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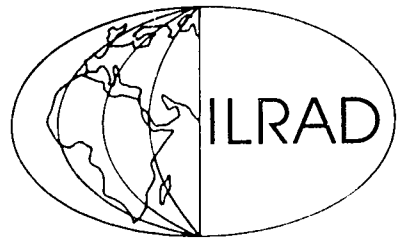
# ILRAD 1993

# Annual Scientific Report




International Laboratory
for Research on Animal Disease

P.O. Box 30709 • Nairobi • Kenya

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# PREFACE

This volume presents summaries of the scientific activities of the International Laboratory for Research on Animal Diseases for 1993. The Laboratory has a global mandate from the Consultative Group on International Agricultural Research, to which it belongs, to conduct research on better ways of controlling livestock diseases which constrain livestock and agricultural productivity in developing countries. The major goal for ILRAD has been to develop new and improved methods for two of the most important disease complexes of the tropics and sub-tropics; trypanosomiasis—a protozoan disease transmitted to animals by tsetse and other biting flies—and tick-borne diseases, including a virulent form of theileriosis. Methods of vaccination, improved chemotherapy and enhanced genetic resistance to disease are being developed or evaluated. As can be seen from the abstracts, the research approach utilizes some of the latest methodologies in the fields of epidemiology, immunology, biochemistry, molecular genetics and computer modelling to address the interactions between the pathogenic organisms, their vectors and the livestock species directly affected.

The major disease research programs are supported by two other important programs: the socioeconomics program seeks to determine the economic and environmental impacts of alternative disease control strategies and the reports of this scientific program are included here. The work of the program for collaborative research, training and information—whilst critical to the research and outreach functions of the Laboratory—is only indirectly in evidence in this report through the numerous collaborative research projects and the number of Research Fellows (postgraduate trainees) who contribute directly to ILRAD's research output.

ILRAD conducts its research in collaboration with many partners. Its modern technological approach to solving the livestock problems of developing countries sets the Laboratory at the heart of an international network. Official and informal linkages help relate the results of basic research from elsewhere (providing techniques and analogies from other human or animal research problems) to refine and augment ILRAD's strategic approach to its mandated diseases. The Laboratory, in turn, helps to bring appropriate technologies, information and advice to scientists of national programs for livestock disease control or international institutes and agencies concerned with the integrated issues of agricultural research and international development. This range of the Laboratory's interactions is apparent from the (non-exhaustive) lists of collaborating and visiting scientists to ILRAD in 1993 and the number of scientific staff seconded by donor nations to the Laboratory.

Research at ILRAD is managed on a project basis and the abstracts are numbered according to their relation to projects in each of the three programs. The full titles of the projects are given on the contents page and abridged versions are given as running heads on each page of the collected abstracts. It should be borne in mind, however, that much of the work represents progress in continuing research programs. Therefore, reference is made to previous reports and to future plans. Further scientific details or background in specific areas can be found in earlier *Annual Scientific Reports* or in the list of recent ILRAD publications. Reporting modern science in abstract form presents a further difficulty; that of technical expressions and abbrevi-

ations. Each branch of science has its own quota of technical terms and commonly accepted abbreviations. Previously, in attempts to make the abstracts readable in their own right, many of these abbreviations have been given in full. In certain cases, especially when fields overlap—as they do for instance in molecular immunology—it is sometimes not feasible to explain the scientific results and the abbreviations to the non-specialist reader in a few lines. For this reason, this report includes a glossary of relevant scientific abbreviations.

The majority of ILRAD's publications are published in refereed scientific journals, and most of these have an ILRAD publication number (in brackets in the publication list). Should the source of the publication not be readily available, developing country scientists requiring copies of these papers are invited to write to the ILRAD Library quoting the reference with its number. Readers wishing for a resume, or less technical accounts of the work of the Laboratory in 1993, are invited to consult the *Highlights* or *ILRAD's Annual Report* for 1993, or the Laboratory's quarterly publication *ILRAD Reports* which deal with selected aspects of the Institute's research and activities. Together, we hope that these publications will assist and inform those with an interest in the work of ILRAD—be they national planners and scientists seeking better methods to alleviate animal disease constraints, the representatives of donor nations who supply material support and a shared vision for improved agricultural productivity or, simply, fellow research workers around the world.

*P.R. Gardiner*
*Information and Planning Officer*
*International Laboratory for Research on Animal Diseases*
*Nairobi, February 1994*

# TICK-BORNE DISEASES

## E1a.1 Defining cloned *Theileria parva* parasites by cytotoxic T lymphocyte responses

**Scientists:** E. Taracha, S. Morzaria
**Research Associate:** P. Spooner

Cloned populations of *Theileria parva* have been characterized by cross-immunity studies, reactivity with monoclonal antibodies and DNA probes. Further studies were designed to examine *in vitro* the cellular immune responses following infection of cattle with clones of *T. parva*. Two polyclonal or cloned parasite-specific MHC class I restricted cytotoxic T lymphocytes (CTL) lines have been used to examine antigen specificities expressed by cells infected with cloned and uncloned *T. parva* strains. These CTL lines were generated from animals immunized with the Muguga strain of *T. parva* parasites. Target cells infected with either of two cloned, or an uncloned, *T. parva* population(s) of the Marikebuni stock were lysed at comparable levels by the polyclonal CTL line. However, when tested with cloned CTL, target cells infected with one of the clones were not recognized, whereas those infected with the other clone, or uncloned parasite populations, were lysed at comparable levels. The CTL lines were also assayed on target cells infected with uncloned Muguga parasites or one clone of this population, and comparable levels of cytotoxicity were observed. These results suggest that within the Marikebuni stocks at least two components exist, with distinct antigenic specificities, causing differential CTL reactivity. On the other hand, the Muguga clone might be well represented antigenically within both parent stocks suggesting that the differences demonstrated at the DNA level between parasites are not directly correlated with antigenicity. Together, these findings demonstrate that using CTL clones it may be possible to define antigenic specificities expressed by different cloned parasites.

## E1a.2 Polymorphic DNA markers for the discrimination of stocks of *Theileria parva*

**Scientists:** R. Bishop, S. Morzaria, V. Nene, P. Toye, P. Wijngard*
**Research Associates:** P. Spooner, E. Gobright

*University of Utrecht, The Netherlands

The currently available polymorphic DNA markers are confined to a limited region of the *T. parva* genome. Additional markers, which are dispersed in the genome, are required for studies on genome mapping and recombination in field populations. Sequences with homology to M13 phage 'minisatellites' have been isolated from *T. parva*, and these are dispersed in the *T. parva* genome and are polymorphic when the *EcoR1*-digested DNA of different stocks is analysed by Southern blotting.

A novel multicopy sequence, LA6, has been identified which distinguishes most *T. parva* stocks by Southern blotting and is predicted to encode a protein with an unusual amino acid composition. The sequence is transcribed in schizonts, unlike the *Tpr* (or *Theileria parva* repetitive) sequences described previously (and see abstract

E1c.2), and LA6 exhibits a mixed genotype in one of the recombinant *T. parva* parasites isolated at ILRAD.

As described previously, the technique of polymerase chain reaction (PCR) amplification using single arbitrary primers (10-mers) has been tested on purified piroplasm DNA from different *T. parva* stocks. Certain arbitrary primers generate amplified fragments which are consistently polymorphic among different stocks. Analysis of three of the polymorphic amplified fragments indicated that one was derived from a repetitive sequence, while the other two were derived from unique sequences. One of the unique sequences was transcribed in schizonts. Due to their dispersion in the genome, it may be possible to associate polymorphisms defined by arbitrary primers with phenotypes of interest in the parasite.

## E1a.3 Polymorphism in the p67 sporozoite antigen gene of *Theileria parva*

**Scientists:** S. Morzaria, V. Nene, R. Bishop, D. Kariuki*
**Research Associates:** P. Spooner, E. Gobright

*National Veterinary Research Centre, Muguga, Kenya

Sequencing of the gene encoding the p67 sporozoite surface antigen from *Theileria parva* stocks derived from cattle or buffalo has revealed a 129 bp insertion in the buffalo-derived stock which is absent from the cattle-derived stock. It is possible that this additional DNA sequence in the p67 gene may provide a marker for buffalo-derived *T. parva*. In order to examine this possibility, various *T. parva* stocks have been screened for polymorphism in their p67 gene.

The parasites used in the study originated from various parts of eastern, central and southern Africa and contained parasite stocks and their clones isolated from cattle and buffalo. All the parasites were characterized by a panel of schizont-specific monoclonal antibodies (MAb) before being examined for allelic differences in the p67 gene. The PCR primers, 246 and 247, flanking the p67 gene were designed and used in a polymerase chain reaction (PCR) assay to amplify the p67 gene from DNAs prepared from the sporozoite, schizont and piroplasms stages of the parasites. Three differently sized PCR products were obtained. All the cattle-derived *T. parva* stocks and clones showed a PCR product of approximately 2100 bp while all the buffalo-derived stocks showed two PCR products of 2250 bp and 2050 bp. A parasite population cloned from the *T. parva* buffalo-derived stock showed only one PCR product of 2250 bp. These results show that the p67 allele present in the cattle-derived *T. parva* is unique and different from those present in the buffalo-derived parasite. The presence of two p67 alleles indicates a mixed parasite population in the buffalo-derived *T. parva*. However, this needs to be supported by the characterization of the PCR band of 2050 bp.

The PCR-based assay provides a simple method of characterizing buffalo- and cattle-derived *T. parva*. More field isolates need to be examined to see if there are other p67 alleles present in the wild population. Since none of the p67 alleles present in the buffalo-derived *T. parva* are present in the cattle-derived *T. parva*, it will be important to investigate if the p67 polymorphism can be exploited to examine the

underlying genetic mechanism involved in the 'transformation' of buffalo-derived to cattle-derived parasite.

### E1a.4 Characterization of recombinant *Theileria parva* parasites

**Scientists:** S. Morzaria, R. Bishop, A. Young, T. Dolan
**Research Associates:** P. Spooner, F. Mwakima, R. Skilton

Cloned recombinant *T. parva* parasites derived from elements of the Muguga/Uganda and Muguga/Marikebuni stocks have been isolated from animals experimentally co-infected with these stocks. Cattle immunized with the Muguga/Uganda recombinant were resistant to challenge with a *T. parva* Muguga bulk stabilate, but breakthrough infections occurred on challenge with a *T. parva* Uganda stabilate. The immunological properties of the recombinant were thus similar to those of the *T. parva* Muguga parent stock. Size-polymorphic telomeric *Sfi*I fragments separated on CHEF gels, together with a series of chromosome internal polymorphic markers were examined in piroplasm DNA from the recombinant parasites. This analysis indicated a high frequency of physical recombination in the chromosomes of the recombinant *T. parva* parasites.

## E1b.1 Diagnostic ELISA development for *Theileria parva*

**Scientists:** S. Morzaria, A. Musoke, V. Nene, P. Toye, T. Dolan
**Research Associates:** J. Katende, C. Nkonge, P. Spooner, R. Skilton

The candidate antigens identified for the development of antibody detection ELISA for *T. parva* are p67, p104, p150 and p85, referred to as the polymorphic immunodominant molecule (PIM). These antigens are present in all *T. parva* isolates tested. They have been well characterized and their genes sequenced. Using the pGEX system, recombinant purified fusion proteins of each of the candidate antigens have been synthesized and comparative antibody detection ELISAs were performed against a set of defined sera from cattle experimentally infected with *T. parva* (Muguga isolate). This comparative analysis revealed that the PIM recombinant antigen provided the highest sensitivity (99%). The PIM antigen has also been tested against field sera from areas endemic and non-endemic for East Coast fever, demonstrating a specificity of greater than 95%. Due to the polymorphic nature of PIM, experiments are being conducted to examine the specificity of the antigen when tested with sera from cattle infected with different field isolates of *T. parva*.

## E1b.2 Diagnostic ELISA development for *Theileria mutans*

**Scientists:** S. Morzaria, A. Musoke, V. Nene, T. Dolan
**Research Associates:** J. Katende, C. Nkonge, P. Spooner, R. Skilton

A 32 kDa antigen has been identified as a candidate diagnostic antigen for *T. mutans*. The antigen has been found to be present in two *T. mutans* isolates from Kipange (Zanzibar, Tanzania) and Intona (Kenya). Sera from cattle infected with other tick-borne organisms such as *Theileria, Babesia* and *Anaplasma* spp. do not cross-react with the 32 kDa antigen. Antibody- and antigen-detection ELISAs have been developed using a native purified antigen and sequential sera collected from experimentally infected animals. The tests were found to be superior in their sensitivity and specificity to the routinely used immunofluorescence tests for *T. mutans*. In order to provide a standard source of the antigen, attempts were made to produce a recombinant antigen. The gene coding the 32 kDa antigen has been cloned and characterized using the pGEX expression plasmid vector. The p32 *T. mutans* antigen (not full length) has been produced as a fusion protein with the 26 kDa glutathione S-transferase (GST) molecule. A simple method of purifying the antigen has been developed using a GST-sepharose affinity chromatography matrix. Experiments are in progress to characterize responsiveness to this antigen with sera from tick-transmitted *T. mutans* infections and sera from areas where *T. mutans* is or is not endemic. The immunofluorescence test and the ELISAs employing crude parasite antigen will be used as the tests for comparison.

## E1b.3. Development of a diagnostic ELISA for *Anaplasma marginale*

**Scientists:** S. Morzaria, A. Musoke, V. Nene, T. Dolan
**Research Associates:** J. Katende, C. Nkonge, P. Spooner, R. Skilton
**Technicians:** A. Maina, J. Kiarie

*Anaplasma marginale* molecules of 23/19 and 75 kDa have been identified as candidate diagnostic antigens. The p23/p19 has been found to be present in Kenyan, USA and Mexican isolates. In collaboration, an antigen detection ELISA has been developed at ILRAD, and a competitive antibody detection ELISA is being developed by the Washington State University, USA. Using sequential sera collected from two animals experimentally infected with *A. marginale* (Kapiti isolate), *Anaplasma*-specific antigen were detected from seven days after infection while patent parasitaemia was detected on day 15. The antigen remained detectable until day 250 when the experiment was terminated. Tne sensitivity and specificity of the antigen-ELISA will be established by comparison with the existing card agglutination test.

## E1b.4 Development of a diagnostic ELISA for *Babesia bigemina*

**Research Fellow:** N. Tebele
**Scientists:** S. Morzaria, A. Musoke, V. Nene, T. Dolan
**Research Associates:** J. Katende, C. Nkonge, P. Spooner, R. Skilton

A 200 kDa antigen of *Babesia bigemina* has been identified as a candidate diagnostic antigen based on the reactivity of (a) sera from field cattle showing high *B. bigemina* antibody titres in the immunofluorescence test (IFAT) and (b) reactivity of sera from experimentally infected cattle in immunoblots against whole parasite lysates of *B. bigemina*. In the latter case the 200 kDa is the first antigen to provoke reactive antibodies following primary infection. Other antigens are recognized following a second challenge. An antibody detection ELISA has been developed using a monoclonal antibody (MAb) to the 200 kDa antigen to immobilize the antigen in the microtitre plate. The ELISA was superior in its sensitivity to the IFAT using sera from experimentally-infected cattle. However, when 200 bovine sera from Tasmania, an area free of *Babesia* spp were tested against the 200 kDa antigen in the antibody detection ELISA, 16% were found to be positive, indicating a possible cross-reactivity with antigens of other organisms. Interestingly, when the 200 sera from Tasmania were tested in an antigen detection ELISA, only three sera tested positive indicating that the MAbs might be reactive with specific regions of the 200 kDa molecule. In contrast, 174 bovine sera, collected from Scotland (supplied by the Centre for Tropical Veterinary Medicine) and tested in the antibody detection ELISA, were all negative. The gene encoding the 200 kDa antigen has been isolated and is being characterized.

## E1b.5. Nucleic acid probes to ribosomal DNA for the detection of *Theileria* species

**Scientists:** R. Bishop, S. Morzaria, A. Young
**Research Associates:** P. Spooner, E. Gobright

Analyses have continued of large and small subunit ribosomal RNA sequences (LSU rRNA and SSU RNA, respectively) for their use in the differentiation of *Theileria* parasites. DNA sequencing reveals species-specific regions within LSU rRNA genes which are larger than those within SSU rRNA genes. Oligonucleotides derived from LSU sequences which differentiate five species of *Theileria* have been synthesized. Multiple non-overlapping oligonucleotides, which are specific for *T. taurotragi* and *T. parva* have been developed. The LSU oligonucleotides do not require high stringency washing conditions for the assay to effectively differentiate between parasite species.

Work has been carried out to adapt SSU oligonucleotide probes developed earlier for non-radioactive use. Oligonucleotides specific for *T. parva* and *T. taurotragi* have been derivatized with alkaline phosphatase. The derivatized oligonucleotides were used successfully to detect polymerase chain reaction-amplified parasite ribosomal DNA and *T. parva* sporozoite rRNA in parasite-infected *R. appendiculatus* salivary glands.

## E1c.1 Comparative restriction maps of *Theileria parva* stocks

**Scientist:** S. Morzaria
**Technician:** T. Ndolo

*Theileria parva* isolates and stocks have been shown to contain mixed parasite populations that display phenotypic and genotypic diversity. The understanding of the mechanism of these polymorphisms may be useful in planning immunization strategies in which strain differences play an important role. A complete restriction map of the *T. parva* (Muguga stock) genome is available which will form the basis for the study of genetic polymorphism. A comparative linking map of other *T. parva* stocks will show whether the polymorphisms are due to mutations, large rearrangements and/or deletions. The construction of a library containing large DNA fragments can be used to provide an ordered cloned library representing the whole of the relatively small sized *T. parva* genome. This, together with the *Sfi*I linking clones as landmarks, will allow accurate alignment of the physical and genetic maps. These maps will facilitate both cloning and location of known genes, and archiving of all information on the structure of the genome. The map will also be useful for studies of sexual recombination in *T. parva*.

Agarose-embedded DNAs from purified piroplasms of the Muguga, Marikebuni, Uganda and Boleni stocks of *T. parva* have been prepared. These four *T. parva* stocks were selected because of their unique immunological and biological characteristics. The *Sfi*I restriction fragment length polymorphisms (RFLP) have been observed in these four stocks. In direct comparison of the polymorphisms in the *Sfi*I fragments it was found that the Uganda stock was the most easily differentiated. The genome size was smaller than the other *T. parva* stocks. A map of the Uganda stock deduced from the *Sfi*I size fragment polymorphism shows that genomic changes were confined to mainly chromosomes 1, 3 and 4. So far no major genes have been localized in any of the deleted regions. The Marikebuni and Boleni stocks show minor differences between the stocks as well as from the reference Muguga stock.

## E1c.2 The *Theileria parva* repetitive (Tpr) gene family

**Scientists:** R. Bishop, A. Musoke, S. Morzaria, V. Nene
**Research Associates:** C. Nkonge, P Spooner

Probes derived from *Tpr* repetitive DNA sequences have been used extensively to define restriction fragment length polymorphisms (RFLPs) among *Theileria parva* stocks. Sequencing of *Tpr* genomic clones reveals the presence of large, partially repeated open reading frames (ORFs) arranged in tandem. The DNA sequences of the 3′ ends of the ORFs are approximately 90% homologous within one genome, but in some instances only 70% homologous among different *T. parva* stocks, suggesting that the proteins which they potentially encode are rapidly evolving. The organization of the repeated ORFs is reminiscent of vertebrate immunoglobulin or troponin T genes and suggests a system designed to produce diversity.

In order to investigate whether the ORFs are expressed as proteins, attempts have been made to express portions of them as fusion proteins in *Escherichia coli*. Antisera raised against a pGEX fusion protein expressing the C-terminal region of a *Tpr* ORF did not react with Western blots of *T. parva* sporozoite, schizont and piroplasm antigens. A construct in the PMG1 vector did not express at sufficiently high levels to be useful for raising antisera. Generation of antibodies is currently being attempted using a synthetic peptide derived from a *Tpr* ORF. Another construct containing a full length *Tpr* ORF has been generated in a baculovirus transfer vector and is being tested for expression. Northern blot analysis using *Tpr* DNA probes reveals a 3.5 kb RNA transcript in the piroplasm, but not the schizont stage, of the parasite. DNA sequence analysis of piroplasm cDNA clones shows at least five different *Tpr*-homologous sequences, derived from four loci in RNA derived from a single *T. parva* stock.

## E1c.3 Characterization of the gene encoding the P-glycoprotein homologue of *Theileria parva*

**Research Fellow:** M. Kibe
**Scientists:** O. ole-MoiYoi, A. Peregrine, R. Neubald*

*Brunel University, UK

The sequence of the 2.3 kb *T. parva*-glycoprotein gene analogue revealed an open reading frame (ORF) of about 1.8 kb. Part of this ORF was cloned in-frame into the baculovirus expression plasmid pAc111. This construct was then co-transfected with wild type virus DNA into Sf 21 insect cells. Among the ten positive clones obtained, two were found to express a recombinant protein of about 65–70 kDa. This protein was purified on preparative polyacrylamide gels for immunization of rabbits.

Genomic DNA from various *T. parva* stocks was cut with restriction enzymes which cut within and outside the *T. parva* P-glycoprotein gene locus. Southern blots of these digests indicate that there are two copies of this gene which are almost identical in stocks of *T. parva* Muguga and *T. parva* Uganda. Further, there is restriction length polymorphism in one copy of this gene in stocks of *T. parva* Mariakani, *T. parva* Marikebuni (2246) and *T. parva* Boleni.

In our initial attempts to isolate and identify a P-glycoprotein gene analogue in *T. parva*, we isolated a 750 bp DNA fragment from genomic libraries of *T. parva*. Sequencing of this 750 bp fragment showed an ORF of about 254 amino acids. Interestingly at the 3′ end of this ORF, there was a TGA stop codon followed by a putative poly A tail. Comparison of the sequence of this 750 bp fragment with the 5.7 *Sal* I genomic fragment shows that the 750 bp fragment is probably a processed pseudogene which was derived by retrotranscription through an RNA intermediate.

## E1d.1 Analyses of factors influencing *Theileria parva* infections in *Rhipicephalus appendiculatus* ticks

**Scientists:** A. Young, T. Dolan, S. Morzaria, R. Norval*, A. Sherriff†, G. Gettinby*
**Research Associates:** F. Mwakima, J. Scott

*University of Florida, USA
†University of Strathclyde, UK

A large database was established from the results of experimental transmission of *Theileria parva* by *Rhipicephalus appendiculatus* ticks. Data from the transmission of parasites from infection with one stabilate of the *T. parva* Muguga stock (3087) using dilutions of between 1/20 and 1/200, one breed of cattle and two stocks of *R. appendiculatus*, Muguga and Ol Pejeta, were used for analysis. Factors associated with the ticks and cattle used and the infections developing in the cattle were studied in relation to the infection variables (prevalence, abundance and intensity) in the tick batches harvested daily from cattle. A General Linear Model (GLM) was used to determine the importance of factors and interactions in influencing the levels of tick infection variables. Analyses of the prevalence (% of tick infected), abundance (mean number of infected salivary gland acini per tick examined) and intensity (mean number of infected salivary gland acini per infected tick) in male and female ticks showed that 14 factors or interactions had a significant effect ($p < 0.05$). Six of these factors were highly significant for all six tick infection variables. These were piroplasm parasitaemia of the animal on the day of harvest, minimum packed cell volume of the animal over the sampling period, age of animal, interaction between stabilate dilution and whether the animal died or recovered, month in which the tick batch was harvested and stabilate dilution administered to the animal. Statistical models were obtained for each of the infection variables. The GLM analyses were found to be useful tools in identifying factors that influence infection levels and in devising methods for producing tick batches with more predictable infections. These findings will be essential for carrying out future immunization studies involving tick challenge and in understanding the transmission of infection in epidemiological studies.

## E1d.2 Correlations between piroplasm parasitaemias, stages of infection and the levels of infections produced in *Rhipicephalus appendiculatus* ticks feeding on *Theileria parva*-infected cattle

**Scientists:** A. Young, S. Morzaria, T. Dolan, G. Gettinby*
**Research Associate:** F. Mwakima
**Research Fellow:** H. Ochanda

*University of Strathclyde, UK

Sixty-four Boran cattle between 6 and 12 months of age were infected with *Theileria parva* (Muguga) sporozoite stabilate (3087) at a 1/20 dilution in groups of four over

a period of one year. These cattle were treated daily from day 10 to 14 with 10 mg/kg body weight of oxytetracycline. This treatment significantly prolonged the infection and therefore allowed the study of the infectivity of *T. parva* to *Rhipicephalus appendiculatus* nymphs feeding on them.

Intraerythrocytic piroplasms were first detected on day 11 after infection and 100% of cattle harboured piroplasm parasitaemia by day 14 after infection. An exponential increase in mean piroplasm parasitaemia was recorded between day 13 and 17 after infection which was followed by a linear increase between day 17 to peak at day 21. The mean piroplasm parasitaemia then decreased. Nymphal ticks were applied on 12 cattle so that they became replete from day 8 onwards and it was found that, using a standard assessment of the salivary gland infection of the resultant adult ticks, the first infected ticks occurred in those which had become engorged on day 13 after stabilate infection. By day 15, all nymphal batches when examined as adults showed infection. The prevalence and abundance of *T. parva* infection in the salivary glands appeared to increase in a linear manner up to day 17 after infection, both in female and male ticks, and levelled off up to day 19. Prevalence remained high in both male and female ticks, but the abundance decreased both in male and female ticks.

One of the objectives of the study was to obtain highly infected ticks for sporozoites studies. Male ticks rarely reached the infection level required for harvesting (a mean abundance of over 80 acini infected per tick). Female ticks reached the required abundance more frequently but usually only if they engorged as nymphs between days 17 to 19 after infection. The nymphal tick application schedule has now been redesigned so that large numbers of ticks drop from cattle from day 17 to 19 after infection. This has been shown to be an efficient method of obtaining large batches of highly infected ticks. There was no direct correlation between parasitaemia and infection levels developing in ticks, except in those which became replete before day 17 after infection. The stage of infection appeared to have an important effect on the level of infection developing in ticks.

## E1d.3 *In vitro* feeding system for *Rhipicephalus appendiculatus* and the transmission of *Theileria parva*

**Scientists:** S. Waladde*, A. Young
**Research Associate:** F. Mwakima

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

Following the initial studies reported last year, a new apparatus for feeding *Rhipicephalus appendiculatus* nymphae *in vitro* was developed using an artificial feeding membrane with added olfactory and tactile stimuli and with heparinized blood supplied above the membrane. The blood meal was replaced every eight hours. The feeding system was incubated at 37 °C in an atmosphere of 2% $CO^2$ and 90% relative humidity. This system allowed more efficient feeding of ticks than in the systems described previously as the ticks reached a mean engorged weight which was comparable to that of ticks fed on cattle for four days, the first day of repletion for both membrane and cattle-fed ticks. The percentage of ticks which moulted from

engorged nymphs fed on membranes were comparable to nymphs which became replete after feeding on cattle for four days. When this system was used to feed nymphal ticks on *Theileria parva*-infected blood, most groups of ticks became infected. The highest infections were obtained from ticks which had fed and became replete on day 4 on membranes but the infection level decreased markedly in ticks which took longer to feed. This was in contrast to ticks which fed and became replete on these infected blood donor cattle where the level of infection increased with longer repletion periods. In addition, the longer the ticks took to feed on membranes, the lower their engorged weights and the lower their moulting success became. This was not observed in tick groups fed on cattle where engorgement weights and moulting success remained constant over time. These problems are now being addressed so that ticks with engorgement, moulting and transmission characteristics similar to those fed on cattle can be produced *in vitro* and the system can be used reliably in experiments *in vitro*.

### E1d.4 Comparative susceptibility of different stocks and instars of rhipicephalid ticks to infection with different stocks and sources of *Theileria parva*

**Scientists:** A. Young, G. Medley*, S. Morzaria, B. Perry
**Research Fellow:** H. Ochanda

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The vector competence, defined as the ability of ticks to transmit *Theileria parva* judged by the level of infection developing in the tick salivary glands, of six stocks of *Rhipicephalus appendiculatus* from Kenya, Zambia and Zimbabwe and one stock of *R. zambeziensis* from Zimbabwe has been compared using *T. parva* (Muguga) from Kenya and *T. parva* (Boleni) from Zimbabwe by feeding these ticks on infected cattle. In a series of experiments it was shown that the vector competence of these tick stocks varied greatly for the different *T. parva* stocks. For example, *R. appendiculatus* (McIlwaine) and *R. appendiculatus* (Muguga) were very efficient vectors of *T. parva* (Muguga) while *R. appendiculatus* (Western Mashonaland) and *R. zambeziensis* were very poor vectors. In contrast, when *T. parva* (Boleni)-infected cattle were used, *R. appendiculatus* (Eastern Province, Zambia) and *R. zambeziensis* were by far the most competent vectors and *R. appendiculatus* (Muguga) and *R. appendiculatus* (Western Mashonaland) were the poorest. The laboratory stocks of *R. appendiculatus* from Muguga and McIlwaine were therefore relatively poor vectors of *T. parva* (Boleni) but were good vectors of *T. parva* (Muguga). This is the first indication that through the range of *R. appendiculatus* and *T. parva* there may be great differences in the parasite/tick relationships—although differences between populations within Kenya had already been noted.

In a second series of experiments, the competence of larval/nymph and nymphal/adult transmission were compared for *T. parva* (Muguga) and *T. parva* (Boleni) stocks. For *T. parva* (Muguga), nymphs had similar prevalence of infection as adults but the abundance and intensity was much higher in adults than nymphs

since adults had 5 to 20 times the abundance. When *T. parva* (Boleni) was used, infections were not detected in nymphs of the Muguga or McIlwaine stocks of *R. appendiculatus*, although the adults had relatively high infections. When larval/nymphal and nymphal/adult transmission were compared using animals which were carriers of *T. parva* Marikebuni as a source of infection, again, the adult *R. appendiculatus* developed relatively high infection compared to nymphs indicating that the nymphal/adult transmission is likely to be much more efficient than the larval/nymphal transmission.

### E1d.5 Estimates of heritability of *Rhipicephalus appendiculatus* stocks to infection with *Theileria parva*

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**Research Associate:** F. Mwakima

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In order to estimate the heritability of susceptibility to infection, full sibling families were produced from two stocks of ticks, the laboratory stock *R. appendiculatus* Muguga and the field stock *R. appendiculatus* Kiambu.

Thirty male and 30 female ticks of each stock were applied individually to animals and allowed to mate and engorge. The individual engorged female ticks were collected in individual tubes. The eggs from each tick hatched and were applied to rabbits, one sibling group per ear. The resultant engorged larvae were collected from the ears and placed separately in tubes. After moulting to nymphs, 19 groups of siblings from *R. appendiculatus* (Muguga) and 17 from *R. appendiculatus* (Kiambu) were tested for their susceptibility to infection. Two cattle were infected with a 1/20 dilution of *T. parva* (Muguga) stabilate 3087 and the infection monitored. Ticks from each sibling group were applied in individual patches on the back of one animal for each tick stock and ticks from individual patches were collected as they became replete. After moulting to adults the infections in the individual sibling groups were assessed using standard procedures. The results for male and female prevalence, abundance and intensity of *T. parva* infection in the salivary glands were obtained from ticks that became engorged on the infected cattle on days 16 and 17 after infection.

Analysis of variance, using the abundance of infection in the sibling groups with a log transformation using Harvey's Least Squares analyses, was undertaken. Heritability estimates obtained were found to be high thereby demonstrating that some of the variability of infection in ticks was under genetic control.

The heritability estimates indicate that selection for high and low susceptibility in ticks should be possible. Family selection is now under way using crosses between full sibling groups which showed low and high infection levels so that selection of lines of greater and lesser susceptibility can be obtained.

## E1d.6 Survival of *Rhipicephalus appendiculatus* and its infection with *Theileria parva* under different conditions

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Investigations of the survival of *Theileria parva* in *Rhipicephalus appendiculatus* were carried out on groups of ticks under different conditions to generate epidemiological data which could be used in modelling transmission. Groups of adult ticks were used which were highly, moderately and lowly infected as well as nymphs which were highly and moderately infected. The highly infected groups of adult ticks were maintained in incubators at (i) 20 °C, or (ii) 24 °C, or at diurnally fluctuating temperatures of (iii) 13–23 °C and (iv) 23–30 °C, all at a relative humidity (RH) of about 85% and (v) under natural conditions at ILRAD. The climatic conditions to which the naturally exposed ticks were subjected were recorded daily. The nymphs and the moderately and lowly infected adult ticks were only exposed to two incubation conditions; 20 °C and 85% RH or natural conditions. The ticks were sampled at monthly intervals to assess their survival and infection rates.

In the highly infected adult tick group, high infections were maintained up to week 10 after moulting for ticks kept at 20 °C, 24 °C, 13–23 °C and 23–30 °C after which the infections declined rapidly. The ticks under natural conditions, however, maintained high infections up to about week 34 before the parasite levels declined. Infection disappeared from all the adult ticks kept at 20 °C, 24 °C and 23–30 °C at between 30 and 34 weeks after exposure. The ticks kept at 13–23 °C however maintained low infections up to 42 weeks after exposure. By week 62, ticks kept under natural conditions were still relatively highly infected.

Tick survival was influenced markedly by temperature. For example, all ticks kept at 24 °C and 23–30 °C had died by week 58 after exposure. Diurnal temperature fluctuation in incubators of 13–23 °C was better for survival than a constant temperature of 20 °C, but survival under natural conditions was much better. The survival of the moderately and lowly infected adult ticks and their *T. parva* infections followed a similar pattern to that of the highly infected ticks. An additional observation has been that female ticks survive longer than male ticks but male ticks appear to maintain infections longer. The survival of highly infected nymphs and their *T. parva* infection showed a much shorter duration than in the adult ticks. The infection in nymphs declined rapidly by week 14 after exposure to 20 °C and infection disappeared between weeks 26 and 30. Under natural conditions, infection levels declined rapidly from week 18 after exposure but after 50 weeks infections could still be detected. The data set when complete will be useful in modelling tick and parasite survival.

## E1d.7 Probability estimates to asses infection rates in *Theileria parva* infected ticks

**Scientists:** G. Gettinby*, A. Young, S. Morzaria, T. Dolan
**Research Associates:** J. Scott, F. Mwakima

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A computer program was developed to use the Monte Carlo method to test the suitability of tick batches infected with *T. parva* for the challenge of immunized cattle. The Monte Carlo method predicts the probability of a certain event occurring and the interest in this application was to determine the proportion of cattle in a group which would become infected if a certain number of ticks were applied and to predict the variation in the number of sporozoites received by the cattle. The minimal infective unit from ticks feeding on cattle is one infected acinus. In previous studies, a tick batch with a prevalence of 23.3% in female ticks, an abundance of 0.97 and an intensity of 4.2 was used to infect cattle. The Monte Carlo method indicated that 20 ticks had to be used to ensure the probability of 100% of 11 cattle becoming infected. As predicted, 100% of the cattle became infected and also, as predicted, the cattle received a lethal challenge. This challenge was considered to be too high compared to what is likely to occur under field challenge. On further study it was found that what was required was an even but relatively low challenge, and that tick batches with low prevalence (3–10%) and an intensity of one would provide a reproducible challenge with little variation in the number of acini (and hence sporozoites) received per animal. Such a challenge would be useful in assessing the degree of protection of immunized cattle. This will be confirmed by the application of predicted numbers of infected ticks from the selected batch to cattle and the variation in infection developing in the cattle will be determined.

## E1d.8 Investigations into factors controlling diapause in *Rhipicephalus appendiculatus* populations

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Engorged female ticks from Eastern Province of Zambia, West Mashonaland, Zimbabwe and Kiambu District, Kenya, were collected from cattle and the offspring bred through to nymphs of the next generation. The engorged nymphs were exposed in quasi-natural situations in nylon gauze columns fixed into the soil in plant boxes at ILRAD and Veterinary Research Laboratory, Harare.

The ticks moulted into adults and their behaviour within the nylon gauze columns were observed daily and on one day a week at hourly intervals. The Kiambu, Kenya, ticks became active in the columns first followed by the Eastern Province, Zambia, ticks. The ticks from Western Mashonaland became active later. The overall pattern was that the Kenyan ticks were active throughout the exposure period (eight months from August 1992 to March 1993) and spent most of their time up the nylon gauze columns questing. The Zambian ticks showed a lower activity than Kenyan ticks but higher than Zimbabwean ticks. There was little evidence of diurnal rhythm in the activity of the ticks. To support the behavioural observations a set of nylon columns was sampled every month and the adult ticks were applied to the ears of rabbits. The rapidity of attachment of the different tick stocks was recorded and success of feeding, as judged by the number of replete females and their weight was determined. The Kenyan ticks attached rapidly to the rabbits and had a feeding success of over 90% on all occasions and reached expected engorgement weight. The Zimbabwean ticks failed to attach to the rabbit ears in many instances and showed a feeding success of less than 20% on all occasions with lower than expected engorged weights. The Zambian ticks showed an intermediate feeding behaviour with a 20–40% feeding success. Hence it was demonstrated that Zimbabwean ticks entered a diapause and Zambian ticks showed evidence of diapause but this was not as strict as in the Zimbabwean ticks. The Kenyan ticks showed no evidence of diapause.

In Harare, it was noted that the Kenyan ticks failed to enter into a diapause although they showed quiescence during the long dry period. It is likely that the Kenyan ticks which were active would have died if the plant boxes had not been watered at weekly intervals. The Zimbabwean and Zambian ticks went into diapause as judged by the lack of activity and ability to attach to rabbits. However, there was strong evidence that this behavioural diapause broke by December in the Zambian and Zimbabwean ticks because of critical photoperiod of about 13.2 hr. At that time they started to climb up the columns and their feeding pattern changed with rapid attachment to rabbits, greatly improved feeding success and increased engorgement weight.

## E1d.9 Differential development and emission of *Theileria parva* sporozoites from the salivary gland of *Rhipicephalus appendiculatus*

**Scientists:** M. Shaw, A. Young
**Research Associate:** F. Mwakima

The onset of tick feeding induces the rapid development of the parasite within the 'e' cells of the Type III acini of the tick salivary glands. Numerous uninucleated sporozoites are produced within four days, which are inoculated with the saliva of the tick into the bovine host. In general, *Theileria* parasites appear to develop in a limited range of salivary gland cells. The predominant cell infected is the 'e' cell of Type III acini, but development in 'd' cells and in Type II acini has been reported. The present study extends these observations and considers the mode of discharge of the mature sporozoites from the 'e' cells.

The paired salivary glands of adult female *R. appendiculatus* consist of approximately 1400 acini of three types (Types I–III). Type I acini are composed of

non-granular cells while Types II and III contain several secretory cell types (a–f). The identification of these different cell types is based on size, staining properties, histochemical composition and also on the ultrastructural characterization of the secretory granules. The types of acini are differentially arranged along the length of each gland with, in particular, the Type III acini occupying a more peripheral position in relationship to the main salivary gland duct.

When the differential rate of development of sporozoite development within individual acini in heavily infected salivary glands was studied, the distribution of infected salivary gland cells along the length of the salivary gland appeared to be random. Examination of sections of infected cells from various sites along the salivary gland from four-day-fed ticks showed that there was considerable variation in the degree of sporozoite development but no clear pattern.

