vitro* and *in vivo* results was not observed regarding diminazene aceturate.

When cattle were infected with *in vitro* produced metacyclics, resistance of *T. vivax* stock CP 2171 was retained to treatment with 0.5 and 1.0 mg/kg isometamidium chloride after continuous *in vitro* propagation for 30 months. The reduced sensitivity of epimastigotes of CP 2171 to isometamidium *in vitro* was similar in cultures initiated from these cattle compared with results obtained in cultures initiated from cattle infected with the original stabilate.

TR1.18 Time-dose-response and pharmacokinetic simulation *in vitro* of *Trypanosoma brucei brucei* to diminazene aceturate (Berenil®)

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Trypanosomes of continuously growing populations were incubated in the presence of 10.0, 1.0 or 0.1 µg diminazene aceturate/ml at 37°C for various time periods. Subsequently, samples were washed and either inoculated in mice or further propagated *in vitro* in drug-free medium and monitored for their growth for the following 10 days. The capability of trypanosomes of drug-sensitive CP 2137 clone 1 to grow *in vitro* was irreversibly damaged after a pulse incubation (< 1 minute) with 10.0 µg or 1.0 µg diminazene aceturate/ml. In contrast, trypanosomes of drug-resistant CP 2469 clone 1 tolerated incubation with 10 µg drug/ml for up to one hour and 1.0 µg drug/ml for up to 24 hr. Differences in drug susceptibility were also detected regarding infectivity to mice and changes in cell volume. The results demonstrated that only a short exposure (minutes) to diminazene aceturate concentrations as they occur in bovine plasma at the initial peak after Berenil® treatment is enough to irreversibly damage drug-sensitive trypanosomes. However, peak concentrations of one to three hours were not sufficient to eliminate drug-resistant trypanosomes. But incubation for two days with 1.0 µg diminazene aceturate/ml also eliminated drug-resistant trypanosomes.

When drug-sensitive trypanosomes were exposed *in vitro* for 24 hr to diminazene aceturate concentrations found in cattle after treatment with 3.5 mg/kg, the majority of the trypanosomes were dead and none of the surviving parasites could be subsequently propagated *in vitro* in the absence of drug for more than two days. Mimic of bovine plasma levels revealed that only a minority of a drug-resistant trypanosome population (CP 2469 clone 1) was not irreversibly damaged. Single surviving trypanosomes were able to establish growing cultures. The addition of feeder-layer cells did not change the outcome of these experiments.

TR1.19 Production of anti-isometamidium antibodies

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Isometamidium chloride (ISMM) (Samorin®, Trypamidium®) is the only compound currently recommended for use in cattle as a prophylactic against trypanosomiasis. Resistance to this compound has been described in the field and the techniques currently available for screening trypanosome populations for resistance take many weeks or months to produce a conclusive result. There is, therefore, a need to develop a rapid test for quantitating resistance to the drug. In an attempt to produce anti-ISMM antibodies, which might be used to design an ISMM enzyme-linked immunosorbent assay (ELISA), ISMM has been conjugated to either human serum albumin (hsa) or baboon haemoglobin (hb) using glutaraldehyde. Balb/c mice and Sprague-Dawley rats were primed with either the hb-ISMM or hsa-ISMM conjugate emulsified in Freund's complete adjuvant, and subsequently boosted with the same drug conjugate, but emulsified in Freund's incomplete adjuvant. Because hb-ISMM bound poorly to the plastic of ELISA plates, whilst good binding was obtained with hsa-iSMM, only plates coated with hsa-ISMM were used to screen serum from immunised rats and mice for anti-ISMM antibodies. Anti-hsa antibodies were absorbed out from sera by a 12-hour incubation in medium containing 5% hsa. After administering the same antigen to rats and mice three times at two week intervals, the anti-ISMM specific antibody titres, using an ELISA technique, were shown to be 1:12,500 in the rat sera and between 1:500 and 1:2,500 in the mice sera. A fifth administration of antigen, one month after the fourth, raised the anti-ISMM specific antibody titres in all rats to 1:62,500, and in all mice to 1:2,500. The immunised mice will be further immunised for eventual production of monoclonal antibodies. Since the anti-ISMM antibody responses were far superior in Sprague-Dawley rats than in mice, Lou rats are being immunised, using a similar protocol to that used for Sprague-Dawley rats, for eventual production of monoclonal antibodies.

TR1.20 The effect of verapamil alone and in combination with trypanocides on multidrug-resistant *Trypanosoma brucei brucei*

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Following previous studies of verapamil reversal of multi-drug resistance in cancer cells and chloroquine resistance in malaria, the effect of the calcium channel blocker verapamil was investigated on multidrug-resistant and -susceptible *Trypanosoma brucei brucei*. Resistance of cloned parasites to diminazene aceturate (Berenil®) and isometamidium chloride (Samorin®) was expressed in a cell-free *in vitro* culture system. Verapamil showed anti-trypanosomal activity against both multidrug-resistant and -susceptible trypanosomes at concentrations above 1 µg/ml. Verapamil did not reverse multi-drug resistance when used at concentrations of 0.1 or 1.0 µg/ml in combination with diminazene aceturate or isometamidium chloride. Results obtained *in vitro* correlate with observations in mice. It is

suggested that multi-drug resistance in African trypanosomes is due to mechanisms other than those occurring in cancer cells, malaria or South American trypanosomiasis.

TR1.21 Molecular and functional characterisation of an extra-chromosomal element in drug-resistant *Trypanosoma brucei brucei*

Student: H.R. Jamnadass
Supervisors: N. B. Murphy, O.K. ole-MoiYoi

A 6-kb extra-chromosomal element was found in a multidrug-resistant *Trypanosoma brucei brucei* field isolate. Progress on the characterisation of this element was greatly hindered by problems in purifying sufficient amounts of 6-kb for analysis. This problem has largely been overcome now by the development of methods to amplify the 6-kb region using the polymerase chain reaction. Experiments using this gel purified 6-kb element as a probe suggest:

1)This element is only present in a multidrug-resistant isolate and clones derived from it.

2)There are genomic copies of this element in both the sensitive and resistant isolates.

3)Using the PCR amplified probe, a genomic library of the resistant isolate was screened. Using the PCR amplified probe of the 16 clones picked, two hybridised weakly to the 6-kb element. These clones were shown to contain repetitive sequences when hybridised to total genomic DNA suggesting the presence of a repetitive sequence on the 6-kb element. The possibility that these repeats may have been previously identified was ruled out as none of the already identified *T. brucei* repeats hybridised to the 6-kb element.

4)By using the PCR probe and the genomic clones as a probe on Northern blots, preliminary results show the presence of three additional transcripts in the resistant long slender bloodstream form when compared to a sensitive isolate. Transcripts have also been identified which appear to be specific to different developmental forms of a drug-sensitive isolate.

5)That the 6-kb element is responsible for the drug-resistant trait is suggested by the observation that the copy number of this element is greatly reduced when parasites are maintained on the absence of drug pressure.

TR1.22 Characterisation of genes encoding multidrug resistance-like proteins in *Trypanosoma brucei* and *Trypanosoma vivax*

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Resistance to anti-trypanosomal drugs shares several features with multidrug-resistance (MDR) in mammalian neoplastic cells. The most notable of these is the induction of resistance to several drugs using a single chemotherapeutic agent. The emergence of widespread resistance to the drugs available for treatment of African trypanosomiasis is grave and occurs at a time when no new drugs are available. It is thus important to try to understand the molecular mechanisms operational in trypanosome drug resistance. In mammalian tumour cells, as well as in some protozoan parasites, MDR genes encode membrane glycoproteins which actively remove drugs from the cell.

In an attempt to identify P-glycoprotein gene-like sequences, we employed oligonucleotides made on the basis of amino acid-conserved domains of available MDR sequences as well as anti-MDR antibodies to screen *Trypanosoma brucei* and *T. vivax* libraries. Screening these libraries with PCR products or an MDR probe from *T. parva* also allowed the identification of several clones which are currently being characterised. On Western blots of lysates prepared from *T. brucei, T. congolense* or *T. vivax*, the anti-MDR antibodies recognised several protein bands. However, several of these are small and fall outside of the Mr range reported for mammalian or other protozoan P-glycoproteins.

TR2.1 The characterisation and analysis of a developmentally regulated cytoskeletal gene from *Trypanosoma brucei brucei*

Student: W. Endege
Supervisor: N.B. Murphy
Research Associate: E. Gobright

In the analysis of cytoskeletal proteins from *Trypanosoma brucei brucei*, fractions enriched for filament forming proteins were used to generate polyclonal antisera in rabbits. This antisera predominantly reacts with proteins of 80-kDa and another of about 15-kDa which was suspected of being a degradation product of the larger protein. Immunolocalisation studies showed clear labeling of the paraflagellar rod as well as distinct labeling between the kinetoplast and basal body. Immunoscreening of a *T. brucei* λ*gt* II expression library with this antisera allowed the isolation of a number of clones, each of which contained a small insert (500 bp or less). Northern analysis showed that hybridising transcripts for this gene are also small (about 650 bp) and probably encode the 15-kDa protein identified in Western blots. This gene is well conserved between the different trypanosome species (more so than actin or tubulin) and stable transcripts are greatly reduced in non-dividing bloodstream forms in comparison to actively dividing bloodstream and insect forms. The developmental regulation of this gene would seem to depend on the expression of different family members as the parasites proceed through the different developmental stages. The complete cDNA sequence of this gene, including the 5' miniexon and 3' polyA tail have been derived. So far, no potential homologues for the encoded protein of this sequence have been identified in the databases, although there are domains showing conservation between it and intermediate filament-forming proteins, as well as a small nuclear protein from trout sperm called a high-mobility-group protein. Some small dispersed repeats of amino acids are also evident within the sequence, but the significance of these repeats is still unknown.

TR2.2 Purification and characterisation of p80, a trypanosome cytoskeletal protein

Student: W. Endege
Supervisor: O.K. ole-MoiYoi

Using differential salt precipitation techniques and detergent lysis, we have isolated from *Trypanosoma brucei brucei* IITat1.1 an 80-kDa protein which is highly insoluble in low and high salt buffers. We raised antibodies against this protein in rabbits. When these antibodies were used for immunofluoresence and immunocytochemistry, they localised this protein within the paraflagellar rod and in the region where the flagellum lies on the main body of the cell. Fluoresence and electron microscopy immnunolocalisation was also observed in this organelle, in dividing trypanosomes. Using the Western blotting technique and immunocytochemistry, this protein has also been shown to be present in the other life cycle stages of the parasite, namely the stumpy, procyclic, epimasgote and metacyclic forms. Recent phosphorylation studies using a purified trypanosome casein kinase II-like enzyme have revealed that p80, which is present in the slender bloodstream forms, can be highly phosphorylated *in vitro*, whereas the same does not apply to the same protein found in the short stumpy forms of the parasite. This would suggest that, *in vivo*, phosphorylation is used as a postranslational modification mechanism in the transition from slender to stumpy forms of the parasite.

The gene that encodes this protein has been isolated from a λ*gt* 11 metacyclic *T. congolense* IL3000 cDNA library. Transcription studies from the different life cycle stages of *T. brucei* (IITat 1.1) have revealed the presence of a 2.2-kb transcript in the long slender bloodstream, intermediate and procyclic forms of the parasite. Short stumpy forms have a significantly diminished or completely absent p80 transcript. This strongly indicates that the switching-off of mRNA synthesis is an additional mode of the differential regulation to that of phosphorylation observed at the protein level. Sequence analysis of this gene has revealed two open reading frames, one on each strand. The open reading frame on the forward strand has one stop codon near the 5' end, which has been proved to be real by sequencing six more independent cDNA clones isolated from the metacyclic specific library. However, this does not rule out the possibility that transcripts from other life cycle stages lack this stop codon. There are between four and six copies of this gene within the *T. congolense* IL3000 genome. The genomic organisation observed on Southern blots of DNA from *T. brucei* (IITat 1.1), *T. congolense* (IL3000) and *T. vivax* (IIDat 1.2) digested using selected restriction endonuclease shows that the gene(s) coding for this protein are arranged similarily in the different trypanosome species.

TR2.3 The trypanosome miniexon-derived RNA (medRNA) is polyadenylated in a stage-dependent manner

Scientists: R. Pelle, N.B. Murphy

The trypanosome life cycle involves changes not only in parasite morphology, infectivity and metabolism, but also in gene expression. The differentiation of the trypanosome through several distinct stages in its host and vector correlates with selective expression of genetic information. In the search for genes involved in the switching of trypanosomes from one form to another, we have isolated clones, using a subtractive hybridisation approach, which hybridised to stumpy form cDNA. Further characterisation of these clones reveal that they all contained similar sequences. Northern analysis revealed the following features of the RNA.

1)The RNA transcript corresponding to this clone is absent in the poly(a)$^+$ fraction of RNA from slender and procyclic forms. In contrast, they are present in the poly(A)$^+$ fraction of RNA from intermediate forms and stumpy forms, but the level of the transcript is about 10 times higher in stumpy than in intermediate forms.

2)This transcript is present at a high level in the poly(a)$^-$ fraction of RNA from all forms of the trypanosomes, without significant differences.

3)The size of the poly(A)$^+$ transcript is larger than that of the poly(A)$^-$ transcript.

In another analysis, we demonstrated that the polyadenylation of the transcript is a post-transcriptional event and sequences from genomic DNA cannot be amplified in the PCR when miniexon spliced leader (SL) and oligo(dT) oligonucleotides are used as primers. Sequence analysis showed that this clone corresponds to medRNA with a poly(A) tail at its 3' end.

All these data suggest that, in addition to the role of the medRNA may play an important role in the control of the trypanosome switching from a dividing to non-dividing

form. A possible mechanism by which medRNA activity is regulated should be its differential post-transcriptional 3' end polyadenylation.

TR2.4 Replicative DNA polymerases of trypanosomes

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In eukaryotic genome replication, the DNA polymerase α holoenzyme synthesises the lagging DNA strand, while DNA polymerase δ synthesises the leading strand. We have cloned and sequenced the *Trypanosoma brucei brucei* pol-α gene coding for the catalytic subunit of DNA polymerase α. This allows study of this enzyme by both genetic and biochemical approaches.

The *T. brucei* DNA polymerase α sequence of 1339 amino acids is 123 and 129 amino acids smaller at the N-terminal part than the human and yeast homologues, respectively. The protein has the six regions that are conserved to a high degree in the central part of the human DNA polymerase α, yeast DNA polymerases α and δ and, to a lesser extent, various viral DNA polymerases. These regions constitute the nucleotide binding domain and the catalytic site of the DNA polymerases. An important structural difference in this domain may be formed by a group of five cysteines in *T. brucei* DNA polymerase α that are not conserved.

T. b. brucei strain 427 displays sequence polymorphisms between the two alleles of the gene. A *Pvu* II polymorphic site within the coding region of pol-α is silent at the amino acid level, but it is separated by only 37 bp from a polymorphism that is not. We investigated the presence of the *Pvu* II polymorphism among ten different *T. brucei* stocks and, although more data are required, our results suggest that there is selection against presence of the *Pvu* II site in homozygous form. The selection would then probably be directed against the nearby non-silent mutation.

T. brucei pol-α is closely linked to an ORF of 442 codons in the same orientation, which we called pol-α associated gene 1 (polaAG1). There is evidence that both genes are transcribed continuously and are part of the same transcription unit. Interestingly, pol-α mRNA is mainly present in dividing cells while polaAG1 is expressed constitutively.

T. brucei pol-α probes corresponding to conserved parts of the protein cross-hybridise weakly with *T. congolense* and *T. vivax* pol-α, indicating that major differences between the DNA polymerases α of these parasites may exist. In *T. congolense*, the pol-α and polaAG1 genes are organised as in *T. brucei*, while in *T. vivax* the genes may not be linked.

TR2.5 Genetic manipulation of trypanosomes

Scientists: N.B. Murphy, H. Hirumi
Research Associate: K. Hirumi

Effective transfection techniques can be used to identify genes by screening for appropriately altered phenotypes (drug resistance, growth regulation) after introduction of a library of DNA fragments. They may also be used for the analysis of gene function by alteration of cloned genes *in vitro* and reintroduction. The development of such techniques for African trypanosomes is at present under study at ILRAD and other laboratories interested in the molecular biology of these organisms. It is our belief that such techniques will greatly accelerate the advance of trypanosome biology in allowing us to elucidate the function of developmentally regulated genes, in identifying factors involved in their regulation as well as studies involved in the control of parasite virulence and drug resistance. We have chosen initially to define systems for transfection with bloodstream forms of *Trypanosoma brucei brucei* since this is the best characterised African trypanosome and transfection systems for bloodstream form parasites would be the most useful. Results to date indicate that it is possible to transfect and express foreign DNA in trypanosomes. It is also possible to use fluorescently labeled DNA and flow cytometry for the separation of positively fluorescing trypanosomes to isolate organisms that have taken up DNA from those that have not. This should prove useful in later experiments for the cloning and analysis of positive transfectants. Results also indicate that it is possible to complement genomic mutations responsible for virulence in mammalian hosts by a shotgun approach. The virulent parasites generated in this experiment are presently being analysed and compared to their non-virulent or attenuated parental stock.

TR2.6 Analysis of the expression sites for metacyclic form and bloodstream form specific VSG genes of *Trypanosoma congolense*

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At the metacyclic stage of their development, the African trypanosomes uniquely activate only a small set of metacyclic form-specific VSG (mVSG) genes. In order to study the regulation of mVSG gene expression during cyclical development of *Trypanosoma congolense*, we have cloned two metacyclic form-specific VSG genes (mVSG1 and mVSG2) of IL3000.

Both mVSG1 and mVSG2 cDNA clones have been sequenced. mVSG1 is 1356 bp and encodes a protein of 48 kDa predicted molecular weight. mVSG2 is 1313 bp and encodes a protein of 47.8 kDa predicted molecular weight. These are the sizes of the proteins (average size 48 kDa) revealed in the lysates of metacyclic form trypanosomes by antibodies specifically immuno-selected by each of the recombinant mVSGs. The two mVSGs show no significant nucletide sequence homologies; however, at the predicted amino acid level they each contain a conserved 7mer peptide (DAAAGDN) in the N-terminal region. Additionally, they have a well-conserved set of eight cysteine residues and a

common CLCV pattern in the middle. None of them has any homologies with any of the published VSG sequences, not even in the C-terminus region.

mVSG1 gene is located on large chromosomes and is activated without an apparent rearrangement. At least 27 kb of the genomic vicinity of this VSG gene is conserved in all the developmental forms of IlNat2, irrespective of whether they express the gene or not. mVSG2 gene has at least one copy which appears to have undergone some alteration in the bloodstream form trypanosomes by the loss of sites for some restriction enzymes present in both the cDNA and the one copy found in the metacyclic form trypanosomes. Whether the alteration is related to the deactivation of this particular mVSG remains to be determined. Each of the mVSG genes is transcribed from a genetic locus with a different physical organisation.