*Theileria parva* appears to develop selectively in 'e' cells of Type III acini and development in Type II acini only occurs in highly infected salivary glands when 200 or more acini become infected. Sporozoite development in Type II acini was in general slower and more variable. The actual process of kinete penetration of the salivary glands has not been described for *Theileria* but as there is cell selection some degree of host cell recognition by the kinete is likely.

The emission of sporozoites from infected 'e' cells is similar to an apocrine secretion and occurs gradually. The apical membrane of the 'e' cells is lost which allows sporozoites free access to the lumen of the acinus and collecting ducts of the salivary gland. The complete loss of the apical membrane is quite different from the normal events of salivary gland droplet emission and probably accounts for the complete emptying of the cell content of the 'e' cell. The host cell nucleus at this stage often shows advanced signs of apostosis (condensation and marginalization of the chromatin). These processes result in the discharge of all the contents of the infected cells, including residual bodies and any residual host cell cytoplasmic organelles. The process of discharge however is gradual since few sporozoites are found in the salivary acinus valve or in the connecting ducts. In addition, it is not possible physically for large numbers of sporozoite to pass simultaneously from individual acini through the valve. These studies indicate that the *T. parva* sporozoite infected is given to animals as a trickle rather than in a burst.

## E1d.10 Characterization of molecules involved in sporozoite entry into lymphocytes

**Scientists:** J. Syfrig, S. Morzaria, A. Musoke, V. Nene, M. Shaw, P. Toye, Y. Yagi, A. Young

New approaches for the detection of receptor molecules on lymphocytes for sporozoites of *Theileria parva* molecules have been explored. Cell adhesion assays with plastic-coated p67 antigen molecules or sporozoites were not sensitive enough to detect specific binding. Recombinant proteins, NS-1p67 and baculovirus-derived p67 coupled to Sepharose beads could precipitate a 45 kDa protein from NP-40 lysates of surface biotinylated peripheral blood lymphocytes (PBL). The same result was obtained with lysates from PBL metabolically labelled with $^{35}$S-methionine. Im-

munoblots of the precipitated molecule with anti-MHC I antibodies were negative. However, the molecule reacted with a monoclonal antibody to actin. Furthermore, the electrophoretic mobility and the isoelectric point of the 45 kDa protein was identical to actin. Since the p67-containing coat of the sporozoite is shed during the entry process, an interaction of p67 with actin *in vivo* could only occur inside the parasite and would therefore be of secondary importance for sporozoite entry. To avoid the possible interference of actin with the physiological binding of the sporozoite ligand(s) to the lymphocyte receptor(s), cross-linking techniques using transfer of a label from the ligand to the receptor by the iodinatable, photoactivatable crosslinker SASD are being employed.

## E2a.1 Stable expression of a soluble form of p67, a sporozoite antigen of *Theileria parva*

**Scientists:** J. Steyaert, V. Nene
**Research Associate:** E. Gobright
**Technician:** S. Wanyonyi

The unfused recombinant theilerial sporozoite antigen, p67, produced in the cytoplasm of *Escherichia coli* is highly unstable. A relatively stable expression of p67 has however been achieved by targeting the protein to the periplasmic space of the bacterium. The eukaryotic signal sequence was replaced with the secretion signal of the *pho A* gene of *E. coli* and the chimaeric gene was placed under the control of the $P_{tac}$ promoter. In addition, the vector system contains the origin of replication of phage f1, allowing easy manipulation of the cloned gene. WK cells ($lacI_Q$) were found to express p67 upon depression of gene expression with IPTG.

A p67 construct lacking sequences that are predicted to form a structure that anchors p67 to the sporozoite membrane was also made. A translation stop codon was engineered into the gene so that translation terminates at residue 622. When compared to p67, the truncated p67 derivative, $p67_{622}$, was expressed at higher levels and it was more efficiently targeted to the periplasmic space. Both p67 and $p67_{622}$ can be released from bacteria by osmotic shock. The recombinant proteins appear to be soluble in standard buffer; no major proteolytic degradation of p67 or $p67_{622}$ is observed in crude lysates.

Current efforts are directed towards purifying large amounts of soluble $p67_{622}$.

## E2a.2 Identification and characterization of oligosaccharide chains of *Theileria parva* sporozoite p67 and insect cell-derived p67

**Scientists:** Y. Yagi, V. Nene, A. Musoke
**Technicians:** S. Wanyonyi, D. Lugo

Previous attempts to determine the carbohydrate moieties on the p67 sporozoite surface molecule following immunoprecipitation of the molecule from sporozoite lysates were unsatisfactory. An alternative approach has been used to identify oligosaccharide chains on p67 which involves fractionation of sporozoite lysates by two-dimensional electrophoresis, transfer of lysate proteins to nitrocellulose and specific staining using the digoxigenin conjugate method (DIG) and gold labelled lectins (Con A, DBA, LPA, PNA, UEA and WGA). Silver-stained gels revealed two major spots of sporozoite proteins at pI 6.2 and about 100 spots between pI 5.0 and 7.5. Using DIG about 50 spots were detected (pI 5.0 to 6.0) and a p67 specific monoclonal antibody (MAb AR 12.4) reacted with some of these spots. Several lectin positive spots were detected but these did not overlap with MAb reactive proteins.

Insect cell-derived p67 (BEVp67) was affinity purified using MAb AR 21.4. When analysed by silver staining of 2-D gels four spots were identified. Three proteins had a molecular weight of 98 kDa and a pI of 5.7, 5.3 and 5.2 while the fourth protein had a pI of 5.2 with a molecular weight of 92 kDa. The 98 kDa protein at pI 5.2 was

the dominant species and only this molecule was clearly positive for DIG staining. This analysis agrees with metabolic labelling studies which have demonstrated that BEVp67 contains a mixture of unmodified and partially glycosylated p67 molecules.

### E2a.3 Epitope mapping of the polymorphic immunodominant molecule of *Theileria parva*. I. Epitopes recognized by antibodies

**Scientists:** P. Toye, J. Scheerlinck, A. Musoke
**Research Associates:** P. Spooner, J. Katende
**Technician:** J. Nyanjui

The precise identification of antibody epitopes of the polymorphic immunodominant molecule (PIM) antigen will determine whether the regions of the molecule important in sporozoite neutralization are in conserved or variant regions, and whether the immunodominance of the molecule is due to a single, strongly immunogenic epitope. To determine these epitopes, we have used a set of overlapping peptides spanning the entire *T. parva* (Muguga) PIM, and deletion constructs of PIM in bacterial (pGEX) and COS cell expression vectors.

Thus far, nine monoclonal antibodies (MAbs), all of which except one (JK12.2) show neutralization activity *in vitro*, have been assayed. The MAbs react with different sets of peptides located in the 5′ constant region (MAb 4, 5, 6, and IL-S40.2), the variant region (MAb 2, IL-S36.5 and JK 12.2) or the 3′ variant region (IL-S32.3).

The peptide sequences recognized by bovine and rodent antisera are mostly located in the variable region of the molecule. In contrast to the mouse MAbs and antisera, both bovine and rat antisera react with the tetrapeptide repeat region (Pro Gln Pro Glu). The antisera react with many peptides throughout the molecule, with the majority of the peptides being in the variant region.

In summary, the results indicate that there are several immunogenic regions throughout the molecule and not a single immunodominant epitope. No correlation has been established between the neutralization activity of antibodies *in vitro* and their ability to recognize particular linear peptide sequences.

### E2a.4 Epitope mapping of the polymorphic immunodominant molecule of *Theileria parva*. II. Localization of immunodominant T cell epitopes

**Scientists:** P. Toye, J. Scheerlinck, A. Musoke
**Research Associates:** P. Spooner, J. Katende
**Technician:** J. Nyanjui

Sequence data derived from different *T. parva* strains indicate that the gene coding for PIM contains three regions. The N and C-terminals appear to be highly conserved and spaced with a more variable region. In view of the potential use of PIM as a vaccine, it would be interesting to determine whether the major T helper epitopes are located within the conserved or the variable region of PIM. Indeed while T helper epitopes located in the variable region might lead to non-cross protective responses,

epitopes located in conserved regions might cross-protect between different strains of *T. parva*. Further, given the immunodominance of the antigen in terms of its ability to induce a strong antibody response in immune cattle, the antigen may contain strong T helper epitopes which could be incorporated into any subunit vaccine. For these reasons, it is important to localize the T helper epitopes in the constant regions and focus the immune response towards these epitopes.

In preliminary experiments using the pGEX expression system, different constructs containing either the full length, the 5′ constant region or the 5′ constant region plus the variable region were used. The fusion proteins were purified and compared for their ability to re-stimulate PBM from PIM-immunized animals *in vitro*. Several animals were screened for the ability of their PBM to proliferate specifically to PIM. Subsequently, in the animals identified as positive, a T helper limiting dilution experiment was performed using the different constructs. Compared to the precursor frequency of T helper cells specific for the full length PIM, the frequency of T helpers specific for the 5′ constant region is low. In contrast the T helper precursor frequency specific for the construct containing the 5′ constant region and the variable region is very similar to that of the full length PIM. Therefore, in this animal the variable region of the PIM molecule appears to contain major T helper epitopes. However, the precursor frequency of T helper cells to the 3′ constant region needs to be included in the analysis. Furthermore, more animals must be examined to cover MHC class II heterogeneity in the cattle population. The ability of overlapping peptides to induce proliferative responses in these animals will also be examined.

## E2a.5 Expression and characterization of genes encoding the p150 and p104 diagnostic antigens of *Theileria parva*

**Scientists:** R. Bishop, H. Hirumi, V. Nene, P. Toye
**Research Associate:** R. Skilton
**Technicians:** S. Wanyonyi, J. Nyanjui

For the optimal development of a diagnostic ELISA for *Theileria parva* (see abstract E1.b1) four antigens (p67, p85, p104 and p150) are under consideration for inclusion in the assay.

The open reading frame (ORF) of the 150 kDa antigen gene of *T. parva* is 4359 bp long and potentially encodes a 165 kDa protein which contains a signal sequence at the N-terminus. A successful expression construct of p150 encodes residues 1181 to 1453, the C-terminus, and accounts for about 19% of the protein. The p150 molecule encodes 1453 amino acid residues and it contains a series of repeat sequence between residues 1227 and 1300; proline is generally repeated at four residue intervals within this domain. Southern blot analysis of DNA from different stocks of *T. parva* revealed an *Eco*RI restriction fragment length polymorphism (RFLP), the site being located in the repeat domain. To determine if this region might be polymorphic in *T. parva*, polymerase chain reaction (PCR) products corresponding to nucleotides 1783–4242 were generated from ten stocks of *T. parva*. Agarose gel electrophoresis of *Eco*RI digested PCR products confirmed the RFLP results. Approximately 800 bp of the 3′ end of the PCR products, which encodes the peptide

repeat, was directly sequenced. The deduced protein sequence was highly conserved except for the sequences within the repeat peptide domain. Ten parasite stocks fell into five groups based on the length and type of sequence repeats.

Sera from cattle experimentally infected with the Muguga, Uganda, Boleni, Marikebuni or buffalo 7014 stock of the parasite, i.e. parasites representative of groups I to IV, were used in an immunoblot analysis. All the sera cross-reacted with the purified recombinant p150 suggesting that there is an antibody response to the conserved sequences of p150. It is, therefore, unlikely that the polymorphic repeat sequences in the expressed portion of p150 would compromise the potential of the recombinant protein as a diagnostic antigen for *T. parva* infection.

In the case of the p104 antigen, the ORF encoding 924 residues was cloned into pGEX1N and near full length fusion proteins were detected by immunoblot analysis. However, it has not been possible to purify the fusion proteins by affinity chromatography. Stable fusions of Sj26 with residues 649–720 and residues 721–924 of the p104 gene, designated BBA and BM-16, respectively, have been produced. Approximately 20 mg of purified protein of each construct has been synthesized for experimental purposes. One baculovirus clone encoding a full length p104 gene has been isolated. The virus directed the synthesis of a p104-sized protein which was recognized by a monoclonal antibody to recombinant p104 from bacteria. This clone is being evaluated as an alternative source of recombinant p104 for diagnosis.

To facilitate cloning of the full length p104 gene, synthetic primers to the 5′ and 3′ end of the gene containing *Eco*RI sites were used to amplify the ORF. To avoid PCR induced errors, the central portion of the cloned PCR product was replaced with a BstXI fragment from the original genomic copy of p104. These restriction enzyme sites are located 70 and 27 bp from the ends of the PCR product. Following replacement, the hybrid construct was sequenced to ensure that the 5′ and 3′ ends of the gene were error free. This replacement method combined with the flexibility of PCR allows easy manipulation of gene sequences with high fidelity and requires minimal sequence analysis. This construct, T5, has also been used to determine cell-mediated immune responses to p104.

Polymorphism in the p104 gene was also investigated. The 3′ end of gene has two stretches of 41 and 22 amino acid residues which contain the amino acid proline repeated at about three residue intervals. An 828 bp fragment of the gene was amplified from nine different stocks of *T. parva* and approximately 770 bp of the PCR product was subjected to direct sequence analysis. In contrast to the p150 gene, DNA sequence polymorphisms in p104 were characterized by base substitutions and the deduced protein sequence was fairly conserved. The parasite stocks were divided into four groups on this basis and in total contained only 22 base substitutions which translated into 16 amino acid replacements. Most of the replacements were located in regions outside the peptide repeats; only one was located in the first repeat and none was in the second. The parasite stocks divided on the basis of the repeat differences fell into the same groups as for the p150 gene.

## E2b.1 Screening of cDNA libraries with *Theileria parva*-specific cytotoxic lymphocytes

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The technique described is a methodological approach to the isolation of schizont cDNAs from infected lymphocytes to search for parasite antigens which may elicit protective cellular responses in cattle.

A size-fractionated cDNA library was constructed in the pCDM8 vector from poly-A+ RNA isolated from the *T. parva* (Muguga)-infected cell line D409 N2 TpM. Randomly primed genomic DNA from piroplasms was used as a probe in colony blot hybridization to identify parasite cDNAs. Two hundred and thirty-four hybridizing colonies were isolated and grown separately in liquid medium. When miniprep DNA from each clone was reprobed with the genomic DNA, 200 of the clones were confirmed to be of parasite origin. These clones were sequenced from both ends.

Comparison of the sequences revealed that the cDNAs originated from 77 separate genes. Homology searches in DNA and protein databases showed that 12 of the genes had high homology with previously identified sequences which included hsp70, hsp82-90, hsp90, valosin/cdc48 (two different genes), a keratin-like protein, rRNA, tRNA, an anonymous *T. brucei* gene, $Ca^{++}$ transporting ATPase, KYBP or SNF2 transcription activator and NADH dehydrogenase. All cDNAs will be assessed to determine if they contain epitopes recognized by immune CTL.

## E2b.2 Isolation and characterization of naturally processed peptides of *Theileria parva*

**Scientists:** A. Musoke, D. McKeever, W. Fish, E. Taracha, D. Hunt*
**Research Associates:** C. Nkonge, G. Lamb
**Technician:** E. Awino

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Reports have described the isolation from cells of naturally processed peptides that constitute epitopes for $CD8^+$ and $CD4^+$ T cells. These reports have been based on soluble antigen and allogeneic and intracellular pathogen specificities, and have used whole cell lysates and immunopurified MHC molecules as sources. In the latter case, it has been possible using microsequencing techniques to identify the peptides and their parent antigens.

Efforts have focused initially on the development of a peptide sensitization assay for identifying high performance liquid chromatography (HPLC) fractions that contain epitopes of *T. parva*-specific cytotoxic T lymphocyte (CTL) clones. To ensure the presence of sufficient material in HPLC fractions, experiments were performed using bulk peptide preparations made from trifluoroacetic acid (TFA) lysates of parasitized cell lines, size fractionated to molecules of < 33 kDa by membrane

filtration. After separation by reverse-phase HPLC using a TFA/acetonitrile gradient, these fractions were lyophilized and stored at –70 °C before use. Target cells which had been kept at room temperature overnight were labelled with $^{111}$Indium oxine and sensitized by incubation with the peptide fractions for 90 min prior to the addition of CTL. A number of sensitization experiments using Concanavalin A blasts as target cells were unsuccessful, and this was attributed to an inconsistent capacity of these cells in class I MHC presentation of peptides, as has been observed in murine systems. Two alternative target systems were employed with success. The first of these is based on a cell line infected with *T. parva* (Marikebuni 3366), a parasite clone that is not recognized by the *T. parva* (Muguga)-specific CTL clone used for screening. The second involves the use of the screening clone itself as a target, and takes advantage of a fratricide effect that occurs following successful sensitization. The sensitivity of the latter system is enhanced by the addition of exogenous β-2 microglobulin of human origin. These assays have allowed the identification of a peptide fraction of *T. parva*-infected cells that contains the epitope of CTL clone T20.40. By pooling the appropriate fractions from a number of identical HPLC runs, it was possible to separate the material under two different gradient systems, and to rescreen these fractions with the clone. A positive fraction was identified in each set, and samples are being subjected to micro sequence analysis at the University of Virginia.

### E2b.3 Generation of CD4+ *Theileria parva*-specific T-cell clones from immune cattle

**Scientists:** D. McKeever, E. Taracha, D. Grab
**Research Associate:** G. Lamb

Following observations reported previously that CD4+ T cells are important in the generation of killing activity in parasite-specific CTL, a panel of CD4+ T-cell clones has been generated from an animal homozygous for the 103 sire MHC locus. This animal has received only one immunizing dose with *Theileria parva*. The clones were selected on the basis of their recognition of intact autologous infected cell lines and have been assessed for phenotype and specificity. The clones are currently being screened for their capacity to recognize *T. parva* antigens presented by uninfected antigen presenting cells with a view to their being used to screen parasite expression libraries. They will also be used in studies of helper function for CTL induction and CD4+ T-cell effector function against *T. parva*.

### E2b.4 Use of Inv+ *Escherichia coli* cells to present parasite peptides to antigen-specific T cells

**Scientists:** V. Nene, D. McKeever
**Technicians:** S. Wanyoni, E. Awino

Soluble recombinant antigens, either as crude *Escherichia coli* cell lysates or purified protein, have been extensively used to pulse antigen presenting cells (APCs) to

measure antigen-specific T-cell responses. The ability to present the antigen using whole bacterial cells would greatly facilitate studies aimed at defining T-cell antigens within a library of bacteria expressing a complex mixture of recombinant antigens.

The *inv* locus of *Yersinia pseudotuberculosis* imparts an invasive phenotype to *E. coli* and should allow efficient access of the cells into a MHC class II antigen presentation pathway. We have initiated a study to test this hypothesis using the *T. b. brucei* ILTat 1.3 VSG antigen and VSG-specific T cells from steer F98 as a model system.

The *inv* locus was subcloned into plasmid pACYC184 to allow compatibility with the more common ColE1 based cloning vectors. *E. coli* $Inv^+$ cells were then transformed with pGEX-VSG which expresses trypanosome variable surface glycoprotein (VSG) as a Sj26 fusion protein. Growth curves for *E. coli* $Inv^+$, *E. coli* $Inv^+VSG^+$ and *E. coli* $Inv^-VSG^+$ cells were established.

Standard T cell proliferative assays were carried out using monocytes as APCs and purified VSG or *E. coli* cells as the antigen source. Monocytes were pulsed with the bacterial cells for one hour prior to addition of peripheral blood lymphocytes. The bacterial cells suppressed the proliferative response to soluble VSG to a small degree but, in two experiments, only APCs pulsed with *E. coli* $Inv^+VSG^+$ induced a proliferative response. This system remains to be tested with VSG gene deletions and T-cell clones which are of known specificity.

Following establishment of optimal assay conditions, a *T. parva* expression library will be screened with established T-cell clones to determine the specificity of this arm of the immune response to parasite infection.

## E2c.1 Characterization of *Theileria mutans in vivo* and *in vitro*

**Scientists:** S. Morzaria, A. Young, T. Dolan
**Research Associate:** F. Mwakima
**Technicians:** J. Kiarie, S. Mwaura

For studies of *Theileria mutans*, including immunization experiments, and standardization and validation of diagnostic tests, a sporozoite stabilate, providing a reproducible challenge, is required. Several attempts to prepare a sporozoite stabilate of the Kipange stock of *T. mutans* with the laboratory stock of *Amblyomma variegatum* (Kiboko) have been unsuccessful. The major problem has been extremely low parasite infection rates in ticks despite apparently optimal tick feeding and pick-up. It was thought that the failure to obtain heavily infected ticks might be due to the relative insusceptibility of the laboratory tick stock to infection with the parasite stock from Zanzibar.

Thus, the infectivity of another *T. mutans* stock for the Kiboko *A. variegatum* was evaluated. A pick-up was made by both the larval and nymphal stages during the piroplasm parasitaemia of between 8–34%. The subsequent nymphal and adult stages were assessed for *T. mutans* infection rates following pre-feeding on rabbits. It appears from the preliminary data that the optimum pre-feeding period for nymphs is four days while that for adults is five days. The adults were more heavily infected than the nymphs. The highest infection rate in *A. variegatum* was 57% and on average each tick had 11.7 infected acini. This is the highest infection rate ever reported of *T. mutans* in the tick vector which is being used to establish and test a sporozoite stabilate.

## E2c.2 Molecular characterization of the gene encoding the 32 kDa *Theileria mutans* antigen and expression of the gene as a fusion protein

**Scientists:** S. Morzaria, A. Musoke, V. Nene
**Research Associates:** R. Skilton, S. Spooner, C. Wells, C. Nkonge
**Technicians:** J. Kiarie, L. Gichuru

The gene for the immunodominant *Theileria mutans* 32 kDa piroplasm antigen, a diagnostic antigen of *T. mutans* infection of cattle, has been cloned and three cDNA variants, clones 9, 21, 23, have been sequenced. At the protein level, clones 9 and 23 are 98% homologous, whereas clone 21 is 90% homologous to clones 9 and 23. These clones have about 60% homology to the published sequences of immunodominant piroplasm molecules of *T. buffeli* and *T. sergenti*, indicating the presence of a family of equivalent molecules within the *Theileria* genus.

Piroplasm DNA from a stock of *T. mutans*, digested with *Hind* III and *Sma*I, give single bands in Southern blots when probed with clone 9 cDNA, suggesting a single copy of the gene. However, some enzymes such as *Kpn*I and *Eco*RI produce multiple bands, indicative of restriction site polymorphism in the antigen gene in the uncloned stock.

Clones 21 and 23 have been expressed in the pGEX bacterial expression system as stable fusions with the C-terminus of Sj26, a 26 kDa glutathione S-transferase (GST). Up to 5 mg of recombinant antigen can be rapidly purified from one litre of bacterial culture. Two monoclonal antibodies have been raised against GST/23 and these bind to *T. mutans* piroplasms. Binding occurs at the piroplasm surface and can be inhibited by competition with GST/23 as shown by immunoelectron microscopy.

A small comparative study has been carried out using a panel of sera from naturally-exposed cattle in East and West Africa in immunoblots of *T. mutans* piroplasm lysates and ELISAs using GST/23. The intensity of binding to a 32 kDa band in immunoblots shows good correlation with ELISA values. Recombinant 32 kDa antigen may, therefore, be useful in determining *T. mutans* infection of cattle.

### E2c.3 Identification and characterization of genes encoding diagnostic antigens of *Babesia bigemina*

**Research Fellow:** N. Tebele
**Supervisors:** A. Musoke, V. Nene, S. Morzaria
**Research Associates:** J. Katende, R. Skilton

Previous studies using immunoblotting have shown that sera from cattle in areas endemic for *Babesia bigemina* react with a 200 kDa antigen of this parasite. Monoclonal antibodies (MAbs) to this antigen have been characterized and some of these are currently being used in an ELISA to detect antibodies to *B. bigemina* infection. The antigen used in this assay is a semi-purified lysate of parasites from cattle with high piroplasm parasitaemia. The quality of this antigen is difficult to standardize and varies from batch to batch. This compromises the sensitivity and specificity of the test. To standardize the antigen, recombinant protein or synthetic peptides corresponding to epitopes recognized by sera from *B. bigemina*-infected cattle are being developed.

A cDNA library of isolated merozoites from the Kikuyu stock of *B. bigemina* was constructed in lambda gt11 and a portion of the library screened using sera from three rats immunized with immunoaffinity purified 200 kDa antigen. Immunoscreening revealed 65 positive clones and 20 of these had DNA inserts that varied in size from 3.8 kb to 600 bp.

One plaque with a 3.8 kb insert reacted strongly with sera from cattle in a *B. bigemina* endemic area, sera from animals experimentally infected with *B. bigemina* merozoites, and a panel of 12 MAbs to the 200 kDa antigen. The insert DNA is a single gene copy and has a transcript size of 4 kb. The 3.8 kb insert was purified and a $^{32}$P-labelled probe made from it hybridized to *B. bigemina* genomic DNA and RNA, but did not hybridize to bovine lymphocyte DNA. The 3.8 kb insert has been cloned into a pGEX expression plasmid vector and a glutathione S-transferase fusion protein produced. The fusion protein was purified using a glutathione sepharose affinity chromatography matrix. A comparative antibody capture ELISA using the fusion protein and parasite lysate as antigen and sera of known reactivity to *B. bigemina* gave similar results. This suggests that the critical diagnostic 200 kDa epitopes are encoded in the 3.8 kb insert.

## E3a.1 Identification of B- and T-cell epitopes on the p67 sporozoite surface antigen of *Theileria parva*

**Scientists:** A. Musoke, D. McKeever, V. Nene
**Research Associate:** J. Katende
**Technicians:** E. Awino, J. Ngugi

In previous experiments to assess the protective capacity for cattle of a recombinant sporozoite surface antigen, NS1-p67, the clinical responses to challenge varied from complete protection with no evidence of disease to severe and fatal reactions. The degree of protection could not be correlated with the titres of antibody as measured by ELISA or the capacity of the sera to effect neutralization of sporozoites infectivity. In search of a correlate, it was investigated whether or not animals that exhibit complete protection produce antibodies reacting with different B-cell epitopes from those that undergo severe reactions. Overlapping peptides covering the entire sequence of the p67 molecule were synthesized by Chiron Mimotopes. The peptides were 15 amino acids in length with an overlap of seven residues. Two peptides from an unrelated theilerial protein served as controls for background reactivities. The peptides were tested by ELISA using neutralizing monoclonal antibodies (MAbs) generated against recombinant and native p67 antigens, and sera collected from cattle immunized with NS1-p67 or exposed naturally in the field.

The neutralizing MAbs recognized four distinct peptides (epitopes), three being recognized by MAbs raised against recombinant p67 and one by antibody TpM12, which was raised against the native molecule. The bovine immune sera recognized only one of the peptides reactive with the MAbs. The rest of the peptides reactive with bovine sera represented sequences at either end of the molecule. No sera reacted strongly with peptides from the middle of the p67 molecule. Field sera produced heavy backgrounds, so that interpretation of the data was not possible.

A statistical analysis was carried out on the data generated from the immunized cattle that were either severe reactors (susceptible) or non-reactors (immune) to challenge. The sample size in each group was eleven. Non-reactors had a significantly higher antibody response to peptides 5, 20 and 74. The significance of this finding is unclear since these sequences do not correspond to those bound by the MAbs.

Further characterization of the bovine immune responses to the p67 antigen has also included efforts to identify T-cell epitopes. A complete set of cleaved 15-mer p67 peptides overlapping by seven residues was purchased from Chiron Mimotopes for use in T-cell proliferation assays. Each peptide is N-terminally acetylated and carries a C-terminal diketopiperazine moiety as a result of the cleavage reaction. Peptides were resuspended in a multipurpose peptide solution buffer recommended by the manufacturers, and added at a final concentration of 10 mg/ml to cultures of peripheral blood monocytes (PBM) derived from an animal immunized with native p67. Cultures were maintained for four days and proliferation was measured by the addition of $^{125}$IUdR for the final eight hours. Two major immunogenic peptide sequences were identified on the molecule, EVADGIWKLSTIPDK (residues 433–447) and ESSFVIKISDMMRRR (residues 529–543). The peptides are currently being used to screen PBM derived from cattle that have been challenged after immunization with NS1-p67 in an effort to determine associations between T cell specificities and protection.

## E3b.1 Generation of improved expression constructs in vaccinia virus for the p67 sporozoite surface antigen of *Theileria parva*

**Scientist:** D. McKeever
**Research Associates:** R. Kennedy, N. MacHugh
**Technician:** W. Mwangi

Two improved p67-based plasmid constructs have been designed and incorporated into recombinant vaccinia viruses. The first of these was designed to give rise to improved surface expression of the antigen on virus-infected cells. It is based on a chimaeric gene in which the final 228 bp of the p67 gene are replaced with the 3′ terminal 85 bp of the *Trypanosoma brucei* ILTat 1.3 variable surface glycoprotein gene, which directs the addition of its glycerophosphoinositol linkage. Cells infected with the recombinant virus (VVp67S2) show good surface expression of the chimaeric antigen, and the virus will be tested in immunization experiments in cattle.

An additional, modified p67 recombinant virus has been made in which the first 66 bp of the gene, which encode the antigen's putative signal sequence, have been deleted. This manipulation should prevent co-translational transport of the gene product into the endoplasmic reticulum, and so enhance its processing for class I MHC presentation. The virus will be used in studies of the inductive requirements of cellular cytotoxic immune responses.

## E3b.2. Effects of molecular modification on the immunogenicity of recombinant vaccinia virus constructs

**Scientists:** M. Juarrero, D. McKeever
**Technicians:** W. Mwangi, F. Mbwika

Two modifications of the Wyeth p67 recombinant virus have been made with deletions of the serpin (SPI and SPII) genes respectively. These viruses were tested in mice for enhancement of cellular and humoral immune responses to the p67 antigen. No clear enhancement was observed, although virus-specific responses appeared stronger in mice immunized with the wild-type parent virus than in those immunized with recombinants.

Recombinant vaccinia viruses incorporating the genes encoding the bovine cytokines IL-2 and IL-6 have been generated. The IL-4 recombinant described previously has been shown to produce biologically active cytokine in infected cell cultures. Cytokine activity in supernates from cultures infected with the IL-2 and IL-6 recombinants has not been evaluated. In two experiments, cattle have been inoculated with the Wyeth p67 recombinant in conjunction with either the IL-2 or IL-4 containing viruses, and their immune responses to the p67 antigen examined. The immunization protocols employed have not revealed an immune-enhancing effect of these cytokine containing recombinant viruses for the p67 antigen, but further immunization experiments will benefit from development of assays of bovine IL-4 function (see abstract T2b.3).

## E3b.3 Novel adjuvant systems as safe and efficient non-recombinant antigen delivery vehicles

**Student:** A. Diaite
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A comparison of complete Freund adjuvant, the RIBI adjuvant system, the pluronic polyol-based Titermax system and ISCOMs for the induction of immune responses in cattle to soluble antigens has been reported previously. Analyses of the humoral responses of groups of cattle immunized with trypanosomal variable surface glycoprotein (VSG) in the context of the different delivery systems have been made. An ELISA has been developed for detection of total VSG-specific antibody and for analysis of the isotype specificity of the response. Titres are determined by comparison of each serum OD reading with a standard curve derived from a hyperimmune serum included in each assay.

These analyses have revealed that antibody responses are similar between the groups, both in quantity and IgG isotype specificity. In light of observations reported previously that T-cell responses were greater in animals immunized with VSG ISCOMs than in the other groups, these results suggest that enhanced T-cell priming by ISCOMs may not be associated with more effective B cell help.

## E3c.1 Inductive requirements of *Theileria parva*-specific cytotoxic T lymphocytes (CTL)

**Scientists:** E. Taracha, D. McKeever
**Technician:** E. Awino

Work with murine and human cells has generated conflicting data suggesting that $CD8^+$ CTL specific for certain antigens can be induced *in vivo* and *in vitro* with or without help from $CD4^+$ T cells. Using highly purified (96–99%) populations of $CD4^+$ and $CD8^+$ T cells co-cultured with autologous *T. parva*-infected lymphoblasts as antigen presenting cells (APC), it has been shown that $CD4^+$ T cells are crucial to the induction of naive and memory bovine $CD8^+$ CTL.

To study the effect of $CD4^+$ T cells, co-cultures of primed $CD4^+$ and primed $CD8^+$ T cells as well as primed $CD4^+$ and unprimed $CD8^+$ T cells with APC were shown to generate $CD8^+$ CTL activity comparable to that of unfractionated immune cells. This activity was higher than that generated in control cultures of $CD4^+$ or $CD8^+$ T cells as responders. Activity in cultures of unprimed $CD4^+$ and primed $CD8^+$ T cells was mid-way between these two. Experiments utilizing $CD4^+$ T cells primed to a non-*T. parva* antigen as helper cells generated *T. parva*-specific $CD8^+$ CTL only from immune but not naive $CD8^+$ T cells. This activity was nearly twofold higher when monocytes (4-cell cluster) presented the non-*T. parva* antigen than when autologous *T. parva*-infected lymphocytes (3-cell cluster) were used as APC.

To define the signals delivered tc $CD8^+$ T cells from antigen-specific $CD4^+$ T cells, a supernatant of T-cell growth factors (TCGF) or recombinant bovine interleukin-2 (rBIL-2) has been added to co-cultures of unprimed or primed $CD8^+$ T cells with APC. CTL activity comparable to that obtained with $CD8^+$ T cells in the presence of $CD4^+$ T cells and APC was observed only with cultures of primed but not unprimed $CD8^+$ T cells as responders. Further experiments to examine whether contact between immune $CD4^+$ and naive $CD8^+$ T cells is an essential requirement were performed using a two-chamber culture plate with a semi-porous membrane. Unprimed or primed $CD8^+$ T cells were co-cultured with APC in the outer chamber and primed $CD4^+$ T cells were co-cultured with APC in the inner chamber. Cytotoxic activity was observed only with cultures of primed but not unprimed $CD8^+$ T cells. Experiments to examine the influence of the APC phenotype on the induction of $CD8^+$ CTL utilized *T. parva*-infected $CD4^+$, $CD8^+$, $\gamma\delta$ T cells and unfractionated infected cells. Co-cultures of primed $CD4^+$ and primed $CD8^+$ as well as primed $CD4^+$ and unprimed $CD8^+$ T cells with infected $CD4^+$ or infected $\gamma\delta$ T cells generated much higher killing levels than those cultures with infected $CD8^+$ T cells or unfractionated infected cells.

It is evident from these results that *Theileria*-immune and naive $CD8^+$ T cells require help from antigen-specific $CD4^+$ T cells to generate CTL activity. However, while TCGF or rBIL-2 can replace the need for $CD4^+$ T cells by primed $CD8^+$ T cells, these factors cannot induce $CD8^+$ CTL from naive $CD8^+$ T cells in the presence of APC. The results further suggest that contact signals from immune $CD4^+$ T cells to naive $CD8^+$ T cells are important for the induction of $CD8^+$ CTL. It also appears from the results that the phenotype of the APC is critical to the induction of $CD8^+$ CTL.

The observed differences between primed and unprimed $CD8^+$ T cells for input from immune $CD4^+$ T cells will be examined. It is also planned to extend these studies to soluble defined antigens presented by various APC to T cells.

### E3c.2 Development of an antigen presentation assay for bovine cytotoxic T lymphocytes (CTL)

**Scientists:** C. Daubenberger, E. Taracha, D. McKeever
**Research Associate:** R. Kennedy
**Technicians:** D. Ngugi, E. Awino

Defined antigens chosen for this work are the 11E3 clone (supplied by Dr. Joachim Lipp of the University of Berne, Switzerland) and the p104 rhoptry antigen of *Theileria parva* sporozoites. The genes that encode these antigens were each cloned in the p1114 vaccinia virus shuttle vector and recombinant viruses have been produced that incorporate the genes, based on the WR vaccinia strain. In the case of the 11E3 antigen, two transfection constructs have been made, both in the pRcCMV plasmid, with expression being driven by the immediate early promoter of cytomegalovirus (CMV). One of these constructs incorporates the intact gene, while the other has had the signal sequence deleted through the use of a strategic restriction enzyme site. The p104 gene has been cloned in the pSG5 plasmid under the control of the SV40 promoter.

The VVp104 recombinant virus has been inoculated into a steer, and humoral and cellular immune responses of this animal to p104 are currently being evaluated. It is hoped to generate p104-specific CTL clones from this animal for use in antigen presentation studies with transfected cell lines.

Bovine fibroblastoid lines generated from bone-marrow biopsies have been evaluated for use as target cells in antigen specific CTL assays. A line derived from animal BK261, which is homozygous for the MHC locus of sire 103, has been transformed by transfection with SV40 elements under the control of the human vimentin promoter. Unlike the parent line, the transformed line grows well in 2% foetal calf serum (FCS) under antibiotic G418 selection, and displays altered growth behaviour. However, FACS analysis has revealed that the transformed line and its parent line are deficient in class I MHC expression, and this is reflected in their failure to act as targets for alloreactive CTL clones. New primary cell lines have now been established from skin and muscle tissue collected from the same animal, and these will be transformed with the SV40 construct.

## E3c.3 Limiting dilution assay for T helper cells in *Theileria parva*-immune calves

**Scientists:** J. Scheerlinck, T. Van de Putte*, B. Goddeeris*
**Technician:** D. Ngugi

*Catholic University of Leuven, Belgium

The intensity of *T. parva*-specific CD4+ T-cell responses in young cattle immunized by infection and treatment is largely unknown. A limiting dilution assay for CD4+ T cells has been established to quantify these responses in calves. The assay was first optimized using fixed *T. parva*-infected cell lines as stimulators but has subsequently been adapted for soluble antigens presented by monocytes. Results obtained using this assay indicate that young animals mount strong parasite-specific CD4+ T-cell responses after infection-and-treatment immunization.

The possibility that a limiting dilution assay of CD4+ T cells represents an alternative to the use of cloned parasite-specific CD4+ T cells for defining the specificity of these responses in antigen screens has been explored. A possible complication is that a proportion of parasite-specific CD4+ T cells may recognize antigens that are exposed only when the parasitized cell has been lysed through other mechanisms, and may therefore exert no effector role against the intact infected cell. To address this question, the frequency of CD4+ T cells responding to fixed *T. parva*-infected cells was compared with that of cells responding to purified schizont antigens presented by blood monocytes and that of cells proliferating in the presence of both antigen presenting systems. It was reasoned that if both types of stimulators present different sets of peptides, the frequency of cells responding to the mixture would equal the sum of the frequencies of those cells responding to each stimulator individually. It was found, however, that all three frequencies were essentially similar, suggesting that fixed *T. parva*-infected cells present the same set of peptides as antigen presenting cells processing purified theilerial schizonts. This suggests that T-helper cells generated against isolated schizont-derived antigens might be restimulated during challenge by peptides presented on the surface of *T. parva*-infected cells during challenge.

## E3c.4 A role for γδ T cells during infection of young cattle with *Theileria parva*

**Scientists:** C. Daubenberger, E. Taracha, D. McKeever
**Technicians:** D. Ngugi, E. Awino

Previous observations have indicated that a proportion of cattle immunized against *Theileria parva* withstand homologous challenge with the parasite in the absence of detectable CD8+ cytotoxic T lymphocytes (CTL). This phenomenon is more frequent in young cattle. T cells bearing the gamma/delta antigen receptor (γδ T cells) constitute up to 50% of T cells in peripheral blood and lymphoid organs of calves below six month old. This prompted us to investigate the involvement of γδ T cells in the immune response of young calves against *T. parva* (Muguga).

Three calves were immunized with *T. parva* at the age of 12 weeks. One was infected with *T. parva* sporozoites and treated 13 days later with buparvaquone, while the two remaining calves were inoculated with *T. parva* sporozoites and treated simultaneously with a long-acting formulation of tetracycline.

A limiting dilution analysis of γδ T cells sorted from peripheral blood of the calf treated with buparvaquone was conducted to determine if the frequency of proliferating γδ T cells changed during the course of a primary infection. Three stimulator cell populations, autologous Con A blasts, autologous heat-stressed (HS) Con A blasts and autologous *T. parva*-infected cell lines were used in the assay. This analysis revealed that *T. parva*-specific γδ T cells were present at low frequencies prior to immunization, while those responding to Con A blasts or HS Con A blasts were not detectable. Frequencies of *T. parva*-specific γδ T cells and those responding to HS Con A blasts increased dramatically during immunization and dropped after treatment with buparvaquone.

Upon challenge at the age of 17 weeks with the immunizing parasite, the calves were assessed for the capacity to generate cytotoxic activity against autologous infected cells. Co-cultures of peripheral blood mononuclear cells (PBMC) and autologous infected cell lines were established and assayed after one week for killing activity. Cytotoxicity was detected only in the calf immunized by infection followed by buparvaquone. Specific activity was as high as 50% at an effector-to-target ratio of 40:1, and was abrogated by the addition of monoclonal antibodies specific for CD8 and class I MHC but not class II MHC molecules. All of the three calves were protected against challenge.

The calves were re-challenged at the age of 20 weeks. Bulk culture analysis of cytotoxic activity during the challenge revealed specific activity between 20 and 50% at effector-to-target ratios of 40:1 in all three animals. Killing activity was parasite specific and class I MHC restricted. Interestingly, cytotoxicity in PBMC derived from the calf immunized by infection followed by buparvaquone treatment was reduced by up to 50% after depletion of γδ T cells from the effector population.

To investigate the basis of the reactivity of bovine γδ T cells for autologous infected cell lines, a panel of γδ T-cell clones has been generated. These cell lines express GB21A$^+$, defined by a monoclonal antibody specific for the bovine γδ T-cell receptor, as well as expressing the WC1 specificities CD3, CD2, CD4, and a proportion are CD8$^+$. The clones proliferate specifically in the presence of autologous *T. parva*-infected cell lines, but it has not yet been established whether they are cytotoxic.

## E3c.5 Identification, purification and partial characterization of immunoreactive hsp 70 protein(s) of *Theileria parva*

**Scientists:** C. Daubenberger, D. Nandan, M. Shaw, Y. Yagi, D. McKeever
**Research Associate:** R. Kennedy
**Technician:** D. Ngugi

Preliminary results suggest that a proportion of bovine γδ T cells are activated specifically by autologous *Theileria parva*-infected cell lines and by heat-stressed Concanavalin A blasts, and that the frequency of these cells increases dramatically during primary exposure to the parasite. It has been established that a proportion of

γδ T cells in mouse and human are activated by stress proteins. We have examined the possibility that *T. parva*-specific γδ T cells recognize stress proteins by characterizing the expression of the heat shock protein (hsp) 70 family of stress proteins in *T. parva*-infected cell lines.

We have isolated members of the *T. parva* hsp 70 family by using their gelatin-sepharose binding properties. This affinity chromatography technique is presumably based on the chaperonin activity of these proteins and has already been used to isolate hsp 70 proteins from trypanosomes, liver cells and fibroblasts.

A number of sera derived from *T. parva*-immune cattle reacted specifically with these isolated stress proteins on immunoblots, while no reactivity was found against commercially available bovine hsp 70 and hsp 70 proteins isolated from Con A blasts.

Rat polyclonal sera have been raised against the *T. parva*-derived hsp 70. One of these sera gives rise to intense punctuate staining of the schizont cytoplasm in immunofluorescence analysis of infected cells; staining of the host cell cytosol is variable and of lower intensity. The latter observation can be explained by cross-reactive epitopes located on bovine and parasitic stress proteins, since hsp 70 proteins are known to be highly conserved. Staining of the schizont is markedly intensified by prior incubation of the infected cells at 42 °C for 3 hr. Host cytoplasm staining is unaffected by this treatment. In an analysis of a number of cell lines infected with the Muguga stock of *T. parva*, staining of the host cytosol has been observed to be highly variable. In no case has staining been observed in the nucleus, even after heat stress.

Immunoprecipitation studies of metabolically labelled *T. parva*-infected cells and HS con A blasts have confirmed the specificity of this serum for hsp 70 proteins. Interestingly, the molecular species present in HS con A blasts is invariably smaller than the parasite-associated molecule. To determine if the elements recognized by this serum are present in a number of parasite stocks, cell lines derived from a T-cell clone infected with the Muguga, Marikebuni, Boleni or Lawrencei stocks of *T. parva* were examined by immunofluorescence. No differences were observed in the staining characteristics of these lines.

The N-terminal sequence of 14 amino acids of one of the isolated *T. parva* stress proteins has been determined and shows 92% homology with the human glucose regulated protein 75.

## E3c.6 Generation of monoclonal reagents specific for bovine T-cell subsets

**Research Associate:** N. MacHugh
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Studies to generate monoclonal antibodies (MAbs) specific for the bovine T-cell receptor (TCR) have employed three different approaches to generate transfected cells expressing bovine TCR molecules on the cell surface for immunization purposes. The extracellular domains of the bovine α and δ TCR chains were cloned in

pCDM8 downstream of the sequences encoding the murine immunoglobulin heavy chain transmembrane domain. Cells transfected with the δ-chain construct did not exhibit surface staining with the putative δ-specific MAb GB21A although the antigen was detected in low quantities in the cytosol. It was not possible to screen cells transfected with the α-chain construct because of the lack of appropriate reagents. In the light of these observations, it was decided that mouse immunizations were unlikely to succeed. In separate experiments, mice were immunized with material immunoprecipitated from bovine peripheral blood monocytes (PBM) with the CD3-specific MAb MM1A. This MAb has been shown to co-precipitate the TCR chains but immunized mice made no detectable antibody responses for this molecule on bovine lymphocytes. In a third approach, in collaboration with Dr. Gerd Pluschke (Ciba-Geigy, Basel, Switzerland), gene sequences encoding the constant region of the bovine TCR β chain have been cloned in an expression vector that allows the expression of a chimaeric TCR on the surface of a murine T-cell hybridoma. Transfected cells will be used to immunize mice in an attempt to generate MAb specific for the β chain.

Further characterization of the MAb GB21A has provided strong evidence that it reacts with the bovine δ TCR chain. Immunoprecipitation studies have confirmed that it reacts with molecules of the appropriate size for the TCR chains, and CD3 is co-precipitated. As already mentioned, the antibody has been shown to react with the bovine δ TCR chain in transfected cells.

### E3c.7 Depletion of T-cell subsets from cattle *in vivo*

**Scientists:** J. Scheerlinck, J. Naessens

Deletion of lymphocytes *in vivo* using monoclonal antibodies (MAb) has been well documented in mice and has played an important role in analysing the role of various lymphocyte subsets in a number of immune mechanisms.

This technology has been applied to cattle to study bovine specific immune mechanisms. Other workers have observed that intravenous administration of MAb specific for T-cell subsets results in significant depletion of the corresponding subsets in peripheral blood but not in other lymphoid compartments. In addition, intravenous inoculation of MAb for prolonged periods is sometimes associated with adverse reactions. Thus, a modified inoculation regime has been developed that allows the administration of larger quantities of MAb with minimal side effects. The extent of depletion has been monitored by fluorescence activated cell sorter analysis of blood and lymph node aspirates. Significant depletion of the appropriate subset was observed in these samples. In addition, the functional significance of $CD4^+$ T-cell subset depletion was examined by measurement of the reactivity of peripheral blood mononuclear cells to Foot and Mouth Disease Virus (FMDV) antigen, against which the animals were immunized prior to depletion.

Analysis of $CD4^+$ T-cell function was performed in two FMDV-immune calves after recovery of normal T-cell counts in peripheral blood following depletion of $CD4^+$ and $CD8^+$ T cells. Specific T-cell proliferation was undetectable in both animals for six weeks after cessation of the depletion process. However, specific

activity was observed to have re-appeared spontaneously in one animal by day 59. Both animals recovered normal proliferative responses after re-immunization with FMDV. These preliminary results suggest that abrogation of CD4+ T-cell memory may be possible by depletion of peripheral CD4+ T cells. Further, studies of the extent of depletion in lymphoid organs and additional optimization of the depletion procedure are required before this technique can be used as an effective tool for the investigation of the role of different lymphocyte subsets in bovine immunity.

## E3c.8 Development of nucleic acid-based typing techniques for bovine class II MHC products

**Scientists:** G. Russell*, D. Fraser*, R. Spooner*, D. McKeever
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To develop nucleic acid-based methods for bovine class II MHC typing polymerase chain reaction (PCR), primers were first designed to amplify bovine class II DRB and DQB polymorphic regions using messenger RNA derived from a panel of nine MHC-homozygous Boran cattle generated at ILRAD. Amplified products were then cloned and sequenced. Data from multiple clones indicate that the major expressed DRB and DQB class II genes of cattle are DRB3 and DQB1, and also reveal a high proportion of new alleles. These sequences were used to design additional PCR primers to amplify segments of these genes for typing purposes. A number of PCR-based typing techniques were examined, and two complementary methods were chosen that would be applicable to a range of class II genes. Types are assigned by analysis of restriction fragment length polymorphism (RFLP) in PCR-amplified segments of the class II gene; the presence or absence of a series of restriction enzyme sites is determined from the sizes of the restriction fragments. The PCR-RFLP assignments are then checked and confirmed by assessment of single-strand conformation polymorphism (SSCP), in which sequence-dependent variation in the migration of the denatured PCR products is used to distinguish different class II genes. These methods have been used on genomic DNA to type the DRB3 alleles carried by the homozygote panel, and both SSCP and PCR-RFLP split the nine animals into the same 5 allelic groups.

Both the PCR-RFLP and SSCP methods for DRB typing will be used at ILRAD to evaluate MHC-related effects on the efficacy of subunit vaccines in field trials. However, since DQB is also expressed by bovine antigen presenting cells, the available tests will not be sufficient for this purpose. Primers have been designed and restriction enzymes selected for DQB typing by the same two methods and these are currently being tested with material from the homozygote panel.

Analysis of DQB cDNAs prepared from the homozygote panel has produced unexpected results. While DRB expression appears to be essentially conserved, it also appears that individual animals express more that one DQB product, and that this variation is the result of gene duplication. This observation has implications for MHC-related effects on the efficacy of subunit vaccines.

# TRYPANOSOMIASIS

## T1a.1 Design of a prevalence study of cattle trypanosomiasis using antigen detection ELISA in Northern Kwazulu Land, Republic of South Africa

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Cattle trypanosomiasis in Northern Kwazulu Land causes substantial financial losses. A recent report estimates that 171,600 (55%) cattle from a population of 313,624 cattle in three districts are at risk of being infected with trypanosomes. A total of 115,788 treatments with ethidium has been administered to cattle since 1990. The total cost of the drug itself is about US$ 115,788.00. The total expenditure increases substantially when costs of administering the drug, vector control and losses in production are added.

Past surveys using thin and thick blood smears from 20 emaciated cattle selected at dip tanks in the area indicated that 10 to 15% of these cattle were infected with *Trypanosoma congolense* and/or *T. vivax*. Even though trypanosome infections were detected, failure to adopt formal random techniques prevented accurate estimation of the prevalence of trypanosomiasis in the region. It is therefore impossible to accurately compare point prevalence estimates before and after the introduction of disease control measures, or to precisely monitor changes in disease occurrence over a period of time. Moreover, the low sensitivity of the method employed in the diagnosis of trypanosomiasis severely limits the accuracy of the results. The objective of this project was to assist the Onderstepoort Veterinary Institute and the Kwazulu Government in designing an unbiased cross-sectional study of cattle trypanosomiasis in Northern Kwazulu Land, using the antigen detection ELISA test.

Each of the three districts at risk is divided into sections and one or more sections is or are assigned to a dip tank. There are 196 sections and 106 dip tanks. If a dip tank is servicing more than one section, cattle from each section visit the dip tank on different days of the week. A multistage sampling design was planned for this study. The primary unit is the section. A list frame of all sections in each district was compiled. Sections were randomly selected, with probability proportional to the number of cattle they contain. The sample size determination of sections is based on the percentage of sections in each district. The secondary unit is cattle. Cattle will be selected systematically as they go through the dip tank. The sample size of cattle is based on a simple random sampling design for each district. The estimated prevalence is 50% with a required precision of 5% for a level of confidence of 95%. Based on these parameters the calculated sample size is 383 cattle per district. Since there will be intra-section correlation, the factor $1+(x-1)\rho$ where x is the mean cattle-section size and $\rho$ is the intra-section correlation coefficient (which measures the non-independence of cattle in same section) to inflate the sample size was used. In this case $x = 1600$ cattle and $\rho$ is estimated at 0.2. The adjusted sample size per district is 703 cattle. The study was designed to provide valid estimates of the extent of trypanosomiasis in the area. These estimates have the advantages that they can be compared in subsequent years to determine the effects of trypanosomiasis control programs and

that they can show changes in the occurrences of trypanosomiasis over a period of time.

## T1a.2 Investigation of high prevalence of trypanosome infections in cattle from an area of Northern Zanzibar thought tsetse-free

**Scientists:** P. Lessard, R. Masake, P. Majiwa
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Tsetse fly eradication has been chosen as the goal of trypanosomiasis control on the island of Unguja, Zanzibar. This decision is based on epidemiological and ecological conditions of the island. The island is protected by a 30 km natural barrier, and only one tsetse species, *Glossina austeni,* is known to be responsible for trypanosome transmission. The current eradication program is designed to attack the problem from two sides: vector and parasite control. Vector control includes (i) the application of synthetic pyrethroid insecticides on living targets (livestock) via pour-on formulations or dip tanks, (ii) the use of insecticide impregnated screens and (iii) the help of the Sterile Insect Technique (SIT). Parasite control includes (i) the treatment of all infected animals with Berenil® and (ii) the control and treatment of imported livestock from the main land.

The impact of the control program on the prevalence of trypanosome infections of cattle was initially monitored using the microhaematocrit centrifuge technique (HCT). However, this parasitological technique which is highly specific is of low sensitivity. Therefore, to better estimate the prevalence of cattle trypanosomiasis on Unguja Island, the antigen-detection ELISA technique was utilized.

In November 1992, blood and serum samples were collected from cattle in the Northern districts. The area was not included in the previous eradication program because *G. austeni* has never been identified, the region has been described by entomologists as ecologically unsuitable for the fly, clinical cases of trypanosomiasis have never been recorded and no prior survey has found the parasite in cattle of the region. Results of the HCT agreed with the clinical evaluation (clinical signs and packed cell volume) of trypanosomiasis in the region. All HCT tests were negative. However, the results of the antigen detection ELISA test estimated the prevalence of trypanosome infections to be approximately 50%. If substantiated by other diagnostic methods such as DNA probes, antibody-detection ELISA and the mouse inoculation technique, the results imply that a high level of transmission is occurring in the area and that the control measures should be extended to the Northern districts.

## T1a.3 African Trypanotolerant Livestock Network (ATLN): tsetse component

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Earlier studies at the ATLN site in the Ghibe Valley, southwest Ethiopia, have demonstrated the occurrence of a high level of resistance in trypanosome isolates, principally *T. congolense,* to all available trypanocidal drugs. As a result of this, from 1986 to December 1990, trypanosome prevalence in cattle was rising annually and the condition of cattle was deteriorating. In January 1991 a trial was started using Cypermethrin-high-cis as a 'pour-on' insecticide to control *Glossina pallidipes, G. fuscipes* and *G. morsitans submorsitans* at the Tolley/Gullele site in the Ghibe Valley. This study was initiated in order to determine the effectiveness of this tsetse control method in reducing trypanosome prevalence in cattle where resistance to trypanocidal drugs was a serious constraint.

Between 2000 and 3000 cattle were treated at monthly intervals with a synthetic pyrethroid (RS-alpha-cyano-3-phenoxybenzyl (IRS)-cis, trans-3-(2.2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate), at a dosage of 1 ml/10 kg body weight. Treatment was given as a pour-on application along the backline, using an automatic drench gun applicator. Ovarian aging of tsetse flies has provided data to estimate the increased mortality in the tsetse population. By September 1993, the tsetse population was reduced by more than 95% compared with the pre-control level. Trypanosome prevalence in cattle was also reduced by up to 75% despite a high level of trypanocide resistance. A significant reduction in numbers of other biting flies also occurred.

Further studies on data from this site are being carried out to examine relationships between tsetse density, mortality, normalized difference vegetation index and climatic variables.

## T1a.4 Maintenance of production colonies of different *Glossina* species

**Scientist:** S. Moloo
**Technicians:** N. Kuria, M. Owino, A. Kafwa

The ILRAD Tsetse Vector Unit maintains breeding colonies of tsetse flies. Trypanosome parasites normally pass through several stages of their life cycle in the tsetse fly. Although it is possible to maintain trypanosomes *in vitro* and to infect laboratory animals and livestock by injecting parasites into the animals, trypanosomes maintained and transmitted artificially can differ from parasites transmitted naturally through the bite of an infected tsetse fly. For this reason, a great deal of the trypanosomiasis research conducted at ILRAD requires trypanosomes that have developed in tsetse flies.

Five tsetse breeding colonies were maintained at ILRAD in 1993. These were *Glossina morsitans centralis* which originated from Singida, Tanzania; *G. pallidipes*

from Shimba Hills, Coast Province, Kenya; *G. pallidipes* from Nguruman, Rift Valley Province, Kenya; *G. brevipalpis* from Kibwezi Forest,Kenya; and *G. longipennis* from Nguruman, Kenya. All five breeding colonies were maintained at 25 °C and were fed five days a week on the ears of half-lop rabbits. *G. m. centralis* and *G. longipennis* were kept at 70% relative humidity and all the others at 80%. The mean numbers of breeding females of *G. m. centralis, G. pallidipes* (Shimba Hills), *G. pallidipes* (Nguruman), *G. brevipalpis* and *G. longipennis* colonies were 8908, 2794, 2754, 1516, 1298; they produced 262,105 (mean wt. 34.5 mg), 72,455 (41.1 mg), 60,380 (41.9 mg), 41,139 (76.0 mg) and 29,479 (76.6 mg) puparia, respectively. A colony of *G. fuscipes fuscipes* was initiated in January 1992 with 500 wild puparia from Mbita, Rusinga Island, Kenya. This colony is at the expanding phase and at the end of August 1993, it had 750 breeding females and had produced 9954 pupae (mean wt. 23.6 mg). A *Glossina austeni* colony was initiated in September 1992 with 200 wild puparia from Muhaka Forest, Shimba Hills, Coast Province, Kenya. The colony reached the target of 1400 breeding females in mid-August 1993 and produced 10,334 pupae (mean wt. 21.7 mg) by the end of August 1993.

The five production colonies provided all the tsetse required for trypanosomiasis research conducted at ILRAD in 1993 and also provided a resource for research groups at the International Centre of Insect Physiology and Ecology (ICIPE), the Kenya Trypanosomiasis Research Institute (KETRI), the University of Nairobi, Kenyatta University, the Kenya Medical Training College, the Universities of Bristol and Wales in the UK, the Swiss Tropical Institute and Texas University, USA.

## T1a.5 Genetics of two colonies of *Glossina pallidipes* originating from allopatric populations in Kenya

**Scientists:** R. Gooding*, S. Moloo
**Technicians:** B. Rolseth*, N. Kuria, M. Owino, A. Kafwa

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Two colonies of *Glossina pallidipes,* each with more than 2500 breeding females, were established recently with material collected from allopatric populations in Kenya. The Shimba Hills colony was established in October 1986 from 119 puparia that were produced by 88 wild-caught females, and the Nguruman colony was established in January 1989 from 447 puparia that were produced by 551 wild females. Most bionomic parameters (insemination rates, female survival rates, puparial weights and emergence rates) did not differ between the colonies. However, fecundity (puparia/female/reproductive cycle) of the Shimba Hills colony females was significantly greater than that of the Nguruman colony females. Tsetse in these colonies differed also in that the Nguruman colony flies were significantly more susceptible to infection with two stocks of *Trypanosoma congolense.*

These two large colonies, originating from allopatric populations of *Glossina pallidipes* from the Shimba Hills and Nguruman, differ biologically and, with respect to vectorial competence, were compared at 14 enzyme-loci using polyacrylamide gel electrophoresis. The colonies had similar levels of genetic diversity with

approximately half of the loci being polymorphic, an average of 1.6 to 1.7 alleles per locus and a mean heterozygosity per locus of approximately 18.4%. However, the colonies differed significantly in allele frequencies at the loci for phosphoglucomutase, glucose-6-phosphate dehydrogenase, xanthine oxidase, octanol dehydrogenase and phosphoglucose isomerase. The results were compared with earlier studies on this species and no evidence was found for selection of specific alleles during establishment or maintenance of colonies of *G. pallidipes*, nor were specific chromosomes, or marker genes, associated with the biological differences between the two colonies.

## T1a.6 Comparative study of the susceptibility of different laboratory strains of *Glossina* species to *Trypanosoma simiae*

**Scientists:** S. Moloo, E. Zweygarth*
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*Trypanosoma simiae* causes hyperacute disease in domestic pigs with death occurring within hours to a few days after trypanosomes appear in the blood. The infection is transmitted to pigs by infected *Glossina* or directly by other haematophagous insects. Cyclical development of *T. simiae* was first studied in *G. morsitans morsitans* and it was found that the cycle undergone by this species is similar to that of *T. congolense*, another *Nannomonas* species. Warthogs, and probably also bush pigs, are natural reservoirs of *T. simiae* and any species of *Glossina* is a potential vector of *simiae* trypanosomiasis. The present study was undertaken to determine if there are differences in the susceptibility of four different laboratory-bred *Glossina* species belonging to the three main taxonomic groups, *fusca*, *palpalis* and *morsitans*, to three stocks of *T. simiae*.

Teneral *Glossina* species from the laboratory reared colonies were fed on four Large White pigs infected with three different stocks of *T. simiae* isolated in Coast Province, Kenya. Thereafter, the tsetse were maintained on goats and dissected on day 28 to determine the trypanosome infection rates. *Glossina brevipalpis* was as susceptible as *G. pallidipes* whilst *G. palpalis gambiensis* was not susceptible to the acute *T. simiae* stock CP 11 which was isolated from a wild *G. austeni*. *Glossina brevipalpis* was as susceptible as *G. pallidipes* to the other acute *T. simiae* stock CP 813 isolated from a wild *G. pallidipes*. *Glossina morsitans centralis* was also as susceptible as *G. brevipalpis* and *G. pallidipes* whilst *G. p. gambiensis* was not susceptible to this *T. simiae* stock. *Glossina morsitans centralis* showed very low susceptibility to the chronic *T. simiae* stock CP 1896 isolated from a bush pig, whilst *G. brevipalpis*, *G. p. gambiensis* and *G. pallidipes* could not be infected by this *T. simiae* stock. Male *Glossina* were generally more susceptible than females to the three *T. simiae* stocks. Thus, *G. brevipalpis*, a *fusca* group tsetse, and *G. m. centralis* and *G. pallidipes*, the *morsitans* group tsetse, were susceptible to the two acute *T. simiae* stocks, whilst *G. p. gambiensis*, a *palpalis* group tsetse, was not susceptible to these stocks. *Glossina morsitans centralis* showed low susceptibility to the *T.*

*simiae* stock, causing chronic infection, whereas *G. brevipalpis, G. p. gambiensis* and *G. pallidipes* could not be infected with the same stock of chronic *T. simiae.*

### T1a.7 A study on the maturation of procyclic *Trypanosoma brucei brucei* in *Glossina morsitans centralis* and *G. brevipalpis*

**Scientist:** S. Moloo
**Technicians:** J. Kabata, C. Sabwa, I. Okumu, F. Kaaria, N. Gitire, J. Muia

Teneral *Glossina morsitans centralis* and *G. brevipalpis* were fed *in vitro* upon medium containing procyclic *Trypanosoma brucei brucei* derived from the midguts of *G. m. centralis* or *G. brevipalpis* which had immature trypanosome infections. The tsetse were then maintained on rabbits and, on day 31, were dissected to determine the infection rates. In *G. m. centralis,* the midgut and salivary gland infection rates by *T. b. brucei* were 46.0% and 27.0% with procyclics from *G. m. centralis,* and 45.4% and 24.7% with procyclics from *G. brevipalpis,* respectively. In *G. brevipalpis,* the rates were 20.2% and 0.0% with procyclics from *G. m. centralis,* and 28.0% and 0.0% with procyclics from *G. brevipalpis,* respectively. Teneral *G. m. centralis* and *G. brevipalpis* were also fed similarly upon procyclic *T. b. brucei* derived from *G. m. centralis* or *G. brevipalpis* on day 31 of infection, the former tsetse species had mature infections while the latter were without salivary gland infections. In *G. m. centralis,* the infection rates in the midgut and salivary glands were 48.9% and 17.0%, and 38.0% and 17.0% when fed on procyclics from *G. m. centralis* and *G. brevipalpis,* respectively. In *G. brevipalpis,* the rates were 21.5% and 0.0%, and 10.7% and 0.0% with procyclics originating from *G. m. centralis* and *G. brevipalpis,* respectively. Thus, procyclic *T. b. brucei* from susceptible *G. m. centralis* could not complete cyclical development in refractory *G. brevipalpis,* whereas those from *G. brevipalpis* developed to metatrypanosomes in the salivary glands of *G. m. centralis.* Teneral and 15-day-old non-teneral *G. m. centralis* were fed *in vitro* upon heparinized goat's blood containing *T. b. brucei* bloodstream trypomastigotes, or upon medium containing procyclic *T. b. brucei* derived from *G. m. centralis* with mature infections. On day 31, their infection rates were determined. The infection rates of *T. b. brucei* in the midgut and salivary glands of *G. m. centralis* fed on the infected blood were 70.4% and 40.4% when fed as tenerals, as against 15.3% and 4.0% when fed as non-tenerals. Those tsetse which were fed on the procyclic-containing medium showed rates of 50.0% and 25.6%, as against 11.6% and 2.5%, respectively. It would appear, therefore, that the maturation of *T. b. brucei* in tsetse is probably not determined by a single lectin-procyclic interaction, but it is the result of a complex interaction between many interrelated physiological factors of both the trypanosome and the tsetse vector.

## T1b.1 Cloning and characterization of a *Trypanosoma congolense*-specific antigen released in the course of infection: identification of a thiol protease precursor

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The gene encoding the *Trypanosoma congolense* species-specific antigen occurs as an imperfect tandem repeat in the genomes of these parasites. The copy number is estimated to be between 20 and 30. Surprisingly no hybridization signals with blotted digests of DNA from *T. simiae* have been detected. This indicates that the gene is conserved in all groups of *T. congolense* but that the probe is a *T. congolense*-specific, rather than *Nannomonas*-specific sequence. These results are consistent with studies at the protein level, where the monoclonal antibody recognizing the protein encoded by this gene failed to react, on Western blots, with protein lysates prepared from *T. simiae*.

However, from hybridization analysis the gene is clearly more abundant in parasites of the 'Savannah' genotype (IL 3000 and IL 1180). In this cloned parasite, the cysteine protease locus has been markedly amplified as seen in *Not*I and *Sfi*I digests of chromosome-sized molecules. The copies of the gene in the chromosomes of the West African Riverine/forest clone appeared linked and clustered on one chromosomal restriction fragment of less than 290 kb. Additionally, the locus or loci in the Savannah-type *T. congolense* IL 3000 may have undergone gene amplification.

Northern blot analysis, using the cDNA insert cloned in the recombinant plasmid pcCCP-1600 as probe, revealed a transcript of 1.8 kb which was present in poly(A)$^+$ RNA prepared from all life-cycle stages of the Savannah-type *T. congolense* IL 3000. A complete nucleotide sequence of the cDNA in the pcCCP-1600 plasmid revealed a full length cDNA encoding a protein of 444 amino acids with a calculated Mr of 48,097 and a pI of 6.47. The sequence also has two potential N-linked glycosylation sites.

A homology search in databases revealed a high degree of identity of the deduced protein sequence to cysteine proteases from both animal and plant sources. Pairwise comparisons between the deduced sequence of *T. congolense*, designated TcCPP (*T. congolense* cysteine protease precursor), with those from other trypanosomes displayed 69–70% identity to the cysteine protease sequences recently reported for *T. brucei* group trypanosomes. The identity between the *T. congolense* TcCPP with its *Trypanozoon* parasite homologues occurred along the entire lengths of the deduced zymogen molecules and required the introduction of only one major gap. There are seven contiguous proline residues interrupted by one threonine at position 347 [$(PPP)_2TP$] in the C-terminal extension of 104 amino acids. This differs from the nine consecutive prolines [$PT(PPP)_3$] in this region of both the *T. brucei* subspecies near the junction with the central domain of the enzyme. The central region of the TcCPP sequence, which, like cysteine protease sequences from other *Kinetoplastida*, has considerable identity (45%) to the cathepsin L class of mammalian enzymes with an

overall overlap of 222 amino acids. This gene is one of two encoding antigens that are either secreted or released, by dying trypanosomes, into the circulation and other tissues of mammalian hosts. Characterization and availability of these antigens will provide standardized reagents for use in studies of trypanosome epidemiology.

### T1b.2 Characterization of *Trypanosoma vivax* and *T. congolense* species-specific recombinant antigens

**Scientists:** R. Masake, O. ole-MoiYoi, T. Urakawa, H. Hirumi
**Research Associate:** S. Minja
**Technicians:** J. Makau, J. Njuguna

Recently, genes encoding antigens specific for the trypanosome species *Trypanosoma vivax* and *T. congolense* were introduced into the Baculovirus expression system and clones secreting recombinant antigens which reacted with the trypanosome species-specific monoclonal antibodies (MAbs) were selected. The *T. vivax* recombinant antigen gave a strong reaction with MAbs Tv27 by antigen-detection ELISA and on Western blotting. The antigen reacted weakly with *T. congolense* and *T. brucei* specific monoclonal antibodies using antigen-detection ELISA but no reactivity was noted in Western blots. Furthermore, when the *T. vivax* recombinant antigen was employed in antibody-detection ELISA, it reacted with antibodies induced in *T. vivax*-infected cattle. On screening serial serum samples from experimentally infected cattle, the optical density (OD) by ELISA closely followed the parasitaemic profile of the animal, with the optical density falling to background levels soon after remission of parasitaemia. To facilitate incorporation of this antigen into ELISA kits for diagnosis of trypanosomiasis, storage and handling conditions were determined. Storage at –70 °C, –20 °C and 4 °C did not cause noticeable reduction in reactivity of the antigen with MAb Tv27, while storage at room temperature or 37 °C led to significant losses in the reactivity of the recombinant antigen as detected by OD levels in ELISA. Freezing and thawing of the recombinant antigen did not adversely affect its reactivity if high temperatures were avoided. The *T. congolense* recombinant diagnostic antigen is being similarly investigated for its suitability for incorporation into diagnostic assays.

### T1b.3 Characterization of trypanosome genus-specific monoclonal antibodies

**Scientists:** R. Masake, O. ole-MoiYoi, E. Authié
**Research Associate:** S. Minja
**Technicians:** J. Makau, J. Njuguna

In an attempt to make antigen-detection ELISA amenable for use in generalized epidemiological surveys of trypanosomiasis, genus-specific monoclonal antibodies (MAbs) were generated. These MAbs were screened for cross-reactivity with other protozoan parasites such as *Leishmania, Theileria, Babesia* and *Anaplasma* and have been shown to be specific for trypanosomes. Two of the trypanosome-specific MAbs

(TPM-1 and TPM-2) were compared for their ability to detect trypanosome infection in experimental animals infected with *Trypanosoma brucei, T. congolense* and *T. vivax*. TPM 1, an IgM, can trap trypanosome antigens in bovine sera as early as three days after infection in some cases, but reliably detects antigens at the same time or after the appearance of trypanosomes in the blood. The TPM 1 antibody traps antigen for a long time during the infection. In comparison to the species-specific antigen-detection ELISAs for the three species, assays incorporating TPM 1 reveals 22% more infected samples harbouring *T. congolense* antigens, 22% more samples of *T. brucei* and 18% more samples harbouring East or West African *T. vivax*. Modification of the procedures for this antigen-detection-ELISA has greatly improved the detection of infection with a concomitant reduction in the number of false negatives. TPM 2, an $IgG_1$, was slightly better than the species-specific MAbs in revealing trypanosome infection. TPM 2, being an $IgG_1$, will benefit from assay procedures using continuous shaking whilst the IgM antibody will be suitable for simpler, unshaken antigen capture assays.

## T1b.4 Characterization of *Trypanosoma congolense* parasites isolated from horses on the Del Monte Farm in Thika

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The Del Monte Co., Kenya Ltd. in Thika has 35 horses which were examined for a period of 14 months to establish the trypanosomiasis prevalence, incidence and to identify the species of trypanosomes involved. Blood samples obtained from the horses showing parasitaemia during this period were cryopreserved, providing 18 stabilates, for characterization of the trypanosomes. This was done on the basis of morphology and the use of DNA hybridization probes. The parasites were all identified as the Savannah-type *Trypanosoma congolense*. Molecular karyotyping of six trypanosome clones from different isolates was carried out using orthogonal field alternation gel electrophoresis (OFAGE) to determine whether they belonged to one or more serodeme. There were five different chromosome profiles displayed among these clones, and they were differentiated on the basis of the size and number of medium-sized chromosomes. The profiles resembled those of the Savannah *T. congolense* and differed from a Kilifi-type *T. congolense* used as a control.

Experimental infection of horses gave rise to the typical signs of trypanosomiasis with both acute and chronic disease being developed. Plasma samples obtained from the experimental horses were tested using the antigen- and antibody-ELISA techniques. Pre-infection sera gave negative antigen and antibody results to *T. congolense*. Following infection most horses began to show the presence of *T. congolense* antigens 2–3 weeks after infection, soon after the appearance of detectable parasitaemia. Antibodies to *T. congolense* were however detected over a variable period, in

the period 3–5 weeks after infection. Fluctuations in parasitaemia, antigenaemia and antibody levels were subsequently evident. No trypanosomes were detectable in these horses following treatment with Berenil® either using parasitological techniques or antigen/antibody detection assays.

### T1b.5 Comparison of antigen-detection ELISA, PCR and parasitaemia in revealing *Trypanosoma congolense* infections

**Scientists:** R. Masake, P. Majiwa, O. ole-MoiYoi, S. Moloo, J. Waitumbi
**Technician:** J. Njuguna

It has previously been shown that using the polymerase chain reaction (PCR) it is possible to amplify Savannah-type *Trypanosoma congolense* DNA in the presence of quantities of up to 10 µg of host DNA. Blood samples have been obtained from *T. congolense*-infected cattle, DNA extracted and used as target DNA in PCR. The target DNA was primed with oligonucleotides specific for either the Savannah or Kilifi genotypes of *T. congolense*. The PCR-based test detected trypanosome DNA in equal numbers of samples as the buffy coat technique for animals infected with *T. congolense*, Kilifi-type. On the contrary, the PCR technique was superior to the buffy coat technique in detecting trypanosome infections with Savannah-type *T. congolense*. PCR-positive blood samples have also been obtained from an animal treated with Berenil®, whereas during this same period there was a drop in optical density on antigen-detection ELISA for *T. congolense* infection.

### T1b.6 Application of *Trypanosoma congolense*-specific DNA probes to the characterization of parasite populations obtained from Burkina Faso

**Scientists:** R. Masake, P. Majiwa, G. Duvallet*
**Technicians:** J. Makau, A. Adema, H. Gathuo

*CRTA, Burkina Faso

Trypanosome isolates of the *Nannomonas* type were examined by DNA probe analysis to determine their subspecific type. The parasites (D ind/80/CRTA(3)s18, Sam/82/CRTA/31 and Sam/CRTA/28/s7), were fixed onto nylon paper and probed with the Savannah- (pg NRE 372), Kilifi- (pg N1K450) and Forest-type (TSW 103) repetitive sequence DNA probes. TSW 103 hybridized to isolate D ind/80/CRTA/3/s18 (IL 3874) while pg NRE 372 reacted with isolates Sam/82/CRTA/31 and Sam/CRTA/28/7. The Kilifi (pg N1K450) probe did not hybridize to any of the trypanosome isolates. Chromosomal DNA of the isolates were separated on OFAGE. The chromosomal profile obtained for the Forest-type lacked intermediate-sized chromosomes while the Savannah-type exhibited few intermediate-sized chromosomes. A further seven *Nannomonas* trypanosome isolates from Burkina Faso had more complicated genetic heterogeneity. Trypanosome isolates SAM 31/c1 and Kaa/83CRTA/69 clone 1 proved to be Savannah-type *Trypanosoma*

*congolense*. Trypanosome isolates Samo-24-3 and Sam 30/2 contained a mixture of parasites responding to Savannah, Kilifi and Forest-type DNA probes while Sam/82/CRTA/33-4 and Sam 29/2 consisted of trypanosomes with reactivities corresponding to the Kilifi and Savannah-type *T. congolense*. These trypanosomes are being cloned to check for the possibility of one trypanosome displaying DNA-sequences unique to more than one *T. congolense* subspecies.

### T1b.7 Serological analysis of bovine and caprine sera from Nord-Kivu Province of Eastern Zaire for evidence of trypanosomiasis

**Scientists:** A. Makumyaviri*, R. Masake
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Trypanosomiasis was reported to occur in man and his domestic animals in western parts of Zaire as early as the middle of this century. In contrast, the status of trypanosomiasis in eastern parts of Zaire is generally not known. The aim of the present study was (i) to determine the prevalence of trypanosomiasis in domestic animals from eastern Zaire and (ii) to investigate whether or not *Trypanosoma congolense* was the most prevalent species.

One hundred and three serum samples were obtained from 97 Brown-Swiss and 6 Ankole cattle randomly chosen and belonging to small-scale farms in three territories of Nord-Kivu province: Masisi (n = 46), Beni (n = 43) and Rutshuru (n = 14). Sera were also obtained from 12 indigenous goats at Nyabibwe and Goma towns. All the sera were stored at –20 °C until they were analysed for the presence of trypanosome antigens and antibodies using a sandwich enzyme-linked immunosorbent assay (ELISA) and an indirect antibody ELISA test respectively.

Analysis of cattle sera revealed that 44% of the cattle were antigenaemic (37% cattle in Masisi, 40% in Beni and 78% in Rutshuru). Interestingly, most of the antigenaemic cattle displayed mixed infection (55.5%) involving *T. brucei* and *T. congolense*. Circulating trypanosome antigens were also detected in 58% of the goats. In contrast to the situation found previously in the Western Province of Zaire, the data from cattle and goats indicate that 40% of all the trypanosome infections were due to *T. brucei*, followed by *T. congolense* (26.9%) and *T. vivax* (5.2%). In the anti-trypanosome antibody ELISA test, 67% of animals were found to have antibodies directed against trypanosomes. When the results of antigen and antibody ELISA tests were combined, it was found that 74% of the cattle had been exposed to trypanosome infection.