Detailed comparative analyses of the genomic loci at which each of these gene transcripts is expressed, and the expression sites of the bloodstream form VSG genes in the same antigenic repertoire, is in progress.

TR2.7 Regulation, rearrangement and amplification of a gene during cyclical development of *Trypanosoma congolense*

Scientists: P.A.O. Majiwa, W.R. Fish
Technician: E. Omolo

Genomes of parasitic protozoa contain various repetitive sequence elements, some of which appear to facilitate the rearrangement of specific genes. The rearrangements and transpositions are mediated by simple repetitive DNA sequences located within both the 5' and the 3' vicinities of the genes affected. In both *T. n. congolense* and *T. n. simiae*, the most highly repetitive DNA sequences identified to date are primarily satelite DNA comprised of tandem repeats not transcribed into steady-state RNA.

We have previously identified a recombinant clone, in a bloodstream form *T. congolense* cDNA library, which contains the transcript of a gene that is transcribed specifically only in the bloodstream form stages of the parasite irrespective of the VSG expressed. Furthermore, the gene is differentially amplified and rearranged in different antigenic repertoires of the parasite, and is apparently not linked to any of the at least three different VSG genes expressed in two distinct antigenic repertoires.

The genomic locus from which the transcript is made has been identified and cloned. A significant portion of the locus is comprised of repetitive sequences more abundant in IL3000 than in IlNat2.1 or other clones. The region transcribed into steady-state mRNA is located on a 1.2-kb internal portion of the genomic clone. Full-length cDNA clones homologous to this locus are being sequenced in order to determine the extent of homologies between the genomic clone and the mature steady-state mRNA transcripts. Transcription analyses of genomic clones, using radiolabelled nascent transcripts, will enable us to determine the point at which its transcription starts. The amplified and rearranged locus has also been cloned in both phage and plasmid vectors. Detailed analysis of this locus is being performed to enable us to determine whether it has the potential for transposition to other genomic loci.

TR2.8 The characterisation of cell division cycle (cdc) genes from African trypanosomes

Scientists: B. Ricard, N.B Murphy

This project is part of the search for genes involved in the slender to stumpy switch in bloodstream form trypanosomes. Since stumpy forms are non-dividing, genes involved in the regulation of cell division are presumed to play a role. Such cell division cycle (cdc) genes have been identified and studied in other species and some of these genes code for protein kinases.

Oligonucleotides were constructed which were specific for cdc kinases based on the conserved amino acid sequences in the catalytic domains of these enzymes. Using the polymerase chain reaction on total genomic DNA from *T. brucei* and *T. congolense* with mixed oligonucleotides showing homology for both DNA strands, some genomic sequences were specifically amplified. After cloning and sequencing, three from *T. congolense* (Tccdcl, Tccdc6, Tccdc8) and one from *T. brucei* (Tbcdc4.3) were chosen for further study.

All the sequences were of similar size, about 300 bp, and all except Tccdc8 included both sequences used for amplification. The three *T. congolense* sequences were different; Tbcdc4.3 was similar to Tccdcl and all four sequences showed homology to cdc2 genes, with Tccdc8 showing the highest degree of similarity. Using the sequences as probes on genomic DNA blots, it was shown that each probe hybridised to different but unique sequences and therefore corresponded to different, single-copy genes. There was little cross-hybridisation between different trypanosome species. Efforts to show differential transcription in different life cycle forms were negative, but need to be repeated.

Genomic DNA libraries from *T. brucei*, *T. congolense* and *T. vivax* were constructed in λ*gt* 11. The *T. conglense* library was probed with Tccdcl, Tccdc6 and Tccdc8; the *T. brucei* library was probed with Tbcdc4.3 and Tccdc8. Clones containing trypanosome DNA inserts of about 7 kbp have been isolated. Partial restriction enzyme maps were constructed to localize the regions to which the cdc probes hybridised to obtain smaller fragments of about 1.6 kbp. These have been subcloned into pBluescript II and sequential deletions generated for DNA sequencing.

TR2.9 An improved feeder-layer-free system for cultivating bloodstream forms of *Trypanosoma congolense*

Scientist: H. Hirumi
Research Associate: K. Hirumi
Technician: J.R. Wando

A bloodstream form (BSF) supporting medium HMI-9, which was developed earlier for cultivating BSFs of *Trypanosoma brucei* (Hirumi and Hirumi, 1989), supported continuous growth of *T. congolense* IL3000 BSFs (ILRAD, Ann. Sci. Rep., TR2.20, 1989). This did not, however, support the growth of BSFs of other tested stocks. Further improvement of the medium was, therefore, carried out. By using a newly modified medium HMI-93, BSFs of four stocks (IL2079, IL2466, IL3266 and CP-81) and two clones (IL3000 and ILNat3.1 derived from IL1180) were successfully cultivated *in vitro* at 34-36°C in the absence of

feeder cell layers. The medium was modified from Iscove's modified Dulbecco's MEM by supplementing with 0.05 mM bathocuproine sulfonate, 1.5 mM L-cysteine, 0.5 mM hypoxanthine, 0.12 mM 2-mercaptoethanol, 1 mM sodium pyruvate, 0.16 mM thymidine, 20% (v/v) heat-inactivated young goat serum and 5% (v/v) Serum Plus™ (Hazleton Biologics, Lenaxa, KS). Trypanosomes obtained from two different sources were used to initiate primary cultures: (I) metacyclic forms (MCFs) which were propagated *in vitro* at 27°C, and (II) BSFs obtained from Balb/c mice which were infected with the BSFs transformed *in vitro* from the MCFs (IMB-derived BSFs). The MCFs placed in 25cm^2 T-type (T-25) flasks rapidly attached to the bottom of the flasks and transformed to BSFs during the initial 24 hr and continued to proliferate. The BSFs isolated from the infected mouse blood by means of diethylaminoethyl cellulose (DE52) column chromatography also continued to proliferate in the flasks. Cultures were maintained by replacing the medium every 24 hr. Every four to five days, the attached BSFs were resuspended in fresh medium by gentle pipetting and then were subcultured. The method was further simplified by initiating primary cultures directly with 10 µl of the tail blood of infected mice in 24-well culture plates and then by subcultivating either in wells or in T-25 flasks. The shortest population doubling time, nine hours, was achieved by seeding subcultures with 10^6 BSFs/ml. The BSFs propagated in this system were morphologically similar to those seen in infected mouse blood, were covered with a surface coat when examined by electron microscopy and were infective to mice. All the IMB-derived BSFs examined were obtained from mice infected with BSFs which were transformed from *in vitro*-propagated MCFs. Thus, it may be necessary to examine further whether stocks which had not undergone the *in vitro* transformation (such as new stocks isolated from naturally infected animals) also have the ability to grow in the *in vitro* system reported here.

TR2.10 The effect of goat transferrin on the growth of bloodstream forms of *Trypanosoma brucei brucei* without feeder layer cells

Scientists: Y. Yabu, H. Hirumi

A feeder layer-free system has been established for cultivating bloodstream forms (BSFs) of *Trypanosoma brucei brucei* using modified Iscove's medium (HMI-9) (Hirumi & Hirumi 1989). In this system, however, many BSFs died during the initial five to seven days, indicating need for further improvements. Although HMI-9 medium also supported the continuous growth of metacyclic (MC)-derived BSFs of *T. congolense* IL3000 in the absence of feeder cells, the medium failed to support the growth of MC-derived BSFs of the other five stocks of *T. congolense* tested. Recently, young goat serum (YGS) replacing foetal bovine serum (FBS) successfully supported the growth of five MC-derived stocks of BSFs of *T. congolense* in the absence of feeder layers (ILRAD, Ann. Sci. Rep. TR2.20, 1989). An attempt therefore, was made to elucidate the difference between FBS and YGS. To date, goat transferrin (GTf) was found to be different from that of bovine in molecular size as examined by means of SDS-PAGE. In order to determine the effect of GTf on the growth of BSFs of *T.b. brucei*, purification of GTf from serum was carried out. Goat sera were precipitated with various amounts of ammonium sulfate, ranging from 250 g/l to 350 g/l. A transferrin-rich fraction was obtained from supernatant at 300 g/l saturation. The supernatant was dialysed in a solution of 0.2 M Tris-HCl buffer, pH 8.0, in 0.07 M NaCl at 4°C. Then it was passed through a DEAE-cellulose column (DE-52). Starting buffer elution was used to elute the pure transferrin. The effect of GTf on the growth of BSFs in the absence of feeder layers was then investigated by supplementing a modified Iscove's medium with

various amounts of GTf ranging from 200 to 0.2 µg/ml. Prior to the examination, Iscove's medium was modified by supplementing it with 50 µM bathocuproine sulfonate, 500 µM L-cysteine, 600 µM hypoxanthine, 30 µM thymidine, 1 mM sodium pyruvate, 5 µM ferrous sulfate, 1 µM ferric nitrate, 60 µM L-ornithine, 10 ml/l myristic acid/bovine serum albumin complex, 10% (v/v) heat-inactivated FBS and 10% (v/v) Serum Plus®. Mouse derived BSFs (5×10^4/ml) were inoculated into each T-25 culture flask containing various concentrations of GTf and incubated at 37°C. In the presence of GTf, BSFs continuously proliferated as long slender forms without transforming to stumpy forms during the initial three days. In contrast, in the absence of GTf, the BSFs rapidly transformed to stumpy forms during the period and died by day five. In the concentration of 50 µg/ml, added GTf showed optimal growth of BSFs. In this culture condition, BSFs increased in number up to $2\text{-}3 \times 10^6$ BSFs/ml by day three after initiation of the culture.

TR2.11 Subcellular fractionation studies on the endocytotic pathway of African trypanosomes

Scientists: D.J. Grab, J.D. Lonsdale-Eccles, W.R. Fish, N. Borowy*, J. Gruenberg**
Research Associate: P. Webster
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Using the enzyme horseradish peroxidase (HRP), we have been able to demonstrate the uptake of this enzyme into flagellar pocket membranes and endosome-like organelles which had been fractionated on both Percoll and sucrose density gradients. Using a relatively new technique, free flow electrophoresis (FFE), we have been able to confirm and extend our initial observations. We found that unlike with density gradients, we were able to subfractionate flagellar pocket membranes from the more positively charged endosome fraction from crude membrane fractions. Using lactoperoxidase, we have been able to iodinate the proteins within these organelles. We have also used FFE to further fractionate highly enriched Golgi and lysosome fractions. Golgi was separated into several galactosyltransferase rich subfractions, one of which contains the trans-Golgi marker acid phosphatase as well as adenyl cyclase. A tentative primary lysosome preparation (L-3) was separated into at least three acid proteinase rich components. These fractions as well as others should be useful in dissecting *in vitro* the biochemical events associated with endocytosis as well as provide useful antigens for possible immunologic intervention.

TR2.12 Identification of a transferrin binding protein in African trypanosomes

Scientist: D.J. Grab
Research Associate: P. Webster
Technician: Y. Verjee

Most of the acid-soluble iron in plasma is bound to a specific carrier protein called transferrin. Like most cells, it appears appears that African trypanosomes require this molecule for normal development within the blood of the mammalian host. Here we present a summary of our attempts to purify a transferrin receptor from these parasites. *Trypanosoma brucei brucei* MiTat 1.52 was isolated from infected rat blood and incubated overnight at 37°C in RPMI-1640 containing 0.3% glucose, 50 µM hypoxanthine, 10% heat inactivated rat serum and either ^{35}S methionine or a mixture of ^{3}H amino acids (Amersham TRK 440). The cells were washed with serum-free medium before lysis in buffer containing Triton X-100, protease and phosphatase inibitors, and ^{125}I bovine transferrin. After centrifugation to remove cytoskeletal complements, the lysate was subjected to Sephacryl S-300 column chromatography and the iodinated transferrin complexes precipitated with monospecific rabbit antisera or IgG. Under reducing conditions, the molecular weight of the trypanosome transferrin binding protein was found to be 82,000 Daltons.

TR2.13 The vacuolar ATPase of African trypanosomes - molecular biological studies

Scientists: W.R. Fish, N.B. Murphy
Research Associate: M.D. Macklin
Technicians: C.W. Muriuki, A. Muthiani

Several clones of the A (catalytic) subunit of a vacuolar ATPase (V-ATPase) initially were selected from a *Trypanosoma congolense* IL 3000 metacyclic cDNA library using a consensus oligonucleotide probe. Sequencing of these clones, and sub-clones derived by a variety of methods, allowed us to nearly complete the sequence. The largest cDNA contained 1970 bp with: 1) a 1776 bp open reading frame, 2) truncated 5' and 3' ends (lacking the mini-exon and a polyA tail) and 3) a 5' untranslated region. The cDNA and deduced amino acid sequences show considerable similarity to the A subunit of other organisms, particularly that of plants. However, differences do exist, the trypanosome A subunit possessing a significantly higher cysteine content and amino acid substitutions in conserved regions involved in catalysis and nucleotide binding.

To obtain the B (regulatory) subunit of the V-ATPase, the polymerase chain reaction technique (PCR) was utilised. Cloning and subsequent sequencing of a PCR product showed it to be virtually identical at the amino acid level to that from other organisms. This DNA fragment was used to probe the same *T. congolense* IL 3000 metacyclic cDNA library. Fifteen cDNA clones were isolated and are presently under analysis.

The smallest subunit of the V-ATPase, which forms the proteolipid proton pore, is also being sought. Presently, PCR products of the correct size for the primers used are under analysis.

Both the full-length A subunit insert and the PCR product for the B subunit will be used as probes to isolate clones from *T. b. brucei* and *T. vivax*. Comparison of the

V-ATPase from these three trypanosomes will allow assessment of their evolutionary relationship both to other organisms and among themselves and may suggest unique chemotherapeutic or immunological targets.

TR2.14 Biochemical properties of the vacuolar ATPase of *Trypanosoma brucei brucei*

Student: W. Kitungu
Supervisor: W.R. Fish

The vacuolar ATPase (V-ATPase) is a large heterooligomeric enzyme that plays a pivotal role in the acidification of various organelles of the endo- and exocytic pathways. We have assayed for the presence of this enzyme in a variety of sub-cellular fractions of bloodstream forms of *Trypanosoma brucei brucei*. One fraction, predominantly of endosomal origin, showed significant total ATPase activity.

This fraction was assessed for the presence of a variety of ATPases by the application of differential inhibitors. The results showed: 1) an absence of the mitochondrial F_0F_1-ATPase, 2) the presence of Na^+/K^+-ATPase (30% of the total ATPase activity) and 3) another, unknown ATPase activity (12% additional activity above that of the Na^+/K^+-ATPase).

N-ethylmaleimide (NEM), to which V-ATPases show a higher sensitivity than other ATPases, inhibited all but 12% the total ATPase activity (IC_{50} = 40 µM). Taken together, the above results suggest that the V-ATPase is present and, curiously, that the trypanosome Na^+/K^+-ATPase may possess a catalytically active -SH group. NEM inhibition of the Na^+/K^+-ATPase is unusual.

In addition, the response of ATPase activity either to added phospholipid or nitrate is diagnostic for V-ATPases. Exogenous phospholipid caused an increase in activity which is comparable with that of other V-ATPases. If V-ATPases are incubated on ice with nitrate, Mg^{++} and ATP, a dramatic time-dependent decrease in activity occurs. A similar phenomenon was observed with the trypanosome enzyme in the presence of nitrate and Mg^{++} alone. However, it is curious that all the activity was lost, indicating that all trypanosome ATPases present might be sensitive to this treatment.

Definitive evidence for the presence of the V-ATPase in *T. b. brucei* was provided by the inhibitory activity of Bafilomycin A_1 (BFA), the only known specific inhibitor of the V-ATPase. ATPase activity which was not inhibited by reagents specific for other classes of ATPases showed BFA inhibition (IC_{50} of 4-10 nmoles/mg protein). This value is approximately ten-fold higher than that shown for pure preparations of the V-ATPase from other organisms. Therefore, it is essential that a method be developed for preparing the trypanosome V-ATPase in high purity. We are presently investigating the efficacy of a variety of such methods that have been successful with V-ATPases from other organisms. Preliminary data show purification of the V-ATPase away from the Na^+/K^+-ATPase, an increase in the specific activity of BFA inhibited ATPase and an IC_{50} for BFA of approximately six nmoles/mg protein. In addition, improved methods will be investigated for the preparation of a more homogeneous sub-cellular fraction with a view towards study of the acidification process in trypanosomes.

TR2.15 Identification and functional characterisation of protein antigens of *Trypanosoma vivax*

Student: B. Burleigh
Supervisors: P.R. Gardiner, M. Clarke*

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Formalin-fixed, uncoated, three-day culture forms of *Trypanosoma vivax* 1392 were used to immunise Balb/c mice for the purpose of monoclonal antibody production. Tissue culture supernatants were screened on Western blots and by immunofluorescence on frozen thick sections (300 nm) of *T. vivax* 1392 bloodstream forms. Although the initial aim was to look for surface reacting monoclonals, some hybridomas producing antibodies that were not surface reactive were also selected and cloned. Of the several obtained, two were characterised further.

The first, MAb 4B11, an IgM, labels the surface of bloodstream form trypanosomes in immunofluorescence and is also localised mainly to the surface on thin sections. This MAb recognises a 36-kDa band in all *T. vivax* isolates tested to date and also recognises the same band in lysates of *T. vivax* 1392 epimastigotes. By Western blot analysis, this MAb does not recognise any protein in *T. brucei* bloodstream or procyclic forms, nor in *T. congolense* procyclics. The protein does not bind to, or at least is not eluted from, Con-A sepharose.

In thin cryosections of *T. vivax* bloodstream forms, the second MAb, 4E1, an IgG_1, localises to the periphery of unidentified intracellular organelles. On Western blots, it recognises a 65-kDa protein which is present in all *T. vivax* isolates tested so far but is not present in *T. brucei*. It is also present in *T. vivax* 1392 epimastigotes. It appears to be a mannose containing glycoprotein as it is bound to and can be eluted from Con-A Sepharose. In one step, Con-A Sepharose significantly enriches for this protein. Preparative gels were run and the protein band excised. The protein was recovered from the gel in a seemingly pure form by elution. This material will be used to raise polyclonal antiserum in rabbits. Endocytosis with BSA gold and co-localisation with MAb 4E1 will be done as an attempt to determine the nature of the organelles in which this protein is found. Structural information will come from N-terminal sequencing, peptide mapping, glycosylation studies and, eventually, DNA sequencing.

TR2.16 Proteases of African trypanosomes

Scientists: J.D. Lonsdale-Eccles, D.J. Grab, W.R. Fish, E. Authié, M.J. Kornblatt
Research Associates: P. Webster, G.W.N. Mpimbaza, I.A. Gumm

A cytosolic trypsin-like peptidase (TTP) and a lysosomal-like endopeptidase (trypanopain) have been isolated from *Trypanosoma brucei brucei* MITat 1.52 and *T. congolense* IL3000. TTP, which has pKa values that probably correspond to an active site histidine and a buried N-terminal amino acid, is optimally active about pH 8.2. Kinetic studies indicate that this trypsin-like enzyme has a highly restricted specificity with binding sites on both sides of the cleavage point. In some respects the specificity of TTP is similar to that of various blood

clotting enzymes, but plasminogen was not activated and blood clotting times were unaffected TTP.

TTP is inhibited by several serine protease inhibitors including diisopropylfluorophosphate (DFP). The rate of DFP inactivation of the enzyme is about is four-fold slower than that of trypsin, but about five-fold faster than that of a plasminogen activator and 100-fold faster than that of factor X_a. Thus, the active site of the enzyme is similar to, but distinct from, that of several trypsin-like enzymes. This unusual enzyme is also inhibited by the cysteine protease inhibitor Z-Leu-Lys-CHN_2.

The kinetics of hydrolysis of small fluorogenic peptides by living parasites suggests that some hydrolytic activity is readily accessible and may be located at or near the surface of the parasite. About 90% of the accessible peptidolytic activity expressed by the live parasites may to be due to TTP, with the remaining 10% being caused by trypanopains. The observation that trypanopains are found in the flagella pocket as well as in lysosome-like, intracellular bodies is consistent with trypanopain hydrolysis of the peptides near the surface of the parasites. Temperature perturbation studies indicate that the flagella pocket can be made inaccessible to large molecules at low temperature, whereas small molecules may continue to have ready access to the pocket. The trypanopains, which are found only in the life cycle stages of *T. brucei*, *T. congolense* and *T. vivax* that have a variable surface glycoprotein, are optimally active at acid pH. Nevertheless, they exhibit considerable activity at neutral and alkaline pH and so may exhibit activity outside the acid lysosomes. Interestingly, the resistance to trypanosomiasis shown by certain bovids has been correlated with the production of antibodies to trypanopain.

Trypanopain activity is increased several-fold in parasites that have been treated with tyloxapol. This indigestible trypanocide alters the density of the lysosomes but free-flow electrophoresis indicates that it has no effect on their surface charge. Tyloxapol destabilises the lysosomes so that there may be as much as a twenty-fold increase in the trypanopain activity in the cytosolic fractions.

TR2.17 The characterisation of a thiol protease in *Trypanosoma congolense*

Student: Z.M. Nkhungulu
Supervisor: J.D. Lonsdale-Eccles

We have purified and partially characterised a cysteine protease from *Trypanosoma congolense*. Recent subsite specificity studies indicate that the enzyme prefers substrates with a basic residue in P1 and a non-polar residue in P2. H-D-Ala-Leu-Lys-NHMec was also found to be a good substrate for the enzyme as well as Z-Phe-Arg-NHMec. Occupation of the P3 position with an appropriate residue is also important for catalysis. Enzyme activity was tested against the diazomethyl ketones which are cysteine-protease-specific inhibitors. Z-Leu-Lys-CHN_2 and Z-Leu-Met-CHN_2 were found to be the best and most potent inhibitors for the enzyme. The enzyme hydrolyses a variety of proteins, including trypanosome variable surface glycoprotein.

Polyclonal antibodies to the enzyme were raised in rabbits and the authenticity of the antibody was ascertained by the antigen capture ELISA technique. Immunolocalisation of the enzyme in the parasite using this antibody labels lysosome-like organelles. We have

shown, using electron dense BSA-gold particles, that these organelles are part of the endocytotic pathway.

Endopeptidase activity at different life cycle stages of three species of African trypanosomes were determined using assay conditions which permitted us to distinguish between lysosomal and non-lysosomal proteolytic systems. In *T. congolense* the lysosomal activity against Z-Phe-Arg-NHMec at acid pH increases as the parasites differentiate from procyclic forms to bloodstream forms. In *T. vivax*, Z-Phe-Arg-NHMec activity is also high in metacyclic and bloodstream forms but undetectable in epimastigote forms. In *T. brucei*, Z-Phe-Arg-NHMec hydrolytic activity in bloodstream forms was greater than in procyclic forms, but also showed further increases as the parasite differentiated from long slender to short stumpy forms. Results obtained with Z-Arg-Arg-NHMec and Z-Gly-Gly-Arg-NHMec hydrolysis were, however, much more difficult to interpret. Thus, during their life cycle, each of these African trypansomes exhibits complex changes of endopeptidase activity suggestive of an induction of lysosomal activity between their insect and mammalian forms.

TR2.18 Peptidases: characterisation by renaturation of peptidase activity of *Trypanosoma (Duttonella) vivax* isolates from East and West Africa, production of polyclonal antibodies to a 92-kDa peptidase

Student: A.I. Ibitayo
Supervisor: P.R. Gardiner

A group of isoenzymes, the peptidases, are currently being studied because they have been shown to be useful in the detection of infection and diagnosis of infection and disease. They are also thought to play a role in the pathogenesis of disease. Preliminary renaturation studies showed that the peptidases (substrate - leucylglycylglycine and leucylalanine) of *Trypanosoma vivax* differed from those of *T. brucei* and *T. congolense*. We therefore correlated the existence of these enzymes with the particular phenotypic characteristics of individual *T. vivax* stocks. By means of SDS-PAGE-separation of parasite proteins and development of enzyme activities in an overlay gel technique, two bands of activity were obtained for each stock, one with a molecular weight of 92 kDa and the other 200 kDa or 220 kDa. Kenyan stocks gave bands at 92 kDa and 200 kDa, while West African and Ugandan stocks gave bands at 92 kDa and 220 kDa. Again, as previously noted from isoenzyme, serological and DNA studies, the Ugandan stocks were more similar to the West African stocks than the Kenyan stocks. All stocks had the 92-kDa band in common.

We have raised polyclonal antibodies in rabbits to the 92-kDa protein and studies are currently in progress to precipitate peptidase activity qualitatively and quantitatively. If this turns out to be successful, it would enable the determination of the sub-cellular localisation of this protein and the possibility of its being released during trypanosome infection in different ruminant and rodent hosts. It is also of interest to determine the functional role of this peptidase in these trypanosomes.

TR2.19 Purification of trypanosome alternative oxidase (TAO)

Scientists: E.J. Bienen, J. Kornblatt
Technicians: F. McOdimba, J. Nthale

The trypanosome alternative oxidase (TAO) and its regulation is one of the most unique features of trypanosome metabolism and as such is a prime target for chemotherapy, since this enzyme is not shared by the host and is the sole terminal oxidase in the bloodstream forms. Purification and characterisation of this very important enzyme can lead to new approaches to chemotherapy.

In an attempt to purify the trypanosome alternative oxidase (i.e., the sole terminal oxidase present in bloodstream stages) we have used detergent solubilisation and affinity chromatography to obtain a fraction enriched for exygen utilisation in the presence of α-glycerophosphate as substrate and phenazine methosulfate, which acts as a mediator for electron transfer. This activity may represent either the oxidase or the dehydrogenase component, due to specific significant data which we have so far failed to obtain. Although the applied techniques and acquired data represent an advance in purification of components of the TAO, more work needs to be done to definitely determine which components are represented by the obtained activity. Even if the activity is the dehydrogenase component, this has not yet been purified from trypanosomes, and although it is expected to be similar to that from other organisms, a purified dehydrogenase component may then be used as a means of purifying the oxidase.

TR2.20 Regulation of the mitochondrial ATPase in pleomorphic bloodstream trypanosomes

Scientists: E.J. Bienen, M.K. Shaw

Previous work has shown that long slender and short stumpy bloodstream trypanosomes show significant differences in mitochondrial metabolism with the potential for intramitochondrial generation of ATP. We have been trying to specifically identify key elements of some of these pathways with a goal towards understanding their regulation and role during pleomorphic differentiation.

We have shown, using enzyme assays and electron microscopy, that the oligomycin-sensitive mitochondrial ATPase is differentially expressed between long slender and short stumpy bloodstream trypomastigotes. Short stumpy forms show a four- to five-fold increase in the oligomycin-sensitive ATPase, with an overall doubling of ATPase activity in a crude mitochondrial fraction. There is a further increase in both ATPase activity and oligomycin sensitivity as the cells differentiate to procyclic forms. Using other ATPase inhibitors, we have shown that there is no cross-contamination with sodium/potassium ATPase and possibly slight contamination with vacuolar ATPase, but the majority of oligomycin-insensitive activity is not significantly sensitive to other ATPase inhibitors. More work is needed to fully establish and re-evaluate the mitochondrial pathways related to energy production and their implications for differentiation and potential chemotherapeutic targets.

TR3.1 Antigen-specific responses of bovine T cells during infection with *Trypanosoma congolense*

Scientist: J.N. Flynn
Technician: R. Saya

In Boran (trypanosusceptible) cattle there was a transient antigen-specific T-cell proliferative response of cells isolated from the spleen and peripheral blood (PBMC) during infection with *T. congolense* IL1180. PBMC from N'Dama cattle exhibited a similar response, but this response was maintained (albeit at a lower level) until termination of the experiment at 40 days post infection (dpi). The highest parasite-antigen driven proliferative responses were seen in N'Dama PBMC isolated at around 20 dpi. At this time the PBMC not only responded to the antigens derived from the infecting clone (IL1180) but also to antigens from a clone of an unrelated serodeme (IL3000), whereas Boran PBMC isolated at this time responded to IL1180 only.

To determine the molecular weight(s) of the antigenic trypanosomal proteins, whole trypanosome lysate was fractionated by SDS-PAGE, electro-blotted onto nitrocellulose membranes and the major protein bands isolated and used directly in T-cell proliferation assays. There were no major differences in the antigen recognition profiles of Boran and N'Dama cattle. The variable surface glycoprotein (VSG) did not induce T-cell proliferation in infected cattle despite the presence of serum antibodies to this variable antigenic type.

TR3.2 Induction of immunosuppression in *Trypanosoma congolense* - infected cattle

Scientists: J.N. Flynn, M.R.J. Sileghem
Technician: R. Saya

We have previously shown that T-cell function is impaired in Boran cattle following primary challenge with *T. congolense* IL1180. Although mononuclear cells isolated from peripheral blood (PBMC) and spleen have depressed responses to mitogenic stimulation, this effect was most marked in cells derived from lymph node tissue (LNC). Accordingly, LNC were chosen for further studies of this phenomenon. In these experiments, pairs of MHC-matched cattle were used. One animal was infected whilst its MHC-matched partner served as a normal uninfected control. LNC were obtained by performing lymph node biopsies on infected (draining bite site) and normal nodes during the third week of infection. Using these cells, it was possible to demonstrate that LNC from infected cattle not only failed to proliferate in response to a mitogenic stimulus, but also suppressed the potential of both normal PBMC and normal LNC to proliferate when they were cultured together. Addition of indomethacin or catalase either alone or together did not result in a restoration of proliferation, indicating that the suppression was mediated by prostaglandin-independent mechanisms. To assess the role of macrophages in the induction of trypanosome-associated immunosuppression, this population of cells was labelled with the monoclonal antibody IL-A24 and depleted by flow cytometry. Depletion of IL-A24 positive cells from infected LNC resulted in a restoration of proliferation in co-cultures, indicating a key role for these cells in the induction of immunosuppression.

TR3.3 Suppression of IL-2 production and IL-2 receptor expression in bovine trypanosomiasis

Scientists: M.R.J. Sileghem, J.N. Flynn
Technician: R. Saya

Tsetse-transmitted *Trypanosoma congolense* infection in Boran cattle causes a state of immunodepression in lymph nodes draining the site of challenge. Co-culture experiments in which the cells from these nodes were mixed with lymph node cells from uninfected MHC-compatible animals have revealed the presence of suppressor cells capable of arresting Con A-induced T-cell proliferation. These suppressor cells were found to act by blocking the secretion of interleukin 2 (IL-2) as well as the expression of IL-2 receptors. Neither function could be restored by inhibition of prostaglandin synthesis or degradation of hydrogen peroxide. This demonstrated a major difference in the action of the bovine suppressor cell population and the equivalent murine suppressor cell population which blocks IL-2 production through prostaglandin release. In both species, however, the suppressor cells were found to co-purify with the macrophage fraction.

TR3.4 Functional activity of bovine monocytes during *Trypanosoma congolense* infection

Scientist: M.R.J. Sileghem
Technician: R. Saya

Monocyte function was measured throughout a tsetse-transmitted *Trypanosoma congolense* infection in tolerant N'Dama and susceptible Boran cattle. To obtain a broad view on the functional status of the cells, different aspects of monocyte activity were recorded.

The uptake of bacteria (*Micrococcus luteus*) and *Trypanosoma congolense* was measured on a fluorocytometer using fluorescent-labelled particles. The killing of internalised bacteria was assessed by culturing the bacteria-fed monocytes for two hours after removal of the non-internalised bacteria with lysozyme The oxidative burst of the cells was measured using the oxidation of 2',7' dichlorofluorescein diacetate. Release of interleukin 1 was titrated using a thymocyte co-stimulation assay, and the presence of transforming growth factor in the supernatants was analysed using blocking antibodies.

In short, uptake and killing of bacteria did not seem to be markedly affected by the infection. The antibody-dependent uptake of trypanosomes also was only marginally increased in some animals. In contrast, the secretion of interleukin 1 by blood monocytes was increased more than 100 times. This activation was found in both Boran and N'Dama cattle. At least in the Boran, a similar but weaker activation was seen in the spleen. Transforming growth factor was found to be either not secreted during the infection or to be present in concentrations which were too low to manifest a biological activity. Finally, an assay has been developed to detect bovine tumour necrosis using monoclonal antibodies. As yet, no data are available.

TR3.5 Phagocytic potential of bovine granulocytes during *Trypanosoma congolense* infection in cattle

Scientist: M.R.J. Sileghem
Technician: R. Saya

A method was developed to measure the uptake of trypanosomes by granulocytes using two-colour fluorocytometry. To this aim granulocytes were incubated with living *T. congolense* labelled with a green fluorescent dye and then stained with a granulocyte specific monoclonal antibody labelled with a red fluorescent dye. Using the FACScan, a gate was set on the granulocyte fraction based on size, granularity and red fluorescence. The emission of green fluorescence by the gated cells was then used to measure uptake of trypanosomes by the granulocytes. Thus we were able to measure the uptake of trypanosomes by blood phagocytes in suspension without separating the unbound parasites from the phagocytes to measure uptake.

It was found that uninfected Boran and N'Dama cattle both manifested a binding of 200 trypanosomes/100 granulocytes. Following tsetse-transmitted infection, the binding was only marginally increased to 500 per 100 granulocytes. In the N'Dama group, all four animals manifested this increase. In the Boran group, two animals retained the pre-infection values whereas two showed an activation similar to the N'Dama. However, all four Boran were equally susceptible to the disease. It would thus appear that the differences occurred between individual animals and this did not correlate with resistance to disease.

The activity of the granulocytes was also probed by measuring the oxidative burst of the cells following addition of a strong trigger *in vitro* (opsonised bacteria). In both breeds, the oxidative burst appeared to remain unaltered during the infection.

TR3.6 Comparative erythrophagocytosis and monocyte surface membrane epitope expression during *Trypanosoma congolense* infection in Boran and N'Dama cattle

Student: V. Taiwo
Supervisors: D.J.L. Williams, L. Logan-Henfrey

Standard chromium 51 (Cr^{51}) release assays were used to compare erythrophagocytosis (phagocytosis and cytotoxicity) by macrophages (MQs) taken from Boran and N'Dama cattle, and one buffalo, following a primary *Trypanosoma congolense* infection. The time course of release of intracellular Cr^{51} by MQs from the two breeds of cattle was also studied. Monocyte (MO) surface membrane antigen expression using monoclonal antibodies (MAbs) IL-A24 (monocyte), IL-A21 (MHC class II), IL-A15 (C'3bi) and IL-A109 (monocyte) was monitored during infection, as a measure of their activation state.

Erythrophagocytosis increased following *T. congolense* infection in both breeds of cattle and in the buffalo. However, it was significantly higher and occurred earlier during infection in the Boran than in the N'Dama, while levels in the buffalo were lower than in the N'Dama. There was a more rapid rate of release of Cr^{51} by MQs of Boran cattle which together with the cytotoxicity results suggest that they engulf more RBC over time compared to N'Dama. The rates at which MQ engulfed RBC from infected or uninfected animals were small and hence difficult to interpret.

Monocytes of both breeds of cattle expressed all the surface markers studied in a similar phasic pattern suggesting that recycling of these molecules occurs with time. More MHC class II molecules were expressed on N'Dama MOs than Boran, whereas more C'3bi receptors were expressed on Boran MOs, but the significance of these observations remains to be studied in more detail. It was noted during this study that MAb IL-A109 may be related to IL-A24 in its staining characteristics in that MQs which were found to be negative for IL-A24 were also negative for this MAb. In addition, both were expressed on the same population of cells.

It was observed that Diff-quick-stained freshly isolated MQs from both breeds of cattle had phagocytosed lymphocytes and RBC, but this occurred earlier in the Boran (14 dpi) than in the N'Dama (28 dpi). Neutrophilic hypersegmentation was noticed in Boran cattle as early as 21 dpi, and throughout the infection.

TR3.7 *In vitro* macrophage activation by soluble trypanosome antigen and bacterial lipopolysaccharide

Student: V. Taiwo
Supervisors: D.J.L. Williams, L. Logan-Henfrey

To investigate the possible mechanism(s) of macrophage (MQ) activation during bovine trypanosomiasis, *Trypanosoma congolense* IL13E3 lysate at 5, 10, 15, 20 and 40 µg/ml and bacterial (*Salmonella enteritidis* and *Serratia marcescence*) lipopolysaccharide (LPS) at 0.1, 1.0 and 10 µg/ml were used to stimulate monocytes (MOs) from uninfected Boran and N'Dama cattle, in culture for 18, 48 and 96 hours. A standard chromium 51 (Cr^{51}) release assay was used to assess cytotoxicity and phagocytosis of autologous red blood cells (RBC) by the MQs. Analysis of the surface membrane antigens on the MQs and MOs was done using monoclonal antibodies (MAbs) IL-A24 (monocyte) and IL-A21 (MHC class II) and flow cytometry by the standard double staining technique.

MQs incubated with trypanosome lysate but not with LPS showed increased phagocytosis and cytotoxicity of RBC. This activation was optimal at 10 µg/ml, which is equivalent to 10^7 trypanosomes/ml. This suggests that trypanosome antigens could be responsible for MQ activation during infection. However, since peak parasitaemias rarely exceed 5×10^5 trypanosomes/ml of blood in the cow, it is possible that other factors may be responsible for the augmentation of MQ activation during infection.