The disparity in the prevalence of different trypanosome species reported in the two provinces could be due to (i) differences in the methods employed for diagnosis, (ii) the vector species found in each province and/or (iii) the use of trypanotolerant breeds of cattle in most of previous studies in western parts of Zaire. Furthermore, the proximity of Nord-Kivu Province grazing paddocks to the Virunga National Park harbouring wild game, which can act as reservoir of infections, may be partly responsible for the high percentage of cattle exposed to trypanosomiasis.

## T1b.8 Identification of trypanosomes in tsetse flies caught in the Central African Republic

**Scientists:** P. Majiwa, D. Cuisance*
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Nylon membranes containing touch blots of *Glossina fuscipes fuscipes* caught in the Central African Republic between 15 and 25 August 1992 were sent to ILRAD from CIRAD-EMVT, Montpellier. In order to identify trypanosomes *(Trypanosoma brucei, T. vivax, T. simiae,* the different types of *T. congolense* and, if possible, *T. grayi)* in the flies, at least six touch blots were made from each fly. It was already suspected that majority of the gut infections could be attributed to *T. grayi*-like trypanosomes but it had been impossible to determine if any of the flies was infected with more than one trypanosome species. Each of the filters was cut into strips and the strips individually hybridized with DNA probes specific for the different trypanosome species or subspecies. Additionally, a strip from each filter was hybridized with a PCR product of the conserved segment of the 16S rRNA gene. Under defined hybridization conditions, the PCR product hybridizes with DNA from all the trypanosomes on which it has so far been tested. It has not been tested on DNA of *T. grayi.*

Out of the 31 samples, only 21 (67%) gave positive signals of hybridization with the positive control probe; of these, 13 (42%) did not react with any of the specific probes. The majority of the samples which were negative with the positive control probe were on a single filter so that samples which were positive with one or more of the specific probes but negative with the control probe were excluded from the analysis.

The Savannah-type *T. congolense* was identified in three samples (2519, 2538 and 25421); the Kilifi-type *T. congolense* was also present in sample 25421. The Tsavo-type *T. congolense* was identified in one sample (2279), *T. simiae* was detected in three samples (2285, 2413, and 2456) and *T. brucei* was detected in only one sample (2594). It is possible that the samples which did not hybridize with the control probe were from trypanosomes with a significantly divergent 16S rRNA gene. It is interesting that the Kilifi-type *T. congolense* and Tsavo-type *T. congolense* were each found in at least one sample from this study site and, as in previous studies, at least one instance of a mixed infection was observed.

## T1c.1 Phylogenetic relationships among *Trypanosoma congolense*

**Research Fellow:** A. Osanya
**Supervisors:** P. Majiwa, P. Kinyanjui*

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At least four different genotypic groups of *Trypanosoma (Nannomonas) congolense* have been described based upon profiles of iso-enzymes, nucleotide composition of the highly repetitive satellite DNA, restriction fragment length polymorphisms (RFLPs) of conserved nuclear and kinetoplast DNA sequences and, more recently, randomly amplified polymorphic DNA (RAPD) patterns. The exact phylogenetic relationships of these morphologically identical but genetically heterogeneous parasites is obscure.

We have designed specific oligonucleotide primers based on conserved nucleotide sequences of 18S ribosomal RNA (18S rRNA) genes of *T. brucei, Leishmania donovani* and *Euglena gracilis,* and used these in the polymerase chain reaction (PCR) to amplify genomic DNA from four different clones each representing a different genotypic group of *T. congolense.* PCR products of approximately 1 kb were generated. The nucleotide sequences of the PCR products were determined by direct sequencing to provide partial nucleotide sequences of the 18S rRNA gene in each of the trypanosomes. Using these sequences and those that have been published for the other trypanosomatids, it will be possible to establish the genetic relationship amongst these protozoan species.

The PCR products of the 18S rRNA gene are sensitive probes for specific identification of trypanosomes. We have used heterologous PCR products as probes in hybridization analyses of genomic DNA and PCR products from the different types of *T. congolense* and from other protozoa. The restriction fragment length polymorphisms observed confirmed heterogeneity of the 18S rRNA gene sequences among the trypanosomes comprising the subgenus *Nannomonas.* Additionally, primers of arbitrary nucleotide sequence have been employed in the PCR amplification of genomic DNA from the four trypanosome clones representing the different *T. congolense* types. The RAPD patterns will be used in the calculation of pairwise genetic similarity indices among these trypanosomes.

## T1c.2 Heterozygous loci in natural populations of *Trypanosoma congolense*

**Scientist:** P. Majiwa
**Technician:** M. Maina

Several oligonucleotides of arbitrary sequence were used to perform polymerase chain reaction (PCR) amplification of DNA from cloned isolates of the Savannah-type *Trypanosoma (Nannomonas) congolense* initially obtained from different geographic locations. In general, clones derived from an antigenic repertoire displayed a similar pattern of randomly amplified polymorphic DNA (RAPD) bands, the only

differences observed, if any, being in the relative amounts of some of the amplification products.

Primers which produced relatively simple patterns of RAPD bands were used in detailed analyses of DNA from *T. congolense* clones representing three different antigenic repertoires. A proportion of the primers revealed cryptic heterozygous alleles among different isolates of the Savannahtype *T. congolense*. Many of the polymorphic as well as non-polymorphic RAPD bands were cloned in T-plasmid vectors and used in Southern blot hybridization analyses of DNA from the same parasite clones. The restriction enzyme fragment length polymorphisms, observed when certain cloned RAPD bands were used in combination with some restriction enzymes, are consistent with the existence of recombinant genotypes or heterozygous loci among natural populations of the Savannah-type *T. congolense*.

### T1c.3 Analysis of Kilifi-type *Trypanosoma congolense* clones for evidence of genetic-recombination

**Scientists:** R. Masake, S. Moloo, P. Majiwa
**Technicians:** A. Adema, H. Gathuo

Genomic analysis of *Trypanozoon* group trypanosomes by others has demonstrated the occurrence of genetic exchange both under natural and experimental conditions. Similar findings have not been reported for the *Nannomonas* and *Duttonella* groups of trypanosomes. Our earlier attempt to find hybrids of *Trypanosoma congolense* utilized co-transmission of two clones derived from Kilifi- and Savannah-type *T. congolense* by *Glossina morsitans centralis*. Screening of over 500 clones prepared from the infected flies did not yield recognizable hybrids. This may indicate the existence of sufficient differences in the genetic make-up of the two types, preventing exchange of genetic material. To avoid this possibility, further studies were conducted using clones only of the Kilifi-type *T. congolense*. DNA from 16 clones was amplified employing five oligonucleotide primers (ILo 509, 872, 907, 1026 and 1053) known to give distinct patterns of randomly amplified polymorphic DNA (RAPDs). Five clones, namely K45/1, K73/1, K59/1, K59/2 and K97/2, yielded DNA products similar to Savannah-type *T. congolense* IL 1180. These clones were subjected to repeated RAPD analysis until consistent DNA product banding was obtained. This eliminated the previously suggested similarities of two of the clones (K45/1 and K73/1) with Savannah-type *T. congolense*. The remaining three clones, namely K59/1, K59/2 and K97/2, were then re-cloned, expanded in mice and their chromosome sized-DNA separated on OFAGE. This revealed a contamination of K59/1 with a Savannah-type *T. congolense*. To date we have not found any evidence for genetic exchange amongst the Kilifi *T. congolense* clones.

## T1c.4 Primary structure of *Trypanosoma congolense* variable surface glycoproteins

**Scientists:** T. Urakawa, P. Majiwa, Y. Eshita*

*Department of Parasitology, Kurume University School of Medicine, Japan

The nucleotide sequences of transcripts which encode five different variable surface glycoproteins of *Trypanosoma congolense* were determined. This brings to seven the total number of different VSGs of *T. congolense* whose sequences have been determined to date. A comparison made of the deduced amino acid sequences of these surface proteins of *T. congolense* reveals strong conservation of several structural domains, particularly cysteine residues located predominantly toward the carboxy-terminal half of the molecules. However, there was no apparent structural correlation when comparisons were made between those VSGs expressed by *T. congolense* arising early in parasitaemia and those expressed by either the other bloodstream forms or metacyclic forms of the parasite. Pairwise or global alignment of the deduced amino acid sequences showed the longest homologous stretches to comprise at most seven amino acid residues. A comparison of the amino acid sequences of these proteins with those of *T. brucei* VSGs revealed no significant homologies other than conserved structural motifs. The data make it possible to predict general primary structural features of *T. congolense* VSGs. The *T. congolense* transcripts, like those of *T. brucei*, have degenerate splice acceptor sites for the miniexon.

## T1c.5 Iso-VATs of *Trypanosoma congolense*

**Scientists:** P. Majiwa, T. Urakawa, H. Hirumi
**Research Associate:** K. Hirumi
**Technician:** M. Maina

Isotypic trypanosomes have been described among different species of trypanosomes of the subgenus *Trypanozoon*. Although suspected to occur on serological grounds, isotypic variants have not been clearly demonstrated among the *Nannomonas* trypanosomes. We have cloned DNA sequences encoding six antigenically distinct VSGs of *Trypanosoma (Nannomonas) congolense* derived from two isolates from different locations. These VSG gene sequences have been used in genomic DNA blot hybridization assays to investigate the possible presence and distribution of homologous sequences in the genomes of cloned isolates of *T. congolense* belonging to other antigenic repertoires. Two of the six VSG cDNAs detected homologous sequences in at least one other antigenic repertoire. The homologous sequences so detected revealed antigenic repertoire-specific and, in some instances, clone-specific restriction enzyme fragment length polymorphisms (RFLPs) which may be useful in parasite characterization. Polyclonal monospecific antiserum raised against an ILNaR2 metacyclic VSG (mVSG1), expressed in the baculovirus/insect cell system, was used to stain Western blots of lysates of metacyclic trypanosomes from heterologous antigenic repertoires. An antigen, similar in size to VSGs and bearing antigenic

epitopes similar to the ILNaR2 mVSG1, was shown to be expressed by metacyclic forms of ILNaR3. Thus, although a majority of the sequences encoding *T. congolense* VSGs are restricted to the genomes of trypanosomes of the homologous antigenic repertoires, the data obtained with ILNat3.3 VSG and ILNaR2 mVSG1 genes indicate that isoVATs exist in *T. congolense*. These observations have important implications for the frequency, dispersion and stability of the VSGencoding sequences within populations of *T. congolense*.

## T1c.6 The use of randomly amplified polymorphic DNAs to identify chromosome-specific hybridization markers

**Research Fellow:** L. Apopa
**Supervisors:** P. Majiwa, P. Kinyanjui*

*Department of Biochemistry, University of Nairobi, Kenya

Chromosome rearrangements that occur in trypanosomes appear to affect mainly the medium-sized chromosomes. A possibility exists that non-duplicative activation of telomeric VSG genes might involve chromosome rearrangements occurring several kilobase pairs upstream from the affected VSG gene, thus remaining undetectable by standard gel electrophoresis and Southern blot hybridizations with the VSG gene probes. Since trypanosomes do not condense their chromosomes at any stage of cell division, this hypothesis cannot be tested by cytological hybridization. Some of the size alterations in chromosomes occur when the trypanosomes are repeatedly passaged, cyclically transmitted through tsetse flies or grown in rodents in the presence of trypanocidal drugs. However, individual chromosome rearrangement are impossible to identify in the absence of a marker specific for the chromosome.

Chromosomal DNA of *Trypanosoma congolense* clone IL 1180 was subjected to pulsed field gradient gel electrophoresis to separate the four medium-sized chromosomes (340, 360, 400 and 500 kb). The four chromosomes were individually purified from agarose gel slices and then amplified by the polymerase chain reaction primed with short oligonucleotides (10-mers) of arbitrary nucleotide sequences. The fingerprints of DNA fragments generated were resolved by standard agarose gel electrophoresis. The majority of primers generated reproducible fingerprints, some of which were polymorphic for the different chromosomes analysed. In this way, a fragment of DNA was identified which hybridizes only to one of the four chromosomes from which it was amplified by a random primer. The approach will be developed to generate chromosome-specific hybridization markers for this parasite.

## T1c.7 Adaptation of plasmid constructs for the transformation of *Trypanosoma congolense*

**Scientists:** T. Urakawa, P. Majiwa
**Technician:** M. Maina

One of the impediments to an effective genetic analysis of parasitic protozoa is the lack of appropriate plasmid vectors for routine and efficient transformation of the organisms. Although several plasmid constructs have been used in the transformation of some parasitic protozoa, including *Trypanosoma brucei,* such plasmids are unlikely to be directly functional in *T. congolense.* With the objective of obtaining constructs which contain selectable markers and *T. congolense* sequences which could mediate their integration by homologous recombination into targeted sites of the *T. congolense* genome, two types of plasmid constructs were made: one with neomycin resistance and the other with hygromycin resistance as the respective selectable markers.

Two different constructs were made, each with the neomycin resistance gene. One, designated 22pHD16Neo/7, was made by insertion into the plasmid pHD16Neo of a 2.0 kb fragment of *T. congolense* genomic DNA. This fragment of DNA was initially identified from earlier studies as constituting a differentially amplified locus in different *T. congolense* clones. The locus is transcribed specifically in bloodstream forms of the parasite. This fragment was inserted in phase immediately upstream of the aldolase splice acceptor site, downstream of which is the neomycin resistance gene. In front of the neomycin gene is located an aldolase polyadenylation signal. This construct has no promoter and therefore would be dependent upon an endogenous promoter at its site of integration. The second construct was made by insertion upstream of the *T. brucei* PARP promoter of a 2.2 kb fragment of DNA at an expression site for an mVSG expressed in two different antigenic repertoires of *T. congolense.* In the *T. congolense* genome, this fragment is located approximately 5.0 kb from the VSG gene, with which it forms a single polycistron. In addition to the *T. brucei* PARP promoter, the construct has the aldolase splice acceptor site, the neomycin resistance gene and the aldolase polyadenylation signal.

The construct containing the hygromycin resistance gene was made by replacing the $\alpha\beta$ tubulin intergenic region of *T. brucei* with the equivalent region from *T. congolense.*

All the constructs have been linearized by digestion with restriction enzymes whose recognition sites are located uniquely within the inserted sequences of *T. congolense* DNA and have been used in the electroporation of both bloodstream and procyclic forms of *T. congolense* IL 3000. Electroporated procyclic forms have been established in media supplemented with 10 µg/ml of G418, an analogue of neomycin.

## T1c.8 Attempts to establish hybridization *in vitro* between clones of *Trypanosoma congolense* expressing different patterns of drug resistance

**Scientists:** H. Hirumi, P. Majiwa, T. Urakawa
**Research Associate:** K. Hirumi
**Technician:** F. Chuma

To facilitate experimental investigation of trypanosomal drug resistance and its fate upon recombination, a culture system has been initiated using epimastigote populations of two *Trypanosoma congolense* clones—IL 2466.1 and IL 3338.2.

The maximum tolerated concentrations of diminazene aceturate and quinapyramine sulphate by *T. congolense* IL 2466.1 are $10^{-2}$ ng/ml (sensitive) and $10^{2}$ ng/ml (resistant), whereas those of *T. congolense* IL 3338.2 are $10^{2}$ ng/ml (resistant) and 1 ng/ml (relatively sensitive) respectively, determined by the *in vitro* drug sensitivity test (see abstract T1e.7).

Epimastigote populations derived *in vitro* from the clones were co-cultivated at 27 °C for 28–34 days. Metacyclic forms (MCFs) which appeared in the mixed cultures were collected by means of DE52 column chromatography and were cultured in HMI-93 medium at 34 °C. The MCFs further transformed to BSFs and continued to proliferate under such conditions.

The BSFs were then maintained axenically in the presence of both diminazene aceturate ($2 \times 10^{2}$ ng/ml) and quinapyramine sulphate (10 ng/ml) for ten days without medium change. Trypanosomes surviving the dual treatment were increased in number in HMI-93 in the absence of the drugs. To date, a total of nine BSF populations which survived the dual treatment have been isolated, and will be examined for molecular evidence of hybridization *in vitro*.

## T1d.1 cDNA cloning of transcripts over-expressed in the non-dividing bloodstream form trypanosome using arbitrary primers

**Scientists:** R. Pellé, N. Murphy
**Technician:** P. Pandit

The development of a method now termed RADES-PCR (for randomly amplified developmentally expressed sequences) has been described previously for the identification of developmentally regulated genes. In this technique, single or double stranded cDNAs from each form of the parasite are amplified by the polymerase chain reaction (PCR) using short oligonucleotide primers (10-mers) of arbitrary sequence. The fingerprints of DNA fragments generated are then examined by agarose or polyacrylamide gel electrophoresis and DNA fragments showing differences between stages are purified and used as probes on Northern blots. Further development to improve the robustness of the RADES-PCR method have been undertaken. In addition to the arbitrary 10-mer primers, longer primers (up to 40-mers) have also been tested, including the universal sequencing primer. At low annealing temperatures, such primers also generated fingerprints which were characteristic of the life-cycle stage from which the template cDNA was derived. Total RNA from different stages of *T. b. brucei* were reverse-transcribed into ss-cDNAs using the universal sequencing oligonucleotide as primer.

The cDNAs were then amplified by PCR using the same sequencing primer in combination with a miniexon oligonucleotide primer. Agarose gel electrophoresis of the PCR products showed that up to six cDNA fragments, ranging between 200 and 600 bp, were generated for each template cDNA. A 250 bp cDNA fragment specific to the non-dividing, stumpy bloodstream forms was purified and used as a probe on Northern blots. This cDNA probe strongly hybridized with two transcripts of approximately 2.4 and 3 kb in the poly(A)$^+$ RNA from the stumpy forms and very weakly with poly(A)$^+$ RNA from other stages. No hybridization signal was detected with poly(A)$^-$RNA. Using this cDNA probe, a genomic DNA clone of 6 kbp was isolated from a λgt11 genomic library. This DNA clone was PCR amplified using an oligonucleotide corresponding to the 3′ end of the 250 bp cDNA fragment as the forward primer and an oligonucleotide specific to the λgt11 vector as reverse primer. A 2 kbp DNA fragment was generated and subcloned in a sequencing plasmid vector. On Northern blots, the 2 kbp fragment hybridized with the 2.4 and 3 kb transcripts only in the stumpy forms. The fact that these transcripts are expressed at a high level in the non-dividing bloodstream form trypanosomes (and only at a trace level in the actively dividing forms) suggests that they may be involved in the process of down regulation of cell proliferation. Partial sequencing of the 250 bp cDNA fragment and the 2 kbp DNA clone did not reveal homology with any known sequence in the GenBank DNA sequence database (release 77).

## T1d.2 *Trypanosoma brucei* VSG mRNA binding proteins: identification of potential binding sites at the 3′ untranslated region

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Recent reports show that several important signals involved in the post-transcriptional control of gene expression in eukaryotes are located at the 3′ untranslated region (3′ UTR) of mRNA molecules. Among these are: all signals determining mRNA localization, signals controlling polyadenylation, mRNA stability, translation initiation and signals controlling growth and differentiation. These signals exercise their control through interactions with regulatory RNA-binding proteins. A method has been developed to identify these RNA-binding proteins and is being applied to African trypanosomes. We have shown that the 300 nt-3′ end of the *T. b. brucei* AnTat 1.10 VSG mRNA forms specific complexes with cytoplasmic proteins from ILTat 1.1 bloodstream forms. Subsequently, oligonucleotides complementary to the conserved '8-mer' and '14-mer' found at the 3′ end of all *T. b. brucei* bloodstream form VSG mRNAs have been used to test if the blocking of these RNA motifs by hybrid formation with these oligonucleotides may prevent or reduce the formation of complexes. The results have shown that: (1) the anti '14-mer' oligonucleotide induces a high level of degradation of the transcript and strongly reduces the formation of the complexes as well as their sizes; (2) the anti '8-mer' oligonucleotide strongly reduces the formation of the complexes without affecting their initial sizes; (3) an oligonucleotide complementary to the region between the conserved '8-mer' and '14-mer' produces effects comparable to those of the anti '14-mer' oligonucleotide; and (4) an oligonucleotide complementary to a sequence upstream of the '8-mer', and downstream of the 3′ end of the stop codon, gives results similar to those obtained when using the anti '8-mer' oligonucleotide; conversely, an oligonucleotide upstream and next to the 3′ end of the stop codon, as well as oligonucleotide ILo 256 complementary to the 3′ end of the medRNA, do not affect the formation of the complexes. In a parallel analysis, we found that RNA transcripts generated using a double mutant construct in which the conserved '8-mer' and '14-mer' (but not the sequence between these two motifs) have been deleted, gave a result similar to the one obtained when using RNA from the non-mutant construct. These results suggest that the sequence between the conserved '8-mer' and '14-mer', as well as the '14-mer' sequence, are strongly involved in the formation of complexes between VSG transcripts and regulatory proteins. It is possible that, for VSG mRNAs that need to be degraded rapidly as the bloodstream forms transform into procyclic form trypanosomes, there is more than one cis-acting RNA regulatory element in the 3′ UTR. In the future, a mutant construct in which the '8-mer' and '14-mer' inter-region is deleted will be used to verify the role played by this domain in the formation of the complexes. In addition, each of the potential RNA regulatory motifs will be cloned in a transcription vector to evaluate their function.

## T1d.3 Characterization of the cytoskeletal elements involved in kinetoplast segregation

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The trypanosome cytoskeleton plays a vital role in cell shape, differentiation and motility. Furthermore, elements of the cytoskeleton are physically attached to the kinetoplast and are essential for kinetoplast segregation during trypanosome cell division. In order to characterize the components involved in kinetoplast segregation cytoskeletal extracts (flagella, basal bodies and proteins isolated on nitrocellulose filters) have been used to raise monoclonal and polyclonal antibodies. Localization studies using these different monoclonal and polyclonal antibodies have been carried out. Those antibodies that bind to proteins located at the kinetoplast/basal body are of particular interest. In initial studies to identify genes encoding such proteins, *Trypanosoma brucei brucei* genomic and cDNA expression libraries have been screened with a number of currently existing antibodies raised against detergent-extracted cytoskeletal organelles of *T. b. brucei*. The first is a polyclonal antibody (named Mix) previously obtained from hybridoma supernatants that react with paraflagellar rod (PFR) and the basal bodies of the axoneme. The second is a monoclonal antibody (BBa4) which reacts with only the basal bodies. The polyclonal serum has identified a number of clones derived from the cDNA library and one clone from the genomic library. These clones will be further characterized by using oligonucleotide probes based on the known DNA sequence of the PFR. Any clones which hybridize with this PFR oligonucleotide will initially be excluded from further study as they will represent sequences encoding PFR proteins which have been previously described. Any non-hybridizing clones will be of interest since they may contain sequences of cytoskeletal genes responsible for proteins involved in attachment of the basal bodies to the kinetoplast and in segregation and mitosis.

## T1e.1 Development of ELISAs for isometamidium and homidium

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An ELISA to detect the trypanocide isometamidium in biological fluids, reported previously, has now been improved and simplified by using an isometamidium-horseradish peroxidase conjugate. The improved assay has the following advantages: (i) purification and biotinylation of IgG is no longer a requirement since whole hyperimmune sheep serum is used, (ii) microtitre plates may be coated with hyperimmune serum in large batches and stored frozen, (iii) the new assay has fewer stages; starting with pre-coated microtitre plates, then an overnight competition stage, and finally a ten-minute colour development stage, (iv) there is less inter-assay variation in absorbance values, (v) there is less of 'matrix effect' due to the inclusion of the test serum sample. The improved assay has been validated using sera from isometamidium-treated Boran cattle and found to give results comparable with the previous method.

In order to increase the amount of available information on the serum concentrations of isometamidium necessary to protect cattle against populations of *Trypanosoma congolense* with different levels of sensitivity to isometamidium, 15 Boran cattle were treated, by intramuscular injection, with isometamidium chloride at a dose of 1.0 mg/kg body weight. Thereafter, the animals were repeatedly challenged at monthly intervals by the application of five *Glossina morsitans centralis* infected with one of two different trypanosome clones; five cattle were challenged with *T. congolense* IL 1180, a drug-sensitive population; ten cattle were challenged with *T. congolense* IL 3343, a derivative of *T. congolense* IL 1180 expressing a moderate level of resistance to isometamidium. All five animals challenged with *T. congolense* IL 1180 were refractory to five monthly challenges. In contrast, seven of the ten animals challenged with *T. congolense* IL 3343 became infected following the second challenge. The three remaining animals became infected following the third challenge. The isometamidium ELISA was used to determine serum drug concentrations in the cattle at regular intervals throughout the experiment. Amongst the animals challenged with *T. congolense* IL 3343, differences in drug pharmacokinetics did not appear to account for the differences in susceptibility to challenge. The data resulting from this and other, similar, experiments will be useful in interpreting the significance of serum isometamidium concentrations measured by the ELISA in cattle in the field.

In an attempt to standardize the isometamidium-ELISA, anti-isometamidium monoclonal antibodies have been produced. Two $IgG_1$-secreting hybridoma cell lines were obtained using spleen cells from balb/c mice immunized with an isometamidium-porcine thyroglobulin conjugate. Supernatants from these cell lines could be used as an alternative to polyclonal IgG obtained from Scottish Blackface sheep immunized with the same conjugate. However, the sensitivity and specificity of the

ELISA using the polyclonal IgG were superior to those obtained using the monoclonal antibodies. However, one of the cell lines showed promise of being potentially useful in an ELISA for the detection of homidium.

Finally, ELISA methods for the determination of concentrations of homidium in sera from treated cattle have been developed and tested using Friesian cattle in Glasgow. Both an indirect competitive assay using a homidium-ovalbumin coating conjugate and biotinylated anti-homidium IgG, and a competitive enzyme immunoassay using whole hyperimmune sheep serum and a homidium-horseradish peroxidase conjugate, were successful in quantifying levels of homidium for several weeks after treatment.

## T1e.2 Membrane transport, accumulation and processing of Samorin by *T. congolense*

**Scientists:** J. Wilkes, H. Hirumi, D. Zilberstein*
**Technicians:** J. Odhiambo, S. Kemei

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The presence of a saturable transport system for the accumulation of the trypanocide Samorin by *Trypanosoma congolense* has previously been reported. The kinetic properties of this system were consistent with a transport molecule present in the plasma membrane, and was characterized in a number of defined populations of *T. congolense*. These populations, demonstrating a $10^3$-fold range in sensitivity to Samorin, showed a clear relationship between the level of this transport activity (the $V_{max}$ for the process) and sensitivity to the drug. Characterization of this transport activity is reported.

Uptake of $^{14}C$-labelled Samorin by the *T. congolense* clone IL 1180 was monitored in the presence of a range of potential substrates for the transport mechanism (amino acids, nucleosides, nucleobases). Only the purine nucleosides adenosine and guanosine gave significant reduction of uptake, indicating competitive inhibition. An inhibitor of purine nucleoside transport in mammalian cells, nitrobenzyl thio inosine, was found to produce a major inhibition of Samorin transport and kinetic analysis demonstrated the inhibition to be non-competitive with a Ki value of 4 mM. Adenosine uptake was monitored in a centrifugation assay. As reported by other workers, uptake was rapid (linear only up to 30s) and mediated by saturable plasma membrane associated carrier systems. Inhibition of adenosine uptake by Samorin was less than 15%, which may indicate the presence of a third transport component responsible for the uptake.

Measurements of trypanosome internal volume and visual observation of pelleted trypanosomes, indicates a high degree of concentration of the drug within the cell (> 50 fold), indicating the process is energized in some way. Replacement of external $Na^+$ with choline did not affect the uptake, indicating $Na^+$-linked processes are not involved. Replacement of $Na^+$ with $K^+$, a treatment producing membrane depolarization, produced a 60–70% inhibition of the uptake. No evidence of efflux of the drug was obtained, accumulated drug was only exchanged

for external drug. This indicates that entry of the drug is electrogenic (involving the passage of positive charge across the membrane) and accumulation is effected by transmembrane potential.

Samorin is a complex mixture of phenanthridines and, with homidium, constitutes a large proportion of the trypanocides in use. These compounds can be visualized and isolated on a preparative basis by thin layer chromatography followed by cation exchange chromatography. These fractions have been tested in an *in vitro* drug sensitivity test (see abstract T1e.7), the different components of Samorin demonstrating a wide range of efficacies. In addition, a high performance liquid chromatography method has been developed using a reverse phase ion pairing separation mechanism, which will be used to identify components of Samorin, and potential metabolites or conjugates in the host and in the trypanosome.

## T1e.3 Identification of isometamidium-binding proteins in *Trypanosoma congolense, T. brucei* and *Leishmania donovani*

**Scientists:** D. Zilberstein*, J. Wilkes, A. Peregrine
**Technician:** J. Odhiambo

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Interaction of the fluorophore isometamidium with intracellular components of bloodstream forms of *T. congolense* enhances its fluorescence. This enhancement is saturable, and titration of the fluorescence response indicates the presence of a single population of high affinity binding sites with Kd = 150–250 ng/ml. This interaction appears to be independent of the parasites' susceptibility to the drug, indicating that the level of the isometamidium-interacting molecule is similar in both susceptible and resistant populations of *T. congolense*.

Similar changes in the fluorescence of isometamidium were also observed in cell lysates of *T. brucei* bloodstream forms and promastigote forms of *Leishmania donovani*, a closely related trypanosomatid. But whereas isometamidium is taken up by both bloodstream and procyclic forms of *T. congolense* and *T. brucei*, *Leishmania* parasites do not have a transport system for this molecule. Therefore, both *T. congolense* and *T. brucei* are sensitive to isometamidium but *L. donovani* can tolerate high concentrations of the drug.

In order to identify isometamidium-binding proteins, the drug was coupled to sepharose-4B beads by means of cyanogen bromide. The resultant conjugate was used to produce an affinity column. Triton X-100 lysates of *T. congolense* IL1180, *T. brucei* ILTat 1.4 and *L. donovani* were incubated with the isometamidium affinity columns. Two protein bands of 24 and 37 kDa from *T. congolense* were eluted from the columns with 2 mM isometamidium. A major protein band of 54 kDa was identified in both *T. brucei* and *L. donovani*. All these isometamidium-binding proteins are positively charged as they bind to cation exchange columns. When they interact with isometamidium, these proteins enhance its fluorescence, resembling the change observed with cell lysates.

## T1e.4 Characterization of homidium uptake in *Trypanosoma congolense*

**Scientists:** A. Peregrine, J. Wilkes, D. Zilberstein*
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Homidium (Novidium®, Ethidium®) is a phenanthridine that is commonly used as a therapeutic agent for bovine trypanosomiasis. It is also used occasionally as a prophylactic agent for the same purpose. Since drug resistance appears to be compromising the efficacy of the compound in the field, a research project has been initiated to determine the molecular basis of resistance to homidium in trypanosomes.

Homidium is a fluorophore. Fluorescence spectroscopy was therefore used in initial studies to determine whether this property could be used to characterize the interaction of homidium with trypanosomes. The molecule was shown to have an absorption $\lambda_{max}$ of 469 nm. Furthermore, when excited at this wavelength, there were two emission $\lambda_{max}$; at 605 nm and 650 nm. However, when incubated with either live *T. congolense* IL 1180 (a homidium-sensitive clone) or a lysate of the same population, no significant alteration was observed in the molecule's emission spectrum when excited at 469 nm. Unlike Samorin®, therefore, the fluorescent property of homidium could not be used to characterize the interaction of the molecule with trypanosomes. The suitability of $^{14}$C-homidium was therefore investigated. Using a centrifugation methodology that was previously developed for determining uptake of Samorin® by trypanosomes, *T. congolense* IL 1180 was shown to exhibit uptake of homidium when incubated at 37 °C in phosphate-saline glucose, pH 7.4. Uptake of $^{14}$C-homidium by *T. congolense* IL 1180 was dependent on the drug concentration in the medium, and saturable over a concentration range of 0.05–2.0 µg/ml. Kinetic data was best modelled using an allosteric plot. However, in contrast to uptake data for Samorin, the Hill coefficient was close to unity. $V_{max}$ for the process was 36 ± 1.9 ng/$10^8$ cells/min (n = 6). $K_m$ for the process was 0.73 ± 0.08 µg/ml (n = 6). A range of potential competitor molecules for the uptake process were investigated: amino acids at 0.1–10 mM, and nucleosides at 0.01–1 mM. None of the amino acids investigated (tryptophan, phenylalanine, lysine, arginine and histidine) altered uptake of homidium over the concentration range used. However, the purine nucleosides adenosine and guanosine inhibited uptake by 19 and 33%, respectively. The similarity of the homidium transport process by trypanosomes will be compared to that responsible for uptake of Samorin.

## T1e.5 Induction and characterization of a quinapyramine-resistant clone of *Trypanosoma congolense*

**Research Fellow:** G. Ndoutamia
**Scientists:** N. Murphy, A. Peregrine

A quinapyramine-resistant clone of *T. congolense* IL 1180 has been derived previously that is 40-fold more resistant to quinapyramine than the parental clone. The

40-fold increase in resistance was shown to be associated with significant cross-resistance to isometamidium, homidium and diminazene in mice. Similar observations were made in goats. Using random primers in a polymerase chain reaction (PCR), we have identified differences between the parental (sensitive) and 40-fold resistant population. Furthermore, one primer (ILo 868) demonstrated differences that appear to correlate with increasing levels of resistance when populations of IL 1180 with intermediate levels of resistance were included in the experiment. This PCR product was cloned into plasmid pBluescript. Eight cross-hybridizing clones were isolated; seven had an internal *Hind* III site and one did not. One of the clones (clone 2) was used to probe Southern blots of genomic DNA from the sensitive and resistant populations and revealed that there was an alteration in size of some hybridizing bands with increasing levels of resistance. Although the PCR product showed differences between sensitive and resistant populations by Southern blot analysis, the PCR was not consistently reproducible. We therefore tested another series of primers; one primer (ILo 1122) gave a reproducible result that again appeared to correlate with increasing levels of resistance to quinapyramine. Three of the PCR products with this primer were shown to consistently vary between the sensitive and resistant populations. All of the products were shown to have sequences in common, but one of the products (of 0.8 kb) has additional sequences that are not contained within the other two sequences of 1 kb and 0.4 kb. These observations are borne out by Southern blot analysis with these PCR products. However, no differences were observed between the sensitive and resistant populations by Southern blot analysis with any of the three PCR products when restriction digests of genomic DNA were used. It is believed that the differences consistently observed in the PCR are due to point mutations in the priming sites in the genome.

In order to map the extent of the alteration in the genome associated with resistance to quinapyramine, the PCR products of primer ILo 868 and ILo 1122 were used to probe Southern blots of separated chromosomes of sensitive and resistant populations. The result has shown that the three products of ILo 1122 hybridized to three chromosomes of approximately 5.7, 3.7 and 1.6 megabases. The product of ILo 868 hybridized to one of the chromosomes that the three PCR products of ILo 1122 hybridized to, but also to two other chromosomes. In order to accurately identify the nature of alterations in the genome which correlate with quinapyramine resistance, the same four PCR products will be used to screen a *T. congolense* genomic library.

### T1e.6 Identification and characterization of an extrachromosomal element in *Trypanosoma brucei brucei*

**Research Fellow:** H. Jamnadass
**Supervisors:** N. Murphy, O. ole-MoiYoi, A. Peregrine

In earlier reports we described the identification and initial characterization of a 6.6 kilobase pair (kbp) extrachromosomal DNA element in a multi-drug resistant isolate of *T. b. brucei:* CP 547. The copy number of the element is reduced or lost on continuous passage of the trypanosomes in the absence of drug pressure. However, the element is regained and retained if the parasites are maintained under drug

pressure. A membrane-bound, polymerase chain reaction (PCR) amplification method was developed for the generation of a specific probe, and nucleotide sequence analysis of cDNA clones selected with this probe revealed the presence of a 108 bp repetitive sequence within the 6.6 kbp element.

The 108 bp repeat is strongly transcribed in actively dividing trypanosomes, generating heterogeneously sized stable transcripts. In a PCR, primers specific for the miniexon sequence and primers specific for the 108 bp repeat were unable to amplify sequences from single-stranded cDNA prepared from the drug-resistant CP 547. This result suggests that the heterogeneous transcripts specific for the 108 bp sequence do not have miniexons on their 5′ ends.

From densitometry, *T. brucei* isolates tested so far have approximately 40,000 copies of the 108 bp repeat per genome, although there appears to be significant variation between families of this repeat of different isolates.

Although the role of the 6.6 kbp element is unknown, the 108 bp minisatellite shows some characteristics of being a retroposon. Results of a PCR amplification of total genomic CP 547 DNA using primers specific for the 108 bp repeat and oligo (dT) revealed that there are probably some genomic copies of the 108 bp repeat close to poly (A) motifs. To determine if the 6.6 kbp element was made up entirely of the 108 bp repeat, or contained unique, non-repetitive sequences, random arbitrary primers were used in a PCR amplification to generate DNA fingerprints of the target DNA. The three target DNAs used were total genomic DNA from *T. brucei* CP 547, an alkaline lysis preparation of CP 547 trypanosomes (known to enrich for the element), and gel-purified 6.6 kbp DNA. Of the sixty arbitrary primers tested, ILo 878 generated an 800 bp fragment which does not hybridize to the 108 bp repeat but hybridizes to the 6.6 kbp element and its multimeric forms.

### T1e.7 Use of an *in vitro* drug-sensitivity test for examination of the resistance of *Trypanosoma congolense* to Samorin

**Scientists:** H. Hirumi, A. Peregrine, J. Wilkes
**Research Associate:** K. Hirumi
**Technician:** F. Chuma

An *in vitro* drug-sensitivity test for quantifying the sensitivity of bloodstream forms (BSFs) of *Trypanosoma congolense* to trypanocidal drugs has been further improved (Hirumi, H., Hirumi, K. and Peregrine, A.S., *Journal of Protozoology Research* 3: 52–63, 1993) and used to determine the minimum effective concentrations of four isomers (B, C, D and E) of isometamidium chloride, separated from Samorin by means of thin-layer chromatography. The concentration of drug fractions required to kill BSFs of *T. congolense* IL 1180 *in vitro* were established; B, $10^4$ ng/ml; C, $10^3$ ng/ml; D, 10 ng/ml; E, $10^3$ ng/ml, whilst whole Samorin was effective at 1 ng/ml. The result revealed that isomer D plays the major role in the trypanocidal activity of Samorin.

A curious, concentration-dependent killing was consistently noted with isomer D which killed the BSFs at an extremely low concentration ($10^{-4}$ ng/ml). Although effective at $10^{-4}$ ng/ml, isomer D proved ineffective at 1, $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-5}$

ng/ml. The effect was consistently demonstrable when the sensitivity test was made by seeding the test wells with $10^5$ BSFs/well ($2 \times 10^5$ BSFs/ml, 500 µl/well) which is slightly lower than the seeding density ($2 \times 10^5$ BSFs/well) employed in earlier reports.