Neither trypanosome lysate nor LPS had any effect on the expression of the two surface antigens studied in this experiment. The antigens were phasic in their expression on both MQs and MOs. This might be due to their state of maturation and/or the expression of the antigens on the cells for specific functions. The expression of the MHC class II antigen appeared to increase as the MQs were cultured on plastic plates.

TR3.8 Interaction between live trypanosomes and specific antibodies to exposed VSG epitopes

Student: D. Russo
Supervisor: D.J.L. Williams

Trypanosomes incubated with variable surface glycoprotein (VSG)-specific antibodies in the absence of complement or phagocytic cells clear these antibodies from the cell surface. Antibody molecules appear to move in association with the VSG to the flagellar pocket and are internalised in endocytic vesicles. *Trypanosoma brucei brucei* (slender and stumpy forms), *T. congolense* and *T. vivax* all demonstrated this ability, irrespective of the source of antibody (monoclonal or polyclonal). Pure Fab_1 prepared from polyclonal rabbit anti-sera was cleared from the trypanosome surface, suggesting that cross-linking of the VSG molecules was not a prerequisite for clearance.

The speed at which antibody molecules were cleared from the trypanosome surface depended on the amount of antibody present and on the isotype of immunoglobulin. IgM was cleared more rapidly than IgG and each antibody had a characteristic pattern of clearance. Protease, protein synthesis and cytoskeletal inhibitors did not prevent clearance of limiting doses of antibody. However, addition of either leupeptin or cycloheximide prevented complete clearance of saturating doses of antibody. Also, very high quantities of polyvalent IgG or the addition of a second step antibody also prevented clearance. Analysis of the effect of antibody clearance on fixation of complement revealed that trypanosomes incubated with IgM or IgG antibodies became refractory to complement-mediated lysis after 30 min and 60 min, respectively.

TR3.9 The signals necessary for bovine B cell activation

Scientists: J. Newson, J. Naessens
Research Associate: J. Magondu
Technician: P. Mucheru

An increase in cytoplasmic Ca^{++} level is the first measurable change following B-cell stimulation. The kinetics of Ca^{++} flux has been analysed for a number of potential stimulants using a fluorescent probe and the FACScan. Pokeweed mitogen, usually considered to be a B-cell mitogen, was ineffective at inducing the first activation step in enriched B cells. However, concanavalin A, considered to be a specific T-cell mitogen, was highly effective, as were anti-bovine light chain and IgM MAbs. Two of these MAbs in combination were more effective than one alone. Other B-cell MAbs, different from Ig, were unable to induce Ca^{++} flux.

The effective stimulants from the Ca^{++} flux analysis were then used, either alone or with conditioned media or irradiated lymphocytes, *in vitro* to induce antibody secretion in enriched B cells. Co-expression of early activation and B-cell markers was analysed. In culture, 90% of the cells died during the first 24 hours. The activation markers analysed were only weakly expressed on activated B cells, compared with strong expression on activated T cells. Using PWM or antigen as stimulant, the very small activated population of enriched B cells secreted a significant amount of antibody during seven days in culture,

despite the fact that after seven days it still constituted only 10-20% of the surviving B cells. Further studies will await access to bovine B-cell growth factors.

TR3.10 Trypanosome-specific and non-specific antibody responses to *T. congolense* infection in N'Dama and Boran cattle

Scientists: J. Newson, J. Naessens, D.J.L. Williams

There is evidence from other parasitic diseases that much of the induced increase in IgM is due to polyclonal activation, since few of the immunoglobulins react with parasite antigens. Humoral responses were therefore investigated in N'Dama and Boran cattle infected with *Trypanosoma congolense* IL1180. Specific antibody was measured using three forms of parasite antigen and non-specific antibody using nine irrelevant proteins.

Serum IgM levels increased in all cattle. The Boran showed the higher parasite-specific IgM activity, especially against the IINat 3.1 VSG, but IgG responses to both the IL1180 lysate and the VSG occurred one week earlier and were maintained at a higher level in the N'Dama than in the Boran. The Boran showed activity, mainly IgM, against seven of the irrelevant proteins. N'Dama showed much less activity against them and uninfected cattle were non-reactive. Sera were taken from the time of peak activity against β galactosidase, one of the irrelevant proteins, and the antibodies were purified on an affinity column. Preliminary results show that trypanosome-specific activity was mainly in the serum fraction that bound to β galactosidase. If this is confirmed, it eliminates the possibility that the non-specific antibodies bind to cross-reactive determinants on the trypanosomes. Thus, IgM antibodies against non-trypanosome antigens are stimulated in *T. congolense* infection. This polyclonal activation is much more marked in Boran than in N'Dama cattle.

TR 3.11 Antibody responses to non-VSG antigens of *T. congolense* in N'Dama and Boran cattle

Scientists: E. Authié, J.D. Lonsdale-Eccles
Student: Z. Mbawa
Research Associate: D. Webster
Technician: D. Muteti

Three immunodominant antigens of 69, 33 and 23 kDa were identified when analysing antibody responses to non-VSG antigens in cattle re-challenged with *T. congolense* IL 2642. The antigens of 69 and 33 kDa were differentially recognised by N'Dama and Boran cattle. In primary infections with *Trypanosoma congolense* IL1180, all animals recognised the 69-kDa antigen. As in the re-challenge experiment, the N'Dama mounted a high anti-69-kDa IgG1 response, whereas the Boran mainly responded with IgM. Monoclonal antibodies have been raised against the 69-kDa antigen, and its molecular characterisation was initiated. No antibodies directed to the 33-kDa antigen were detected in the sixteen Boran following primary infection with *T. congolense*. In contrast, the 33-kDa antigen was recognised by all trypanotolerant animals (fifteen N'Dama cattle and three buffalo). Anti-33-kDa antibodies were detected between 25 and 45 days postinfection (dpi) in thirteen out of the eighteen trypanotolerant animals, and between 45 and 70 dpi in the remaining five. Together with

the data from the re-challenge experiment, these results indicate that antibody response to the 33-kDa antigen of *T. congolense* might be associated with a degree of resistance to pathogenic effects of trypanosomes. Immunoelectron microscopy showed that the 33-kDa antigen is located in lysosome-like organelles of the parasite. It has been reported previously that the lysosomal compartment of *T. congolense* contains a cysteine protease of approximately 32 kDa. To determine whether the two proteins are the same, a monoclonal antibody was raised against the 33-kDa antigen. This monoclonal antibody (MAb 4C5) bound to the biochemically purified cysteine protease. Thus, the 33- kDa antigen recognised by trypanotolerant animals is likely to be the cysteine protease of *T. congolense*. In a sandwich ELISA using MAb 4C5 and a rabbit anti-protease antibody, the plasma from infected Boran and N'Dama cattle was found to contain circulating protease from the day the parasitaemia was first detected. Experiments are on-going to assess the role which the parasite protease might play in pathogenesis, and to evaluate the immunoprotective potential of this antigen.

TR3.12 Complement activity and C3 levels in Boran and N'Dama cattle during primary infection with *Trypanosoma congolense*

Scientist: E. Authié
Technician: D.K. Muteti

Complement activity decreases in trypanosome-infected cattle, and the severity of hypocomplementemia is related to the level of parasitaemia. However, the data from a re-challenge experiment with *T. congolense* IL2642 indicated different individual and breed abilities to maintain complement levels during infection. An haemolytic assay for the bovine alternative complement pathway, and a sandwich ELISA using a monoclonal antibody against bovine C3, have been developed. Complement activity (HC_{50}) and C3 levels in plasma were monitored in three N'Dama and four Boran cattle following primary infection with *T. congolense* IL 1180. HC_{50} and C3 decreased in all animals from the first detection of trypanosomes in blood, thus confirming that complement is primarily activated through a direct interaction with the parasite and that the presence of antibodies is not required. The four Boran experienced a continuous decrease in HC_{50}, which fell by 60% in 45 days of infection. Two out of three N'Dama began to limit hypocomplementaemia by 25 days postinfection (dpi) and tended to regain pre-infection levels. In the remaining N'Dama, HC_{50} fell by 50% in 45 days of infection, and thereafter increased towards the pre-infection level. Since parasitaemia was similar in all animals until 30-40 dpi, differences in complement kinetics are not explained only by different efficiencies in parasitaemia control. N'Dama cattle appear to have an ability to limit hypocomplementaemia during primary infection, but there are individual variations in this ability. Whether these variations are related with the degree of trypanotolerance, as was observed in the trypanotolerant Baoulé breed, should be investigated. Kinetics of plasma C3 generally paralleled those of HC_{50}. However, in some instances these two parameters were not closely correlated, indicating that other complement components may be affected by trypanosome infection. An ELISA for factor B, using monoclonal antibodies raised against bovine factor B, is being developed.

TR3.13 Trypanosome-specific antibody responses in trypanotolerant and susceptible breeds of cattle following a *T. congolense* infection

Scientists: D.J.L. Williams, S.K. Moloo
Research Associate: J. Newson
Technicians: F. McOdimba, M. Opollo

A study was undertaken to determine whether trypanotolerant N'Dama cattle mounted superior variable surface glycoprotein (VSG)-specific responses compared to trypanosusceptible Boran cattle, following a tsetse fly-transmitted primary infection with *T. congolense* IINat 3.1.

The N'Dama group controlled both the anaemia and the parasitaemia. In contrast, the Boran group became severely anaemic and highly parasitaemic and three of the four were drug-treated before day 56 post infection (dpi), to prevent possible death.

Three assays were used to detect antibodies specific for antigens exposed on the surface of living trypanosomes. A neutralisation of infectivity test (NIT) was used to detect antibodies specific for the metacyclic variable antigenic types (mVATs) and a complement lysis assay (CLA) and an ELISA were used to detect antibodies specific for eleven populations of parasites isolated from the peripheral blood of three N'Dama and two Boran, 14, 17 and 21 dpi. The results of these assays showed that there was no difference between the two breeds in the timing of the variant-specific antibody response. Antibodies became detectable to the mVATs 18 dpi and to the bloodstream form VATs 7-14 dpi. Also, there was no difference in the isotype of variant specific antibodies which were predominantly IgM. Low levels of IgG_1 were occasionally detectable in both Boran and N'Dama. However, significant differences between the two breeds were identified in the quality of the antibody response. Higher and more persistent titres of IgM were detected in the N'Dama using the ELISA, and sera from the N'Dama lysed a greater percentage of the parasite populations tested in the CLA compared to the Boran.

These results suggest that the N'Dama mounted superior antibody responses specific for the surface antigens of the VSG. It is generally accepted that antibodies specific for the conformational epitopes of the VSG are protective. Thus, the N'Dama may have controlled the levels of parasitaemia through their superior antibody responses. How this affects the N'Dama's ability to control the course of the disease remains to be determined.

TR3.14 Mouse L-cells transfected with the bovine interleukin 2-receptor chain

Scientists: P.G. Toye, N.B. Murphy, J. Naessens
Research Associate: N.D. MacHugh
Technician: J. Nyanjui

To facilitate the production of mouse monoclonal antibodies to the IL-2 receptor, we have transfected the gene encoding the bovine IL-2 receptor chain (the bovine Tac antigen, CD25) into mouse L fibroblasts ($H\text{-}2^K$).

A plasmid containing a cDNA copy of the gene (Weinberg *et al.*, 1988, Immunology, 63:603) was provided by Dr. R. Reeves (University of California, USA). A rabbit antiserum

to the bovine IL-2 receptor was a gift from Dr. Dirk Dobbelaere (Institut fur Genetik, Karlsruhe, Germany).

The cDNA copy of the gene was subcloned into the pCDM8 eukaryotic expression vector to allow transcription of the gene under the control of the cytomegalovirus long terminal repeat promoter. The resulting plasmid was transfected into COS-7 cells, in which transient expression of the receptor was detected with a specific rabbit antiserum. Stable transfectants were established by co-transfection of this plasmid and a plasmid containing the thymidine kinase gene into L(tk$^-$) cells. Positive transfectants were selected by incubation in HAT medium, followed by FACS analysis using the specific rabbit antiserum. Cloned cell lines were established by limiting dilution. The transfected cell line has provided strong evidence that monoclonal antibody CACT 116A (Dr. W. Davis, Washington State University, Washington, USA) is specific for the Tac antigen. FACS analysis has shown that the monoclonal antibody reacts with the transfected L-cells, but not the Ltk$^-$ parent cell line. Nevertheless, the transfected L-cell has been inoculated into C3H (H-2^k) mice from which we hope to produce monoclonal antibodies which react with the active site (inhibiting IL-2 binding) and other epitopes (not inhibiting IL-2 binding). Transfection of bovine genomic DNA into the transfectant expressing Tac, followed by selection with labelled IL-2, may allow us to isolate a transfectant expressing the high affinity (75/55) receptor. This transfectant would be used to generate monoclonal antibodies against the high affinity receptor.

TR3.15 Bovine leucocyte activation antigens

Scientist: J. Naessens
Technician: J. Nthale

Clustering of MAbs for activated leucocyte antigens, has been improved by biochemical analysis. In some experiments, we have included MAbs obtained from Dr. W.C. Davis, Washington State University, Pullman, Washington.

One antigen (gp 125) detected by a cluster of four MAbs has a MW of 125,000 under reducing and 185,000 under non-reducing conditions. Endoglycosidase F treatment reduced the MW by approximately 20,000, giving us a minimum estimate of 16% carbohydrate. The antigen had a precursor of MW 115,000 that matured into the higher MW form after about one hour. It appeared on the surface late after lectin-stimulation (at least 3 weeks) and was not found on transformed bovine cell lines. Sequential precipitations proved that two MAbs, one from ILRAD and one from Washington State University, detected the same antigen of MW 120,000, but which was different from the gp 125. It gradually appeared during the first week, but it was never strongly expressed. Its apparent MW remains unchanged before and after reduction and could be homologous to human CD26 or CD30. A third antigen was composed of a light chain of MW 125,000 (similar only in MW to the other two antigens) and a heavy chain of MW 165,000. Since it is also expressed on other cell types (granulocytes), its function is not directly linked to cell activation. MAbs were selected which appeared early (one day) after activation and which were present on activated T and B cells. One of these, CACT 116A, precipitated under reducing conditions a band of 56,000, similar in MW to the alpha chain of the IL-2 receptor in human and mouse (see also report TR3.14).

TR4.1 Development of a bovine trypanotolerance resource population

Scientists: A.J. Teale, S.J. Kemp, D. Kennedy

A resource population based on N'Dama (*Bos taurus*) and Boran (*Bos indicus*) founder animals, which is expected to segregate genetic resistance to trypanosomiasis, is being developed. Four trypanotolerant N'Dama bulls are being bred with trypanosusceptible Boran cows using embryo transfer technology in order to produce one family of 30 full-sibling crossbred F1 offspring from each bull. It is planned to eventually produce an F2 population, the construction of which will depend on the trypanotolerance/sensitivity status of the F1 animals.

The first F1 calves were born in January 1990. Two of the four families are now nearing completion. The other two are expected to be completed in 1991.

Beginning in January 1991, the first of the F1 animals will be challenged with *Trypanosoma congolense* IL1180 by tsetse fly bite, when they will be one-year old. Further animals will then be challenged on a monthly basis as they reach one year of age. Prior to and following challenge, animals will be monitored for PCV, serological responses to variant, invariant and irrelevant antigens, body weight change, total and differential leukocyte levels, complement activity and erythrophagocytosis. Following challenge, they will also be monitored for parasitaemia and antigenaemia.

DNA will be prepared for genotyping at polymorphic marker loci and serum and plasma will be stored against future needs. Three months post-challenge, or before as indicated by disease status, animals will be treated to eliminate infections. Selected individuals will be maintained to form the basis of the F2 population.

TR4.2 Studies of population genetics of N'Dama cattle

Scientists: R. Suzuki, T. Shotake*, S.J. Kemp, A.J. Teale

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This study is an attempt to identify markers capable of distinguishing trypanotolerant N'Dama cattle from relatively trypanosusceptible breeds. The genetic differences between N'Dama (*Bos taurus*), Boran and Maasai (*Bos indicus*) cattle were assessed by analysis of plasma proteins. Two hundred and forty-seven animals (192 N'Dama; 56 Boran; 99 Maasai cattle) were analysed at six loci by polyacrylamide gel electrophoresis. Five of these - albumin (ALB), transferrin (TF), group specific component (CG), post-transferrin-1 (Pot-1) and post transferrin-2 (Pot-2) - showed polymorphism. The prealbumin (PALB) locus appeared monomorphic. ALB, TF, GC and Pot-2 polymorphisms were effective in discriminating between N'Dama cattle and other breeds.

A previously undescribed allele, *Do*, was found at the TF locus. Its gene frequency was over 30% in N'Dama but was 0% and 0.5% in Boran and Maasai cattle, respectively. Also, the *As* allele, which was found in South Asia Zebu cattle but not in European cattle in a previous study, was detected in N'Dama, Boran and Maasai animals.

On the basis of these data, the genetic distances (Nei, 1975) between N'Dama and Boran and between N'Dama and Maasai cattle were 0.26 and 0.25, respectively. The Nei's genetic distance between N'Dama and Zebu breeds suggests that there are probably other protein polymorphisms distinguishing N'Dama from Boran and Maasai cattle.

We are studying other protein polymorphisms by starch gel electrophoresis. Investigation of protein polymorphism differences between trypanotolerant and trypanosusceptible breeds may have significance for studies of the mechanisms of, and genetic basis for trypanotolerance.

TR4.3 Bovine MHC class I gene expression and polymorphism on two haplotypes of Boran cattle

Senior Research Fellow: P.S. Gwakisa
Scientist: A.J. Teale

In an attempt to detect and characterise products of bovine class I MHC genes in addition to those detected by classical serology, monoclonal antibodies (MAb) were raised against products of KN8 and w25 haplotypes. Family and population studies, tissue distribution of epitopes and SDS-PAGE analysis provided evidence that the derived MAb are directed against MHC class I products. FACS analysis, sandwich ELISA, transfection of bovine MHC genes into mouse fibroblasts and Northern blot analysis were used to study the genetic control of the epitopes seen by the MAb. FACS analysis of binding inhibition with PBM suggested that 4 MAb raised against products of this haplotype, IL-A91, IL-A92, IL-A93 and IL-AIII, were detecting epitope(s) on one molecule. Cloned murine cell transfectants expressing a class I gene product, after transfection of total genomic DNA from a homozygous animal, were reactive for all the MAb. Northern blot analysis of the transfectants using locus-specific probes showed that the transfected gene codes for a BoLA-A molecule.

Two MAb, IL-A89 and IL-A94, generated against products of the w25 haplotype detected two distinct epitopes. Population studies revealed that Boran cattle carrying the w25 haplotype have epitopes for both MAb, although the epitopes occur independently on different haplotypes. BoLA serotyping of cattle of different breeds showed that reactivity of MAb IL-A94 correlates perfectly with that of an alloantiserum EBAIII, which clusters in the group of reagents defining the w25 BoLA-A class I specificity. Sandwich ELISA indicated broader reactivity of MAb IL-A94. When MAb IL-A94 was first coated to plastic, after addition of cell lysate, MAb IL-A89 and IL-A94 could bind as second biotinylated layer. Neither of the two MAb could bind when IL-A89 was used as first layer. Total genomic DNA from a w25 homozygous animal transfected into mouse L cells resulted in two clones, one positive for MAb IL-A94 only, the other positive for both MAbs, IL-A89 and IL-A94. One interpretation of these data is that at least two genes are expressed on the w25 haplotype, the product of one is able to bind both MAb, IL-A94 and IL-89, whereas the product of the other binds only MAb IL-A94. Another possibility is that the clone bearing an epitope for MAb IL-A94 alone is defective in expression of the IL-A89 determinant.

TR4.4 The bovine lymphocyte antigen typing service

Scientists: S.J. Kemp, A.J. Teale
Technician: J. Mwakaya

Animals and cell lines of defined MHC type are required by ILRAD scientists working in a number of areas. The BoLA-typing service is able to provide these and is also conducting research into the relationship between BoLA and performance under trypanosome challenge in Gabonese N'Dama cattle.

Approximately 400 animals or cell lines were BoLA-typed in 1990. The majority of these were from frozen lymphocyte samples from Gabonese N'Dama cattle. In addition 118 ILRAD cattle were typed for use by other ILRAD scientists and 108 cattle from other institutions were typed as part of collaborative studies. Thirty-four *Theileria*-infected cell lines were typed for ILRAD scientists as a means of confirming their identity.

ILRAD also participated in the 4th BoLA comparison test in which blood samples were shipped from the University of Illinois to a number of participating laboratories around the world. This provides a check on the accuracy of BoLA assignments. The final analysis of this exercise is still awaited, but it is already clear that ILRAD's ability to define the products of the BoLA-system of European cattle compares well with that of other laboratories ILRAD's ability to define the MHC of African cattle remains unrivalled.

TR4.5 Polymorphic microsatellites as components of a bovine gene map

Scientists: S.J. Kemp, A.J. Teale
Technician: J. Mwakaya

It is intended to evolve a bovine gene map for use in further understanding the phenomenon of heritable trypanotolerance. This requires a large number of evenly spread, polymorphic genetic markers.

Recently a class of simple two or three nucleotide repeats which occur with a high frequency throughout the genome of mammals has been described. These regions are highly polymorphic with respect to the number of repeats they contain and are thus a potentially valuable source of markers in the construction of a linkage map. These regions have been termed *microsatellites*.

The exploitation of microsatellites has been made possible by use of the polymerase chain reaction (PCR). Synthetic oligonucleotides are prepared which uniquely hybridise with regions flanking a microsatellite. These serve as PCR primers to produce many labelled copies of the microsatellite region. The length polymorphism of these is revealed by reducing polyacrylamide gel electrophoresis.

We have begun exploitation of microsatellites by studying such regions in three published sequences - those occurring in the bovine genes BOVIRBP, BOVP45C21 and BOVFSHB. Oligonucleotide primers were selected to act as PCR primers, using a simple purpose-made computer program and PCR conditions were optimised for each primer pair.

All three microsatellites were found to be highly polymorphic in length and Mendelian inheritance was demonstrated in all cases. One of these genes (BOVP45C21) encodes bovine steroid 21-hydroxylase and is known to be linked to the MHC on bovinechromosome 23. We have observed the co-inheritance of the BOVP45C21 microsatellite and MHC type in a family of N'Dama embryo-transfer calves. This provides a potentially valuable tool for studies of the MHC.

There seems no doubt that PCR-based typing of polymorphic microsatellites is capable of providing the distribution of markers needed for a bovine gene map. They are present at a high density, are highly polymorphic and are readily exploited. Furthermore, exchange of markers between laboratories is possible merely by the exchange of two short primer sequences.

TR4.6 *Trypanosoma congolense* Infection in Boran cattle: histopathology and immunocytochemistry

Scientists: L. Logan-Henfrey, J. Naessens
Student: V. Taiwo
Research Associate: N.D. MacHugh
Technicians: F. McOdimba, J. Kamau

Immunocytochemistry has proved to be a valuable tool in studies of disease processes in human medicine. Similarly, immunocytochemistry in conjunction with bovine specific monoclonal antibodies (MAbs) has greatly improved our understanding of the distribution of cells in normal bovine lymphoid organs. With the increasing availability of bovine cell type-specific monoclonal antibodies, studies of the immunopathology of bovine diseases are now possible.

The purpose of this study is to examine temporal changes in tissues collected from cattle infected with *T. congolense*. Eight six-month old Boran calves were infected with *T. congolense* (IL-1180) by allowing 5 infected *Glossina morsitans* to feed on the left flank of each animal. One infected calf was killed weekly, 7-49 days postinfection (dpi); one calf was treated with Berenil® 43 dpi and killed on 56 dpi. Two Boran calves served as controls. A post-mortem was performed on each animal and tissue samples collected in formalin and OCT embedding medium. A panel of bovine specific MAbs was identified which had proved useful in identifying cell populations with a fluorescence activated cell sorter (FACS). Twenty eight monoclonal antibodies were screened by immunoperoxidase staining of normal bovine lymph nodes and spleens. Optimal dilutions of the MAbs were selected and distributions of positive cells were noted. A panel of sixteen MAbs were chosen which marked the following cell types: 1. T cells: CD2, CD4, CD8; 2. null cells: γ/δ leucocytes; 3. B cells; 4. monocyte/macrophage/antigen presenting cells; and 5. lymphocyte activation markers: IL 2 receptor and $CD5^+$ cells. The sequence of events in the left prefemoral lymph node (node draining the tsetse fly bite site) and the spleen are being examined by histological and immunoperoxidase staining.

TR4.7 Bone marrow cell cultures

Scientist: J. Naessens
Technician: P. Muiya

To study possible influences of trypanosomes on haemopoietic cell differentiation, we need assays to measure the number of different cell types in their different maturational stages and their dependence on various growth factors.

Erythroid and myeloid progenitor cells were measured in a short-term culture system in methylcellulose. The frequency of the precursor cells that developed into colonies depended on the cell density in the culture plate. Several cell dilutions were used to establish the optimum cell density and the maximum number of precursor cells.

Using two-colour immunofluorescence (?lineage$^-$, MAb VC15^{+++}) and a window for high forward and low side scatter, we could enrich for progenitor cells. Populations of 70-80% progenitors were obtained, most of which were erythroid progenitors (90%). These purified cells were then used to produce monoclonal antibodies, which were screened on bone marrow cells for a small positive subpopulation. Several MAbs were selected and the cells they detect will be determined.

Stem cells can be indirectly measured in a long-term bone marrow culture system. On a preformed stromal layer, bone marrow cells were cultured with horse serum and the number of progenitors in the non-adherent fraction measured at intervals in the short-term culture. The erythroid progenitors disappeared fast, but myeloid progenitors were present until week fourteen. We do not know whether the myeloid progenitors were derived from stem cells in the culture or survived in the culture from its onset.

We looked into the possibility of measuring bovine colony forming units in spleens from SCID/beige mice. In a preliminary experiment, six mice were irradiated with 900 rad and repopulated with different doses of bovine bone marrow cells. Unfortunately, all mice died on day six, either from the irradiation or disease. Further tests will be carried out.

TR4.8 Absence of diurnal rhythm of cortisol in Boran (*Bos indicus*) cattle

Student: G. Abebe
Supervisors: R. Eley*, O.K. ole-MoiYoi

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Circadian patterns of cortisol secretion have been identified in a variety of mammals with the highest level during morning (03.00-10.00h) and lowest in early evening (15.00-19.00h) for those which are active in the day time. The reverse occurs in nocturnal animals, with the peak coinciding with onset of darkness.

Studies pertaining to diurnal rhythm in cortisol concentrations in cattle are scanty and somewhat conflicting. As there is no information available on Boran (*Bos indicus*) cattle, an experiment has been performed on eight Boran calves (four female and four male), age 8-9 months, using the standard procedure for diurnal rhythm study. In brief, the animals were

kept in strict photoperiodic condition (12 hours light and 12 hours dark), ten days prior to the experiment. Blood samples were collected at hourly intervals throughout the 24 hr cycle via jugular indwelling cannulae. Plasma cortisol concentration were measured by radioimmunoassay kits.

Results indicated no apparent diurnal rhythm in cortisol. The lack of diurnal rhythm of cortisol in Boran cattle can be possibly explained by the dependence of cortisol on a sleep-wake schedule. Generally, ruminants do not enter the deep state of sleep, a characteristic of man and other domestic animals. Sleep periods in ruminants are linked directly to the digestive need of the animals since rumination requires both time and consciousness.

4.9 Effect of age on function of the pituitary-adrenal axis of Boran (*Bos indicus*) cattle

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Puberty in *Bos indicus* cattle in the tropics under conditions of good management is reported to begin of 12-15 months. In order to assess the effect that this physiological event could have on the ongoing studies, an experiment was conducted on five female Boran (*Bos indicus*) cattle to assess the effect of age on the pituitary-adrenal axis. These animals were challenged with ACTH and CRF one week apart on three occasions, i.e., at 11, 14 and 17 months of age. Ten venous samples (two before and eight after challenge) were collected from each animal for ACTH cortisol measurements.

Results indicated that there was no apparent age effect on pituitary-adrenal axis response to CRF challenges. At different times of CRF challenge, all animals responded in a similar way as measured by total ACTH and pattern of release. However, ACTH challenge at 17 months of age caused a higher response than at 11 and 14 months, suggesting an age-related increase in adrenal response. Several investigators have proposed that age-related increases in plasma corticosteroid levels contribute to many phenomena associated with biological ageing, though, 17 months of age in cattle is not considered a time of biological ageing, but it is an important phase leading to puberty.

EPIDEMIOLOGY AND SOCIOECONOMICS

ESE1 The definition and characterisation of the impact of livestock disease and its control

Scientists: B.D. Perry, A.W. Mukhebi, J.M Delehanty, J.J Curry
Research Associate: R. Kruska
Technicians: R. Kisiara, E. Mussukuya

This programme, although directed towards the impact of livestock diseases and their control in general, continues to focus on tick-borne diseases in the countries of eastern and southern Africa in which theileriosis occurs. The control of tick-borne diseases is mainly carried out by the use of acaricides to kill the vector ticks, but this method is becoming increasingly unreliable due to many factors, including the rising cost of acaricides (generally in scarce foreign exchange), the development of resistance to acaricides, and the constraints on veterinary services in the maintenance of effective acaricide delivery by dips and sprays. The emergence of effective procedures for the immunisation of cattle against East Coast fever (ECF) using broadly immunogenic stocks of *Theileria parva* in the infection-and-treatment method, and the prospect of genetically engineered vaccines for this and other tick-borne diseases in the near future, means that more effective tools for tick-borne disease control will soon be at the disposal of veterinary departments, veterinarians and farmers.

The principal justification for tick-borne disease control is the adverse effect of these diseases on livestock productivity. Thus, in situations of endemic stability, in which most cattle acquire immunity to tick-borne disease through exposure under circumstances of reduced susceptibility (for example when young, and when in less susceptible Zebu [*Bos indicus*] breeds), and there is little or no effect on production, minimal justification for control exists. Where endemic stability does not exist as the result of low or intermittent tick challenge, where control of ticks by acaricides or pasture management is ineffective, or where exotic (*Bos taurus*) cattle are kept, control may be strongly justified.

Circumstances vary considerably in Africa and the differences in livestock types, livestock production systems, disease epidemiology and disease control practices provide a complex matrix of disease risk to farmers. The criteria for identifying target populations for tick-borne disease control by immunisation will therefore not only be disease prevalence, but also the current and potential role of livestock in socioeconomic terms. With the growing trend for partial and total cost recovery of veterinary services, and their privatisation in some areas, it is important that the socioeconomic impact of diseases on different livestock production systems is assessed as part of the identification of target populations for immunisation. This will ensure that disease control programmes are compatible with farmers priorities and objectives, and available resources, and thus sustainable in the long term.

The research activities of the programme have five main areas of focus.

1.Consideration of the macro socioeconomic environment in which livestock occurs in the region in which *Theileria parva* exists (ESE2). During 1990, a methodology and model for assessing the national and region cost of theileriosis control has been developed. In addition, further detailed assessments of the cost of current tick-borne control in Kenya and Zimbabwe will be undertaken.

2.The assessment of the impact of East Coast fever control at the farm level in two contrasting areas of Kenya (ESE3). During 1990, further definition of target populations for

immunisation has been made in both districts based on disease prevalence (ESE3), farm enterprise economics (ESE4), livestock production system characterisation (ESE5) and farmers' understanding of disease and its control (ESE6).

3.The development of national and continental computerised geographic databases and GIS operating environments to study the epidemiology, economics and control of tick-borne diseases of cattle in Africa (ESE7).

4.The assessment of the impact of current and alternative tick and tick-borne diseases control strategies at a national level in Zimbabwe (ESE8).

5.The modelling and projection of disease impact under differing characterised circumstances in the region (ESE9).

This covers the areas of:
a) Geographical models
b) Economic models
c) Disease models

ESE2 Macro socioeconomic statistics of countries affected by East Coast fever

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Research Associate: R. Kruska

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Assessment of the economic impact of improved theileriosis control at national and regional levels requires country level in addition to farm/production system level socioeconomic data. These data are also important for assessing the role of livestock in national economies and the economic importance or cost of ECF.

ECF affects eleven countries in eastern, central and southern Africa. These countries had an estimated human population of 175 million (29% of Africa) in 1988. The land area in which ECF occurs and cattle are reared is estimated to be 156 million ha (19% of the total). However, this varies widely from less than 1% in Sudan to 22% in Kenya, 58% in Zimbabwe and virtually 100% in Burundi. The affected countries had an estimated cattle population of 63 million in 1988 (35% of Africa's total), of which 24 million (38%), or 13% of Africa's total, were in ECF-affected areas. In ten countries (excluding Mozambique), livestock contributed 21% of agricultural gross domestic product (GDP) of US$16 billion, or 8% of the national GDP amounting to US$42 billion in 1986. This contribution varied substantially from about 5% of agricultural GDP in Burundi, Rwanda and Malawi to 31% in Tanzania and Zambia and to 35% and 39% in Zimbabwe and Kenya, respectively.

Compiled macrostatistics were used to make a preliminary estimate of the direct regional cost or economic importance of ECF in the affected countries in 1989 using a computer spreadsheet model. The model is based upon several parameters and assumptions regarding the size and general structure of the herds affected by ECF, the impact of the disease on cattle production (beef, milk, traction and manure), farm gate and shadow

prices for valuing product losses, and treatment, control, research and extension costs due to the disease.

The regional cost of ECF was estimated to be US$168 million in 1989, and the disease caused an estimated mortality of 1.1 million cattle. This loss amounted to US$7.02 per head of cattle or US$1.08 per ha in ECF-affected areas. Milk loss accounted for 47% of the total, 90% of which was due to morbidity and 10% due to mortality loss. Beef loss constituted 12%, loss of animal traction was 13% and acaricide application costs were 20% of the total cost. The cost of manure loss, treatment, research and extension accounted for 8% of the total cost.

Ex-ante economic analysis of immunisation against ECF at a cost of US$2.50 per animal targeted at all calves and 5% of adults introduced into the affected areas showed high economic returns. The regional cost of the disease was reduced by 26, 31 and 36% at a 0, 25 and 50% reduction in acaricide application, and the benefit/cost ratio was 9, 12 and 17, respectively. A sensitivity analysis of immunisation cost indicated that immunisation would remain economically viable up to a cost of US$4.75, 6.25 and 7.75 per animal with 0, 25 and 50% reduction in acaricide use respectively.

ESE3 The impact of theileriosis control in Kilifi and Uasin Gishu districts of Kenya

Scientists: B.D Perry, A.W. Mukhebi, J.M. Delehanty, A.S. Young*, W. Thorpe*, S. Maloo*, S. Mining*, A.S. Maritim*
Research Associate: R. Kruska
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In a collaborative research programme in Kaloleni Division of Kilifi District, Coast Province, three institutes (KARI, ILRAD and ILCA) are pooling their expertise and resources to meet different but closely interacting objectives.

The common denominators of these institutional objectives have been to assess and identify target populations for ECF immunisation, and gather baseline data to allow subsequent evaluation of the efficacy, impact and appropriateness of its delivery. In order to identify target populations for immunisation, a structured and step-wise procedure has been followed:

(a)Testing the efficacy of the infection-and-treatment method under field conditions at the coast.

A series of studies have been done by KARI on government and parastatal farms which have shown high levels of efficacy as measured by the development of serum antibodies and the reduction in disease incidence. There have generally been low numbers of clinical reactors in the studies (1-4%).

(b)Estimating livestock population size and distribution.

A livestock census has been carried out in the division by the ILCA/KARI project. There were a total of 57,516 cattle in 1,552 herds. Of these, 889 (1.5%) were classified as grade cattle (defined as those showing some evidence of genetic improvement). A detailed breakdown of cattle numbers by type, age, location, herd size and agroecological zone is available.

(c)Assessing and quantifying the role of livestock in agriculture and society.

In a stratified sample of 77 farms, a farm enterprise study of inputs, outputs, gross margins and productivity by land, labour, capital, etc., was carried out. Although this clearly demonstrates the returns from grade dairy cattle kept under zero-grazing management in the high rainfall area, it also highlights the important contribution to household income made by Zebu cattle in the drier (CL5) zone. This will be reported in more detail (ESE4).

(d)Assessing the prevalence of *Theileria parva* infection.

A cross-sectional study of the prevalence of antibodies to *T. parva* was carried out in three age strata of the two major cattle types in the three management systems (zero-grazing, semi-zero grazing and free range). This demonstrated the widespread prevalence of *T. parva* infection across all strata, suggesting a high level of population immunity, which, in the face of continued reports of ECF, suggests that only those animals which have survived ECF infection remain. The high prevalence of antibodies in both grade and Zebu cattle in animals of all age groups, including those under 1.5 years of age, suggests that infection, and thus mortality, occurs early in life.

(e)Assessing the age at which infection with *T. parva* occurs (by season) and the case-fatality rate in susceptible calves.

A prospective study is currently under way to determine the maximum age at which immunisation should be carried out in order to avoid mortality, and what is the case-fatality following infection.