## T2a.1 Cloning, sequencing and expression of the gene encoding the 69 kDa immunodominant trypanosome antigen

**Research Fellow:** A. Boulangé*
**Scientist:** E. Authié*

*Visiting scientists part supported by EMVT-CIRAD, France

Cattle infected with *Trypanosoma congolense* mount an immune response to a 69 kDa parasite antigen, but N'Dama (trypanotolerant) and Boran (susceptible) cattle differ in their antibody responses to the 69 kDa antigen. This protein therefore is potentially a useful tool in the characterization of the immune responses of the two breeds and efforts have been made to clone and express the gene.

The sequence of a 1.94 kbp cDNA encoding 548 amino acids of this antigen identified the 69 kDa protein as a member of the heat shock protein (hsp70) family. Close homology was observed at the amino acid level with the immunoglobulin heavy-chain binding protein (BiP). BiP is a member of a hsp70 subgroup which act as chaperones in the endoplasmic reticulum (ER) of many eukaryotic cells. A *T. congolense* cDNA metacyclic library was screened with the 1.94 kbp cDNA, and a 2.35 kbp clone containing the complete coding sequence of the gene was isolated. This clone encodes a protein of 653 amino acids with a predicted molecular mass of 71 kDa and a pI of 5.03. The deduced amino acid sequence of the N-terminal region confirmed the homology with BiP. Moreover, the trypanosome 69 kDa antigen shares common features with other related BiP-molecules, i.e. an hydrophobic N-terminal leader sequence (2 kDa) and a C-terminal tetrapeptide, MDDL. The former probably targets the protein to the ER, while the latter acts as a retention signal for ER-resident proteins.

Southern blot analysis of genomic DNA suggested that the gene exists in several copies which are probably not organized in tandem repeats, unlike other trypanosome hsp70s described. Mapping and partial sequencing of large genomic clones confirmed that there are at least three copies of the gene. They were identified in a region of 15 kbp, where they present the same 5′–3′ orientation and are separated by arrays of 2 kbp and 5 kbp.

Two cDNA inserts of 1.9 kbp and 0.8 kbp encoding truncated proteins of 63 and 28 kDa, respectively, were subcloned into the pMAL expression vector. The recombinant proteins, R63 and R28, purified by immuno-affinity chromatography, have been used for epitope recognition and other immunological studies (see abstract T2b.1). Synthesis of the full length recombinant protein was attempted in bacterial expression systems as neither disulphide bonds nor glycosylation sites were predicted from the amino acid sequence. Two oligonucleotides were designed for use in the polymerase chain reaction (PCR). One is homologous to the 3′-end of the open reading frame (ORF) and introduces a *BamH1* site just after the stop codon. The other is homologous to the sequence encoding the N-terminus of the mature protein and introduces an *EcoR1* site immediately before the first codon. This was designed to allow directional cloning and expression of the resulting PCR product into the pMAL vector. However, the DNA fragment coding for the complete ORF proved to be extremely unstable, irrespective of the bacterial strain and the plasmid vector used.

A eukaryotic system such as yeast or baculovirus might be required for the expression of the full length, recombinant 69 kDa protein.

## T2a.2 The identification, purification and partial characterization of an endoplasmic reticulum protein with potential calcium binding sites from African trypanosomes

**Scientists:** D. Nandan, T. Pearson*
**Research Associate:** C. Wells
**Technician:** D. Ndegwa

*Department of Biochemistry and Microbiology, University of Victoria, B.C., Canada

A 44 kDa membrane protein has been purified to homogeneity from bloodstream forms of *T. b. brucei*. Purified protein was used to raise polyclonal antibodies in rats. Western blot assays using rat polyclonal serum revealed a doublet of 44/45 kDa in parasite lysates. The total solubilized membranes from *T. b. brucei* bloodstream forms and a total cell lysate of *T. b. brucei* procyclic forms showed identical results. A single band of 53 kDa was found in almost equivalent amounts in all the life cycle stages of *T. congolense* IL 3000. However, *T. vivax* bloodstream forms showed a doublet of 53/56 kDa. No cross reactivity was observed with total cell lysates of rat and bovine liver or bovine fibroblasts. This observation suggests that a trypanosome-specific protein has been isolated.

Indirect immunofluorescence using affinity purified antisera to the 44 kDa protein revealed labelling of the perinuclear area and of a reticular system extending through the middle and posterior regions of the trypanosomes. Immunoelectron microscopy revealed the protein to be present in the endoplasmic reticulum and perinuclear reticulum of all cells. No staining of the Golgi apparatus, cell surface or the flagellar pocket was detected. Protease protection experiments demonstrated that the protein is not exposed to the cytoplasmic side of the endoplasmic reticulum. Antibody-combining epitopes are thus buried in the membrane or oriented towards the lumen of the endoplasmic reticulum.

An amino-terminal sequence of the 44 kDa protein showed significant homology to haemoglobin alpha-T4 chain and a cation binding motif. Unsuccessful efforts were made to assess the possible iron binding nature of the protein. However, binding of ruthenium red by the 44/45 kDa protein was specifically inhibited by 50 mM $CaCl_2$.

Although the role of this protein has yet to be determined it is a useful marker of the endoplasmic reticulum and may perhaps function, in calcium homeostasis, intracellular signalling or protein folding.

## T2a.3 Possible chaperone-like molecules of African Trypanosomes

**Scientists:** D. Nandan, T. Pearson*
**Research Associates:** C. Wells, G. Mpimbaza
**Technician:** C. Muriuki

*Department of Biochemistry and Microbiology, University of Victoria, B.C., Canada

A simple one-step procedure using gelatin-agarose has been used for the purification of two constitutively expressed molecular chaperones from African trypanosomes. The molecular masses of the proteins are 68 kDa and 72 kDa respectively. The 68 kDa protein reacted with a monoclonal antibody which also reacts with members of a family of 70 kDa heat shock proteins from a wide variety of species. The other protein appears to be similar to a trypanosome protein which is an immunodominant antigen in cattle infected with *T. congolense* and has been identified as an HSP 70 homologue of mammalian BiP (see abstract T2a.1).

The amino-terminal sequence of the 72 kDa protein showed identity to the amino terminal sequence determined for the 69 kDa antigen. A monospecific polyclonal antisera against the 68 kDa protein has been raised in rats. Immunoblot analysis of various life cycle stages of *T. congolense* IL 3000 revealed developmental regulation of this protein. Immunoelectron microscopy using the same antiserum showed localization in lysosomal-like organelles in trypanosomes. It has been previously shown that the 72 kDa protein is loocalized both in the endoplasmic reticulum and lysosomal like organelles. Although the 72 and 68 kDa proteins belong to a family of stress proteins, they differ sufficiently to evoke different immunological responses. A comparison of the biological and structural properties of these proteins is in progress.

## T2a.4 Phosphatases of *Trypanosoma congolense*

**Research Fellow:** M. Tosomba
**Supervisor:** J. Lonsdale-Eccles

The secretion, cellular location and properties of acid phosphatase activity in *T. congolense* have been examined. Whilst evidence of extracellular acid phosphatase (AcP) activity has been reported for a number of parasitic protozoa, growing bloodstream forms of *T. congolense* were not found to release AcP into the growth medium. Electron microscopic localization using a modified Gomori technique produced lead phosphate deposition in the flagellar pocket and vesicles of intact trypanosomes. Speculatively, the enzyme contained in the vesicles may be destined for endocytosis, while the activity being transported or secreted in the flagellar pocket may be for purposes of extracellular digestion of molecules. However, further experiments are required to support these suggestions.

Although cytochemical experiments did not demonstrate the presence of 'free' and 'bound' activities, biochemical analysis revealed activity in the high speed pellet

(135,000 × g) extracted with Triton X-100 and the high speed supernatant. The activity in the extract (57%) is higher than that found in the high speed supernatant (10%).

Three AcPs were separated through ion exchange chromatography (DEAE cellulose) and gel filtration (S-300) from cell extracts. A comparative study of their properties indicated that the enzymes were distinct. Their purification and the raising of antibodies for definitive functional and localization studies are in progress.

## T2a.5 Characterization of casein kinase II of *Trypanosoma congolense*

**Research Fellow:** R. Janoo
**Supervisors:** O. ole-MoiYoi, P. Majiwa, N. Murphy

Alignment of casein kinase II (CKII) amino acid sequences from a variety of organisms has allowed the identification of conserved domains for this enzyme, and construction of oligonucleotides for use in the polymerase chain reaction (PCR). Using *T. congolense* IL 3000 genomic DNA as target, these primers have enabled the amplification of sequences of about 400 bp. The PCR products were cloned into plasmid vectors and used to transform competent bacterial cells. The transformants were selected by Southern blot analysis, and the DNA was isolated and sequenced. A homology search in the Gene/Protein database revealed a high degree of identity of the deduced protein sequence to the casein kinase $\alpha(\alpha')$ subunits from both eukaryotes and plants. The deduced sequence displayed a 64% identity to chicken CKII and a 61.4% identity to *Theileria parva* CKII $\alpha$ subunit. However, surprisingly, the deduced nucleotide sequence also showed a 62.7% identity to the mRNA sequence for CKII of *Zea mays*. Of the 133 amino acid residues encoded by this fragment, all the amino acids within the conserved domains of CKII reported so far were identical and all the amino acid replacements were mostly conserved though some radical replacements were also present.

In order to obtain the full length gene of *T. congolense* casein kinase II, a sized EMBL 3A subgenomic library was screened. On Southern blot analysis, a 1.2 kb fragment, obtained upon the digestion of phage DNA with *Xho* I was identified and subcloned into a plasmid (pgNCK II). Sequence data obtained confirmed the earlier observations and comparison of the *T. congolense* sequence with those from higher eukaryotic $\alpha$ and $\alpha'$ sequences reveal that it is most identical with the $\alpha$ subunit.

Northern blots of total and poly ($A^+$)RNA reveal a single transcript of 1.55 kb. Southern blot analysis of gels of chromosome-sized molecules separated by pulsed field gel electrophoresis showed that the gene encoding CKII is present within the megabase chromosomes, which were not resolved under the voltage and pulse interval conditions employed in the analysis. This observation is in keeping with the chromosomal localization of transcribed non-VSG genes of trypanosomes.

## T2a.6 The cysteine protease of *Trypanosoma (Nannomonas) congolense*

**Scientists:** W. Fish, J. Steyaert
**Technicians:** C. Muriuki, D. Ndegwa

The cDNA sequence of a cysteine protease from *Trypanosoma (Nannomonas) congolense* metacyclic forms has been completely sequenced. The deduced amino acid sequence gives a predicted molecular mass of 48.1 kDa for the prepro-enzyme and 33.9 kDa for the processed enzyme. The greatest similarity of the deduced amino acid sequence is to those of other trypanosomatid parasites. Catalytic and structural motifs are conserved amongst the parasite enzymes and the plant enzyme papain and human cathepsin L. The unique poly-proline tract observed in other salivarian trypanosomes is present, as is the unique carboxy-terminal extension seen in all trypanosomatid parasites so far sequenced. Interestingly, in the latter area there is virtually complete conservation of cysteine residues, suggesting that the C-terminal extension is extensively cross-linked with disulphide bonds. This is reminiscent of the C-terminal portion of *Trypanosoma brucei* sub-group VSG molecules. This may render the antigen difficult to process immunologically since reduction of disulphide bonds is a prerequisite in preparing antigens for processing by the variety of proteases found in antigen-presenting cells.

## T2a.7 The vacuolar ATPase of *Trypanosoma (Nannomonas) congolense*

**Scientists:** W. Fish, N. Murphy
**Technicians:** C. Muriuki, D. Ndegwa

The B (regulatory) subunit of the vacuolar ATPase of *Trypanosoma (Nannomonas) congolense* has been completely sequenced. Comparison of the deduced amino acid sequence with that of other organisms shows the most similarity to insects, mammals and plants. These relationships are similar to those previously determined for the A (catalytic) subunit. Interestingly, the latter subunit of *T. congolense* is less similar to that of another obligate blood parasite, *Plasmodium falciparum*, than to the equivalent subunit of metazoan organisms.

Sequence analysis of the proteolipid subunit (PLS) has not been possible as either PCR amplification or screening with sequences from the equivalent subunit of other organisms has not been successful. A portion of both the bovine and *Drosophila* PLS cDNAs, containing the most conserved segments, is now being used for screening. It is probable that African trypanosomes are significantly different in the sequence of the vacuolar ATPase proteolipid subunit from organisms so far examined.

## T2b.1 Bovine T-cell responses to invariant antigens of *Trypanosoma congolense*

**Scientists:** V. Lutje, E. Authié, A. Boulange
**Technicians:** D. Muteti, B. Gichuki

Proliferative responses of bovine lymphocytes to invariant antigens of *T. congolense* (the cysteine protease and 69 kDa BiP-homologue), following tsetse-transmitted challenge, were analysed in several cattle. The antigen preparations used were E64-treated cysteine protease (CP) and R63—a 63 kDa recombinant protein expressed by a partial cDNA encoding the BiP homologue (see Abstract T2a.1). On some occasions, R25—a 25 kDa recombinant protein containing only the C-terminal part of the BiP homologue—was also tested. This fragment, which corresponds to the non-conserved part of the molecule, is likely to contain its T-cell epitopes. No proliferation to these antigens was detected in peripheral blood leukocytes (PBL) from Boran and N'Dama cattle at any stage of infection after treatment of the animals with Berenil, or after the addition of recombinant IL-2 to the culture system. However, significant proliferative responses were observed in cells obtained from lymph nodes (LN) draining the site of infection, which were surgically removed from the animals or obtained immediately after death. Limited proliferative responses to the CP, with stimulation indices (S.I.) of 2.6 and 2.8, were detectable during the first week of infection (day 4/day 7); maximal responses were observed during the second week of infection (S.I. of 147 and 69 on day 11). The response to CP declined (S.I. of 14 on day 19 of infection; S.I. of 5 on day 33), but it remained detectable (S.I. between 3 and 6.5) in LN cells from animals with chronic infections. Although individual variations were observed, the pattern of the response was reproducible between experiments. A similar pattern was observed in the response of LN cells to R63. Highest proliferative responses were detected during the second week of infection (S.I. of 10–30); these responses declined after day 19 (S.I. of 4.8) and remained at levels of 1.6–5.0 during chronic infection. When proliferative responses to R25 were measured in chronically infected cattle, higher S.I. than those obtained with R63 were observed. As the majority of these experiments were conducted on Boran cattle, LN cells from two N'Dama cattle were also tested. On days 11 and 19 of infection, low proliferative responses to the invariant antigens were observed (S.I. of 1.3 on day 11 and 8.3 on day 19 to the CP, and S.I. of 1.0 and 1.9 to R63).

The role of distinct T-cell subsets in the response was then analysed by their selective depletion through fluorescence activated cells sorting. No proliferative responses to the CP, R63 or R25 were detected after depletion of $CD4^+$ or $CD8^+$ T cells. The two T-cell types might be equally responsive to these antigens, by proliferation or by the production of factors which in turn might influence the response of other subsets. The proliferative response was similarly abrogated after depletion of MHC class II positive cells, indicating their probable role in the presentation of these antigens to the relevant T cells.

## T2b.2 Bovine T-cell responses to the variable surface glycoprotein(s) of *Trypanosoma congolense*

**Scientist:** V. Lutje
**Technicians:** D. Muteti, B. Gichuki

No variable surface glycoprotein (VSG)-specific T-cell responses have been described so far in cattle during *Trypanosoma congolense* infection. This has led to the conclusion that the anti-VSG antibody response is T-cell independent. However, both T-cell independent and T-cell dependent B-cell responses to the VSG have been described in mice and, recently, the T-cell response in this species has been shown to be limited to the peritoneal T-cell population.

The proliferative response of bovine lymphocytes to soluble VSG of *T. congolense* IL 1180 (the infecting clone) was analysed in several cattle following tsetse-transmitted challenge with *T. congolense*. Cells were isolated from peripheral blood (PBL) and from lymph nodes (LN) which were surgically removed or obtained immediately after death. No proliferation to the VSG was detected in PBL at any stage of infection. However, a significant proliferative response was observed in LN cells after 11 days of infection, with stimulation indices (S.I.) of 69 and 128 in two different animals; the response then declined rapidly, with S.I. of 2 on day 19 of infection. Proliferation to the VSG was unaffected by the depletion (by fluorescence activated cell sorting) of $CD8^+$ T cells but totally abrogated after the depletion of $CD4^+$ T cells.

To examine the relation between VSG-specific T cells and B cells during *T. congolense* infection, and to verify whether a preferential stimulation of TH1 or TH2 subsets leads to alterations in antibody isotype switch, it is necessary to have access to a large number of homogeneous T cells (lines or clones). Thus, one of a pair of identical twin Boran calves was immunized with soluble VSG. Serum antibody responses to this antigen were mainly directed toward internal VSG epitopes. Strong proliferative responses to the VSG were detected in PBL and were shown to be MHC class II-restricted. T-cell lines were derived after repeated stimulation of PBL *in vitro;* these lines displayed a $CD4^+$ phenotype. The production of VSG-specific T-cell clones is currently under way.

## T2b.3 Development of bovine cytokine reagents to study T- and B-cell responses in bovine trypanosomiasis

**Scientist:** B. Mertens
**Research Associate:** K. Taylor
**Technicians:** K. Tikolo, Y. Verjee, P. Muiya

Interleukin-4 (IL-4) is one of the factors involved in B-cell differentiation, Ig-isotype switch and T cell-dependent immune responses. The expression of bovine IL-4 in *Escherichia coli* has been previously reported, using the pGEX-system. The recombinant fusion protein, rIL-4-GST, was tested for biological activity in an assay based on the T-cell growth factor activity of IL-4 (TCGF assay). Proliferative responses were determined by measuring incorporation of $^{125}$I-UDR into concanavalin A-acti-

vated bovine peripheral blood lymphocytes cultured for three days in the presence of rIL-4-GST. Maximum proliferation occurred with 50 µg/ml rIL-4-GST. No responses were induced by the GST carrier alone.

The recombinant protein, either as fusion protein or as factor Xa cleaved IL-4, was used as an immunogen to raise polyclonal and monoclonal antibodies. Two hybridoma fusions have been carried out. Supernatants from the hybridoma cultures were tested for their ability to bind rIL-4 in an ELISA assay, using either IL-4-GST or GST as the antigen in the solid phase. Eight clones were selected that recognized IL-4-GST, but not GST. All eight monoclonal antibodies (MAbs) are of the $IgG_1$ isotype. Their ability to block the biological activity of rIL-4-GST and rIL-4 expressed in the baculovirus system is being tested at ILRAD and by Dr. B. Collins at the Institute of Animal Health, Compton, UK. On Western blotting, two of the MAbs react with unreduced rIL-4-GST. Development of an immunoassay to measure IL-4 is in progress.

We have also designed and produced 24-mer phosphorothioate antisense oligonucleotides complementary to the translation initiation regions of the mRNAs for bovine IL-4 and bovine IL-2. These oligonucleotides will be tested for their ability to block specifically the synthesis of the corresponding cytokine. These reagents will be useful for future studies of B-cell responses *in vitro*, and for analysing the participation of T-helper cell subsets in bovine immune responses.

### T2b.4 B-cell activation *in vitro*

**Research Associate:** K. Taylor
**Scientists:** D. Williams, V. Lutje
**Technician:** B. Gichuki

Following infection with *Trypanosoma congolense*, cattle experience an increase in the number of peripheral blood B cells and a concomitant increase in the expression of the CD5 antigen on those B cells. There is also a rise in the total serum IgM and in trypanosome antigen-specific IgM. Infections in trypanosusceptible Boran cattle are also characterized by an increase in polyspecific IgM antibodies but a poor antigen-specific $IgG_1$ response. In contrast, trypanotolerant N'Dama cattle produce higher levels of specific $IgG_1$ and polyspecific antibodies are rarely detected.

To determine the significance of this phenomenon to trypanotolerance, techniques are required to further characterize bovine B cells—in particular their state of activation and the nature of the antibodies produced. Previous attempts to detect bovine antibody secreting cells *ex vivo* have been unsuccessful. A method has therefore been developed in which bovine B cells can be maintained in culture and stimulated to differentiate into antibody-secreting cells.

Peripheral blood mononuclear (PBM) cells were isolated from peripheral blood and spleen of ten Boran cattle (four uninfected, three on a single occasion with *T. congolense* and three infected on two or more occasions with *T. congolense*). The cells were cultured in medium alone, or with lipopolysaccharide (LPS), pokeweed mitogen (PWM) and/or recombinant bovine IL-2 for three or six days. The cells were

analysed for their proliferative activity, expression of activation markers and for their capacity to secrete immunoglobulin.

No antibody-secreting cells (ASC) were detected in fresh PBM from either infected or uninfected cattle. The expression of an epitope thought to be associated with the transferrin receptor, recognized by monoclonal antibody (MAb) IL-A77 on B cells was weak. However a higher percentage of total B cells and B cells expressing the Mac1 antigen were detected in infected than uninfected cattle. Significant levels of ASC were observed in fresh spleen cells from all cattle tested. The percentage of IL-A77+ B cells was higher in spleen cells than PBMs.

After three days in culture, no ASC were detected in PBM incubated in medium alone, and the percentage of IL-A77+ B cells was low. However ASC were observed in PBMs cultured with LPS, IL-2 or PWM. The highest numbers of ASC were observed in cultures stimulated with LPS. The addition of IL-2 to LPS-stimulated cells made no significant difference to the number of ASC detected; however the addition of IL-2 to PWM-stimulated cultures appeared to reduce the number of ASC. Higher numbers of ASC were detected in cells from trypanosome-infected cattle. There appeared to be a correlation between the number of ASC and the percentage of IL-A77+ B cells.

Higher numbers of ASC were detected in cultured spleen cells than cultured PBMs, with LPS being the most efficient stimulator of ASC. After three days in culture, although a significantly higher percentage of B cells from infected cattle expressed the Mac1 antigen than from uninfected cattle, there was no significant difference between treatments. Similarly, there was no change in the percentage of B cells expressing the CD5 antigen following culture. PWM was the most efficient stimulator of proliferation in PBMs from uninfected and primary infected cattle. But in animals which had received a multiple infection, the responses of PBMs to PWM was poor. Spleen cells had similar proliferative responses to both LPS and PWM. In PBMs and spleen cells cultured for six days with LPS or PWM, with and without IL-2, higher numbers of ASC were detected, but the overall pattern was similar to the three day cultures.

Trypanosome antigen-specific ASC were detected in spleen cells from trypanosome-infected cattle but not from uninfected animals. Thus, it will now be possible to monitor the phenotype, activation state and antibody profile of B cells in trypanosusceptible and trypanotolerant cattle during trypanosome-infection.

## T2b.5 Alpha-2-macroglobulin: development of reagents and interaction with *T. congolense* cysteine protease (congopain)

**Research Associate:** K. Taylor
**Scientist:** E. Authié
**Technicians:** D. Muteti, B. Gichuki

Alpha-2-macroglobulin (α2M) is a unique serum protein which irreversibly binds most classes of proteases, thus inhibiting their activity on large molecular weight substrates. Upon binding a protease, α2M undergoes a conformational change to a more immunologically active form which is capable of binding a variety of cytokines.

The 'fast' form is rapidly removed from circulation by macrophages through receptor-mediated endocytosis. In trypanosome infected cattle, circulating *T. congolense* cysteine protease (congopain) can be detected by antigen ELISA. This study was undertaken to elucidate the interactions of α2M and congopain and their possible effect on the immune response.

Bovine α2M was purified and polyclonal and monoclonal antibodies were developed. Incubation of purified α2M with congopain resulted in the conversion of α2M from its 'slow' (185 kDa) form to its more immunologically active 'fast' form (85 kDa). This conversion was totally inhibited in the presence of E64 (an inhibitor specific for cysteine proteases) but not in the presence of EDTA and PMSF, thus confirming that conversion of α2M was associated with cysteine protease activity. Conversion at pH 7.0 and 37 °C was nearly complete after three minutes in the presence of an excess of congopain. The stoichiometry of congopain binding to α2M was estimated to be approximately 1.0.

To understand the immunological consequences of α2M-congopain complex formation, the ability of the complex to bind bovine cytokines and the effect of the α2M-congopain complex on cellular responses of *T. congolense*-infected cattle *in vitro* will be determined.

### T2b.6 Depletion of CD8+ T cells in cattle

**Scientists:** J. Naessens, M. Sileghem, J. Scheerlinck, D. Kennedy
**Technicians:** J. Nthale, R. Saya

We have used intravenous injections of mouse monoclonal antibodies to deplete CD8+ T cells in cattle. Depletion of CD4+ T cells has already been achieved by others.

Shock reactions were noted following the first injections into cattle—probably because of agglutination of cells in the blood. However, once CD8+ cells were no longer present in the blood no serious clinical signs were observed. To avoid undue distress, a schedule was adopted in which the amount of antibody is gradually increased with time. Milder hypersensitivity reactions did occur in some animals at later stages.

Doses of 1 mg antibody were enough to clear CD8+ cells from the blood. Depletion was seen within half an hour. However, CD8+ cells reappeared overnight in low concentrations, probably because they leaked from tissues. Analysis confirmed that CD8 depletion in lymph nodes was incomplete after administration of mg quantities of antibody, and a minimum of 50 mg of antibody per week was needed to maintain complete depletion of CD8+ cells in blood and lymph nodes.

Two Boran cattle were depleted of CD8+ cells and infected with *T. congolense* IL 1180. During the course of infection (six weeks) no difference was observed in parasitaemia and PCV between the depleted and two control animals. CD8+ T cells do not therefore seem to contribute to the pathology of the disease.

## T2c.1 Activation of macrophages by *T. congolense:* uptake and destruction of erythrocytes *in vitro*

**Senior Research Fellow:** A. Makumyaviri
**Scientist:** M. Sileghem
**Technicians:** R. Saya, P. Mucheru

Trypanosomiasis in cattle is associated with a severe anaemia which is largely attributed to phagocytosis of erythrocytes by macrophages. In a previous study, an *in vitro* assay was used in which blood monocytes were purified and incubated with $^{51}$Cr-labelled erythrocytes. Subsequently, radioactivity was detected in the supernatant and cell pellet. The activity associated with the cell pellet was assumed to be the direct result of erythrophagocytosis and the activity found in the co-culture supernatant was proposed to result from erythrocytes destroyed intracellularly. It was suggested that $^{51}$Cr release could be used as a measure of erythrophagocytosis. Further, it was proposed that $^{51}$Cr release could be used to screen parasite molecules which are responsible for induction of anaemia as the erythrophagocytosis could be simulated by the activation *in vitro* of macrophages by *Trypanosoma congolense* antigens. The aim of the present study was (i) to reveal the optimal conditions for erythrocyte uptake and destruction by macrophages *in vitro* and (ii) to confirm whether or not $^{51}$Cr release can be used as a measure of erythrophagocytosis.

Six Boran cattle (female, one-year-old) were infected with *T. congolense* clone IL 1180 by tsetse challenge. One Boran animal of the same age was used as a control. Cells were collected weekly. Erythrocytes were labelled with $^{51}$Cr or with fluorescein isothiocyanate (FITC) and incubated with monocytes and/or macrophages in the following combinations: blood monocytes and splenic macrophages from infected and non-infected animals; cells purified with autologous (infected) and normal plasma; different pre-incubation time (from 1 to 24 hours), activation of cells *in vitro* with *T. congolense* lysates, with IFNγ or with both, co-culture (from 2 hours up to 48 hours) with auto- or heterologous erythrocytes and variation in the monocyte-erythrocyte ratio. None of the conditions was suitable to detect a release of $^{51}$Cr above the spontaneous $^{51}$Cr release by erythrocytes, but a marked incorporation was detected. The effector-target ratio, as well as the incubation time, had an influence on incorporation. Incorporation could be observed from a 2 hr incubation and peaked and remained stable between 24 and 48 hr. With blood monocytes, the incorporation was elevated during infection, peaked at week 2 after infection, remained increased until week 5 and then decreased. Splenic macrophages manifested a high level of pre-infection $^{51}$Cr incorporation but no transient increase was seen during infection. When the macrophages or monocytes were separated from the labelled erythrocytes by a 0.4 μm semi-permeable membrane, the non-specific $^{51}$Cr incorporation was negligible. The addition of *T. congolense in vitro* did not trigger incorporation of labelled erythrocytes by monocytes and priming of the monocytes with IFNγ prior to addition of the lysate failed to have a stimulating effect. The erythrocyte uptake by macrophages was also demonstrated by flow cytometry using fluorescein-labelled erythrocytes. This approach obviated the need for monocyte purification which reduced the risks of introducing artefacts.

## T2c.2 Production and assay of TNFα during trypanosomiasis in cattle

**Scientist:** M. Sileghem
**Research Associate:** L. Gaidulis
**Technician:** R. Saya

Cells from the macrophage/monocyte lineage are known to release a variety of cytokines which might affect the immune response at different levels and might have an impact on the pathology of the disease. Of particular interest is TNFα, a cytokine which induces erythrophagocytosis and dyserythropoiesis in murine malaria and causes cachexia in a variety of infections. Initially, plasma of cattle infected with *Trypanosoma vivax* IL 2337 was analysed for the presence of bovine TNF by an enzyme immunoassay but only a low activity was detected. Therefore, an alternative approach was used in which TNF production was measured *ex vivo*. Monocytes from *T. vivax* IL 2337-infected cattle manifested a strong *ex vivo* TNF production. In contrast, TNF production was not detected from monocytes of cattle infected with *T. congolense* ILNat 3.1. Trypanosomiasis due to these parasites differs in the degree of anaemia as indicated by packed cell volume (PCV). *T. vivax* IL 2337 causes a severe, acute fall in PCV whereas *T. congolense* ILNat 3.1 causes a more gradual fall in PCV.

As cytokine production *ex vivo* does not necessarily reflect the amount produced *in vivo*, mRNA was isolated from either cultured or freshly prepared monocytes and TNFα gene expression analysed. Slot blot hybridization with a riboprobe was hampered by the insufficient amount of mRNA obtained from monocytes of infected cattle. Therefore, polymerase chain reaction (PCR) analysis was employed in which the TNFα mRNA was amplified by bovine TNFα-specific oligonucleotides. TNFα message was induced during infection of cattle with both *T. congolense* ILNat 3.1 and *T. vivax* IL 2337 but, early in infection, the expression was elevated in the *T. vivax*-infected animals. This superior response was observed in monocytes cultured for 4 hr, but was also observed in freshly prepared monocytes purified by negative selection to avoid the possibility of causing monocyte activation through the preparation. Hence, the reported differences were not due to an *in vitro* artefact and suggest a correlation between levels of TNF induction with the severity of trypanosomal anaemia.

## T2c.3 Identification of macrophage-activating factors from *Trypanosoma congolense*

**Scientists:** M. Sileghem, J. Naessens, P. De Baetselier*
**Technician:** R. Saya

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Macrophage activation is crucial for the induction of the pathology associated with trypanosomiasis. The direct interaction of parasites with the macrophages is considered to be an important early event initiating the cascade of activation. As a conse-

quence, macrophage-activating molecules derived from parasites might be a first target for the design of anti-disease vaccines. As a first approach, immunosuppression and TNFα production were used as indicators to identify such factors.

We have shown previously that lymph node macrophages from cattle infected with *Trypanosoma congolense* are capable of suppressing T-cell proliferation. Addition of a *T. congolense* lysate to a mouse macrophage hybridoma *in vitro* was found to trigger the cells to acquire a suppressive activity and the macrophage-activating fraction from *T. congolense* could be further enriched using ammonium sulphate precipitation and size exclusion chromatography. In trypanosome infections of mice, suppression of proliferation was entirely due to an inhibition of interleukin-2 receptor (IL-2R) expression. Secretion of interleukin-2 (IL-2) was not affected and secretion of interferon γ (IFNγ) was in fact increased. Blocking of IFNγ with neutralizing antibodies furthermore prevented suppression. In cattle, both IL-2 secretion and IL-2R expression were found to be suppressed. Secretion of IFNγ was slightly increased but addition of neutralizing antibodies had no effect on suppression. Therefore, a bioassay was set up in which bovine lymph node cells pulsed with a *T. congolense* lysate were found to block proliferation but not IFNγ secretion. Preparation of the trypanosome lysate in the presence of protease inhibitors abrogated this activity. Following enrichment of the lysate by ammonium sulphate precipitation, the preparation was further fractionated by high performance liquid chromatography using a Superose 12 size exclusion column. Column elution times for proteins were from 20 and 80 min, and the activity was found in different fractions with an elution time ranging from 39 to 45 min. The fractions contained a heterogeneous protein mixture, with dominant common bands detected around 70, 50, 40, 30 and 20 kDa.

Another macrophage function, TNFα production, could be simulated *in vitro*. Addition of soluble variable surface glycoprotein (VSG) to bovine monocytes was found to cause secretion of TNFα when the monocytes were optimally primed with IFNγ prior to addition of the lysate, but not when unstimulated monocytes or sub-optimally primed monocytes were used. In contrast, the membrane form of VSG induced secretion of TNFα when added to unprimed cells and caused a potent TNFα response when added to sub-optimally primed cells. These experiments identify the glycosyl phosphatidylinositol anchor of the VSG, which is not present in soluble VSG, as a potentially potent macrophage-activating moiety.

## T2c.4 Expression and purification of biologically active bovine tumour necrosis factor α

**Research Associate:** L. Gaidulis
**Scientist:** M. Sileghem

Inhibition of certain aspects of macrophage function is the most direct approach to determining the significance of macrophage activation in susceptibility to trypanosomiasis. For different macrophage-derived cytokines, this can be achieved by administration of neutralizing antibodies *in vivo*. Since TNFα has been suggested to be associated with the severity of the disease, this cytokine was considered of primary

importance for such neutralization studies. To produce antibodies for neutralization, biologically active recombinant TNFα was required in large quantities.

The full length coding region for TNFα has been isolated and cloned as reported previously. Oligonucleotide primers were designed to amplify a TNF fragment at the nucleotide sequence coding for the first amino acid of the mature protein. The amplified product was first sub-cloned into Bluescript vector to verify the restriction sites and subsequently into the pMAL-P expression vector to allow for secretion of the protein. *Escherichia coli* cells were transformed with this construct and the resulting transformants induced to produce recombinant protein. The presence of TNF was indicated by the appearance of a strong fusion protein band of 59 kDa which reacted in a specific manner with a polyclonal anti-TNF serum. In the pMAL-P expression system, the protein is fused to a maltose binding protein, allowing protein purification by adhesion to an amylose resin followed by elution with maltose. Through the presence of a cleavage site for the protease Factor Xa, the protein of interest can be released from the carrier protein. In this fashion, one litre of culture yielded about 7–12 mg of relatively pure fusion protein. Cleavage with Factor Xa protease yielded a fraction containing three bands: a 59 kDa uncleaved fusion protein, a 42 kDa maltose binding protein band and a 17 kDa recombinant TNF band. Following confirmation of the presence of biological activity and the capacity of the monoclonal antibody BC9 to bind the active recombinant TNF molecule, the mixture was further purified by affinity chromatography. Uncleaved fusion protein, which also reacts with the antibody, could be excluded by increasing the cleavage efficiency by addition of 0.02 % sodium dodecyl sulphate. Alternatively, the active, mature TNF could be purified from cleaved TNFα by size exclusion chromatography in a high performance liquid chromatograph. The purified TNFα spontaneously formed trimers, which is the predominant form of TNFα in its native conformation.

## T2d.1 Sequential changes in the bone marrow of Boran cattle re-challenged with *Trypanosoma congolense* (IL13-E3)

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**Research Associate:** C. Wells
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To evaluate the efficiency of haemopoiesis affecting the development and maintenance of anaemia in trypanosome-infected cattle, weekly bone marrow samples were collected from five adult Boran cattle prior to reinfection with *Trypanosoma congolense* (IL13-E3) and for 14 weeks subsequently. Peripheral blood haematology, histology and bone marrow cytology were monitored in conjunction with clonogenic assays. The onset of parasitaemia was detected between two and three weeks post-infection (pi). Two animals became severely anaemic: one was euthanized at week 8 (the animal was also leucopaenic and the bone marrow was later shown to be aplastic) and one was treated at week 9. The erythrocyte indices, i.e. mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) did not change from the baseline levels during the first 10 weeks despite the continued drop in mean packed cell volumes (PCV). The anaemia during this period was normocytic normochromic (indicating the non-responsiveness of the bone marrow). Three of the cattle developed chronic anaemia during weeks 11–14 with PCV fluctuating between 18–19%. During this period the MCV progressively rose and the MCHC gradually decreased indicating that the bone marrow response was improving. Similarly, cytology, histology and clonogenic assays provided evidence of an increased erythroid response. The improved bone marrow response allowed the cattle to maintain a stable PCV of 18–19% but not to raise this level. Three weeks following treatment the PCV had increased by only 8% and it did not return to baseline levels for three to five months. Four of the five cattle were periodically leucopaenic during the infection. The mean number of monocytes in blood remained below baseline levels throughout the infection. The mean neutrophil numbers in blood remained below baseline except for peaks at weeks 3 and 10.

Two weeks pi (prior to the onset of parasitaemia) the percentages of lymphocytes ($P < 0.025$), plasma cells ($P < 0.025$) and macrophages ($P < 0.025$) in the bone marrow had significantly increased over baseline, while the percentage of erythroid and myeloid cells remained unchanged. Similarly the diameter ($P < 0.001$) and surface area ($P < 0.001$) of macrophages had significantly increased suggesting that the onset of an immunological reaction in the bone marrow preceded the appearance of detectable parasites in the blood. Cytophagia was not noted in the bone marrow until day 29 at the first peak of parasitaemia. Between weeks 3–7 pi the PCV dropped from $35.8 \pm 2.6\%$ to $24.2 \pm 4.0\%$. Simultaneously there was a marked proliferation of lymphoblasts, lymphocytes, plasma cells and macrophages, yet the erythroid:myeloid ratio remained unchanged. Between 7–14 weeks pi the PCV dropped further from $22.6 \pm 4.2\%$ to $18.0 \pm 0.1\%$. Erythroid precursors increased progressively, granulocytic precursors decreased progressively, macrophages remained elevated and the percentage of lymphoblasts, lymphocytes, and plasma

cells declined. Many macrophages in the bone marrow were heavily laden with haemosiderin (iron).

## T2d.2 Kinetics of haemopoietic progenitor appearance in N'Dama and Boran cattle during a primary *Trypanosoma congolense* infection

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**Technicians:** P. Muiya, M. Opollo, J. Kamau

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The reported study was undertaken to determine if the ability of the N'Dama cattle to control better the anaemia associated with trypanosome infection resides in a superior bone marrow response. Using clonogenic assays, the kinetics of haemopoietic progenitors were compared in three adult trypanotolerant N'Dama cattle and three age-matched trypanosusceptible Boran cattle during the first 17 weeks of a primary infection with *Trypanosoma congolense* clone IL 1180. While the parasitaemia decreased in N'Dama cattle as the infection progressed, the parasitaemia remained high throughout the infection in the Boran cattle. When compared to the N'Dama cattle, the Boran cattle had a more dramatic drop in the packed cell volume (PCV) from 25 days post-infection (dpi) onwards resulting in their PCV stabilizing at lower levels. An increase in mean corpuscular volume (MCV) levels from 46 dpi in the Borans and 63 dpi in the N'Damas was indicative of the responsive nature of the anaemia. This erythropoietic response was confirmed in assays *in vitro* by the occurrence of peaks above baseline of both the early (burst-forming units erythroid, BFU-E) and late (colony-forming units erythroid, CFU-E) erythroid progenitors in cultures from both breeds of cattle. These peaks of erythroid cells were of similar magnitude in the two breeds. However, when the erythroid response was related to the degree of the anaemia, then it appeared that the bone marrow of the N'Dama cattle responded at higher threshold levels of PCV. From 98 dpi onwards, despite the low PCV, the MCV returned to normal and low erythroid progenitor levels were observed in the two breeds. The decline in the levels of the granulocyte-macrophage progenitors (CFU-GM) between 7–42 dpi might account for the decreased numbers of neutrophils and monocytes in the blood observed in both breeds. Thereafter, a further significant drop in the CFU-GM levels was observed in the Borans which may explain the continued decrease in neutrophil and monocyte counts in their blood. In contrast, significant peaks of CFU-GM above baseline were observed in N'Dama cattle at 49 dpi, which could explain the subsequent partial recovery of the neutrophil and monocyte numbers in their blood. This study indicated that following a primary trypanosome infection, the N'Dama cattle were able to increase effectively both the erythroid and myeloid progenitors during the acute phase of the disease and that this might correlate with their ability to maintain both higher red and white cell numbers in blood.

In a previous study, using a *T. congolense* clone IL 13E3 re-challenge infection in five adult Boran cattle, the red cell indices (MCV and MCHC) were unchanged until

week 10 post-infection and low erythroid progenitor levels were observed in the bone marrow. From week 11–14 post-infection, a progressive rise in MCV was associated with a gradual decrease in MCHC, a CFU-E peak above baseline was noted at week 12 and BFU-E appeared in the peripheral blood culture of two animals. During the primary infection with *T. congolense* clone IL 13E3 three years earlier, the same animals developed a non-responsive normocytic normochromic anaemia.

Together, these two studies seem to suggest that the responsive or non-responsive nature of the anaemia occurring during *T. congolense* infection depends on the *T. congolense* clone used. Consequently, a third study is under way to compare the kinetics of haemopoietic progenitors in N'Dama and Boran cattle following infection with *T. congolense* clone IL 13E3.

### T2d.3 Simultaneous challenge of N'Dama and Boran cattle with several clones of *Trypanosoma congolense*

**Scientists:** E. Authié, D. Moloo, L. Logan-Henfrey
**Technicians:** D. Muteti, Z. Javer

A group of three adult N'Dama and four adult Boran cattle challenged and treated two years earlier with *Trypanosoma congolense* IL 1180 were simultaneously re-challenged with four different isolates of *T. congolense* (IL 2642, IL 3281, Farakoba/80 and Nguruman). Ten tsetse infected with each clone were allowed to feed on the left flank of an individual animal. Haematology and parasitaemia were followed over a 15-week period. Parasitaemia was detected in one N'Dama on day 8, one N'Dama and one Boran on day 9 and the remaining animals on day 10. The Boran cattle became leucopaenic within one week following onset of parasitaemia and were all intermittently leucopaenic until treatment. Their packed cell volume (PCV) dropped at a more rapid rate than those of the N'Dama. The Boran cattle had high parasitaemias throughout a short acute infection which required the animals to be treated on days 40, 42, 43 and 61. The anaemia of the Boran cattle, as characterized by the red cell indices, was normocytic normochromic, thus demonstrating a lack of a compensatory bone marrow response. The N'Dama cattle were persistently parasitaemic through 15 weeks of infection. The PCV of two N'Damas fell below 20% on day 57 and stabilized at 18–19% between 8 and 15 weeks, and in the third animal fluctuated between 22–25%. Each of the three N'Damas were leucopaenic for varying lengths of time; from days 48–105, 82–89 and on day 85, respectively. None of the N'Dama recovered their preinfection PCV or leucocyte counts by week 15. The anaemia of the N'Damas was initially normocytic normochromic but became progressively more responsive in two animals resulting in a true macrocytic hypochromic anaemia. Despite the evidence of a responsive bone marrow in two of the three N'Dama cattle, the PCVs failed to improve during the 15 weeks of infection. This multiple clone challenge more closely mimics trypanosomiasis infections in the field where parasitaemias are unabated during the acute infection, animals develop leucopaenia, and zebu cattle require treatment in the acute phase of the disease. In this experiment two of three N'Dama were severely and persistently anaemic and in response developed a macrocytic anaemia.

## T2d.4 Effects of bone marrow adherent cells on the growth *in vitro* of haemopoietic progenitors from *Trypanosoma congolense*-infected cattle

**Scientists:** A. Andrianarivo, L. Logan-Henfrey
**Technicians:** P. Muiya, M. Opollo

The mechanisms underlying the ineffective haemopoiesis associated with *Trypanosoma congolense* infection in cattle are not known. Any possible disturbance in the bone marrow microenvironment could result in an imbalance between the stimulatory and the inhibitory cytokines, leading to dyshaemopoiesis. Since bone marrow adherent cells are the main source of these cytokines, the effects of these cells on the growth of the progenitor cells *in vitro* from three N'Dama and three age-matched Boran cattle were examined during a *T. congolense* (clone IL 1180) infection. Bone marrow mononuclear cells (BMMNC) were cultured before and after removal of the adherent cells and the yields of progenitor cells were compared. In most cases, removal of the adherent cells prior to the infection resulted in either no change or a decrease in erythroid progenitor levels. However, following the infection, a marked but inconsistent increase in erythroid progenitor levels was observed mainly beyond day 56 in the nonadherent cell population as compared with the BMMNC. The growth of granulocyte-macrophage progenitors *in vitro* was not affected by the removal of the adherent cells both prior to and during the infection.

## T2d.5 Enrichment of bovine haemopoietic progenitor cells by negative immunomagnetic selection using monoclonal antibodies

**Technician:** P. Muiya
**Scientists:** J. Naessens, L. Logan-Henfrey, A. Andrianarivo

Monoclonal antibodies (MAbs) have been used to enrich for early bovine bone marrow progenitor cells by negative selection using magnetic beads. MAbs that react with the more mature precursors of bone marrow cells have been raised. Other MAbs were selected from the second International Bovine Antibody Workshop panel.

Two fusions for antibody production have been carried out, one using a spleen from a mouse immunized with large transferrin receptor (TfR)-negative bovine bone marrow cells and another using a spleen from a mouse immunized with large IL A99/24 negative bovine bone marrow cells. The cell population in the large TfR negative cell fraction contains lymphocytes, myeloid cells and the burst forming unit erythroid (BFU-E) cells. The cell population contained by sorting the large IL A99/IL A24 negative cells contains erythroid lineage cells (CFU-Es and BFU-Es). Screening for positive hybridomas was done by flow cytometry. New MAbs raised to these fractions include 4F10, which reacts with its antigen on granulocytes only, and 5B11 and P2C5, which are not yet characterized. MAb P5B3 raised against large IL A99/24 negative bone marrow cells reacts with antigen on erythroid cells.

Trials were carried out to determine the optimum magnetic beads:cell ratio and whether non-specific binding removes colony forming progenitor cells. A cell to beads ratio of 1:20 gave the lowest non-specific binding (26%). Despite non-specific

binding, no change was observed in the number of colony forming cells detected in clonal assays.

Low density mononucleated bone marrow cells pooled from 6–10-week-old Boran calves were enriched for progenitor cells using a selected panel of MAbs. These were seeded in clonogenic assays and BFU-Es counted on day 10. A two-fold enrichment of BFU-Es was observed with a mixture of MAbs IL-A51, IL-A43 and IL-A12, which depletes T-lymphocytes. IL-A137, which reacts with an erythroid antigen, and IL-A149, which reacts with the TfR antigen, each gave a two- to three-fold enrichment of BFU-Es. No enrichment was observed with MAbs IL-A110 and IL-A118, both reacting with antigens on myeloid cells.

### T2d.6 Screening for survival of bovine haemopoietic stem cells in SCID/SCID.Bg/Bg mice

**Technicians:** P. Muiya, J. Kamau
**Scientists:** L. Logan-Henfrey, J. Naessens, S. Kemp

The spleens of sub-lethally irradiated SCID/Bg mice inoculated intravenously with $2 \times 10^5$ enriched (passed over two ficoll gradients followed by selection for transferrin positive cells) bovine bone marrow progenitor cells developed nodules 12 days later. Histological examination of these nodules revealed the presence of erythroid cells with occasional megakaryocytes and neutrophils, suggesting that these cells might originate from a pluripotent stem cell (CFU-S). However, no bovine DNA was detected in the spleens with nodules by polymerase chain reaction (PCR). Characterization of the cells in the nodules using monoclonal antibodies (MAbs) which react with bovine peripheral blood leucocyte and bone marrow cell antigens did not give conclusive results due to non-specific staining.

Twenty-five SCID/Bg mice were irradiated with 400 rads each. Ten mice were inoculated with $10^6$ separated bovine bone marrow cells and ten mice were inoculated with $1.5 \times 10^7$ cells. Five irradiated mice served as controls. Concanavalin A-conditioned medium has been shown in clonogenic assays to stimulate growth of CFU-GM *in vitro*. In order to increase the number of bovine granulocytes and monocytes within the SCID/Bg splenic nodules, Con A-conditioned medium (0.2 ml) was injected into each of five mice on days 0, 2, 4, 6, 8 and 10. On day 13, the spleens of five control mice, five mice given cells, and five mice given cells and Con A were examined. None of the spleens was enlarged and no splenic nodules were detected in any of the mice. The experiment will be repeated using bovine bone marrow populations enriched or depleted of transferrin positive cells.

## T2d.7 Cloning of bovine Stem Cell Factor cDNA

**Scientist:** B. Mertens
**Research Associate:** E. Gobright

Stem Cell Factor (SCF), a recently described haemopoietic growth factor (also known as c-Kit Ligand, Mast Cell Growth Factor or Steel Factor), is an essential regulator for normal haematopoiesis, melanogenesis, gametogenesis and mast cell growth and development. In human and mouse it has been shown that SCF exists in both membrane and soluble forms.

To clone the bovine analogue of the cDNA portion coding for soluble SCF, oligonucleotide primers were designed based on interspecies homologies in gene sequences. RNA was isolated from bone marrow mononuclear cells of Boran cattle. The RNA was reverse transcribed into cDNA and this was used as a template in the polymerase chain reaction (PCR). The amplification resulted in DNA fragments of various lengths. The PCR products were subcloned and sequenced. The sequence of the bovine cDNA contains an open reading frame up to the end of the available fragment which would encode 174 amino acid residues. A 25 amino acid leader peptide similar to that in human SCF is present. Homology with human SCF at the amino acid and nucleotide level is 85 and 90%, respectively. Preservation of cysteine residues at positions 4, 43, 89 and 139 is thought to result in disulphide bonding identical to that seen in the rodent and primate SCFs. At amino acid position 130, the bovine sequence contains an additional codon not present in human or rodent SCFs, but present in the canine analogue. Experiments are in progress to express the truncated bovine cDNA coding for soluble SCF, up to alanine residue 165. The availability of recombinant SCF will be a useful tool to study mechanisms of ineffective haematopoiesis in bovine trypanosomiasis.

## T2d.8 Tumour necrosis factor-α gene expression in cattle infected with *Trypanosoma congolense* (IL 1180)

**Research Associate:** L. Gaidulis
**Scientists:** L. Logan-Henfrey, B. Mertens, A. Andrianarivo
**Technician:** P. Muiya

To study the role of TNF-α in the anaemia observed in cattle infected with trypanosomes, synthetic oligonucleotides were designed and used in a polymerase chain reaction (PCR) to analyse TNF-α gene expression in peripheral blood mononuclear cells (PBMC) and bone marrow mononuclear cells (BMMNC) during a *Trypanosoma congolense* (IL 1180) infection.

Three N'Dama and three Boran cattle were infected and samples of PBMC and BMMNC collected once a week for sixteen weeks. Total messenger RNA (mRNA) was isolated and stored at –70 °C until analysis. The mRNA of weeks 1 to 9 after infection and weeks 1 to 3 prior to infection was reverse transcribed into single stranded cDNA and used as a template in the PCR.

Preliminary results of the PCR analysis indicate that TNF-α mRNA transcripts could be detected in the PBMC and BMMNC of both breeds as early as one week after infection and up to four weeks following infection. Constitutive preinfection TNF-α expression was observed in the PBMC of all the animals and in five of six BMMNC. It is presently difficult to determine whether the pre- and post-infection levels of TNF-α expression differ quantitatively; therefore, no conclusions as to breed differences can be drawn based on these preliminary experiments in a small group of cattle. It appears, however, that there is a marked individual variation in TNF-α expression in cattle infected with *T. congolense*. A procedure to determine quantitative differences in cytokine expression RT-PCR has not yet been established. The mRNA and cDNA collected from this experiment will also serve as a useful source for analysis of the expression of other cytokines during a trypanosome infection.

## T2d.9 Cloning of bovine interleukin-3 cDNA

**Research Fellow:** S. Mwangi
**Supervisors:** B. Mertens, L. Logan-Henfrey

Interleukin-3 (IL-3) is a cytokine expressed by activated T lymphocytes and natural killer cells. *In vitro*, IL-3 supports the growth of early haemopoietic progenitors, and synergistically acts with more restricted cytokines to promote erythroid, myeloid, mixed and megakaryocytic colonies. IL-3 is highly species-specific in its activity. In order to study the role of IL-3 in haemopoietic stem cell differentiation, and to study the aetiology of the anaemia associated with bovine trypanosomiasis, a source of recombinant bovine IL-3 is required.

Using primers based on the 5′ and 3′ untranslated regions of the ovine IL-3 cDNA (courtesy of the Moredun Research Institute, Edinburgh, UK), we have amplified, by the polymerase chain reaction (PCR), a 533 bp cDNA fragment from cDNA made from mRNA isolated from 72h PHA- and PHA/PMA-stimulated PBL from Boran cattle. This cDNA has been cloned into the pGEM-T vector (Promega) and sequenced. The sequence of the cDNA contains a single open reading frame, 432 nt long, which codes for 144 amino acids. The first 17 residues of this polypeptide include a very hydrophobic sequence that is characteristic of a protein signal peptide. The dipeptide Ala-Pro found at residues 18–19 has been found at the amino terminus of several lymphokines, including human and ovine IL-3 and GM-CSF We therefore predict that the Ala 18 is the amino terminus of the mature bovine IL-3 protein. The mature protein would comprise 127 amino acids and have a molecular mass of 14.5 kDa. The cDNA has high homology with the ovine IL-3 sequence (85.4% at the amino acid level, 90.5% at the nt level), and low, but significant homology with the murine (25.7% at the amino acid level, 47.75% at the nt level) and human (35 and 55.8%, respectively) IL-3 sequences. Work is under way to subclone the bovine cDNA and to express the recombinant protein.

## T2d.10 Cloning and sequencing of bovine erythropoietin cDNA

**Research Fellow:** H. Suliman
**Supervisors:** B. Mertens, L. Logan-Henfrey

One of the possible causes of the impaired erythropoiesis associated with anaemia in trypanosomiasis may be an inappropriate erythropoietin (EPO) response. EPO is the principal regulator of erythropoiesis in mammals. Its main site of production is the kidneys in adults while the liver is the main source of EPO during foetal life.

Frozen kidneys and livers were obtained from anaemic Boran steers infected with *Anaplasma marginale*. Fresh kidney and liver were taken from an anaemic Boran and an N'Dama steer infected with *T. congolense*. Cytoplasmic RNA was extracted from these tissues, and both the total and poly A+ RNA were reverse transcribed into cDNA and used as a template in polymerase chain reaction (PCR) assays. Four oligonucleotide primers were designed based on the conserved sequences in the translated and the untranslated region of the human, monkey and mouse EPO cDNA. Depending on the primers used, 0.5 kb and 0.9 kb fragments were amplified. On Southern blot, these fragments hybridized with a monkey EPO cDNA probe and with each other. The two fragments were subcloned and further characterized by sequencing. cDNA from the N'Dama kidney was used to construct a cDNA library according to the ZAP-cDNA system protocol. This kidney cDNA library was screened with the 0.9 kb PCR product. Positive plaques have been detected and full length inserts of bovine EPO cDNA are being sought for expression of the recombinant hormone.

## T2e.1 Microsatellite identification and analysis

**Scientists:** S. Kemp, O. Hishida, A. Teale

Microsatellites are highly polymorphic stretches of simple sequence repeats; there are estimated to be many thousands of such regions in the bovine genome. Many may be uniquely characterized by means of their flanking sequences to provide polymorphic, sequence-tagged sites. These markers are highly informative and easy to apply to large numbers of animals.

In the search for bovine polymorphic microsatellite sequences, 260 clones containing the microsatellite (dG-dT)n.(dC-dA)n have been isolated and sequenced from male N'Dama *(Bos taurus)* subgenomic libraries. The subgenomic libraries were constructed as follows: genomic DNA was restriction endonuclease digested with SAU3A 1, separated by agarose gel electrophoresis, size selected by excision from the gel of a band corresponding to 500–650 bp, purified by *GeneClean*, ligated into the BamH1 site of Puc19 and used to transform competent *Escherichia coli*.

The resulting libraries were screened with a $^{32}$P-labelled $(dG\text{-}dT)_{10}$ oligonucleotide. Positive colonies were re-cloned and an aliquot of each was grown on a gridded membrane. These membranes were screened sequentially with both $^{32}$P labelled $(dG\text{-}dT)_{10}$ and with $^{32}$P-labelled size-selected genomic DNA from N'Dama ND7. Those colonies which hybridized strongly with the $(dG\text{-}dT)_{10}$ oligonucleotide but weakly with genomic DNA were selected for sequencing. In this way, clones containing microsatellites within larger repetitive elements were excluded. This eliminated the problem of uninterpretable multi-locus amplification products which had occurred in approximately 40% of clones screened only with $(dG\text{-}dT)_{10}$.

Two-hundred-and-sixty-two clones in total have now been sequenced. To date, primers to amplify the microsatellite region from a total of 118 of these sequences have been designed. Whilst not all of the 262 sequences were suitable for primer design, approximately 100 further primer pairs will be designed and synthesized on the basis of the remaining sequences. Forty-two polymorphic markers have so far been characterized and extrapolation from current success rates indicates that approximately 70 additional polymorphic markers will result from the already sequenced clones. This will bring the total number of ILRAD microsatellite markers to approximately 100.

Linkage data is concurrently being amassed on these microsatellites by typing the two, large, full sibling N'Dama × Boran families at ILRAD. These families are part of the International Bovine Reference Panel (see Abstract T2e.5) and the rest of this panel (approximately 300 animals) will be typed when the complete set of DNA is available. Chromosome assignments of the ILRAD microsatellites are being made by collaborators Dr. J. Womack (Texas A & M University, USA) and Dr. G. Guerin (INRA, Paris, France) and by ourselves using the bovine/rodent hybrid panel provided by Dr Womack. To date 18 markers have been assigned to chromosomes. New markers have also been screened against sheep and goat DNA and approximately 60% have been found to be useful in these species. These are being used by groups developing a genetic map of sheep.

## T2e.2 Polymorphic markers in specific genes

**Research Fellow:** M. Agaba
**Scientist:** S. Kemp

To correlate the genetic maps of mammalian species requires the identification of polymorphic markers within sequences which are conserved between species. Such markers are known as *type I* or *anchor* loci. The 3′-untranslated (3UT) region of expressed genes is a potential source of such markers. Consequently, DNA sequence differences between N'Dama and Boran cattle in the 3UT regions of selected genes are being characterized and polymerase chain reaction (PCR)-based assays are being developed for rapid genotyping. Oligonucleotide primers defining the 3UT regions of these genes have been designed and used to initiate PCR amplification of DNA from a Boran cow and an N'Dama bull. The amplification product from each animal is then examined for polymorphism either by single-strand conformation polymorphism (SSCP) or by sequencing. Finally, a robust assay has been established to detect this polymorphism routinely; three types of assay are being used for this purpose: SSCP, PCR-restriction fragment length polymorphism (RFLP) and allele-specific amplification (ASA).

We have previously assayed polymorphism in the gamma crystalline and parathormone genes by means of ASA. Here we report polymorphism in eight other loci. The clathrin alpha chain gene is found on bovine synthetic group U25. SSCP analysis revealed polymorphism between the PCR product of N'Dama and Boran cattle. A similar assay revealed polymorphism in the β-arrestin and cartilage proteoglycan genes which have not yet been assigned to bovine chromosomes or synthetic groups.

The myoglobin and osteonectin genes are located on bovine chromosomes 5 and 7 respectively. Sequence differences in these genes were detected by sequencing the PCR product and PCR-RFLP assays were applied to screen for these polymorphisms. The enzyme MboII detects a polymorphism in the PCR product of the myoglobin gene and NlaIII detects a polymorphism in the osteonectin gene. There is strong breed association of the allele frequencies.

The fibronectin gene is on bovine synthetic group U17. A T/C substitution at a single nucleotide position has been identified in the PCR product, the N'Dama bull and Boran cow have T and C respectively at this position. The homozygous state of these alleles make them ideal markers in the N'Dama/Boran F2 generation. An ASA assay is being developed to screen for this marker.

The genes for the glycoprotein hormone alpha subunit and the protamin gene are on synthetic group, U1 and U8 respectively and ASA assays have been developed for both genes. However, they both have a low degree of polymorphism.

Insulin-like growth factor 1, growth hormone and somatostatin genes are not polymorphic in the populations studied. Interleukin-6, interleukin-7, CD8 antigen, prion protein, clathrin beta, superoxide dismutase 2 and chromogranin genes are under investigation.

The target is to identify at least one polymorphic type I locus for each bovine chromosome and to integrate these into the microsatellite-based linkage map by screening the International Bovine Reference Panel with both types of marker. The

anchor loci will then serve as points of cross-reference between the bovine and human and murine maps. Preliminary population studies will also be performed to determine the allele frequencies for each gene in samples representing different breeds of cattle from Africa.

### T2e.3 Genes controlling survival time following *Trypanosoma congolense*-challenge of inbred mouse strains

**Scientists:** S. Kemp, A. Teale

It has been demonstrated previously that different mouse strains have dramatically different survival times following challenge with *Trypanosoma congolense* (e.g. Morrison, W.I., *et al., Clinical Experimental Immunology* 32: 25–40, 1978.) In these experiments A/J, Balb/C and C57/Bl6 mouse strains showed a mean survival time of 16 days, 50 days and 110 days, respectively, following infection with *T. congolense.* An understanding of this phenomenon may provide insight into the processes responsible for trypanotolerance in cattle and suggest intervention strategies—whether or not equivalent mechanisms are involved. Advances in gene-mapping technology have made a genetic-analysis approach to this question feasible.

A Balb/C × C57 crossbred population was therefore established and challenged with *T. congolense* (IL 1180—$10^4$/mouse). The mean time to death of the parental stocks was 68 days for the Balb/C and 142 days for the C57. The F2 (399 mice) showed a mean time to death of 91 days and the backcross to the Balb/C parent (376 mice) had a mean survival time of 82 days.

Interestingly, a significant number of mice became non-parasitaemic and by day 208 all surviving mice (30% of the C57, 3% of the F2 and 0.2% of the back cross) were aparasitaemic. All of these individuals had shown prolonged periods of parasitaemia early in the infection. DNA is now being prepared from animals at the extremes of the F2 phenotype and these will be analysed both by bulked segregant analysis using randomly amplified polymorphic DNA (RAPD) markers and by a commercial murine microsatellite-based map. It is planned to perform an equivalent experiment in an A/J × C57 cross. This cross is expected to show a larger range of phenotype due to the extreme susceptibility of the A/J strain.

### T2e.4 Cattle breed and population-specific markers

**Scientists:** A. Teale, P. Gwakisa* S. Kemp

*Sokoine University of Agriculture, Morogoro, Tanzania

The need for conservation of livestock genetic resources, whether based on *in situ* or *ex situ* approaches, is recognized world-wide. The number of potential target populations is very great. We believe, therefore, that for conservation programs to be effective and economical, there is a requirement for technologies which will enable conservationists and breeders to compare the gene pools of different breeds and

populations of animals, and to obtain sound estimates of genetic distances between the different groups. Such information, together with data on phenotypic characteristics, including productivity and disease resistance capacities, will serve to focus conservation efforts and thus maximize the return on the investment in global conservation.

We have continued to develop and apply randomly amplified polymorphic DNA (RAPD) analysis of pooled DNA samples for the identification of breed/population-specific DNA polymorphisms (reported previously). Because genetic erosion of N'Dama *(Bos taurus)* populations in West Africa is believed to be occurring on an increasing scale through crossbreeding of N'Dama with zebu *(B. indicus)* animals, we have focused our efforts on detection of zebu-specific DNA polymorphisms.

A panel of 80 10-base primers was used to screen DNA pools constructed with samples from individuals of a number of African, European and Caribbean breeds of cattle. Two primers revealed clear and informative polymorphisms and were selected for further development.

Primer ILo 526 generated a 498 bp product from the DNA of all individuals of Boran, Brahman, Maasai zebu, and Tanzanian zebu origin tested, but not from the DNA of any Friesian or N'Dama animals (the latter being derived from herds in both The Gambia and Gabon). The 498 bp product has been cloned and sequenced, and attempts are currently being made to establish the chromosomal position(s) of the locus (loci). The RAPD product appears to be closely related to satellite sequences present in the genomes of several species of ruminants. It is therefore likely that the zebu-specificity which we detect is a result of a mutation in the RAPD priming site which has occurred subsequent to the divergence of the two main cattle sub-species.

Primer ILo 1065 generated an 1150 bp product from the DNA of all male animals of zebu origin tested to-date, but not from the DNA of males of five European *B. taurus* breeds, or females of either cattle subspecies. This Y chromosome polymorphism provides a simple means to detect instances in which zebu bulls have been used with *B. taurus* cows for crossbreeding purposes. Attempts are being made to clone the 1150 bp product and to determine its precise nature.

As the ILo 526 marker detects a subspecies-specific polymorphism in what is possibly a widely dispersed autosomal sequence, and the ILo 1065 marker, which is similarly subspecies specific, is on the Y chromosome, a combination of the two provides a particularly powerful means to detect introgression of zebu genetic material into *B. taurus* populations.

### T2e.5 Bovine reference and resource populations

**Scientists:** A. Teale, D. Kennedy
**Research Associate:** S. Leak

In 1992–93, the bovine genome mapping community, acting under the auspices of the International Society for Animal Genetics, selected a number of bovine families to form an international bovine reference family panel. The purpose of the panel is to serve as a source of standardized material for development of a global consensus linkage map of the bovine genome. The panel comprises 311 animals in full-sibling

families in the USA, Europe, Australia and at ILRAD. The ILRAD contribution is two large full-sibling N'Dama × Boran families (37 and 29 offspring) which provide nearly 25% of the international reference bovine DNA. DNA from the families has been supplied to distributing laboratories in the USA, Australia and Europe. The material is currently being used in the laboratories of the International Bovine Genome Project for marker mapping, and data collection and analysis have been centralized at CSIRO-MAGEC (Brisbane). A steering committee (which has an ILRAD representative) has produced an agreement which governs DNA exchange, submission of results and marker assignation to the map between collaborating laboratories.

Development of a trypanotolerance resource population of N'Dama × Boran cattle, has continued with the third and fourth full-sibling F1 families completed in July 1993, with 22 and 21 offspring born or *in utero*. The first F2 animals, produced by intercrossing the first two F1 families, were born in November 1992. By the end of 1993, 47 F2s had been born and 56 more are confirmed *in utero*. Among these, three major families are developing, on which work will continue to bring two of them to 30–40 calves and one to 20–30 calves. Further development of F2 families will then be achieved by intercrossing between the third and fourth F1 families when animals reach breeding age.

Additionally, 31 F2 embryos in one family have been cryopreserved. Work will continue to establish an appropriately sized bank of N'Dama × Boran F2 embryos in several families. Challenge of F2s with *Trypanosoma congolense* to establish their degree of trypanotolerance was initiated before the end of 1993.

# SOCIOECONOMICS

## SEP1.1 Quantitative assessment of the effect of the dynamics of tick-borne infections in livestock populations on productivity

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Tick-borne infections are among the most widely prevalent of livestock infections worldwide, yet their effect on productivity varies considerably by location, production system, climate and cattle type. Under some circumstances, particularly (but not only) those involving improved breeds of livestock, tick-borne infections cause heavy losses in terms of mortality and morbidity, a situation termed *endemic instability*. Under other circumstances, such as those involving indigenous livestock kept in climates virtually permanently suitable for the tick-vectors, they may cause little or no mortality and morbidity, a situation termed *endemic stability*. Numerous intermediate situations exist. The future application of a new generation of TBD vaccines will require careful targeting to those situations where they are most warranted if production levels of milk, meat and other livestock products are to be significantly increased.

Endemic stability is thought to be determined by: host factors (such as innate immunity, passively and actively acquired immunity, and piroplasm load to vectors); parasite factors (such as stock/strain heterogeneity, composition and virulence); and vector factors (such as total vector burden, fluctuations in vector burden and vector infection rates). What is required is a greater understanding of: a) the combined roles of these factors to the development of endemic stability by experimental studies; b) the relationships between these factors and the variables influencing them (notably climate, vegetation, livestock breed/type and livestock management); and c) the distribution and qualities, current and future, of the driving variables.

A mathematical model of endemic stability to *Theileria parva* infection was developed and validated using data collected in the Trans Mara region of Kenya (Medley, Perry and Young, previously reported). This model is considered to be appropriate to apply to different latitudes, cattle types and production systems to better understand the effect of varying infection dynamics on cattle productivity. A dataset of a longitudinal study of *T. parva* infection in Eastern Province of Zambia,

developed by D. Berkvens (University of Antwerp, Belgium), is being assessed using the same model by G. Medley at Warwick University to examine the effect of seasonality on transmission dynamics and production loss.

A case-study of factors influencing the distribution and dynamics of *T. parva* infections in Zimbabwe has continued. The study has examined the efficacy of two different modelling techniques to predict the spatial occurrence of *T. parva* infections. With one of the techniques, discriminant analysis, results obtained using datasets widely available within the continent were compared with those obtained when additional, less widely available data were included. A discriminant analysis procedure was used to rank the discriminating capacity of independent variables to predict the distributions of *Rhipicephalus appendiculatus, R. zambeziensis* and theileriosis outbreaks, and calculate the predictive values obtained with different combinations of independent variables. These were assessed by calculating the sensitivity and specificity of different combinations of independent variables. Results indicated that discriminant analysis was at least as good as the model CLIMEX in predicting tick distributions in Zimbabwe. Furthermore, it provides more biological insight into the important variables limiting the distribution of ticks. Using this analysis it is relatively easy to predict how distributions might change given different degrees of environmental change brought about by human activity, either directly of indirectly.

## SEP1.2 Regional assessment of the economic impact of theileriosis and its control

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A computer spreadsheet model has been developed and applied to estimate the direct economic cost (or losses) caused by theileriosis and assess the economics of an alternative control strategy based on immunization by the infection-and-treatment method at a regional level; results have been reported previously. The model estimated that in the 11 countries affected by theileriosis in eastern, central and southern Africa, about 24 million head of cattle are at risk from the disease annually, resulting in a direct economic loss of at least US$ 168 million (calculated for 1989). The actual economic losses are of course considerably larger than the direct losses when the indirect costs of the disease are taken into account.

An *ex-ante* economic assessment of the infection-and-treatment method at the regional level has been conducted. Results showed that immunization at a cost of US$ 2.50 per animal would increase total net income from cattle production by a range of US$ 133–307 million per year, and yield a benefit:cost ratio (BCR) in the range of 9–17, assuming 0–50% reduction in acaricide use following immunization. Break-even immunization cost ranged from US$ 4.96 with no change in acaricide use following immunization to US$ 8.22 with 50% reduction in acaricide use. The estimated target annual cattle population for immunization in the whole region is estimated to be seveb million head, at a cost of US$ 18 million. The target population and cost for immunization varies considerably among the affected countries depending on the proportion of the national herd at risk and the actual average cost of immunization per animal.

The computer spreadsheet model has been applied, in collaboration with scientists in national agricultural research systems, to estimate the cost of theileriosis and assess the economics of its control by the infection-and-treatment method immunization at several selected case-study sites in the affected region. Five case studies have been conducted in Kenya, Tanzania, Uganda and Zimbabwe between 1991 and 1993. The study sites included smallholder and large scale beef and dairy farms.

In all these studies, immunization against theileriosis was evaluated against the current control strategy based on acaricide application. Various levels of reduction in acaricide use ranging from 0 to 100% following immunization were assessed. Immunization at a cost ranging from US$ 4 to 25 in the different study sites with 75% reduction in acaricide use yielded the best economic results at the various sites.

The annual economic cost per animal in the different study sites varied from US$ 5 to 14 in indigenous cattle and US$ 11–105 in cross-bred (including taurine) cattle under the current control strategies. An immunization-based control strategy would reduce the economic cost of the disease by an estimated 43% in the whole region. In the various study sites, the cost would decline by a range of 20–67% in indigenous cattle and 34–82% in the crosses. Cattle production in all the study sites appears to be a profitable enterprise (all the calculated BCRs and net income per animal are positive and greater than unity) for both indigenous and cross-bred cattle. The BCR range is 1.83–5.29 and 1.37–4.28 in the indigenous and cross-bred cattle, respectively.

The immunization-based control strategy would generate varied economic results. On the basis of the BCR, immunization at the cost levels used in the analyses, although financially viable in the different production systems (i.e. with a BCR of greater than one), would not be the intervention of choice in zebu cattle at the two Kenyan study sites as the BCRs are not greater than those for the acaricide-based control interventions. The immunization cost would have to decline by at least 6% (from US$ 16) and 24% (from US$ 25) to reach the break-even levels in Uasin Gishu and Kilifi sites respectively. In all the other study sites and for all cattle types (including cross-bred cattle in the Kenyan sites), immunization would be the choice of control intervention, as the BCRs are greater than those for the acaricide-based control strategies. Regional and site/country case studies similar to those conducted for theileriosis will be conducted, with an adapted model, for heartwater.

## SEP1.3 A conceptual model for the assessment of impact of the trypanosomiasis and its control at the local, national and continental levels

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A general conceptual model of the links between trypanosomiasis and actual land use has been developed to guide research and to clarify interdisciplinary linkages. Like many others in ecology and the social sciences, this conceptual model attempts to maximize generality and realism, in order to ensure its appropriateness at the various case-study sites chosen for field investigation. Such flexibility is important in that the significant variables and their relationships in the model are expected to change as the research focus changes among field sites, and from the local level to the national and continental levels of investigation.

In the model, the physical environment consists of a set of initial conditions and external driving variables (e.g., geomorphological, edaphic, topographic and climatic features) that shape a stock of natural resource assets which include vegetation structure, biodiversity and soil fertility. These in turn determine the range of potentially viable land-use systems for a given site. Livestock production, and therefore livestock disease control, form an important component of the land-use system. Similarly, a set of external socioeconomic variables (e.g., human demography, tradition and cultural values, production and exchange relationships—including markets, political structures and processes, policies, etc.—determine the characteristic structure of the socioeconomic environment in a given area. Features of this socioeconomic environment which are important for land-use systems include property rights, available production technology and the local human population structure. Operating within this environment, people make choices from a range of technical and socioeconomic options that determine the actual land-use system. Creation of the actual land-use system subsequently influences both the local epidemiology of trypanosomiasis and the stock of natural assets. Such systemic feedbacks can potentially alter the range of plausible land-use systems and the array of choices regarding land use available to the human population. Moreover, changes in land-use result in a series of human welfare outcomes which can be measured by changes in such indicators as access to resources by individuals and social groups, levels of food consumption, nutritional status, morbidity rates and levels of income and savings.

At each case-study research site, the model will be used as a starting point for understanding system interactions and guiding research design. It will then be revised to reflect new understanding of site-specific processes. Next, the model will be used to test whether system components and interactions are similar across the three scales of investigation: local, national and continental. Finally, the conceptual model will serve as a template for integration of economic, ecological, epidemiological and social computer models.

## SEP1.4 GIS database development and acquisition to aid determination of the impacts of trypanosomiasis control

**Scientists:** R. Reid, B. Perry
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To establish the strength of the link between tsetse distribution and land use, and eventually to predict the effects of tsetse control on ecological properties, data are required for a variety of climatological, environmental, demographic and land-use variables at the continental scale.

Database acquisition has involved establishing contact with organizations that have developed the databases and exchanging data with them. Database development has required collection of secondary data at as fine a resolution as possible and digitizing them into Geographical Information System (GIS) data layers at ILRAD. These new databases show the distribution and magnitude of environmental and demographic variables at three scales: local, national and continental. Datasets were acquired by developing or continuing collaborative relationships with several organizations. First, collaboration has continued with UNEP/GRID with the acquisition of continent-wide data layers of human population density and three different data layers showing the distribution of vegetation. These data layers have been used to determine whether there is a strong relationship between tsetse distribution, human population and vegetation. A new collaboration was established with FEWS (Famine Early Warning System) and data on land-use intensity developed from satellite imagery was acquired for four countries. These data layers were used to establish the strength of the link between tsetse distribution and land use. Another new collaboration was developed with WCMC (World Conservation Monitoring Centre), the data depository of the World Wide Fund for Nature (WWF) and the International Union for Conservation of Nature (IUCN). WCMC provided ILRAD with continental digital data of the distribution and status of protected areas, the distribution and type of wetlands and lists of endangered species for selected African countries. These data will be used to determine the association of tsetse with conservation areas and what areas might be under threat of development if tsetse are successfully controlled. The International Food Policy Research Institute (IFPRI) provided ILRAD with a digital elevation model of Ethiopia so that the Ghibe study site could be put in a national context. The Food and Agriculture Organization (FAO) provided land suitability data layers for Kenya; environmental, tsetse and land-use data layers for Togo; non-digital cattle population data for Ethiopia; and administrative boundaries and soils for the continent. The United States Geological Survey is attempting to use low resolution satellite imagery to conduct an historical analysis of land-use change at the Ghibe field site in exchange for a reclassified version of a new satellite image acquired by ILRAD. ILRAD is developing three major GIS data layers: a cattle population data layer for Africa, a human population data layer for Kenya, and high resolution elevation, roads, towns and rivers data layers for the Ghibe Valley, Ethiopia. When

completed, these data layers will be used in analyses of the social and economic impacts of trypanosomiasis control.

## SEP 1.5 Preliminary use of GIS data layers to examine the relationships between tsetse and land use

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It has been suggested that more effective control of tsetse-transmitted trypanosomiasis may open vast areas of Africa to livestock production, both increasing food production potential and endangering reservoirs of biodiversity on the continent. Removal of the constraint of trypanosomiasis is predicted to allow the expansion of agriculture through increased use of animal traction. If true, there should be a strong inverse relationship between the distribution of tsetse and the distribution of cultivated land or agricultural land use in Africa. The first objective of the Geographical Information System (GIS) analysis was to determine whether such a relationship exists between the distributions of tsetse and agricultural land use for Africa. If such a relationship exists, the second objective was to determine the strength of the link between tsetse and agricultural production relative to other factors that might be related to land use (i.e., human population, rainfall, elevation).