A further study to relate the presence of circulating antibodies to *T. parva* schizont antigen in the division to susceptibility to the Marikebuni stock of *T. parva* by challenge will be conducted shortly.

In Uasin Gishu District, activities are at an earlier stage. Two studies have been carried out on stratified samples of Land Registration Units in the district to characterise livestock production and management. The results of these studies were used to create a sampling frame of farms with cattle to select herds for serological studies of *Theileria parva*, *Babesia bigemina* and *Anaplasma marginale*. This sampling is currently in progress.

ESE4. Farm-level financial and economic effects of current and alternative East Coast fever control strategies in Kaloleni Division of Kilifi District and in Uasin Gishu District of Kenya

Scientists: A.W. Mukhebi, B.D. Perry, J.M. Delehanty
Collaborating Scientists: W. Thorpe*, E. Mussukuya

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The broad objectives of this study are to quantify the baseline farm household economies as a base for financial and economic assessment of alternative East Coast fever (ECF) control strategies particularly immunisation and to determine the role of livestock in the household economies. Specific objectives include quantification of farm resource base (land, labour and capital), farm enterprise (crop and livestock) income, and the level and composition of household (farm and off-farm) income. Farm-level information generated from this study will serve as inputs into economic models for assessing the financial and economic impacts of alternative ECF control strategies under various farm level production circumstances.

Farm-level data were collected through a farm survey of a sample of 77 farms in Kaloleni Division of which 58 farms had cattle and 19 had no cattle. There were wide variations among zones, cattle types, grazing systems and cattle ownership. Mean farm size was 8.14 ha (± 6.91) owned in 2.71 parcels (± 1.45). Mean household size was 12 persons (± 8) which supplied 1200 man-days of labour (± 445) per year. Mean farm capital investment (value of machinery, equipment, tools, facilities, stores, tree-crops and livestock) was KShs. 98,000 (±80,000) or US$4261 (±3522). On average, farms with livestock owned 11.95 cattle (± 8) and 10 sheep and goats (± 9).

The cattle enterprise generated a mean annual gross margin (profit) of KShs.8,000 (± 7,000) or US$348 (± 304) per herd. In grade cattle herds, milk accounted for 72% of the cattle output, animal disposal 27%, and animal traction, manure and hides 1%. In contrast, in Zebu cattle herds, milk accounted for 29% of the output, animal disposal 69%, and animal traction, manure and hides 2%. While milk is the dominant output in grade cattle herds, animal output dominates in Zebu cattle herds.

Mean total farm-generated (crop plus livestock) income per farm was KShs.22,500 (± 17,000) or US$978 (± 739), while mean total household (farm plus off-farm) income per farm was KShs.75,000 (± 66,500) or US$3,261 (± 2,891). Of the farm generated income, crops contributed 58% and livestock 42%; and of the total household income, farm income contributed 30% and off-farm income (employment and business) 70%. The mean per capita (per person) income was KShs.7,000 (± 4,500) or US$304 (± 196).

The role of livestock in the household economy is highlighted by the contribution of livestock to farm investment and farm and household income. Livestock contributed 49% of farm investment (range 21-61%), 42% of farm-generated income (range 15-65%) and 13% of the total household income (range 9-26%). There was a wide variation in the contribution of livestock among agroecological zones, cattle herd types, grazing systems and between farms with and without cattle. In general, the contribution of livestock to investment and income tended to be higher in lower (drier) than in higher (wetter) rainfall zones, Zebu than in mixed or cross-bred cattle farms, lesser (free-range) than in more intensive (zero) grazing systems, and farms with than in those without cattle.

Mean farm resource productivity (profit per unit of resource) was KShs.5620 (± 4,500) or US$244 (± 196) per ha of land, KShs.96 (± 88) or US$4.17 (± 3.83) per man-day of family labour, KShs.1682 (± 1546) or US$73 (± 67) per tropical livestock unit, and KShs.19 (± 24) or US$0.83 (± 1.04) per KSh of variable costs. In general, resource productivity was higher in the higher than lower rainfall zones, in grade than Zebu cattle farms, in zero than free-range grazing systems and on farms with than those without cattle. The differences were generally statistically significant ($p \geq 0.05$) between the extreme rainfall zones (700 mm and 1200 mm per month, between the extreme cattle types (cross-breeds and Zebu herds) and between those farms with and without cattle.

A similar methodology will be followed and similar farm-level data will be collected in Uasin Gishu District to provide a contrast to the results obtained from Kaloleni Division farming systems.

ESE5 Socioeconomic and environmental characterisation in Uasin Gishu District, Kenya

Scientists: J.M. Delehanty, A.W. Mukhebi. B.D. Perry, J.J. Curry
Research Collaborator: R.O. Kisiara

In preparation for field trials of theileriosis immunisation by infection and treatment in Uasin Gishu District, a project is under way to describe farming systems there in order to portray both spatially and across the socioeconomic spectrum the variability in cattle management conditions and strategies likely to impinge on theileriosis risk and its effective control.

Phase I, completed in 1989, involved the collection and analysis of general farm data specific to each of Uasin Gishu District's 640 Land Registration (LR) units. These date were gathered from key informants in the district, and entered in a computerised database and in a GIS program (ArcInfo). Using this database, the 640 LR units were stratified according to four key variables: (a) agroecological zone, (b) farm size, (c) land tenure and (d) cattle types reported. Weighted representation of major strata in the resulting matrix led to the selection of a random sample of 53 LR units for further (Phase II) research.

Phase II data, collected in 1990, have given program scientists the capacity to produce detailed descriptions of farming system and livestock production variability in Uasin Gishu District. Factor-specific tabulations are available across a great array of variables. For example, of the LR units featuring very small farms (their locations are known), 75% contain pure Zebu cattle and 60% report that cattle are occasionally or regularly herded off the farm. By contrast, of the LR units featuring very large farms (greater than 100 acres), only 14 percent contain Zebu cattle, and only 5% report off-farm herding. Considerations such as these will be key in the strategic implementation of improved tick-borne disease control programmes.

These data have also led to a provisional typology of farming/livestock production systems in Uasin Gishu District. ECF control strategies may be tailored to meet the characteristics and apparent needs of these system types. The typology disaggregates LR units according to environmental zone and modal farm size, and the salient features of each type have been summarised.

ESE6 Delivery, adoption and sustainability of animal health interventions on the farm: Ethnoveterinary studies in Kilifi District, Kenya

Scientists: J.M. Delehanty, B.D. Perry, A.W. Mukhebi
Research Collaborators: M.S. Baya, R. Kisiara

Sustainable development implies adjustment to preexisting farm practice. Before a new animal disease control programme is introduced in the field, it is important to learn what farmers in the area already know or believe about diseases, particularly the ones targeted. In preparation for field trials of the infection-and-treatment method of theileriosis control in Kaloleni Division, an ethnoveterinary survey was administered to gauge the depth of local livestock disease knowledge and aid in the implementation of this and subsequent disease control programmes.

Interviews were conducted with 158 cattle-keepers. Each informant provided: (1) baseline information on his livestock operation, (2) a list of all cattle diseases or health problems (i.e., *syndromes*) familiar to him, and (3) detailed information on several of the reported syndromes. The syndrome-specific information included: type, age and sex of cattle affected; seasonality and other conditions; the progression of clinical signs; effects on production; perceived cause; preventive strategies; traditional and modern veterinary treatments and their perceived efficacy; and details of the farmer's most recent case. The quantification and statistical analysis of these data represents a potentially significant advance over methods commonly employed.

The 158 farmers volunteered a total of 515 identifiable syndromes, naturally with considerable duplication. Discrete syndrome *names* totalled 144. A disease lexicon was produced, listing and indexing local disease terms. The lexicon revealed considerable malleability and overlap in local disease classification. This classificatory ambiguity suggests that any campaign directed at controlling a given disease should be identified to the public as a campaign aimed at the entire range of locally defined syndromes likely to correspond with the disease in question. As far as possible, a campaign should elucidate the clinical signs of the disease in question, since some farmers will not have precise definitions of some diseases beyond their most obvious clinical manifestations.

Four major theileriosis descriptors were identified, the most common of which is used restrictively to describe acute theileriosis in calves. The study found marked differences in the ethnoetiologies of the various theileriosis-related syndromes and, in general, poor understanding of the role of ticks in theileriosis transmission. Bridging the various theileriosis cognates - pointing out their etiological relatedness - should be an essential first step in any campaign to implement a new theileriosis control programme in Kaloleni.

ESE7 The development of geographic information system databases for studying the epidemiology, economies and control of tick-borne diseases of cattle

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establishment of geographic information systems (GIS) within ESEP has resulted in the development of several spatial databases relevant to the study of the epidemiology, economics and control of ticks and tick-borne diseases of cattle.

The majority of the GIS database development has been conducted on IBM PS/2 386 microcomputers. GIS software currently installed on the system include pcARC/INFO (a vector-based GIS) and IDRISI (a grid-cell based GIS/image processing system). A newer acquisition within the programme is a Sun Sparcstation 1 graphics workstation, which will become the preferred GIS operating environment. The Sun supports GRASS, a grid-cell based GIS and image processing system. Conversions between pcARC/INFO, IDRISI and GRASS formats is currently being evaluated.

Collaboration with UNEP's Global Resource Information Database (GRID) continues. GRID continues to provide data entry (digitising) support for database development. GRID's Microvax III minicomputer also provides the necessary environment for data- and processing-intensive models such as the climate matching programme CLIMEX. Continental climatic databases have been utilised in the CLIMEX model for the tick species *R. appendiculatus*, *R. zambeziensis* and *A. variegatum*.

Collaboration with the Zimbabwe Deptartment of Veterinary Services to study alternative tick and tick-borne disease control strategies has involved the development of an extensive GIS database. This database, as well as IDRISI GIS, have been installed on an IBM PS/2 within the department in Zimbabwe.

The socioeconomic and environmental characterisation study of Uasin Gishu District, Kenya, has utilised the GIS to describe the variation of farming systems in a spatial context and how this might affect theileriosis risk and control efforts. The GIS has also been useful in identifying the areas most suitable for serological studies.

A spatial database is also being developed for Kaloleni Division, of Kilifi District, Kenya, to incorporate the baseline studies being conducted there on animal disease, productivity and socioeconomic conditions. Inclusion of this data in the GIS should help sort out the complexity of the socioeconomic and epidemiological factors in the division.

GIS database development will continue at the continental, country and district levels. Thus far, inclusion of remote sensing data has been limited; the Sun Workstation should provide an adequate processing environment for image processing and will be a valuable tool in assessing tick habitat.

ESE8 Assessment of the impact of current and alternative tick and tick-borne disease control strategies at a national level in Zimbabwe

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Research Associate: R. Kruska

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Regular compulsory dipping has been in force in Zimbabwe since 1914, initially introduced to control East Coast fever. Following its eradication in 1954, compulsory dipping continued, directed against theileriosis (January disease and Corridor disease), babesiosis, anaplasmosis, heartwater and the effect of ticks *per se*.

Following a breakdown in dipping services in the 1970s during the independence war, heavy losses from tick-borne diseases were experienced. Since independence, there has been a steady resumption of compulsory dipping services in the Communal Lands, which are now as intensive in many parts of the country as they have ever been. This has been augmented by the steady replacement of Delnav® as the main acaricide by Amitraz®, which has considerable residual activity. The financial and administrative responsibility for tick-borne disease control in the Communal Lands is borne entirely by the government, at an estimated cost of Z$18,489,000 for the financial year 1988/89. In a report to the Director of Veterinary Services, Zimbabwe, it was proposed that alternative tick-borne disease control strategies, which make less intensive use of expensive acaricides and rely more on controlled immunisation and the development of natural immunity to tick-borne diseases, a policy which is potentially more sustainable in the long-term, should be considered. Two alternative strategies are proposed:

a)Reduced dipping, involving fortnightly dipping during the summer months and monthly dipping for the rest of the year (equivalent to 21 acaricide immersions annually).

b)A combination of strategic (weekly dipping during the summer months, equivalent to 12 acaricide immersions, supplemented by natural or artificially induced herd immunity to tick-borne diseases) and minimal (equivalent to 4 acaricide immersions) dipping. The choice of strategic or minimal, and the selection of diseases for immunisation, varies by region in Zimbabwe, and a provisional division of the Communal Lands into four zones appropriate for different tick-borne disease control regimes was developed.

For each control strategy, target cattle populations have been identified by zone, and projections of likely consequences made. It was assumed that there would be no significant changes in disease risk or the effect on livestock production by moving from current to either of the alternative control strategies. It was also assumed that strategic and minimal dipping would be applied after a two year transitional period of reduced dipping.

Resources (acaricides, personnel costs, facilities and overheads) expended under each strategy were costed and projected over a twenty year period. Both the reduced and the strategic minimal dipping strategies were found to be more cost-effective than the current practice. They represent a total present value cost-saving of Z$15-20 million at a 10% discount rate. They would potentially reduce the cost of tick and tick-borne disease control in present value terms by 46%, from Z$2.50 to Z$1.35 per animal per year for reduced dipping, and by 68%, from Z$2.50 to Z$0.81 per animal per year for strategic and

minimal dipping (at a 10% discount rate). The technical, epidemiological, production, policy and land-use implications of the alternative strategies have been considered in the report to the DVS. Finally, six broad recommendations were made as to how further study may be carried out.

ESE9 The modelling and projection of disease impact under differing circumstances in Africa

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There are three areas of activity in this project: geographical models, economic models and disease models.

a)Geographical models

In order to further define the parameters controlling the distribution and abundance of the tick vectors of theileriosis, three new modelling approaches have been made. In addition, the model CLIMEX has been applied to the previously developed interpolated climate database to examine the potential continental distribution of tick *Amblyomma variegatum*, vector of heartwater.

i)An attempt has been made to further estimate the model CLIMEX as a predictor of *R. appendiculatus* distribution in southern Africa using a high resolution (approximately 8 km^2) interpolated climate database acquired from CSIRO, Australia. It has previously been shown that due to the strictly seasonal activity of *R. appendiculatus* in southern Africa, and the extended period of unsuitability in the cool dry winter months, annual E1 values do not reflect tick abundance and may not reflect true or potential distribution. The model has been applied to the new database during different seasons and compared with geographical databases of tick presence and disease outbreaks over the period 1979-89.

ii)It has been observed that *R. appendiculatus* is absent from large areas of Communal Lands in Zimbabwe which are climatically suitable for the tick, due to severe overgrazing and thus lack of suitable habitat. A study was carried out to explore the role of satellite derived normalised difference vegetation index (NDVI) global area coverage (GAC) data in predicting habitat suitability for the tick. The first step was to calibrate NDVI on the basis of the six natural regions of Zimbabwe. NDVI GAC was acquired for all of 1989 and composited into mean monthly maximum coverages. Representative Communal Lands were chosen with the aid of Landsat MSS imagery and analysed on the basis of the six natural regions of Zimbabwe.

The 1989 NDVI data have been very useful in defining seasonal vegetation differences between Communal Lands in different natural regions. Comparison of NDVI values between commercial and communal grazing areas has also been valuable in detecting differences which may be attributable to grazing intensity. Calibration of NDVI values within different zones on the basis of *R. appendiculatus* presence will be an important next step to aid decisions on future tick and tick-borne disease control strategies.

iii)In a further attempt to model current and predicted ranges of vector tick species, the model BIOCLIM is being evaluated for the ticks *R. appendiculatus* and *R. zambeziensis*. A selection of georeferenced points throughout the distributions of these ticks was taken where ticks had been collected and accompanying altitude data recorded. For each point, BIOCLIM generates 24 primary climatic attributes and from these are derived a set of indices that summarise annual and seasonal mean conditions, extreme values and intra year seasonality. Selected thresholds and limits of each of the indices are matched across a geographic grid to predict potential distribution.

Early results indicate a good overall fit of potential distribution of both ticks, with considerable local variation. Problems of fit are seen with *R. appendiculatus*, particularly in highland eastern Africa. These may be a function of the considerable altitude variation in eastern Africa and the small number (16) of index points, errors in altitude data accompanying tick records and the poor quality climatic data. Interestingly, the large areas of potential suitability in western Africa predicted by CLIMEX are not predicted by BIOCLIM, although the suitability of highland Ethiopia is predicted by both models.

iv)The climatic suitability for *Amblyomma variegatum* has been evaluated by running CLIMEX on the interpolated databases previously developed. This shows a very close correlation with published distributions of *A. variegatum* and *A. hebraeum*; evaluations of the correlation with abundance are under way.

b)Economic models

A memorandum of understanding was signed between ILRAD and Texas Agricultural Experiment Station (TAES) in June 1990 for a collaborative research project to develop a farm-level simulation model for ex-ante economic analysis and prediction of the likely impacts of improved control of livestock diseases.

A spreadsheet model (TELEM) has been developed by ESEP for estimating the economic importance of theileriosis and the productivity of cattle under existing circumstances. Initial results of its application are reported in ESE2.

c)Disease models

In collaboration with Imperial College, University of London, two models of endemic theileriosis have been developed. The first is a simple model of a cohort of susceptible individuals constructed to estimate the rate of infection (which could be age or exposure dependent). The second is a full transmission model constructed to investigate the dynamic consequences of manipulating infection by immunisation and breed changes. This model was also used to study the effect of a carrier state on disease dynamics and its interaction with a seasonally changing rate of infection.

PUBLICATIONS

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Publications 1989 not included in *Annual Scientific Report* 1989

Aboagye-Kwarteng, T., ole MoiYoi, O.K. and Lonsdale-Eccles, J.D. (1989). Protein phosphorylation in *Trypanosoma brucei*. In: Lonsdale-Eccles, J.D. and Lenahan, J.K., eds. *Protein Traffic in Parasites and Mammalian Cells: Proceedings of a Workshop, Nairobi, Kenya, 29 August to 1 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 1-6 (863).

Bensaid, A., Kaushal, A., Young, J.R., Kemp, S.J. and Teale, A.J. (1989). Organisation genomique du complex majeur d'histocompatibilité du bovin. *Annals de Recherche Vétérinaire 20: 389-394 (1989).*

Black, S.J. and Vanderweerd, V. (1989). Trypanosomiasis: host susceptibility and *Trypanosoma brucei* lipid uptake. In: Lonsdale-Eccles, J.D. and Lenahan, J.K., eds. *Protein Traffic in Parasites and Mammalian cells: Proceedings of a Workshop, Nairobi, Kenya, 29 August to 1 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 30-34 (864).

Brown, W.C. and Grab, D.J. (1989). Lymphokine production by bovine T cells infected with *Theileria parva*: implications for the pathogenesis of East Coast fever. In: *Proceedings of the Eighth Veterinary Hemoparasite Disease Conference, St. Louis, Missouri, 10-12 April 1989.* Washington, D.C.: United States Agency for International Development, pp. 107-120 (897).