However, no continental, geo-referenced database exists that shows the distribution of land use, so two alternative approaches were used. First, tsetse distribution was related to human population density, using the latter variable as a surrogate for the unavailable land-use data. Second, land-use data were obtained for three African countries (Burkina Faso, Mali and Zambia) and these data were related to tsetse distribution. To link the two approaches, the hypothesis that human population density is a proxy for land-use intensity was tested for the three countries. The analysis was conducted by overlaying the data layers of interest (tsetse distribution, human population, rainfall and elevation) and then conducting multivariate statistical analyses (discriminate analysis and categorical modelling) to quantify the relationships.

For the continental analysis, the presence of tsetse was a marginally better discriminator (73% correct) of human population density than rainfall (67% correct) or elevation (65% correct). Even when the effect of rainfall was removed from the analysis, results revealed that for the most part, fewer people live in tsetse-infested than tsetse-free zones. This does not demonstrate a cause and effect relationship, but it suggests that tsetse may be an important constraint to human land use. As anticipated, there was a strong positive relationship between land-use intensity and human population density for Burkina Faso, Zambia and Mali. It was hypothesized that there would be a threshold of human population density below which one would find little or no human land use. This threshold could then be used so that human population density could predict human land use. This lower threshold of human population

density below which there was little to no cultivation varied between 7–25 people/km$^2$ for the three countries studied.

For the country analysis, land use was expected to be low in the presence of tsetse. This was true in Zambia but not in Mali and Burkina Faso. In Mali, the presence of tsetse was associated with both high and low land-use intensity, depending on the rainfall zone. It is likely that areas with high land-use intensity that also have tsetse in Mali are areas where groundwater is particularly high along the Niger River. Moreover, in Burkina Faso, tsetse presence was associated consistently with more intensive land use. In this case, the intensity of land use may be strongly associated with soil type, irrespective of the presence or absence of tsetse. The hypothesis that high human land use no longer overlaps the tsetse distribution will be tested in the next stage of analysis. Similarly, land use is undoubtedly affected strongly by soil types and fertility, availability of groundwater and proximity to coastal zones. These data will be incorporated into the analysis.

## SEP1.6 Comparative case studies of the economic cost of trypanosomiasis

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The computer spreadsheet model (see abstract SEP 1.2) is being adapted for assessing the economic impact of trypanosomiasis and the economics of its alternative control strategies in selected countries in Africa affected by tsetse.

It was earlier reported that an estimated 117,000 Gambian N'Dama cattle (37% of the national herd) are at risk from trypanosomiasis annually. The annual economic cost of the disease in The Gambia (discounted at 10% over a 20-year period) was calculated to be US$ 279,000 per year or US$ 1.30 per head of cattle at risk. Virtually all the costs of the disease (99.9%) were attributed to production losses, with the remaining component being the cost of about 1,000 chemotherapeutic treatments administered to livestock annually. The main production loss (42%) was in decreased milk yield. The other production losses were due to decreases in draught power (26%), herd growth (18%), offtake (10%) and manure (5%). The high livestock densities and high level of draught power in The Gambia imply that indirect benefits from changes in grazing patterns or a shift towards draught power are unlikely if the disease incidence is reduced.

It was estimated that, due to extensive tsetse control, only 4% of Zimbabwe's cattle population (in 1991, 241,000 of a total of 6.1 million) are at risk annually from trypanosomiasis. (Following the 1991/92 drought, the cattle population is estimated

to have fallen to 5.1 million.) Most of these cattle are located in communal grazing areas in the north and northeast.

The direct economic cost of trypanosomiasis in Zimbabwe, when discounted at 10% over 20 years, was estimated to be US$ 37 million, which is an average of US$ 1.8 million per year, or US$ 7.60 per head of cattle at risk. (The *un*discounted annual cost averages US$ 4.3 million at the 1991 exchange rate.) Most of this is the cost of disease control (about 98%). Of the direct cost of trypanosomiasis in Zimbabwe, 68% constitutes the cost of controlling tsetse populations with targets, 27% the cost of dipping and pour-on deltamethrin applications and 3% the cost of treating infected cattle with trypanocides. The remaining 2% of the cost of trypanosomiasis is due to production losses.

In Côte d'Ivoire, 100% of the national herd (estimated at 1.1 million head in 1991) is thought to be at the risk of trypanosomiasis. However, about 69% of the herd is subject to a major national tsetse control project. The annual (discounted) economic cost was estimated to be US$ 5.4 million, or US$ 4.88 per head at risk. About 90% of the cost is from production losses and 10% from control costs. The composition of the economic cost is as follows: herd growth, 25%; draught power, 24%; milk production, 24%; off-take, 17%; tsetse control, 6%; and trypanocidal treatments, 4%.

Zimbabwe's minor production losses contrast markedly with those in The Gambia, where most of the disease costs are due to production losses. The most notable feature of the Zimbabwean production losses is the overwhelming importance of draught power, estimated to constitute 61% of the total, making this the dominant output of the country's communal (traditional) livestock production.

Zimbabwe and The Gambia represent strongly contrasting examples of livestock production systems and the economic impacts of trypanosomiasis in Africa. The cost of the disease per head of cattle at risk is six times higher in Zimbabwe than it is in The Gambia. This is due to the fact that Zimbabwe's farming areas are subject to strong tsetse invasion pressure, requiring the government to spend relatively large sums of money to protect these areas from reinvasion by the fly. The Gambia, in contrast, with its low level of tsetse infestation throughout most of the country and its almost entirely trypanotolerant cattle population, has a low disease cost per head of cattle at risk.

Côte d'Ivoire falls between Zimbabwe and The Gambia situations in that the economic cost consists of both control costs and production losses. The cost of the disease per head of cattle is lower than that of Zimbabwe but higher than that of The Gambia. Furthermore, effective tsetse control could allow expansion of cattle production in the southern part of the country. In many countries with more widespread tsetse infestations, notably those in coastal and central Africa, reducing the risk of trypanosomiasis by employing more effective control methods may well increase the areas available for livestock and crop production.

## SEP1.7 The environmental impacts of trypanosomiasis control: a case study at Ghibe Valley, Ethiopia

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Ghibe Valley, Ethiopia, was selected as an eastern Africa site in which to study the environmental impacts of trypanosomiasis control. The site has similarities to other tsetse-infested areas of the region in terms of elevation and fly species and the African Trypanotolerant Livestock Network has collected long-term data on trypanosomiasis at Ghibe and recently initiated a tsetse-control program there. Field studies are being designed to answer the following two global research questions: Is tsetse control linked to land-use change? If so, how does change in land use influence ecological properties?

A study design was devised to incorporate a comparison of areas where there has and has not been effective tsetse control in the Ghibe Valley. The non-intervention (no tsetse control) areas were selected to be physically and ecologically comparable to the areas where the control has been successful. Several experimental protocols were developed to assess the existence and strength of the link between tsetse control and land use change. A household survey was designed to measure the effects of the control within control and no control zones. The survey will measure recent changes (1990–1992) in herd sizes and composition, and the dimensions of areas cultivated. Because it appears that one of the principal effects of tsetse control on land use will be an expansion of the size of farmers' fields, a verification exercise was implemented to quantify the size of fields estimated by farmers during the household survey. This has been conducted with a sub-sample of surveyed farmers for intervention areas in 1992 and will be conducted for both intervention and non-intervention areas for 1993–1994.

Preliminary surveys of farmers were conducted to develop a history of recent ecological changes in the valley and the important drivers of those changes. This was used to put tsetse control in perspective and to anticipate confounding factors affecting land use change. Factors other than tsetse control that appear to affect land use and ecological properties are related to changes in human population density in the valley. The hope is to control for changes in human population in the study design to isolate the effect of tsetse control by conducting studies in adjacent areas where there has and has not been successful tsetse control.

A study is also being designed to answer the question: how does land use change affect ecological properties? Initially, ecological properties that will be measured are biodiversity and landscape/vegetation structure. These were chosen to reflect the nature of the controversy about the tsetse; namely, that the fly is guardian of wildlife and its habitat on the continent. The range of biodiversity studied has been restricted to large mammal and bird species. The study will quantify both inter- and intra-habitat

differences in vegetation/landscape structure in such a way that they can be used to model ecological impacts by GIS and a Thematic Mapper Landsat satellite image from which a map of current land use/land cover will be developed. Species numbers and distributions will be related to this map in an effort to define relationships that allow prediction of how future changes in land use will affect biodiversity.

## SEP 2.1 Constraint identification and the enhancement of disease awareness: the case of trypanosomiasis in Busia District, Kenya

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The use of baited traps and targets to control tsetse has the potential for successful and sustainable trypanosomiasis control by local communities. However, important considerations with community-based programs are the organizational capacity of the community, the incentives needed to initiate and sustain programs and intra-community distribution of program benefits. A collaborative, multidisciplinary study in Busia District, Kenya, has been investigating these and other issues, using participatory, focus group and formal survey methods.

Focus group interviews in the study villages found that residents attributed far greater importance to the production impacts of livestock trypanosomiasis (especially the loss of draught animals) than was previously expected. There were, however, generally low levels of awareness concerning the causal agent of trypanosomiasis, the role of tsetse in disease transmission, the clinical signs of the disease in both humans and animals and the ability of persons to distinguish tsetse from other flies. Consequently, educational activities were organized in the study villages to disseminate information on the disease, its mode of transmission, and the need for community participation in disease control. These activities were conducted to improve awareness of these subjects in anticipation of the assessment, during the household survey, of willingness to contribute resources to community-based control programs. Educational activities on these topics included a lecture and poster presentation in Kiswahili, a drama in the local language performed by area residents and a *baraza* on the issues raised in the lecture and play.

Factors identified from focus group interviews as potential barriers to the organization of an effective community control program were grouped into three general categories: a) physical structure of the village, b) leadership and organizational features of the village, and c) values and attitudes of villagers vis-a-vis the disease. Important aspects of the physical structure of the village include village size, accessibility, degree of nucleation/dispersion of settlement, size and location of tsetse habitat and degree of closure of village boundaries. The leadership and organization aspects of village social life important as potential catalysts for community participation would include the number of organizations in the village and the experiences of villagers working together in these organizations; the types of organizations that are found and their *raison d'etre;* and the social attributes of leaders, actual and

potential, especially their capacity to mobilize individuals for collective action. The attitudes and values found to be important in understanding people's perceptions of the disease and its control include explanations of causality (especially witchcraft); disease ranking and the relative importance of the trypanosomiases to other diseases; experiences with both human and veterinary trypanosomiasis, especially experiences with control measures/programs; and collective and individual attitudes towards the ability of individuals and communities to control the disease. Analysis of results of the household survey will evaluate these findings at the individual and household levels and will determine the willingness of individuals to contribute resources (especially labour and cash) to community-based control programs.

## SEP3.1 Estimated returns to ILRAD's theileriosis research expenditures

**Scientists:** A. Mukhebi, B. Perry

ILRAD's research programs have been focusing on the two disease complexes of livestock, theileriosis and trypanosomiasis. An attempt was made to estimate returns to ILRAD's research expenditures on theileriosis based upon the results of the estimated regional benefits from immunization against theileriosis using the infection-and-treatment method.

A number of assumptions was made regarding the proportion of ILRAD's total annual operational costs allocated to theileriosis research, the proportion of regional incremental net income from theileriosis immunization attributed to ILRAD's research effort, and the average rate of immunization adoption over the period. Benefit cost analysis using a 10% rate of discount (a low discount rate was used because ILRAD's budget is stated in US dollar terms) was applied to estimate the returns to ILRAD's research expenditures on theileriosis over a 40-year period (1974 to 2013). A long-term period was chosen because of the long-term nature of research investment whereby the major expenditures are incurred in the early years and the major benefits accrue in the latter years. The shorter the period analysed, the less the benefits are captured. (Clearly, not all benefits that would be generated from the adoption of immunization would be due to ILRAD's research. Furthermore, the adoption of immunization would be gradual over time and would be unlikely to reach 100% in all production systems and countries.)

The base scenario analysed assumed that 40% of ILRAD's expenditures would be allocated to theileriosis research between 1974 to and including 1997 (when laboratory testing of the first genetically engineered vaccine is assumed to be complete) and 5% per year as field testing and subsequent maintenance research beyond 1997. The base scenario also assumed that 50% of the incremental net income due to theileriosis immunization at the regional level would be attributed to ILRAD's research, and that the adoption rate for immunization would average 30% over the 40-year period. The base scenario yielded a benefit:cost ratio (BCR) of 3 and an internal rate of return (IRR) of 48%.

Sensitivity analysis with a range in the proportion of ILRAD's annual operational expenditures allocated to theileriosis research of 30–100% between 1974 and 1997 yielded a BCR range of 3–1 and an IRR range of 49–16%, respectively. With a range

of incremental net income from theileriosis immunization attributable to ILRAD's research of 20–100%, the BCR range was 1–5 and the IRR range was 10–49%, respectively. And with an immunization adoption rate of 15–100%, the BCR range was 1–8 and the IRR range was 41–50%. It is worth noting from these estimates that even if all of ILRAD's research costs to 1997 were attributed to theileriosis research (which is clearly not the case), the estimated rate of return at 16% would still be substantial when related to the rate of discount used in the analysis. This implies that the development of an effective theileriosis vaccine alone could underwrite or offset all of ILRAD's total research costs since its establishment over the last 20 years. The actual benefits and returns would be much greater when indirect benefits such as ILRAD's contributions to scientific knowledge, the enhancement of the national agricultural research capacity and environmental advantages of immunization over the use of chemical acaricides were considered.

# PUBLICATIONS

## PUBLICATIONS 1993

**Abebe, G., Eley, R.M.** and **ole-MoiYoi, O.K.** Reduced responsiveness of the hypothalamic-pituitary-adrenal axis in Boran *(Bos indicus)* cattle infected with *Trypanosoma congolense. Acta Endocrinologica* 129: 75–80 (1037).

**Abebe, G., Shaw, M.K.** and **Eley, R.M.** *Trypanosoma congolense* in the microvasculature of the pituitary gland of experimentally infected Boran *(Bos indicus)* cattle. *Veterinary Pathology* 30: 401–409 (0974).

**Agaba, M.** and **Kemp, S.J.** Characterization of the 3′ untranslated region of bovine parathyroid hormone gene in N'Dama and Boran cattle. *Animal Genetics* 24: 325–326 (1136).

**Aliu, Y.O., Mamman, M.** and **Peregrine, A.S.** Pharmacokinetics of diminazene in female Boran *(Bos indicus)* cattle. *Journal of Veterinary Pharmacology and Therapeutics* 16: 291–300 (953).

**Allsopp, B.A., Baylis, H.A., Allsopp, M.T.E.P., Cavalier-Smith, T., Bishop, R.P., Carrington, D.M., Sohanpal, B.** and **Spooner, P.** Discrimination between six species of *Theileria* using oligonucleotide probes which detect small subunit ribosomal RNA sequences. *Parasitology* 107: 157–165. (1212).

**Authié, E., Duvallet, G., Robertson, C.** and **Williams, D.J.L.** Antibody response to a 33 kDa cysteine protease of *Trypanosoma congolense:* relationship to 'trypanotolerance' in cattle. *Parasite Immunology* 15: 465–474 (1127).

**Authié, E., Muteti, D.K.** and **Williams, D.J.L.** Antibody responses to invariant antigens of *Trypanosoma congolense* in cattle of differing susceptibility to trypanosomiasis. *Parasite Immunology* 15: 101–111 (947).

**Bembridge, G.P., Parsons, K.R., Sopp, P., MacHugh, N.D.** and **Howard, C.J.** Comparison of monoclonal antibodies with potential specificity for restricted isoforms of the leukocyte common antigen. *Veterinary Immunology and Immunopathology* 39: 129–136.

**Bishop, R., Sohanpal, B., Spooner, P., Dolan, T., Allsopp, B.** and **Morzaria, S.** Detection of polymorphisms among *Theileria parva* stocks using repetitive, telomeric ribosomal DNA probes and anti-schizont monoclonal antibodies. *Parasitology* 107: 19–31 (1123).

**Bishop, R., Baylis, H., Allsopp, B., Toye, P., Nene, V., Dolan, T.T., Spooner, P., Kibe, M.** and **Morzaria, S.P.** Genomic polymorphisms in *Theileria parva.* In: Morzaria, S.P., ed. *Genome Analysis of Protozoan Parasites: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 11–13 November 1992.* Nairobi: The International Laboratory for Research on Animal Diseases, pp. 61–66 (1226).

**Bishop, R., Sohanpal, B.** and **Morzaria, S.** *Theileria parva:* detection of genomic polymorphisms by polymerase chain reaction amplification of DNA using arbitrary primers. *Experimental Parasitology* 77: 53–61 (1134).

**Biwi, K.M., Rabia, A.R.** and **Dolan, T.T.** Immunisation of cattle against East Coast fever: experience in Zanzibar. In: Kategile, J.A. and Mubi, S., eds. *Future of Livestock Industries in East and Southern Africa: Proceedings of the Workshop Held at Kadoma Ranch, Hotel Zimbabwe, 20–23 July 1992.* Addis Ababa: International Livestock Center for Africa, pp. 113–117 (1235).

**Brezinsky, L., Kemp, S.J.** and **Teale, A.J.** ILSTS005: a polymorphic bovine microsatellite. *Animal Genetics* 24: 73 pp. (1021).

**Brezinsky, L., Kemp, S.J.** and **Teale, A.J.** ILSTS006: a polymorphic bovine microsatellite. *Animal Genetics* 24: 73 pp. (1022).

**Brezinsky, L., Kemp, S.J.** and **Teale, A.J.** Five polymorphic bovine microsatellites (ILSTS010-014). *Animal Genetics* 24: 75 pp. (1062).

**Burleigh, B.A., Wells, C.W., Clarke, M.W.** and **Gardiner, P.R.** An integral membrane glycoprotein associated with an endocytic compartment of *Trypanosoma vivax:* identification and partial characterization. *The Journal of Cell Biology* 120: 339–352 (1149).

**Codjia, V., Mulatu, W., Majiwa, P.A.O., Leak, S.G.A., Rowlands, G.J., Authié, E., d'Ieteren, G.D.M.** and **Peregrine, A.S.** Epidemiology of bovine trypanosomiasis in the Ghibe Valley, southwest Ethiopia. 3. Occurrence of populations of *Trypanosoma congolense* resistant to diminazene, isometamidium and homidium. *Acta Tropica* 53: 151–163 (1044).

**Darji, A., Sileghem, M., Heremans, H., Brys, L.** and **De Baetselier, P.** Inhibition of T-cell responsiveness during experimental infections with *Trypanosoma brucei:* active involvement of endogenous gamma interferon. *Infection and Immunity* 61: 3098–3102 (1210).

**Davis, W.C., MacHugh, N.D., Park, Y.H., Hamilton, M.J.** and **Wyatt, C.R.** Identification of a monoclonal antibody reactive with the bovine ortholog of CD3 (BoCD3). *Veterinary Immunology and Immunopathology* 39: 85–91 (1255).

**De Martini, J.C., MacHugh, N.D., Naessens, J.** and **Teale, A.J.** Differential *in vitro* and *in vivo* expression of MHC class II antigens in bovine lymphocytes infected by *Theileria parva. Veterinary Immunology and Immunopathology* 35: 253–273 (1033).

**Deem, S.L., Perry, B.D., Katende, J.M., McDermott, J.J., Mahan, S.M., Moloo, S.K., Morzaria, S.P., Musoke, A.J.** and **Rowlands, G.J.** Variations in prevalence rates of tick-borne diseases in zebu cattle by agroecological zone: implications for East Coast fever immunization. *Preventive Veterinary Medicine* 16: 171–187 (1053).

**Delehanty, J.** Spatial projection of socioeconomic data using geographic information systems: results from a Kenya study in the strategic implementation of a livestock disease control intervention. In: Dvorak, K.A., ed. *Social Science Research for Agricultural Technology Dimensions: Proceedings of an International Institute of Tropical Agriculture (IITA) Rockefeller Foundation Workshop, 2–5 October 1990, Ibadan, Nigeria.* Wallingford: CAB International, pp. 37–50 (1219).

**Dirie, M.F., Otte, M.J., Thatthi, R.** and **Gardiner, P.R.** Comparative studies of *Trypanosoma (Duttonella) vivax* isolates from Colombia. *Parasitology* 106: 21–29 (1039).

**Dirie, M.F., Murphy, N.B.** and **Gardiner, P.R.** DNA fingerprinting of *Trypanosoma vivax* isolates rapidly identifies intraspecific relationships. *Journal Eukaryotic Microbiology* 40: 132–134 (1119).

**Dolan, T.T.,** ed. *Ticks and Tick-Borne Disease Control: Proceedings of a Joint OAU, FAO and ILRAD Workshop Held in Kampala, Uganda, 12–14 September, 1991.* Nairobi, International Laboratory for Research on Animal Diseases, 49 pp.

**Dolan, T.T.** and **McKeever, D.J.** Current and future vaccines against theileriosis. In: Padney, R., Hoglund, S. and Prasad G., eds. *Veterinary Vaccines.* New York: Springer-Verlag, pp. 318–337. (1005).

**Eisler, M.C., Gault, E.A., Smith, H.V., Peregrine, A.S.** and **Holmes, P.H.** Evaluation and improvement of an enzyme-linked immunosorbent assay (ELISA) for the detection of isometamidium in bovine serum. *Therapeutic Drug Monitoring* 15: 236–242 (1072).

**Ellis, J.A., Godson, D., Campos, M., Sileghem, M.** and **Babiuk, L.A.** Capture immunoassay for ruminant tumor necrosis factor: comparison with bioassay. *Veterinary Immunology and Immunopathology* 35: 289–300 (1155).

**Ellis, J.A., Fish, W.R., Sileghem, M.** and **McOdimba, F.** A colorimetric assay for trypanosome viability and metabolic function. *Veterinary Parasitology* 50: 143–149 (1120).

**Flynn, J.N., Sileghem, M.** Immunosuppression in trypanotolerant N'Dama cattle following *Trypanosoma congolense* infection. *Parasite Immunology* 15: 547–552 (1193).

**Gardiner, P.R.** An introductory review of the important trypanosomes of livestock. In: Morzaria, S.P., ed. *Genome Analysis of Protozoan Parasites: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 11–13 November, 1992.* Nairobi: The International Laboratory for Research on Animal Diseases, pp. 79–85 (1227).

**Grab, D.J., Shaw, M.K., Wells, C.W., Russo, D.C.W., Webster, P., Verjee, Y., Naessens, J.** and **Fish, W.R.** The transferrin receptor in African trypanosomes:

identification, partial characterization and subcellular localization. *European Journal of Cell Biology* 62: 114–126 (1061).

**Gray, M.A., Kimarua, R.W.** and **Peregrine, A.S.** Drug sensitivity screening *in vitro* of populations of *Trypanosoma congolense* originating from cattle and tsetse flies at Nguruman, Kenya. *Acta Tropica* 55: 1–9 (1223).

**Gray, M.A.** and **Peregrine, A.S.** An *in vitro* assay for drug sensitivity of *Trypanosoma congolense* using *in vitro*-derived metacyclic trypanosomes. *Acta Tropica* 54: 291–300 (1084).

**Hirumi, H., Hirumi, K.** and **Peregrine, A.S.** Axenic culture of *Trypanosoma congolense:* application to the detection of sensitivity levels of bloodstream trypomastigotes to diminazene aceturate, homidium chloride, isometamidium chloride and quinapyramine sulphate. *Journal of Protozoology Research* 3: 52–63 (1171).

**Howard, C.J.** and **Naessens, J.** Summary of workshop findings for cattle. In: Naessens J. and Howard, C.J., eds. *Leukocyte Antigens of Cattle and Sheep. Proceedings of the Second International Workshop on Leukocyte Antigens of Cattle and Sheep, Veterinary Immunology and Immunopathology* 39: 25–48. (1256).

**Kaminsky, R., Mamman, M., Chuma, F.** and **Zweygarth, E.** Time-dose-response of *Trypanosoma brucei brucei* to diminazene aceturate (Berenil) and *in vitro* simulation of drug-concentration time profiles in cattle plasma. *Acta Tropica* 54: 19–30 (0826).

**Kaminsky, R., Chuma, F.** and **Zweygarth, E.** *Trypanosoma congolense: in vitro* susceptibility of bloodstream forms to diminazene and isometamidium. *Experimental Parasitology* 76: 213–215 (1079).

**Kemp, S.J.** Markers for mapping trypanotolerance genes. In: Morzaria, S.P., ed. *Genome Analysis of Protozoan Parasites: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 11–13 November, 1992.* Nairobi: The International Laboratory for Research on Animal Diseases, pp. 137–138 (1228).

**Kemp, S.J., Brezinsky, L.** and **Teale, A.J.** ILSTS008: a polymorphic bovine microsatellite. *Animal Genetics* 24: 74 pp. (1023).

**Kemp, S.J., Brezinsky, L.** and **Teale, A.J.** A panel of bovine, ovine and caprine polymorphic microsatellites. *Animal Genetics* 24: 363–365 (1142).

**Kemp, S.J., Maillard, J.C.** and **Teale, A.J.** A polymorphism in the bovine gamma-S-crystallin gene revealed by allele-specific amplification. *Animal Genetics* 24: 125–127 (1054).

**Leak, S.G.A.** and **Mulatu, W.** Advance of *Glossina morsitans submorsitans* and *G. pallidipes* along the Ghibe River system in Southwest Ethiopia. *Acta Tropica* 55: 91–95 (1173).

**Leak, S.G.A., Mulatu, W., Authié, E., d'Ieteren G.D.M., Peregrine, A.S., Rowlands, G.J.** and **Trail, J.C.M.** Epidemiology of bovine trypanosomiasis in the Ghibe Valley, Southwest Ethiopia. 1. Tsetse challenge and its relationship to trypanosome prevalence in cattle. *Acta Tropica* 53: 121–134 (1043).

**Leak, S.G.A., Kemp, S.J.** and **Teale, A.J.** Responses of N'Dama × Boran crossbred cattle to experimental infection with *Trypanosoma congolense. OAU/ISCTRC 21st Meeting, Yamoussoukro, Cote d'Ivoire, October 1991*, p. 112 (1257).

**MacHugh, N.D., Awino, E.O.** and **McKeever, D.J.** A monoclonal antibody specific for bovine CD45RO differentiates memory from naive CD4 positive bovine T cells. *Veterinary Immunology and Immunopathology Supplement* 35: 56 pp. (1160).

**MacHugh, N.D., Taracha, E.L** and **Toye, P.G.** Reactivity of workshop antibodies on L cell and COS cell transfectants expressing bovine CD antigens. *Veterinary Immunology and Immunopathology Supplement* 39: 61–67 (1258).

**MacHugh, N.D., Wijngaard, P.L.J., Clevers, H.C.** and **Davis, W.C.** Clustering of monoclonal antibodies recognising different members of the WC1 gene family. *Veterinary Immunology and Immunopathology Supplement* 39: 155–160 (1259).

**Mahan, S.M.** and **McKeever, D.** Modulation of MHC class I and II antigens on bovine endothelial cells by *Cowdria ruminantium* infection. In: *United States Department of Agriculture, 2nd Biennial Meeting: American Society of Tropical Veterinary Medicine, 2–6 February, 1993, The Anchorage Hotel, Guadeloupe, West Indies*, 25 pp. (1169).

**Maillard, J.C., Naves, M., Palin, C., Kemp, S., Demangel, C.** and **Bensaid, A.** An attempt to correlate cattle origins and resistance to dermatophilosis. In: *United States Department of Agriculture, 2nd Biennial Meeting: American Society of Tropical Veterinary Medicine, 2–6 February, 1993, The Anchorage Hotel, Guadeloupe, West Indies*, 28 pp. (1170).

**Majiwa, P.A.O.** Variability of *Trypanosoma congolense*. In: Morzaria, S.P., ed. *Genome Analysis of Protozoan Parasites: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 11–13 November 1992*. Nairobi: The International Laboratory for Research on Animal Diseases, pp. 87–92 (1229).

**Majiwa, P.A.O., Omolo, E.O.** and **Osaso, J.** Mosaic transcripts in *Trypanosoma (Nannomonas) Congolense. Discovery and Innovation* 5: 323N–330N (1057).

**Majiwa, P.A.O., ole-MoiYoi, O.K.** and **Nantulya, V.M.** New techniques for diagnosis of the African trypanosomiases. *AgBiotech News and Information* 5: 115N–120N (1025).

**Majiwa, P.A.O., Maina, M., Waitumbi, J.N., Mihok, S.** and **Zweygarth, E.** *Trypanosoma (Nannomonas) congolense:* molecular characterization of a new genotype from Tsavo, Kenya. *Parasitology* 106: 151–162 (1056).

**Mamman, M., Aiiu, Y.O.** and **Peregrine, A.S.** Comparative pharmacokinetics of diminazene in noninfected Boran *(Bos indicus)* cattle and Boran cattle infected with *Trypanosoma congolense. Antimicrobial Agents and Chemotherapy* 37: 1050–1055 (1096).

**Mamman, M., Katende, J., Moloo, S.K.** and **Peregrine, A.S.** Variation in sensitivity of *Trypanosoma congolense* to diminazene during the early phase of tsetse-transmitted infection in goats. *Veterinary Parasitology* 50: 1–14 (1075).

**McKeever, D.** The role of class I MHC-restricted cytotoxic T cells in protection of immune cattle from *Theileria parva. Veterinary immunology and Immunopathology Supplement* 35: 34–35 (1159).

**McKeever, D.** Progress towards a vaccine against *Theileria parva:* relevance for heartwater research. *Revué Élevage de Médicine Véterinaire de Pays Tropicaux* 46: 231–235 (1265).

**Medley, G.F., Perry, P.B.** and **Young, A.S.** Preliminary analysis of the transmission dynamics of *Theileria parva* in eastern Africa. *Parasitology* 106: 251–264 (1058).

**Mgheni, M., Mukhebi, A.M., Setshwaelo, L.L., Tsiresy, R.** and **Nyathi, P.** Synthesis of constraints to livestock research and development and recommendations. In: Kategile, J.A. and Mubi, S., eds. *Future of Livestock Industries in East and Southern Africa: Proceedings of a Workshop held at Kadoma Ranch Hotel, Zimbabwe, 20–23 July 1992.* Addis Ababa: International Livestock Center for Africa, pp. 219–223 (1236).

**Moloo, S.K., Gettinby, G., Olubayo, R.O., Kabata, J.M.** and **Okumu, I.O.** A comparison of African Buffalo, N'Dama and Boran cattle as reservoirs of *Trypanosoma vivax* for different *Glossina* species. *Parasitology* 106: 277–282 (1074).

**Moloo, S.K.** A comparison of susceptibility of two allopatric populations of *Glossina pallidipes* for stocks of *Trypanosoma congolense. Medical and Veterinary Entomology* 7: 369–372 (1048).

**Morzaria, S.P., Katende, J.M., Kairo, A., Nene, V.** and **Musoke, A.J.** New methods for the diagnosis of *Babesia bigemina* infection. *Memoirs do Instituto Oswaldo Cruz* 87: 201–205 (1180).

**Morzaria, S.P.** and **Young, J.R.** Genome analysis of *Theileria parva. Parasitology Today* 9: 388–392 (1180).

**Morzaria, S.P., Young, J.R., Spooner, P.R., Dolan, T.T.** and **Bishop, R.P.** *Theileria parva:* a restriction map and genetic recombination. In: Morzaria, S.P., ed. *Genome Analysis of Protozoan Parasites: Proceedings a Workshop Held at ILRAD, Nairobi, Kenya, 11–13 November 1992.* Nairobi: The International Laboratory for Research on Animal Diseases, pp. 67–73 (1230).

**Muiya, P., Logan-Henfrey, L.** and **Naessens, J.** Expression of antigens on haemopoietic progenitor cells in bovine bone marrow. *Veterinary Immunology and Immunopathology* 39: 237–248 (1251).

**Mukhebi, A.W.** and **Perry, B.D.** Economic implications of the control of East Coast fever in eastern, central and southern Africa. In: Kategile, J.A. and Mubi, S., ed. *Future of Livestock Industries in East and Southern Africa: Proceedings of a Workshop Held at Kadoma Ranch Hotel, Zimbabwe 20–23, July 1992.* Addis Ababa: International Livestock Centre for Africa, pp. 107–112 (1237).

**Mukhebi, A.W., Morzaria, S.P., Perry, B.D., Dolan, T.T.** and **Norval, R.A.I.** Costing of East Coast fever vaccine production and delivery. In: *Proceedings of the 10th Small Ruminant-CRSP Scientific Workshop Held at ILRAD, Nairobi, Kenya, 26–27 February 1992,* pp. 35–39 (1260).

**Mulatu, W., Leak, S.G.A., Rowlands, G.J.** and **d'Ieteren, G.D.M.** Preliminary results of a tsetse control campaign using deltamethrin impregnated traps to alleviate a drug resistance problem in the Ghibe Valley, Southwest Ethiopia. *OAU/ISCTRC 21st Meeting Yamoussoukro, Cote d'Ivoire, October 1991,* p. 112 (1257).

**Murphy, N.B., Muthiani, A., Pandit, P., Kemei, S.** and **Moloo, S.K.** The identification of genes of importance in *Trypanosoma brucei brucei* through the use of transfection techniques. In: Morzaria, S., ed. *Genome Analysis of Protozoan Parasites: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 11–13 November 1992.* Nairobi: The International Laboratory for Research on Animal Diseases, pp. 109–121 (1190).

**Murphy, N.B., Muthiani, A.M.** and **Peregrine, A.S.** Use of an *in vivo* system to determine the G418 resistance phenotype of bloodstream-form *Trypanosoma brucei brucei* transfectants. *Antimicrobial Agents and Chemotherapy* 37: 1167–1170 (1133).

**Musoke, A.J., Nene, V.** and **Morzaria, S.P.** A sporozoite-based vaccine for *Theileria parva. Parasitology Today* 9: 385–388 (1177).

**Musoke, A.J., McKeever, D.J., Morzaria, S.P., Nkonge, C.** and **Nene, V.** A recombinant vaccine for *Theileria parva.* In: Talwar, G.P., ed. *Recombinant and Synthetic Vaccines.* New Delhi: Narosha Publishing House, pp. 15–20.

**Mutugi, J.J., Young, A.S., Kariuki, D.P., Maritim, A.C.** and **Orinda, J.** Challenges posed by haemoparasitic diseases in livestock development. In: *Agricultural Research in Kenya: Achievements, Challenges and Prospects.* Nairobi: Kenya Agricultural Research Institute, pp. 141–148 (1262).

**Naessens, J.** and **Howard C.J.**, eds. In: *Leukocyte Antigens of Cattle and Sheep. Proceedings of the Second International Workshop on Leukocyte Antigens of Cattle and Sheep. Veterinary Immunology and Immunopathology* 39: V1–10 (1250).

**Naessens, J.** 2.1 Nomenclature. In: *Leukocyte Antigens of Cattle and Sheep. Proceedings of the Second International Workshop on Leukocyte Antigens of Cattle and Sheep. Veterinary Immunology and Immunopathology* 39: 11–12 (1249).

**Naessens, J.** and **Nthale, J.** Biochemical characterization of three non-lineage antigens defined by workshop antibodies. *Veterinary Immunology and Immunopathology* 39: 217–223 (1248).

**Naessens, J., Olubayo, R.O., Davis, W.C.** and **Hopkins, J.** Cross-reactivity of workshop antibodies with cells from domestic and wild ruminants. *Veterinary Immunology and Immunopathology* 39: 283–290 (1246).

**Naessens, J., Muiya, P.** and **Logan-Henfrey, L.** Bovine CFU-S in irradiated SCID/Beige mice. *Veterinary Immunology and Immunopathology Supplement* 35: 66–67 (1161).

**Ndoutamia, G., Moloo, S.K., Murphy, N.B.** and **Peregrine, A.S.** Derivation and characterization of a quinapyramine-resistant clone of *Trypanosoma congolense. Antimicrobial Agents and Chemotherapy* 37: 1163–1166 (1129).

**Nene, V., Bishop, R., Dolan, T.T., McKeever, D., ole-MoiYoi, O.K., Morzaria, S.P., Musoke, A.J., Shaw, M.K., Toye, P.** and **Young, A.S.** *Theileria parva:* current status. In: Morzaria, S.P., ed. *Genome Analysis of Protozoan Parasites: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 11–13 November 1992.* Nairobi: The International Laboratory for Research on Animal Diseases, pp. 55–60 (1232).

**Nthale, J.M.** and **Naessens, J.** Characterization of a late activation antigen defined by monoclonal antibodies of cluster BoWC8 (TC23). *Veterinary Immunology and Immunopathology* 39: 201–208 (1247).

**ole-MoiYoi, O.K., Brown, W.C., Iams, K.P., Nayar, A., Tsukamoto, T.** and **Macklin, M.D.** Evidence for the induction of casein kinase II in bovine lymphocytes transformed by the intracellular protozoan parasite *Theileria parva. The Embo Journal* 12: 1621–1631 (760).

**Orinda, G.O., Wright, I.G., Leatch, G.** and **Young, A.S.** Human interferon alpha fails to inhibit the development of *Babesia bigemina* and *Anaplasma marginale* infections in cattle. *Veterinary Parasitology* 47: 149–155 (1175).

**Pellé, R.** and **Murphy, N.B.** *In vivo* UV cross-linking hybridization: a powerful technique for isolating RNA binding proteins. Application to trypanosome mini-exon derived DNA. *Nucleic Acids Research* 21: 2453–2458 (1126).

**Pellé, R.** and **Murphy, N.B.** Northern hybridization: rapid and simple electrophoretic conditions. *Nucleic Acids Research* 21: 2783–2784 (1065).

**Pellé, R.** and **Murphy, N.B.** Stage-specific differential polyadenylation of mini-exon derived RNA in African trypanosomes. *Molecular and Biochemical Parasitology* 59: 277–286 (0975).

**Pellé, R.** The use of electrophoretic profile of RNA to differentiate *Trypanosoma brucei* from *T. congolense. Parasitology Today* 9: 96–97 (1164).

**Peregrine, A.S.** and **Mamman, M.** Pharmacology of diminazene: a review. *Acta Tropica* 54: 185–203 (1156).

**Perry, B.D.** and **Young, A.S.** The naming game: the changing fortunes of East Coast fever and *Theileria parva. The Veterinary Record* 133: 613–616 (1181).

**Read, L.K., Jacob, A.N.K., Fish, W.R., Muthiani, A.M.** and **Stuart, K.** Sequences of the three *Trypanosoma congolense* maxicircle genes allow prediction of regions encoding transcripts that undergo extensive RNA editing. *Molecular and Biochemical Parasitology* 60: 337–342 (1211).

**Reid, R.S., Kruska, R.L., Perry, B.D., Ellis, J.E.,** and **Swallow, B.M.** Ecological implications of controlling tsetse-transmitted trypanosomiasis in Africa: GIS case studies. *Bulletin of the Ecological Society of America* 74: 230.

**Rowlands, G.J., Mulatu, W., Authié, E., d'Ieteren, G.D.M., Leak, S.G.A., Nagda, S.M.** and **Peregrine A.S.** Epidemiology of bovine trypanosomiasis in the Ghibe Valley, Southwest Ethiopia. 2. Factors associated with variations in trypanosome prevalence, incidence of new infections and prevalence of recurrent infections. *Acta Tropica* 53: 135–150 (1174).