Bull, R.W., Lewin, H.A., Wu, M.C., Peterbaugh, K., Antczak, D., Bernoco, D. Cwik, S., Dam, L., Davies, C., Dawkins, R.L., Dufty, J.H., Gerlach, J., Hines, H.C., Lazary,S., Leibold, W., Leveziel, H., Lie, Q., Lindberg, P.G., Meggiolaro, D., Meyer, E., Oliver, R., Ross, M., Simon, M., Spooner, R.L., Stear, M.J., Teale, A.J. and Templeton, J.W. (1989). Joint Report of the Third International Bovine Lymphocyte Antigen (Bola) Workshop, Helsinki, Finland, 27 July 1986. *Animal Genetics* 20: 109-132 (822).

Clarkson, A.B., Biennen, E.J., Poltakins, G. and Grady, R.W. (1989). Trypanocidal CoQ analogues: their effect on other mitochondrial systems. *Comparative Biochemistry and Physiology* 94B: 245-251 (839).

Conrad, P.A., Brown, W.C., Baldwin, W.C., Iams, K., Dolan, T.T., Sohanpal, B., Stagg, D.A., Young, A.S., Grootenhuis, J.G., Leitch, B.L. and ole-MoiYoi, O.K. (1989). Application of DNA probes in the characterization of *Theileria* parasites from cattle and African buffalo. In: *Proceedings of the Eighth National Veterinary Hemoparasite Disease Conference, St. Louis, Missouri, 10-12 April 1989.* Washington, D.C.: United States Agency for International Development, p. 409 (899).

Conrad, P.A., Chen, P. and Dolan, T.T. (1989). Detection of *Theileria para* in the salivary glands of infected *Rhipicephalus appendiculatus* ticks with DNA probes. In: *Proceedings of the Eighth National Veterinary Hemoparasite Disease Conference, St. Louis, Missouri, 10-12 April 1989.* Washington, D.C.: United States Agency for International Development, p. 207 (898).

Previous Page Blank

Dolan, T.T., ed. (1989). *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop on East Coast Fever Immunization, Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, 191 pp. (873).

Dolan, T.T. and Mutugi, J.J. (1989). The fertility of Boran heifers immunized against buffalo-derived *Theileria parva*. In: Dolan, T.T., ed. *Theileriosis in Eastern Central and Southern Africa: Proceedings of a Workshop Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 68-70 (877).

Fish, W.R., Grab, D.J. and Lonsdale-Eccles, J.D. (1989). The cross-reacting determinant of the variable surface glycoprotein of metacyclic *Trypanosoma congolense*. In: Lonsdale-Eccles, J.D. and Lenahan, J.K., eds. *Protein Traffic in Parasites and Mammalian Cells: Proceedings of a Workshop, Nairobi, Kenya, 29 August to 1 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 42-44 (865).

Flach, E.J., Shambwana, I., Dolan, T.T., Kassim, A., Pedersen, H., Glass, M. and Morzaria, S.P. (1989). East Coast fever immunization trials on Unguja Island, Zanzibar. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 77-79 (878).

Gettinby, G. (1989). Data handling for theileriosis immunization studies. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 149-154 (885).

Grab, D.J. and Verjee, Y. (1989). Localization of a variable surface glycoprotein phosphatidylinositol-specific phospholipase-C in *Trypanosoma brucei brucei*. In: Lonsdale-Eccles, J.D. and Lenahan, J.K., eds. *Protein Traffic in Parasites and Mammalian Cells: Proceedings of a Workshop, Nairobi, Kenya, 29 August to 1 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 65-70 (866).

Grootenhuis, J.G. (1989). The role of wildlife in the epidemiology of cattle theileriosis. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop on East Coast Fever Immunization, Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 129-136 (900).

Hahn, N.E., Perry, B.D., Hansen, J.W. and Turner, E.C. (1989). Role of blackflies in the epidemiology of Potomac horse fever. *Veterinary Record* 125: 273-274 (901).

Hirumi, H. and Hirumi, K. (1989). Continuous cultivation of *Trypanosoma brucei* bloodstream forms in medium containing a low concentration of serum protein without feeder cell layers. *Journal of Parasitology* 75: 985-989 (729).

Irvin, A.D. and Morrison, W.I. (1989). Vaccines against *Theileria parva*. In: Wright, I.G., ed. *Veterinary Protozoan and Hemoparasite Vaccines.* Boca Raton: CRC Press, pp. 115-130 (544).

Kaminsky, R., Zweygarth, E., Gumm, I.D. and Chuma, F. (1989). *In vitro*-assays zur bestimmung der medikamenten-resistenz bei afrikanischen trypanosomen. (Determination of African *Trypanosoma* drug resistance by means of in vitro assays.) *Mitteilungen der Oesterreichischen Gesellshaft für Tropenmedzin und Parasitologie.* 11: 47-54 (684).

Kemp, S.J., Hazelwood, S., Gettinby, G. and Teale, A.J. (1989). *BoLA-PC: A Package for the Storage and Analysis of Animal Tissue-Typing Data on a Microcomputer System.* International Laboratory for Research on Animal Diseases/University of Strathclyde, 29 pp. (894).

Koch, H.T., Kambeva, L., Ocama, J.G.R., Munatswa, F.C., Dolan, T.T. and Norval, R.A.I. (1989). Immunization of cattle against *Theileria parva bovis* in Zimbabwe. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop on East Coast Fever Immunization, Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 90-93 (880).

Lonsdale-Eccles, J.D. and Lenahan, J.K., eds. (1989). *Protein Traffic in Parasites and Mammalian Cells: Proceedings of a Workshop Held at the International Laboratory for Research on Animal Diseases, Nairobi, Kenya, 29 August to 1 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, 160 pp. (862).

Lonsdale-Eccles, J.D., Mpimbaza, G.W., Verjee, Y. and Webster, P. (1989). Proteolysis and endocytosis in *Trypanosoma brucei.* In: Lonsdale-Eccles, J.D. and Lenahan, J.K., eds. *Protein Traffic in Parasites and Mammalian Cells: Proceedings of a Workshop, Nairobi, Kenya, 29 August to 1 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 94-99 (867).

Maillard, J.C., Kemp, S.J., Leveziel, H., Teale, A.J. and Queval, R. (1989). Le complex majeur d'histocompatibilité de bovine quest Africains: types d'antigenes lymphocytaires (BoLA) du Burkina Faso (Afrique occidentale). (The major histocompatibility complex of West African cattle; lymphocyte antigen [BoLA] typing on Baoule taurines [*Bos taurus*] and Sudanian Zebus [*Bos indicus*] in Burkina Faso [West Africa]. *Revue Elevage de Médicine Vétérinaire des Pays Tropicaux* 42: 275-381 (831).

Morzaria, S.P. (1989). A systematic approach to East Coast fever immunization in the Kilifi District of the Kenya coast. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop on East Coast Fever Immunization, Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 46-52 (875).

Morzaria, S.P. (1989). Identification of *Theileria* species and characterization of *Theileria parva* stocks. In: Dolan, T.T., ed. *Theileriosis in Eastern. Central and Southern Africa: Proceedings of a Workshop Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 102-110 (881).

Mukhebi, A.W., Morzaria, S.P. and Perry, B.D. (1989). Economics of an East Coast fever immunization trial at the Kenya coast. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 60-61 (876).

Mukhebi, A.W., Morzaria, S.P., Perry, B.D., Dolan, T.T. and Norval, R.A.I. (1989). Production and delivery costs of the infection-and-treatment vaccine against East Coast fever. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 155-157 (886).

Mutugi, J.J., Young, A.S., Maritim, A.C., Ndungu, S.G., Linyonyi, A., Nguni, P.N., Mining, S.K., Leitch, B.L., Stagg, D.A., Grootenhuis, J.G., Kariuki, D.P., Morzaria, S.P. and Dolan, T.T. (1989). Parasite stocks used for East coast fever immunization in Kenya. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 39-45 (875).

Norval, R.A.I. (1989). Tick control in relation to the epidemiology of theileriosis. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop on East Coast Fever Immunization, Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 111-120 (882).

ole-MoiYoi, O.K., Iams, K., Nayar, A., Brown, W.C., Sugimoto, C., Grab, D.J. and Conrad, P.A. (1989). Protein kinase activation in *Theileria*-infected cells. In: In: Lonsdale-Eccles, J.D. and Lenahan, J.K., eds. *Protein Traffic in Parasites and Mammalian Cells: Proceedings of a Workshop, Nairobi, Kenya, 29 August to 1 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 127-129 (868).

Pearson, T.W. and Jenni, L. (1989). Detection of hybrid phenotypes in African trypanosomes by high resolution two-dimensional gel electrophoresis. *Parasitology Research* 76: 63-67 (510).

Perry, B.D. (1989). The oral immunization of animals against rabies. In: Grunsell, C.S.G., Hill, F.W.G. and Raw, M.E., eds. *The Veterinary Annual, Twenth-Ninth Issue.* Guildford: Butterworth, pp. 37-47 (622).

Perry, B.D., Lessard, P., Mukhebi, A.W. and Norval, R.A.I. (1989). Epidemiology and decision-making in theileriosis control. In: Dolan, T.T., ed. (1989). *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 137-143 (883).

Perry, B.D., Lessard, P., Norval, R.A.I., L'Eplattenier, R., Dolan, T.T., Grandin, B.F. and Irvin, A.D. (1989). The risk of East Coast fever to livestock in Africa on a geographic basis. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 144-148 (884).

Perry, B.D., Schmidtmann, E.T., Rice, R.M., Hansen, J.W., Fletcher, M., Turner, E.C., Robl, M.G. and Hahn, N.E. (1989). Epidemiology of Potomac horse fever: an investigation into the possible role of non-equine mammals. *The Veterinary Record 125: 83-86 (902).*

Sugimoto, C., Conrad, P.A., Mutharia, L., Dolan, T.T., Brown, W.C., Goddeeris, B.M. and Pearson, T.W. (1989). Phenotype characterization of *Theileria parva* schizonts by two-dimensional gel electrophoresis. *Parasitology Research* 76: 1-7 (641).

Webster, P. (1989). Coated vesicles from the protozoan parasite *Trypanosoma brucei:* purification and characterization. *Journal of Protozoology* 36: 344-349 (670).

Webster, P. and March, M. (1989). Endocytosis by African trypanosomes. In: Lonsdale-Eccles, J.D. and Lenahan, J.K., eds. *Protein Traffic in Parasites and Mammalian Cells: Proceedings of a Workshop, Nairobi, Kenya, 29 August to 1 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 156-160 (869).

Woodford, J.D., Gayer, J.G., Dolan, T.T. and Morzaria, S.P. (1989). An East Coast fever immunization trial on Pemba Island, Zanzibar. In: Dolan, T.T., ed. *Theileriosis in Eastern, Central and Southern Africa: Proceedings of a Workshop Held in Lilongwe, Malawi, 20-22 September 1988.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 80-82 (879)

Zweygarth, E. and Kaminsky, R. (1989). Chemotherapie medikamenten-resistenter Trypanosomenstämme (*Trypanosoma brucei brucei*) mit DL-α-difluormethylornithin. (Chemotherapy of drug-resistant *Trypanosoma brucei brucei* using DL-α-difluoremethylornithine.) *Mitteilungen der Oesterreichischen Gesellschaft für Tropenmedizin und Parasitologie* 11: 55-64 (758).

1990 Publications

Bensaid, A., Kaushal, A., MacHugh, N.D., Shapiro, S.Z. and Teale, A.J. (1990). Biochemical characterization of activation-associated bovine class I major histocompatibility complex antigens. *Animal Genetics* 20: 241-255 (715).

Brown, W.C., Lonsdale-Eccles, J.D., DeMartini, J.C. and Grab, D.J. (1990). Recognition of soluble *Theileria parva* antigen by bovine helper T cell clones: characterization and partial purification of the antigen. *Journal of Immunology* 144: 271-277 (800).

Clarkson, A.B., Jr., Bienen, E.J., Pollakis, G., Saric, M., McIntosh, L. and Grady, R.W. (1990). Trypanosome alternative oxidase. In: Peregrine, A.S., ed. *Chemotherapy for Trypanosomiasis: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 21-24 August 1989.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 43-48 (941).

Clevers, H., MacHugh, N.D., Bensaid, A., Dunlap, S., Baldwin, C.L., Kaushal, A., Iams, K., Howard, C.J. and Morrison, W.I. (1990). Identification of a bovine surface antigen uniquely expressed on $CD4^-CD8^-$ T cell receptor gamma/delta$^+$ T lymphocytes. *European Journal of Immunology* 20: 809-817 (825).

Dobbelaere, D.A.E., Prospero, T.D. Rotidi, I.J., Keike, C., Baumann, I, Eichhorn, M., Williams, R.O., Ahmed, J.S. Baldwin, C.L., Clevers, H. and Morrison, W.I. (1990). Expression of Tac antigen component of bovine interleukin-2 receptor in different leukocyte populations infected with *Theileria parva* or *Theileria annulata. Infection and Immunity* 58: 3847-3855 (924).

Dwinger, R.H., Murray, M. and Moloo, S.K. (1990). Parasite kinetics and cellular responses in goats infected and superinfected with *Trypanosoma congolense* transmitted by *Glossina morsitans centralis. Acta Tropica* 47: 23-33 (710).

Dwinger, R.H., Rawlings, P. Jeannin, P. and Grieve, A.S. (1990). Experimental infection of N'Dama cattle with trypanosomes using *Glossina palpalis gambiensis* caught in the wild. *Tropical Animal Health and Production* 22: 37-43 (711).

Fasogbon, A.I., Knowles, G. and Gardiner, P.R. (1990). A comparison of the isoenzymes of *Trypanosoma (Duttonella) vivax* isolates from East and West Africa. *International Journal of Parasitology* 20: 389-394 (778).

Flach, E.J., Woodford, J.D., Morzaria, S.P., Dolan, T.T. and Shambwana, I. (1990). Identification of *Babesia bovis* and *Cowdria ruminantium* on the island of Unguja, Zanzibar. *The Veterinary Record 126:* 57-59 (772).

Fritsch, G. and Nelson, R.T. (1990). Bovine erythroid (CFU-E, BFU-E) and granulocyte-macrophage colony formation in culture. *Experimental Hematology* 18: 195-200 (732).

Goddeeris, B.M. (1990). A bovine class I major histocompatibility complex molecule plus a *Theileria parva* antigen resembles mouse H-2K^d. *Animal Genetics* 9: 87-95 (801).

Goddeeris, B.M., Morrison, W.I., Toye, P.G. and Bishop, R. (1990). Strain specificity of bovine *Theileria parva*-specific cytotoxic T cells is determined by the phenotype of the restricting class I MHC. *Immunology* 69: 38-44 (686).

Grootenhuis, J.G., Dwinger, R.H., Dolan, R.B., Moloo, S.K. and Murray, M. (1990). Susceptibility of African buffalo and Boran cattle to *Trypanosoma congolense* transmitted by *Glossina morsitans centralis. Veterinary Parasitology* 35: 219-231 (823).

Hansen, J. and Perry, B.D. (1990). *The Epidemiology, Diagnosis and Control of Gastro-Intestinal Parasites of Ruminants in Africa: A Handbook.* Nairobi: International Laboratory for Research on Animal Diseases, 121 pp. (871).

Iams, K.P., Young, J.R., Nene, V., Desai, J., Webster, P., ole-MoiYoi, O.K. and Musoke, A.J. (1990). Characterisation of the gene encoding a 104-kilodalton microneme-rhoptry protein of *Theileria parva. Molecular and Biochemical Parasitology* 39: 47-60 (773).

Iams, K.P., Hall, R., Webster, P. and Musoke, A.J. (1990). Identification of lambda/gt11 clones encoding the major antigenic determinants expressed by *Theileria parva* sporozoites. *Infection and Immunity* 58: 1828-1834 (776).

Jordt, T. and Lorenzini, E. (1990). Multiple superovulations in N'Dama heifers. *Tropical Animal Health and Production* 22: 178-184 (740).

Kaminsky, R. (1990). *In vitro* techniques for assessment of drug resistance in trypanosomes. *AgBiotech News and Information* 2: 205-210 (812).

Kaminsky, R. (1990). *In vitro* techniques for identifying and quantifying drug resistance in *Trypanosoma brucei brucei.* In: Peregrine, A.S., ed. *Chemotherapy for Trypanosomiasis: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 21-24 August 1989.* Nairobi: International Laboratory for Research on Animal Diseases, pp. 73-77 (942).

Kaminsky, R., Gumm, I.D., Zweygarth, E. and Chuma, F. (1990). A drug incubation infectivity test (DIIT) for assessing resistance in trypanosomes. *Veterinary Parasitology* 34: 335-343 (709).

Karluki, T.M., Grootenhuis, J.G., Dolan, T.T., Bishop, R.P. and Baldwin, C.L. (1990). Immunization with *Theileria parva* parasites from buffaloes results in generation of cytotoxic T cells which recognize antigens common among cells infected with stocks of *T. parva parva, T. parva bovis* and *T. parva lawrencei. Infection and Immunity* 58: 3574-3581 (769).

Katende, J.M. Goddeeris, B.M., Morzaria, S.P., Nkonge, C.G. and Musoke, A.J. (1990). Identification of a *Theileria mutans*-specific antigen for use in an antibody and antigen detection ELISA. *Parasite Immunology* 12: 419-433 (799).

Kemp, S.J., Tucker, E.M. and Teale, A.J. (1990). A bovine monoclonal antibody detecting a class I BoLA antigen. *Animal Genetics* 21: 53-160 (783).

Koch, H.T., Kambeva, L., Norval, R.A.I., Ocama, J.G.R., Masaka, S., Munatswa, F.C., Honhold, N. and Irvin, A.D. (1990). Age resistance to *Theileria parva bovis* infection in calves. *Veterinary Parasitology* 37: 197-206 (934).

Koch, H.T., Kambeva, L., Ocama, J.G.R., Munatswa, F.C., Franssen, F.F.J., Ullenberg, G., Dolan, T.T. and Norval, R.A.I. (1990). Immunization of cattle against *Theileria parva bovis* and their exposure to natural challenge. *Veterinary Parasitology* 37: 185-196 (933).

Lessard, P., L'Eplattenier, R., Norval, R.A.I, Kundert, K., Dolan, T.T., Croze, H., Walker, J.B., Irvin, A.D. and Perry, B.D. (1990). Geographical information systems for studying the epidemiology of cattle diseases caused by *Theileria parva. The Veterinary Record* 126: 225-262 (792).

McOdimba, F.A. (1990). The effect of temperature and storage on the infectivity and motility of African animal trypanosomes in the blood of different hosts. *Acta Tropica* 47: 53-60 (754).

Majiwa, P.A.O. and Otieno, L.H. (1990). Recombinant DNA probes reveal simultaneous infection of tsetse flies with different trypanosome species. *Molecular and Biochemical Parasitology* 40: 245-253 (817).

Moloo, S.K. (1990). Large-scale rearing of *Glossina longipennis* in the laboratory. *Acta Tropica* 48: 159-160 (815).

Moloo, S.K. and Kutuza, S.B. (1990). Expression of resistance to isometamidium and diminazene in *Trypanosoma congolense* in Boran cattle infected by *Glossina morsitans centralis. Acta Tropica* 47: 79-89 (749).

Morrison, W.I. and Goddeeris, B.M. (1990). Cytotoxic T cells in immunity to *Theileria parva* in cattle. *Current Topics in Microbiology and Immunology* 155: 80-93 (728).

Morzaria, S.P. and Nene, V. (1990) Bovine theileriosis: progress in immunisation methods. *International Journal of Animal Science* 5: 1-4 (666).

Morzaria, S.P., Spooner, P.R., Bishop, R.P., Musoke, A.J. and Young, J.R. (1990). *Sfi* I and *Not* I polymorphisms in *Theileria* stocks detected by pulsed field gel electrophoresis. *Molecular and Biochemical Parasitology* 40: 203-211 (793).

Mukhebi, A.W., Morzaria, S.P., Perry, B.D., Dolan, T.T. and Norval, R.A.I. (1990). Cost analysis of immunization of East Coast fever by the infection and treatment method. *Preventive Veterinary Medicine* 9: 207-219 (770).

Musoke, A.J. and Nene, V. (1990). Development of recombinant antigen vaccines for the control of theileriosis. *Parasitologia* 32: 73-85 (804).

Naessens, J., Newson, J., McHugh, N., Howard., C.J., Parsons, K. and Jones, B. (1990). Characterization of a bovine leucocyte differentiation antigen of 145,000 MW restricted to B lymphocytes. *Immunology* 69: 525-530 (888).

Nantulya, V.M. (1990). Trypanosomiasis in domestic animals: the problems of diagnosis. *Revue Scientifique et Technique de l'Office International des Epizooties* 9: 357-367 (847).

Nene, V., Gobright, E., Musoke, A.J. and Lonsdale-Eccles, J.D. (1990). A single exon codes for the enzyme domain of a protozoan cysteine protease. *Journal of Biological Chemistry* 265: 18047-18050 (811).

Newson, J. (1990). The optimization of immunoglobulin secretion *in vitro* by mouse spleen cells and hybridoma cells. *Immunology Letters* 23: 293-298 (713).

Newson, J., Mahan, S.M. and Black, S.J. (1990). Synthesis and secretion of immunoglobulin by spleen cells from resistant and susceptible mice infected with *Trypanosoma brucei brucei* Gutat 3.1. *Parasite Immunology* 12: 125-139 (682).

Norval, R.A.I. and Perry, B.D. (1990). Introduction, spread and subsequent disappearance of the brown ear-tick, *Rhipicephalus appendiculatus*, from the southern lowveld of Zimbabwe. *Experimental and Applied Acarology* 9: 103-111 (688).

ole-MoiYoi, O.K., Nayar, A., Iams, K.P., Musoke, A.J. and Yilma, T. (1990). Molecular aspects of *Theileria parva* and approaches to vaccine development for animals. *New York Academy of Sciences* 569: 174-182 (794).

Peregrine, A.S., ed. (1990). *Chemotherapy for Trypanosomiasis: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 21-24 August 1989.* Nairobi: International Laboratory for Research on Animal Diseases, 177 pp. (939).

Peregrine, A.S., Sutherland, I.A., Mutharia, L., Jamnadas, R., Gray, M.A., Codjia, V., Allu, Y.O., Mamman, M., Sllayo, R., D'Ieteren, G.D.M., Moloo, S.K. and Murphy, N.B. (1990). Overview of the chemotherapy research program at ILRAD. In: Peregrine, A.S., ed. *Chemotherapy for Trypanosomiasis: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 21-24 August 1989. Nairobi: International Laboratory for Research on Animal Diseases, pp. 3-8 (940).*

Perry, B.D., Lessard, P., Norval, R.A.I., Kundert, K. and Kruska, R. (1990). Climate, vegetation and the distribution of *Rhipicephalus appendiculatus* in Africa. *Parasitology Today* 6: 100-104 (802).

Shaw, M.K. (1990). Fine structural localization of lipid material in *Theileria parva* (Muguga) schizonts. In: Institute of Physics Conference Series, No. 98. London: Institute of Physics Publishing, pp. 719-722 (790).

Spooner, P.R. (1990). The effects of oxytetracycline on *Theileria parva in vitro*. *Parasitology* 100: 11-17 (763).

Spooner, P.R. (1990). Oxytetracycline inhibition of mitochondrial protein synthesis in bovine lymphocytes infected with *Theileria parva* or stimulated by mitogen. *Parasitology* 101: 387-393 (819).

Talwo, V.O., Nantulya, V.M., Moloo, S.K. and Ikede, B.O. (1990). Role of the chancre in induction of immunity to tsetse-transmitted *Trypanosoma (Nannomonas) congolense* in goats. *Veterinary Immunology and Immunopathology* 26: 59-70 (781).

Teale, A.J. (1990). Genes controlling disease resistance as targets for bovine genome research with particular reference to trypanotolerance. In: Womack, J.E., ed. *Mapping the Genomes of Agriculturally Important Animals: Report of a Meeting Sponsored by the Institute of Biosciences and Technology and Technology, Texas A & M University. College Station, Texas: Texas A & M University*, pp. 65-72 (948).

Toye, P.G., MacHugh, N.D., Bensaid, A.M., Alberti, S., Teale, A.J. and Morrison, W.I. (1990). Transfection into mouse L cells of genes encoding two serologically and functionally distinct bovine class I MHC molecules from a MHC-homozygous animal: evidence for a second class I locus in cattle. *Immunology* 70: 20-26 (762).

Vandeweerd, V. and Black, S.J. (1990). Selective inhibition of the uptake by bloodstream form *Trypanosoma brucei brucei* of serum lipoprotein-associated phospholipid and cholesteryl ester. *Molecular and Biochemical Parasitology* 41: 197-206 (765).

Vos, G.J. and Gardiner, P.R. (1990). Antigenic relatedness of stocks and clones of *Trypanosoma vivax* from East and West Africa. *Parasitology* 100: 101-106 (708).

Vos, G.J. and Gardiner, P.R. (1990). Parasite-specific antibody responses of ruminants infected with *Trypanosoma vivax*. *Parasitology* 100: 93-100 (707).

Webster, P., Russo, D.C.W. and Black, S.J. (1990). The interaction of *Trypanosoma brucei* with antibodies to variable surface glycoproteins. *Journal of Cell Science* 96: 249-255 (777).

Webster, P. and Shapiro, S.Z. (1990). *Trypanosoma brucei:* a membrane associated protein in coated endocytotic vesicles. *Experimental Parasitology* 70: 154-163 (844).

Williams, D.J.L., Newson, J. and Naessens, J. (1990). Quantitation of bovine immunoglobulin isotypes and allotypes using monoclonal antibodies. *Veterinary Immunology* 24: 267-283 (746).

Zweygarth, E. and Kaminsky, R. (1990). Direct *in vitro* isolation of *Trypanosoma brucei brucei* and *T.b. evansi* from disease hosts with low parasitaemia. *Tropical Medicine and Parasitology* 41: 56-58 (820).

Zweygarth, E., Kaminsky, R. and Webster, P. (1990). *Trypanosoma brucei evansi*: dyskinetoplasia and loss of infectivity after long-term *in vitro* cultivation. *Acta Tropica* 48: 95-99 (842).

Zweygarth, E. and Kaminisy, R. (1990). Evaluation of an arsenical compound (RN 110, mel Cy, Cymelarsan) against susceptible and drug resistant *Trypanosoma brucei brucei* and *T.b. evansi. Tropical Medicine and Parasitology* 41: 208-212 (843).

Zweygarth, E., Kaminsky, R., Sayer, P.D. and Van Nieuwenhove, S. (1990). Synergistic activity of 5- substituted 2- nitroimidazoles (Ro 15 - 0216 and Benznidazole and DL-α-difluoromethylornithine on *Trypanosoma brucei brucei. Annales de la Société Belge de Médecine Tropicale* 70: 269-279 (892).

STAFF PRESENTATIONS AT INTERNATIONAL MEETINGS

E. Authié

VIIth Congrès International de Parasitologie (ICOPA), August 1990, Paris, France. Paper presented: *Reconnaissance des antigenes communs de* Trypanosoma congolense: *corrélation avec la résistance à la maladie chez les bovins.* E. Authié et D.J.L. Williams.

European Federation of Immunological Societies, 10th Meeting, Edinburgh, September 1990, Edinburgh, UK. Paper presented: *Antibody response to non-variant antigens of* Trypanosoma congolense: *correlation with resistance to disease in bovines.* E. Authié and D.J.L. Williams

A. Bensaid

25th International Meeting on Animal Genetics, August 1990, Michigan State University, East Lansing, USA. Papers presented:

1. *Sfi I defines extensive polymorphism in the bovine MHC region.* A. Bensaid, A. Kaushal, J.R. Young, S.J. Kemp and A.J. Teale.
2. *Nucleotide sequence of two BoLA cDNAs encoded by two BoLA-A and -B genes.* A.J. Teale, A. Kaushal and A. Bensaid.

J. Delehanty

Annual Meeting of the Association of American Geographers, April 1990, Toronto, Canada. Papers presented:

1. *What a geographer is and is not doing in a veterinary research laboratory (social science at ILRAD, with illustrations from the Kenya Highlands).*
2. *The meaning of sahelian weather (drought and hausa metaphysics).*

Conference of Rockfeller Foundation Postdoctoral Fellows in Social Science and Agriculture, October 1990, Ibadan, Nigeria. Paper presented: *Spatial extrapolition of human ecological point data using geographic informations systems.*

J.J. Doyle

Advances in Biotechnology, Swedish Council for Forestry and Agricultural Research/Swedish Recombinant DNA Advisory Committee, March 1990, Stockholm, Sweden. Paper presented: *Transgenic livestock: opportunities and constraints.*

H. Hirumi

USA/Japan Joint Conference on Parasitic Diseases, July 1990, Nagasaki, Japan. Paper presented: *Present status of the cultivation of metacyclic forms of three salivarian trypanosomes:* Trypanosoma brucei, T. congolense *and* T. vivax. H. Hirumi and K. Hirumi.

32nd Annual Conference of Japanese Society of Tropical Medicine, October/November 1990, Yokohama, Japan. Paper presented: *Chemotaxis of elongated bloodstream forms of* Trypanosoma vivax *(IL1392) against pollen grains of* Fushsia hybrida *and* Saintpaulia ionantha. H. Hirumi and K. Hirumi.

L. Innes

10th Meeting of the European Federation of Immunological Societies, September 1990, Edinburgh, UK. Paper presented: *Identification of* T. parva *schizont antigens recognized by immune T cells.*

S.J. Kemp

XXII International Conference on Animal Genetics, Michigan State University, August 1990, East Lansing, USA. Presentations:

1. *Studies of population genetics of N'Dama cattle.* R. Suzuki, T. Shotake, S.J. Kemp and A.J. Teale.
2. *Bovine MHC class I gene expression and polymorphism in Boran cattle.* P.S. Gwakisa, A. Bensaid, S.J. Kemp, P. Toye and A.J. Teale.
3. *Polymorphic microsatellites as landmarks in a bovine gene map.* S.J. Kemp and A.J. Teale.

R. Kruska

International Conference on Applications of Remote Sensing to Epidemiology and Parasitology, June 1990, Baton Rouge, Louisiana, USA. Presentations:

1. *The use of climate data interpolation in estimating the distribution of* Amblyomma variegatum *in Africa.* R.A.I. Norval, B.D. Perry, R. Kruska and K. Kundert.
2. *Evaluation of grazing lands in Zimbabwe using the AVHRR normalised difference vegetation index.* R. Kruska and B.D. Perry.

J.D. Lonsdale-Eccles

Biochemical Protozoology as a Basis of Drug Design, August 1990, University of Stirling, Scotland. Paper presented: *Proteases of African trypanosomes.*

D.J. McKeever

10th International Conference on Lymphatic Tissues and Germinal Centres in Immune Reactions, July 1990, Compiegne, France. Paper presented: *Functional studies of bovine afferent lymph veiled cells.*

4th World Congress on Genetics Applied to Livestock Production, July 1990, Edinburgh, UK. Paper presented: *Bovine immune responses to parasite antigens.*

S.K. Moloo

7th International Congress of Parasitology, August 1990, Paris. Paper presented: *Susceptibility of different* Glossina *species for pathogenic trypanosomes.*

A.W. Mukhebi

The Biennial Meeting of the African Small Ruminant Research Network, December 1990, Nairobi. Paper presented: *The role of small ruminants in the coastal sub-humid region of Kenya.* W. Thorpe, L. Reynolds, A. Mukhebi, E. Mussukuya and R. Nyambaka.

O.K. ole-MolYol

Committee on Genetic Experimentation (COGENE), January 1990, Paris. Paper presented: *Potential benefits for developing countries and their participation in the generation of genome maps.*

Molecular Parasitology, September 1990, Woods Hole, Massachusetts, USA. Paper presented: *Activation of casein kinase II in* T. parva*-infected bovine lymphocytes.*

Environmental Biosafety for Developing Countries, December 1990, Sweden. Paper presented: *Approaches to Biosafety for Developing Countries.*

H.R. Pereira

22nd International Conference on Animal Genetics, August 1990, East Lansing, Michigan, USA. Paper presented: *T helper cell responses to soluble 67 kDa protein in MHC homozygous animals.* H.R. Pereira and A.J. Teale.

B.D. Perry

International Conference on Applications of Remote Sensing to Epidemiology and Parasitology, June 1990, Baton Rouge, Louisiana, USA.
Papers presented:

1. *The use of climate data interpolation and remote sensing in estimating the distribution and abundance of* Rhipicephalus appendiculatus *in Africa.* B.D. Perry, R. Kruska, P. Lessard, R.A.I. Norval and K. Kundert.
2. *Geographic information systems for the development of tick-borne disease control strategies in Africa.* P. Lessard, R.A.I., Norval, B.D. Perry, K. Kundert, T.T. Dolan, J.B. Walker and A.D. Irvin.

E.N. Taracha

Meeting of the British Society for Immunology, April 1990, London, UK. Paper presented: *Evidence that cytotoxic T-cell responses are important in immunity of cattle to* Theileria parva. E.L.N. Taracha, B.M. Goddeeris and W.I. Morrison.

A.J. Teale

25th International meeting on Animal Genetics, August 1990, Michigan State University, East Lansing, USA. Paper presented: *Nucleotide sequence of two BoLA cDNAs encoded by two BoLA-A and -B genes.* A.J. Teale, A. Kaushal and A. Bensaid.

VISITING SCIENTISTS

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Visiting Scientists - Shortterm 1990

Name	Project	Dates
Dr. G. Gettinby Strathclyde University Glasgow, UK	Consultant in biostatistics and modelling	7/5/90-20/5/90 8/9/90-29/9/90
Dr. B. Allsopp Dept. of Biochemistry Cambridge University Cambridge, UK	Characterisation of *Theileria* genes	26/5/90-30/6/90
Dr. D. Zilberstein Israel Institute of Technology Haifa, Israel	Differential expression of glucose transporters in *T. brucei*	3/7/90-24/9/90
Prof. N. Citri The Hebrew University Jerusalem, Israel	Biochemistry/ diagnostic techniques	2/11/89-21/4/90
Mr. B. Byrom Strathclyde University Glasgow, UK	ECF epidemiology models	25/8/90-8/9/90
Dr. F. Kooy Dept. of Trop.Vet. Med. Utrecht University The Netherlands	Diploidy in metacyclic African trypanosomes	16/11/89-31/1/90
Dr. J.A. Kornblatt Concordia University Montreal, Canada	Trypanosome alternative oxidase	30/12/89-31/7/90
Dr. M.J. Kornblatt Service de Biospectroscopie Institut de Biologie Physico-Chimique Paris, France	Endosomal/lysosomal system, trypanosomes	30/12/89-31/7/90
Dr. S. Inumaru National Institute of Animal Health Ibaraki, Japan	Immuno-characterization of *Theileria*	12/1/90-3/3/90
Dr. J. Hackett Dept. of Microbiology University of Adelaide Adelaide, Australia	Consultant on recombinant *Salmonella* as vectors for *T. parva* genes	14/1/90-28/1/90

Ds. M. Longeri Institutuo di Zootecnica Veterinaria, University of Milan, Italy	Bovine cellular immune responses	31/1/90-31/4/90
Prof. M. Mahmoud Vice Chancellor University of Juba Khartoum, Sudan	Library facilities for editing *Parasitic Protozoa*	1/3/90-31/3/90
Dr. J.J. Turner Dept. of Clin. Vet. Med. Cambridge University Cambridge, UK	Establishment of interferon assay	20/4/90-30/5/90
Dr. T. Jordt Vroending Dyreklinik Soendre Molgervej 52 8762 Flemming, Denmark	Embryo transfer and training	1/5/90-6/6/90
Ms. B. Sabin School of Vet. Med. University of California Davis, Cal., USA	Drug-resistance-like sequence in *T. vivax* and *T. brucei*	25/6/90-31/8/90
Dr. M. Schulz Institute of Pathology University Hospital Zurich, Switzerland	Transfection technology to identify *T. parva* antigens	1/7/90-30/9/90
Dr. T. Tsukamoto Biochemistry Lab. Dept. of Applied Biological Sciences Saga University, Saga, Japan	Role of protein phosphatases in trypanosomes. Differentiation and purification of *Theileria* encoded casein kinase II	25/7/90-18/9/90
Dr. T. Shotake Primate Research Inst. Kyoto University Inuyama City, Aichi Japan	Genetic and biochemical characterisation of cattle	3/8/90-22/8/90
Ms M. Schutte Dept. of Clin. Immunol. University Hospital Utrecht, The Netherlands	Analysis of VH gene repertoire of bovine CD5 versus CD5 B cells	18/8/90-30/9/90

Dr. G. Duvallet C.R.T.A. Burkina Faso	Diagnostic probes, ELISA in trypanosome infections	12/11/90-6/12/90
Dr. R. Brun Swiss Tropical Institute Switzerland	Collaborative research in trypanosomiasis	19/11/90-3/12/90
Professor R. Terry Brunel University Uxbridge, Middlesex England	Supervision of Ph.D. students	1/9/90-7/9/90
Prof. R.N. Coulson Dept. of Entomology Texas A & M University College Station, Texas, USA	Tsetse disease models	8/9/90-21/9/90