**Rowlands, G.J., Mulatu, W., Authié, E., d'Ieteren, G.D.M., Leak, S.G.A., Nagda, S.M., Peregrine, A.S.** and **Rarieya, M.** A statistical model for distinguishing new and recurrent trypanosome infections in cattle at Ghibe, Ethiopia. *OAU/ISCTRC 21st Meeting, Yamoussoukro, Cote d'Ivoire, October 1991*, p. 270.

**Rumberia, R.M., Eley, R.M., Young, A.S., Rowland, A.C.** and **Watson, E.D.** The effect of high and low dose *Theileria parva* infection on the reproduction function of Boran/Friesian heifers. *Theriogenology* 40: 977–986 (1240).

**Shaw, M.K., Tilney, L.G.** and **McKeever, D.J.** Tick salivary gland extract and interleukin-2 stimulation enhance susceptibility of lymphocytes to infection by *Theileria parva* sporozoites. *Infection and Immunity* 61: 1486–1495 (1145).

**Shaw, M.K.** and **Moloo, S.K.** Virus-like particles in *Rickettsia* within the midgut epithelial cells of *Glossina morsitans centralis* and *Glossina brevipalpis. Journal of Invertebrate Pathology* 61: 162–166 (872).

**Sileghem, M.R., Flynn, J.N., Saya, R.** and **Williams, D.J.L.** Secretion of co-stimulatory cytokines by monocytes and macrophages during infection with *Trypano-*

*soma (Nannomonas) congolense* in susceptible and tolerant cattle. *Veterinary Immunology and Immunopathology* 37: 123–134 (965).

**Sileghem, M.R., Flynn, J.N., Saya, R.** and **Williams, D.J.L.** Monocyte activation during bovine trypanosomiasis: different modulation of cytokine production and phagocytosis. *Veterinary Immunology and Immunopathology* 39: 123–134 (1261).

**Taylor, K.A., Paskewitz, S.M., Copeland, R.S., Koros, J., Beach, R.F., Githure, J.I.** and **Collins, F.H.** Comparison of two ribosomal DNA-based methods for differentiating members of the *Anopheles gambiae* complex (Diptera: Culicidae). *Journal of Medical Entomology* 30: 457–461 (1184).

**Teale, A.** Bovine genome research. In: Morzaria, S.P., ed. *Genome Analysis of Protozoan Parasites: Proceedings of a Workshop Held at ILRAD, Nairobi, Kenya, 11–13 November 1992.* Nairobi: The International Laboratory for Research on Animal Diseases, pp. 17–20 (1233).

**Teale, A.J.** Improving control of livestock diseases: animal biotechnology in the Consultative Group on International Agricultural Research. *Bioscience* 43: 475–483 (1114).

**Voigt, W.P., Young, A.S., Mwaura, S.N., Nyaga, S.G., Njihia, G.M., Mwakima, F.N.** and **Morzaria, S.P.** *In vitro* feeding of instars of ixodid tick *Amblyomma variegatum* on skin membranes and its application to the transmission of *Theileria mutans* and *Cowdria ruminantium. Parasitology* 107: 257–263 (1148).

**Waithanji, E.M., Nantulya, V.M.** and **Mbiuki, S.M.** Use of antigen-capture tube enzyme-linked immunosorbent assay for the diagnosis of *Trypanosoma evansi* infections in dromedary camels *(Camelus dromedarius). Revue Scientifique et Technique Office International des Épizooties* 12: 665–672 (1213).

**Waitumbi, J.N.** and **Nantulya, V.M.** A comparison of the antigen detection ELISA and parasite detection for diagnosis of *Trypanosoma evansi* infections in camels. *Veterinary Parasitology* 49: 159–178 (1215).

**Waitumbi, J.N.** and **Murphy, N.B.** Inter- and intra-species differentiation of trypanosomes by genomic fingerprinting with arbitrary primers. *Molecular and Biochemical Parasitology* 58: 181–186 (0845).

**Waladde, S.M., Young, A.S., Ochieng, S.A., Mwaura, S.N.,** and **Mwakima, F.N.** Transmission of *Theileria parva* to cattle by *Rhipicephalus appendiculatus* adults fed as nymphae *in vitro* on infected blood through an artificial membrane. *Parasitology* 107: 249–256 (1147).

**Williams, D.J.L., Taylor, K., Newson, J.** and **Naessens, J.** Specificity of serum antibodies derived from *Trypanosoma congolense*-infected cattle. *Veterinary Immunology and Immunopathology Supplement* 35: 189 pp. (1162).

**Young, A.S., Morzaria, S.P., Bishop, R., Perry, B.D., Dolan, T.T.** and **Gettinby, G.** New methods of studying parasite transmission by ticks. In: *Proceedings of the 3rd Asian Congress of Parasitology, Lucknow, India, 18–21 February, 1993*, p. 7 (1263).

**Young, A.S., Cummins, J.M., Orinda, G.O.** and **Wright, I.G.** Cytokines in tick-borne protozoal infections and their use in therapy. In: Maheshwari, R.K., ed. *Proceeding of the 3rd Asian Congress of Parasitology, Lucknow, India, 18–21 February, 1993*, pp. 13–14 (1264).

**Zilberstein, D., Wilkes, J., Hirumi, H.** and **Peregrine, A.S.** Fluorescence analysis of the interaction of isometamidium with *Trypanosoma congolense*. *Biochemical Journal* 292: 31–35 (1027).

**Zweygarth, E., Moloo, S.K.** and **Kaminsky, R.** *Trypanosoma simiae: in vitro* studies on drug susceptibility. *Acta Tropica* 54: 301–308 (1221).

# STAFF PRESENTATIONS AT INTERNATIONAL MEETINGS

**M. Agaba**

Joint Pan-African Veterinary Association, Regional Commonwealth Veterinary Association Conference, November, 1993. Paper presented: *Understanding trypanotolerance for improved livestock production in Africa: a molecular genetic approach.*

**A. Andrianarivo**

ILRAD/ILCA workshop: Towards the Increased Utilization and Adoption of Trypanotolerance—Current Status of Research and Future Directions, ILRAD, Nairobi, Kenya, April, 1993. Paper presented: *Comparative bone marrow responses during* Trypanosoma congolense *infection in N'Dama and Boran cattle.* A.G. Andrianarivo, P. Muiya, M. Opollo and L. Logan-Henfrey.

**J. Curry**

Ninety-Second Annual Meeting of the American Anthropological Association, Washington, DC, November, 1993. Paper presented: *Identifying factors affecting community participation in tsetse control in human sleeping sickness foci in Busia District, Kenya.* J.J. Curry, D. Kamara and B. Swallow.

Twenty-Second Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), October, 1993. Paper presented: *Assessment of socioeconomic factors affecting implementation of community-based tsetse control, Busia, Kenya.* D. Kamara, P. Echessah, J.J. Curry and B. Swallow.

Workshop: A Veterinary Social Research Program to Improve Community Welfare and Development, Foundation for Research and Development, Pretoria, South Africa, September, 1993. Paper presented: *Socioeconomic aspects of animal disease control.*

Annual Meeting of the Rural Sociological Society, Orlando, Florida, August, 1993. Paper presented: *Household demographic structure, agricultural production systems and land degradation in Swaziland.* C. Stokes, W. Schutjer, R. Warland and J.J. Curry.

KETRI Second Annual Internal Review, KETRI, Muguga, Kenya, May, 1993. Paper presented: *Assessment of the socioeconomic factors affecting implementation of community-based tsetse control in Busia, Kenya.* D. Kamara, P. Echessah, J.J. Curry, B. Swallow and S. Ashley.

Workshop for socioeconomists in KARI, Silver Springs Hotel, Nairobi, Kenya, May, 1993. Paper presented: *Anthropological and sociological research issues at headquarters and the centres.*

ILRAD/ILCA workshop: Towards the Increased Utilization and Adoption of Trypanotolerance-Current Status of Research and Future Directions, ILRAD, Nairobi, Kenya, April, 1993. Paper presented: *Ecological, social and economic impacts of trypanotolerance: collaborative research in Central and West Africa.* R. Reid, J.J. Curry, B. Swallow, A. Mukhebi, B. Perry and J. Ellis.

**C. Daubenberger**

Seminar to the Department of Veterinary Immunology, Tieraeztliche Hochschule, Hannover, Germany, June, 1993. Paper presented: *Veterinary immunology at ILRAD.*

**T. Dolan**

Workshop: Novel Immunization Strategies Against Protozoan Parasites, Nairobi, Kenya, November, 1993. Paper presented: *ILRAD research on theileriosis and tick-borne diseases.*

**J. Doyle**

Veterinary Faculty, University of Utrecht, Utrecht, The Netherlands, May, 1993. Paper presented: *Increasing animal agriculture productivity in the developing world—constraints and opportunities.*

The National Agricultural Biotechnology Workshop, Safari Park Hotel, Nairobi, Kenya, September, 1993. Paper presented: *International biotechnology and improved livestock productivity.*

IBS Meeting on International Biotechnology Programs Providing Opportunities for National Participation, ISNAR, The Hague, Netherlands, November, 1993. Paper presented: *ILRAD and animal biotechnology.*

**A. Gray**

An international scientific meeting by CABI, the Systematics Association, and the Committee on the Application of Science to Agriculture, Forestry and Aquaculture: The Need for Identification Services (Veterinary), Bakeham Lane, Egham, Surrey, UK, June 1993: Paper presented: *The ecological foundations of sustainable agriculture; identification and characterization of pest organisms.*

**D. Hirumi**

The IX International Congress of Protozoology, Berlin, Germany, July, 1993. Paper presented: *Axenic cultivation of bloodstream forms of African trypanosomes.* Hirumi H. and Hirumi, K.

**S. Kemp**

European Bovine Mapping Workshop, Munich, Germany, March, 1993. Paper presented: *ILRAD's genome mapping program.*

Annual Small Ruminant-CRSP workshop, Nairobi, Kenya, March, 1993. Paper presented: *The specificity of bovine microsatellite primers in other ruminants.* M. McIsaac, S.J. Kemp and A. Teale.

ILRAD/ILCA workshop: Towards the Increased Utilization and Adoption of Trypanotolerance—Current Status of Research and Future Directions, ILRAD, Nairobi, Kenya, April, 1993. Paper presented: *Markers for mapping trypanotolerance genes.*

**D. Kennedy**

IFS Scientific Workshop for Eastern African Grantees, Kampala, Uganda, April, 1993. Paper presented: *Embryo transfer at ILRAD.*

**S. Leak**

Twenty-Second Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), October, 1993. Papers presented:

1. *A trial of a cypermethrin 'Pour-on' insecticide to control* Glossina pallidipes, G. fuscipes *and* G. morsitans submorsitans (Diptera: Glossinidae) *in southwest Ethiopia.* S.G.A. Leak, W. Mulatu, G. Rowlands and G. d'Ieteren.
2. *Evaluation of a pour-on by Ethiopian farmers.* B. Swallow, W. Mulatu and S.G.A. Leak.

ILRAD/ILCA workshop: Towards the Increased Utilization and Adoption of Trypanotolerance-Current Status of Research and Future Directions, ILRAD, Nairobi, Kenya, April, 1993. Papers presented:

1. *Factors affecting the estimation of tsetse challenge and the expression of trypanotolerance.* G. d'Ieteren, S.G.A. Leak and G. Rowlands.
2. *Variations in susceptibility to the effects of trypanosomiasis in East African zebu cattle.* G. Rowlands, G. d'Ieteren, E. Authié, S. Leak, W. Mulatu and S. Nagda.

Nairobi Cluster Meeting, Nairobi, Kenya, March, 1993. Paper presented: *A trial of a cypermethrin 'Pour-on' insecticide to control* Glossina pallidipes, G. fuscipes *and* G. morsitans submorsitans (Diptera: Glossinidae) *in southwest Ethiopia.* S.G.A. Leak, W. Mulatu, G. Rowlands and G. d'Ieteren.

**L. Logan-Henfrey**

Workshop: Novel immunization strategies against protozoan parasites, Nairobi, Kenya, November 1993. Paper presented: *Livestock trypanosomiasis: the disease.*

**P. Majiwa**

National Workshop on Science and Technology, Management policies for Development, Kisumu, Kenya, June 1993. Paper presented: *Contribution to workshop on biotechnology and biosafety considerations for Kenya.*

The IX International Congress of Protozoology, Berlin, Germany, July, 1993. Paper presented: *Application of modern techniques to probe population genetic structure of* Trypanosoma congolense.

Twenty-Second Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), October, 1993. Paper presented: *Methods and reagents for detection of trypanosomes in the saliva of live tsetse flies and buffycoat samples from antigenaemic but aparasitaemic livestock.*

**R. Masake**

Conference: Women in Science and Technology, Embu, Nairobi and Mombasa, Kenya, January and February, 1993. Paper presented: *Role of women in agriculture.*

The IX International Congress of Protozoology, Berlin, Germany, July, 1993. Paper presented: *Characterization of* Trypanosoma vivax *species-specific antigen and its gene.*

Twenty-Second Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), October, 1993. Paper presented: *Antigen capture ELISA gives evidence of high prevalence of* Trypanosoma gambiense *human sleeping sickness focus.* T. Asongany, R.A. Masake and V.M. Nantulya.

**D. McKeever**

Second Biennial Meeting of the American Society of Tropical Veterinary Medicine, Guadeloupe, West Indies, February, 1993. Paper presented: *Progress towards a vaccine against* Theileria parva: *relevance for heartwater research.*

Keystone Symposium, Taos, New Mexico, February, 1993. Paper presented: *Emerging principles for vaccine development: antigen processing and presentation.*

Workshop: Novel Immunization Strategies Against Protozoan Parasites, ILRAD, Nairobi, Kenya, November, 1993. Paper presented: *The role of class I MHC-restricted responses in immunity to* Theileria parva.

Seminar to the Department of Dr. Adrian Hill, John Radcliff Hospital, Oxford, UK, August, 1993. Paper presented: *Immune responses of cattle to* Theileria parva.

**B. Mertens**

Eighth Symposium, Molecular Biology of Haematopoiesis, Basel, Switzerland, July, 1993. Paper presented: *Cloning of bovine interleukin 2 cDNA.* S. Mwangi, L. Logan-Henfrey and B. Mertens.

ILRAD workshop: Novel Immunization Strategies Against Protozoan Parasites, ILRAD, Nairobi, Kenya, November 1993. Paper presented: *Development of bovine cytokine reagents at ILRAD.*

**S. Morzaria**

Proceeding of the FAO Expert Consultation on the Use of Applicable Biotechnological Methods for Diagnosis of Haemoparasites, Merida, Mexico, October, 1993. Paper presented: *Modern biotechnological methods for diagnosis of bovine theileriosis and future trends.* S. Morzaria, V. Nene, A.J. Musoke, and R. Bishop.

Ninth International Veterinary Haemoparasite Disease Conference, Merida, Mexico, October, 1993. Paper presented: *Analysis of sexual crosses between* Theileria parva *stocks.* S. Morzaria, J. Young, P. Spooner, R. Skilton, T. Dolan, A. Young and R. Bishop.

**N. Murphy**

Workshop on Genomic Fingerprinting, Instituto Juan March de Estudios e Ivestigaciones, Madrid, Spain, October, 1993. Papers presented: *Application of arbitrarily primed PCR fingerprinting to molecular studies on trypanosomes.*

**A. Musoke**

Expert Consultation on the Use of Applicable Biotechnological Methods for Diagnosing Haemoparasites, Merida, Mexico, October, 1993. Papers presented:

1. *Progress towards development of an antibody detection ELISA for the diagnosis of* Theileria parva. A.J. Musoke, J. Katende, P. Toye, R. Skilton, K. Iams, V. Nene and S. Morzaria.
2. *Modern biochemical methods for diagnosis of bovine theileriosis and future trends.* S. Morzaria, A.J. Musoke, V. Nene and R. Bishop.

**S. Mwangi**

Eleventh Tanzania Veterinary Association conference, Arusha, Tanzania, November, 1993. Paper presented: *Cloning of a bovine interleukin cDNA.* S.M. Mwangi, L. Logan-Henfrey and B. Mertens.

**V. Nene**

IX International Congress of Protozoology, Berlin, Germany July 1993. Paper presented: *Vaccines against* Theileria parva. V. Nene, S. Morzaria, D. McKeever, A. Musoke.

**O. ole-MoiYoi**

Keystone Symposia on Molecular and Cellular Biology: B- and T-cell lymphomas, Copper Mountain, Colorado, USA, April, 1993. Paper presented: *Induction of casein kinase II in lymphocytes transformed by the intracellular protozoan parasite* Theileria parva.

**A. Peregrine**

The 14th International Conference of the World Association for the Advancement of Veterinary Parasitology, Cambridge, UK, August, 1993. Papers presented:

1. *Chemotherapy and delivery systems: haemoparasites.*
2. *Improvement of chemoprophylactic strategies for the control of African bovine trypanosomiasis: use of the isometamidium ELISA.* P. Holmes, M. Eisler and A.S. Peregrine.

British Society for Parasitology, 27th Trypanosomiasis Seminar, London, UK, September, 1993. Paper presented (by Dr. Eisler): *Improvements of chemoprophylactic strategies for the control of African bovine trypanosomiasis: use of the isometamidium ELISA.* M. Eisler, A.S. Peregrine and P. Holmes

**B. Perry**

Nairobi Cluster: Veterinary Association Scientific Meeting, National Museums of Kenya, Nairobi, March 1993. Paper presented: *Geographical information systems and models in the control of vectors of livestock diseases.*

Seminar at EMVT, Paris, France, March 1993. Paper presented: *Research at ILRAD on the impact of vector-borne diseases in Africa.*

KVA Symposium on Rabies, ILRAD, Nairobi, April, 1993. Paper presented: *Dog ecology: implications for rabies control.*

Southern African Rabies Group Symposium, Pietermaritzburg, South Africa, April, 1993. Paper presented: *Dog ecology: implications for rabies control.*

Seminar to the Veterinary Faculty, Sokoine University, Morogoro, Tanzania, September, 1993. Paper presented: *The epidemiology of tick-borne diseases in eastern and southern Africa.*

Seminar to the Board and senior staff of ILCA, Addis Ababa, Ethiopia, October, 1993. Paper presented: *The role of GIS studies on the impact of vector borne disease at ILRAD.*

Expert consultation: The Need for Information Systems to Strengthen Veterinary Services in Developing Countries, FAO, Rome, November, 1993. Paper presented: *Information management technology to strengthen veterinary services: an appraisal of the systems available and their uses in developing countries.* B. Perry and R. Kruska.

Meeting of the Panel of Experts on Ecological Technical and Developmental Aspects of the Program for the Control of African Animal Trypanosomiasis and Related Development, FAO, Rome, December, 1993. Paper presented: *The development and use of geographic information systems to assist trypanosomiasis control.* B. Perry, R. Kruska and R. Reid.

**R. Reid**

ILRAD/ILCA workshop: Towards the Increased Utilization and Adoption of Trypanotolerance—Current Status of Research and Future Directions, ILRAD, Nairobi, Kenya, April, 1993. Paper presented: *Ecological, social and economic impacts of trypanotolerance: collaborative research in Central and West Africa.* R.S. Reid, J. Curry, B. Swallow, A. Mukhebi, B. Perry and J. Ellis.

Twenty-Second Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), October, 1993. Paper presented: *Environmental impacts of trypanosomiasis control: conceptual model, approach and preliminary results of studying indirect effects through changes in land use.* R.S. Reid, R. Kruska, B. Perry, J. Ellis and C. Wilson.

Annual Meeting of the Ecological Society of America, Wisconsin, USA, July, 1993. Poster presented: *Ecological, social and economic impacts of trypanotolerance: collaborative research in Central and West Africa.* R.S. Reid, R. Kruska, B. Perry, J. Ellis and B.M. Swallow.

**R. Skilton**

British Society for Parasitology Autumn Symposium, City University, London, September, 1993. Paper presented: *Functional molecules on the surface of protozoan parasites.*

Department of Biochemistry, University of Witwatersrand, Johannesburg, South Africa, August, 1993. Paper presented: *Molecular cloning and expression of a diagnostic antigen of* Theileria mutans.

**J.-P. Scheerlinck**

Modern Approaches to New Vaccines Including the Prevention of AIDS, Cold Spring Harbour, New York, USA, September 1993. Poster presented: *Depletion of CD4 and CD8 memory in young cattle does not abrogate immunity against* Theileria parva. J.-P. Scheerlinck, S. Morzaria, D. McKeever and J. Naessens.

Workshop: Novel Immunization Strategies Against Protozoan Parasites, Nairobi, Kenya, November 1993. Paper presented: *Depletion of T-cell subsets in cattle.* J.-P. Scheerlinck and J. Naessens.

**E. Taracha**

1993 EMBO Workshop on Cell-Mediated Cytotoxicity, Neve Ilan, Israel. Paper presented: *Inductive requirements of cytotoxic T lymphocytes specific for the protozoan* Theileria parva.

Workshop: Novel Immunization Strategies Against Protozoan Parasites, Nairobi, Kenya, November, 1993. Paper presented: *Inductive requirements of cytotoxic T lymphocytes specific for the protozoan* Theileria parva.

**A. Teale**

ILRAD/ILCA Workshop: Towards the Increased Utilization and Adoption of Trypanotolerance—Current Status of Research and Future Directions, ILRAD, Nairobi, Kenya, April, 1993. Paper presented: *Progress in molecular genetic Characterization of cattle populations, with emphasis on African breeds.*

Australasian Gene Mapping Workshop, University of Adelaide, Adelaide, South Australia, July 1993. Paper presented: *Towards an understanding of the genetic and molecular basis of trypanotolerance.*

Twenty-Second Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), October, 1993. Papers presented: *Aspects of current and future strategies for trypanosomiasis research and control related to ILRAD's mandate.*

CSIRO-Molecular Animal Genome Center, St. Lucia, Queensland, Australia; CSIRO Division of Tropical Animal Production, Longpocket Laboratories, Indooroopilly, Queensland, Australia; Queensland Department of Primary Industries Agricultural Biotechnology Center, St. Lucia, Queensland, Australia. July 1993. Paper presented: *Genetic Characterization and differentiation of cattle subspecies.*

**P. Toye**

Keystone Symposium, Taos, New Mexico, March, 1993. Papers presented:

1. *Molecular immunology of virus infections.*
2. *An assay for the identification of antigens recognized by cytotoxic T cells, based on the transient transfection of COS cells.*

**D. Williams**

Twenty-Second Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), October, 1993. Paper presented: *Antibody responses to the variable surface glycoprotein coat of* Trypanosoma congolense *in N'Dama and Boran cattle.* D.J.L. Williams, K. Taylor, B. Gichuki and E. Authié.

**A.S. Young**

Nairobi Cluster: Veterinary Association Scientific Meeting, National Museums of Kenya, Nairobi, March, 1993. Paper presented: *Integrated control of trypanosomiasis and theileriosis.*

Third Asian Congress of Parasitology. Central Drug Research Institute, Lucknow, India, February, 1993. Papers presented:

1. *New methods of studying parasites transmitted by ticks.* A.S. Young, S.P. Morzaria, R. Bishop, B.D. Perry, T.T. Dolan and G. Gettinby.
2. *Cytokines in tick-borne protozoal infections and their use in therapy. An assay for the identification of antigens recognized by cytotoxic T cells, based on the transient transfection of COS cells.* A.S. Young, J. Cummins, G. Orind and I.G. Wright.

# SHORT-TERM VISITING SCIENTISTS

| Name | Project | Dates |
|---|---|---|
| A. Boulangé<br>Université of Bordeau<br>CIRAD-EMVT,<br>Paris, France | Visiting Research Fellow<br>Heat shock proteins of trypanosomes | From 1992 |
| Prof. T. Taira<br>Weseda University<br>Tokyo, Japan | Biomedical sciences research | 8/12/92–10/1/93 |
| Prof. L. and Mrs. M. Tilney<br>Pennsylvania University<br>Philadelphia, USA | EM investigation of secretory apparatus of early *Theileria* | 1/2/93–15/3/93 |
| Dr. D. Zilberstein<br>The Technion Institute<br>Haifa, Israel | Collaborative research on the action of isometamidium | 2/2/93–2/3/93<br>17/8/93–28/9/93 |
| Dr. S.J. Campbell<br>Orbis Institute<br>Ottawa, Canada | Computer consultant for system and database framework | 22/2/93–5/3/93 |
| Dr. G. Martin<br>Orbis Institute<br>Ottawa, Canada | Computer consultant for system and database framework | 22/2/93–5/3/93 |
| Dr. G.C. Russell<br>IAPGR/AFRC, Roslin<br>Midlothian, Scotland | Bovine MHC typing techniques | 26/2/93–23/3/93 |
| Dr. H. Van Dam<br>Wageningen University<br>The Netherlands | Trypanosomiasis | 12/3/93–27/3/93 |
| Dr. A. Spickett<br>Veterinary Research Institute, Onderstepoort<br>South Africa | Tick biology | 16/3/93–18/3/93 |
| Ms. S. Howe<br>P.O. Box 10<br>Limuru, Kenya | Visiting Editor | 17/3/93–30/3/93 |
| Dr. M. Nosé<br>Pharmaceutical Faculty<br>Nagoya City University<br>Nagoya, Japan | Identification of trypanosome attractants | 20/3/93–27/3/93 |

| Name | Project | Dates |
|---|---|---|
| Dr. T. Takeda<br>Pharmacognosy Dept.<br>Nagoya City University<br>Nagoya, Japan | Trypanosome attractants | 20/3/93–27/3/93 |
| Dr. B. Wilkie<br>Ontario Veterinary College<br>Guelph, Canada | Antibody avidity in trypanosome infections of cattle | 1/9/92–30/4/93 |
| Prof. J. Ellis<br>Winrock International<br>Morrilton<br>Arkansas, USA | Ecological implications of tsetse control | 6/4/93–30/4/93<br>12/8/93–18/12/93 |
| Prof. G. Gettinby<br>Dept. of Statistics<br>and Modelling Sciences<br>Strathclyde University<br>Glasgow, Scotland, UK | Consultant Statistician<br>Disease and herd production models<br>Development of division support systems | <br>11/5/93–24/5/93<br>11/12/93–18/12/93 |
| Dr. C. Robertson<br>Dept. of Mathematics<br>Strathclyde University<br>Glasgow, Scotland, UK | Consultant Statistician<br>Data analysis—statistics and training | 11/5/93–24/5/93 |
| Mr. C. Revie<br>Dept. of Statistics<br>and Modelling Sciences<br>Strathclyde University<br>Glasgow, Scotland, UK | Decision-support systems modelling | 11/5/93–20/5/93 |
| Dr. M. Szulczynski<br>Dept. of Biochemistry<br>Kenyatta University,<br>Nairobi, Kenya | Genetic basis of resistance to isometamidium in trypanosomes | 17/5/93–1/8/93 |
| Dr. J.B. Malloy<br>Animal Research Institute<br>Queensland, Australia | Candidate antigens for ELISAs for tick-borne diseases | 4/6/93–6/7/93 |
| Dr. D. Robinson<br>Manchester University<br>Manchester, England | Cytoskeletal morphogenesis in trypanosomes | 6/6/93–10/11/93 |

| Name | Project | Dates |
|---|---|---|
| Dr. D. Cuisance<br>EMVT/CIRAD Centre<br>ORSTOM, Montpellier<br>France | Consultant<br>Economics of<br>trypanosomiasis | 15/6/93–2/7/93 |
| Dr. M. Eisler<br>Veterinary Faculty<br>Strathclyde University<br>Glasgow, Scotland, UK | Collaborative research<br>for development<br>of ELISA for<br>isometamidium | 27/6/93–21/7/93<br>7/10/93–24/10/93 |
| Mrs. E. Gault<br>Veterinary Faculty<br>and Modelling Sciences<br>Strathclyde University<br>Glasgow, Scotland, UK | Collaborative research<br>for development of<br>ELISA for isometamidium | 27/6/93–21/7/93 |
| Dr. M. Upton<br>Reading University<br>England | Consultant—Economics<br>of trypanosomiasis and<br>Research Fellow supervision | 27/6/93–2/7/93 |
| Dr. E. Anderson<br>ODA Wildlife Project<br>Zimbabwe | Discussions on<br>PCR-based DNA tests<br>for *Theileria* spp. | 28/7/93–29/7/93 |
| Dr. M. Longeri<br>Faculty of Veterinary<br>Medicine, University of<br>Milan, Milan, Italy | Bovine genetics | 23/8/93–30/10/93 |
| Ms. A. Rink<br>Institute for Parasitology<br>Justus-Liebig University<br>Giessen, Germany | Visiting Research Fellow<br>Genetic characterization<br>of trypanotolerant<br>livestock | 23/8/93–12/11/93 |
| Dr. P. Wijngaard<br>Clinical Immunology<br>Academic Hospital<br>Utrecht, The Netherlands | cDNA clones for<br>*Theileria* | 28/8/93–28/10/93 |
| Dr. B. Goddeeris<br>Catholic University<br>of Leuven, Belgium | Immune responses<br>to tick-borne disease<br>organisms | 10/9/93–21/9/93 |
| Prof. V. Anosa<br>University of Ibadan<br>Nigeria | Bone marrow changes<br>during trypanosomiasis<br>in cattle | 15/9/93–15/12/93 |

| Name | Project | Dates |
|---|---|---|
| Dr. G. Duvallet<br>CRTA<br>Bobo-Dioulasso<br>Burkina Faso | Diagnostic reagents for trypanosomiasis | 1/11/93–13/11/93 |
| Prof. Y.O. Aliu<br>Ahmadu Bello University<br>Nigeria | Supervisor of Research Fellow in trypanosome chemotherapy | 28/8/93–11/8/93 |
| Dr. R. Spooner<br>IAPGR, Edinburgh<br>Research Station<br>Roslin, Scotland | Collaborative research discussions for bovine MHC typing | 11/10/93–17/10/93 |
| Dr. D. Rebeskie<br>IAEA, Vienna<br>Austria | Conjugation of monoclonal antibodies | 23/10/93–12/11/93 |
| Dr. S. Ellis<br>Institute for Animal Health, Compton<br>Berkshire, England | Analysis of bovine Class I MHC | 1/11/93–9/11/93 |
| Dr. R. Silayo<br>Sokoine University of Agriculture, Tanzania | Research Fellow supervision Improved cryopreservation of *Theileria parva* | 1/11/93–4/11/93 |
| Dr. H. Stauss<br>MRC Tumour Immunology Unit<br>UCL, London, UK | Discussion on techniques for isolation of schizonts of *Theileria parva* | 8/11/93–9/11/93 |
| Dr. I. McKendrick<br>University of Strathclyde,<br>Glasgow, Scotland | Collaborative research for generic decision support system | 15/11/93–4/1/94 |
| Prof. H. Clevers<br>University Hospital<br>Utrecht, The Netherlands | Collaborative research on molecular genetics of *Theileria parva* antigens | 16/11/93–23/11/93 |
| Dr. D. Berkvens<br>Institute of Tropical Medicine, Antwerp<br>Belgium | Discussions on collaborative research in tick-borne diseases | 18/11/93–21/11/93 |

| Name | Project | Dates |
|---|---|---|
| Dr. D. Geysen<br>Institute of Tropical Medicine, Antwerp<br>Belgium | Discussions on collaborative research in tick-borne diseases | 18/11/93–21/11/93 |
| Dr. S. Kamwendo<br>Tick-Borne Disease Vaccine Production Centre,<br>Lilongwe, Malawi | Discussions on collaborative research in tick-borne diseases | 26/11/93–26/11/93 |
| Dr. C. Quiroga<br>Tick-Borne Disease Vaccine Production Centre,<br>Lilongwe, Malawi | Discussions on collaborative research in tick-borne diseases | 26/11/93–26/11/93 |
| Dr. K. Tjornehoj<br>Tick-Borne Disease Vaccine Production Centre,<br>Lilongwe, Malawi | Discussions on collaborative research in tick-borne diseases | 26/11/93–26/11/93 |
| Dr. R. Pegram<br>FAO Regional Tick and Tick-Borne Disease Program,<br>Harare, Zimbabwe | Discussions on collaborative research in tick-borne diseases | 28/11/93–30/11/93 |
| Dr. A. Bensaid<br>EMVT-CIRAD<br>Maison-Alforts,<br>France | Discussions on collaborative studies on immune responses to heartwarter | 1/12/93–8/12/93 |
| Dr. D. Brown<br>CTVM, Edinburgh<br>UK | Discussions on collaborative projects in tick-borne diseases | 7/12/93–8/12/93 |
| Dr. D.S. Williamson<br>CTVM, Edinburgh<br>UK | Discussions on collaborative projects in tick-borne diseases | 7/12/93–8/12/93 |
| Dr. S. Mahan<br>University of Florida/ USAID/Zimbabwe,<br>SADCC Heartwater Project, Zimbabwe | Discussions on collaborative research on immunity to heartwater | 20/12/93–23/12/93 |

# ABBREVIATIONS, ACRONYMS AND SYMBOLS

| | |
|---|---|
| AcP | acid phosphatase |
| ALVC | afferent lymph veiled cells |
| APC | antibody presenting cells |
| ASA | allele-specific amplification |
| ASC | antibody secreting cells |
| ATP | adenosine triphosphate |
| ATPase | adenosine triphosphatase |
| AnTat | Antwerp *Trypanozoon* antigenic type |
| B-cells | a class of lymphocytes which can be stimulated to produce antibodies |
| BamH1 | a restriction enzyme |
| BCR | benefit:cost ratio |
| BFA | Bafilomycin A1 |
| BFU-E | burst-forming units-erythroid. Colonies of early red blood cell precursors which arise in some bone marrow cultures |
| BiP | immunoglobulin binding protein |
| BMMNC | bone marrow mononuclear cells |
| bp | base pairs (of DNA) |
| BPV | bovine papilloma virus |
| BSA | bovine serum albumin |
| BSF(s) | bloodstream form(s) (of trypanosomes) |
| $^{14}C$ | a radioactive isotope of carbon |
| $^{51}Cr$ | a radioactive isotope of chromium |
| C-terminal | the carboxy-terminus of a protein or peptide |
| CD | curative dose |
| CD-ROMs | compact disks-read only memory |
| CD4 | surface antigen defining a class of T-lymphocytes predominantly concerned with 'helper' functions |
| CD5 | a leukocyte surface antigen predominantly associated with a subclass of B cells |
| CD8 | surface antigen defining a class of T-lymphocytes predominantly concerned with 'cytotoxic' functions |
| cDNA | complementary deoxyribonucleic acid |
| CFU-E | colony-forming units-erythroid. Colonies of late red blood cell precursors which arise in some bone marrow cultures |
| CFU-GM | colony-forming units of granulocyte/macrophage progenitors |
| CFU-S | pluripotent stem cell colonies |
| CGIAR | Consultative Group on International Agricultural Research |
| CKII | casein kinase II |
| CLA | complement lysis assay |
| CMV | cytomegalovirus |
| Con A | concanavalin A—which can act both as a lectin (binding to sugar residues) and a mitogen (causing some lymphocytes to divide) |
| 'congopain' | a cysteine protease purified from *T. congolense* |
| CP | cysteine protease |
| CSIRO | Commonwealth Scientific and Industrial Research Organisation |
| CTL(s) | cytotoxic T lymphocyte(s) |

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| CTLp | cytotoxic T-lymphocyte precursor(s) |
| DA | diminazene aceturate |
| DBA | *Dolichus biflorus* agglutinin—a lectin |
| DEAE | diethylaminoethyl (cellulose) |
| DFMO | DL-a-difluoromethylornithine |
| DFP | diisopropylfluorophosphate |
| DIG | digoxigenin conjugate method |
| DNA | deoxyribonucleic acid |
| dpi | day(s) after infection |
| dT | deoxythymidine |
| E-64 | a protease inhibitor specific for cystein proteases |
| $EC_{50}$ | the concentration of drug in culture that reduces parasite growth by 50% compared to control cultures |
| ECF | East Coast fever |
| EcoR1 | a restriction enzyme |
| ELISA | enzyme-linked immunosorbent assay |
| EPO | erythropoietin |
| ER | endoplasmic reticulum |
| FACS | fluorescence activated cell sorter |
| FAO | Food and Agriculture Organization of the United Nations |
| FBS | foetal bovine serum |
| FCS | foetal calf serum |
| FEWS | Famine Early Warning System |
| FFE | free-flow electrophoresis |
| FITC | fluorescein isothiocyanate |
| g | gram |
| GAC | global area coverage |
| GIS | geographic information system(s) |
| GLM | General Linear Model |
| GRID | Global Resource Information Database (United Nations Environment Program) |
| GST | glutathione-S-transferase |
| GuTat | Glasgow University *Trypanozoon* antigenic type |
| HCT | haematocrit centrifuge technique |
| Hind III | a restriction endonuclease |
| HMI-93 | a liquid medium for supporting trypanosome growth *in vitro* (devised by H. Hirumi and colleagues at ILRAD) |
| HPLC | high-performance liquid chromatograph(y) |
| hr | hour(s) |
| HRP | horseradish peroxidase |
| HSP | heat shock protein |
| HSS | high-speed supernatant |
| IAEA | International Atomic Energy Agency |
| IFAT | indirect fluorescent antibody test |
| IFNγ | interferon gamma—a cytokine |
| IFPRI | International Food Policy Research Institute |
| Ig | immunoglobulin |

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| IgG | immunoglobulin gamma |
| $IgG_1/IgG_2$ | immunoglobulin gamma, classes 1 and 2 |
| $^{125}I$ | a radioactive isotope of iodine |
| IL-1–IL-6 | interleukins one to six (or cytokines) |
| IL-2R | Receptor for interleukin-2 |
| ILCA | International Livestock Centre for Africa (Addis Ababa) |
| ILDat | ILRAD *Duttonella* antigenic type |
| ILNat | ILRAD *Nannomonas* antigenic type |
| ILo | ILRAD oligonucleotide |
| ILRAD | International Laboratory for Research on Animal Diseases |
| ILTat | ILRAD *Trypanozoon* antigenic type |
| IM | intramuscular |
| INRA | Institut National de la Recherche Agronomique, France |
| IPTG | Isopropyl-β-D-thiogalactopyranoside—an inducer of the lac operon for production of β-galactosidase |
| IRR | internal rate of return |
| iso-VAT(s) | antigenically similar variable antigen type(s) of trypanosomes |
| IUCN | International Union for Conservation of Nature |
| IV | intravenous |
| IVDST | *in vitro* drug sensitivity test |
| $K^+$ | potassium ion |
| KARI | Kenya Agricultural Research Institute |
| kb | kilobase(s) (of DNA) |
| Kd | dissociation constant |
| kDa | kilodalton(s) (molecular weight) |
| KETRI | Kenya Trypanosomiasis Research Institute |
| kg | kilogram |
| Ki | inhibition constant |
| km | kilometre |
| Km | rate constant |
| $LD_{50}$ | the dose at which 50% of animals are killed (50% lethal dose) |
| LN | lymph node |
| LNC | cells derived from lymph node tissue |
| LPA | *Limulus polyphemus* agglutinin—a lectin |
| LPS | lipopolysaccharide(s) |
| LR | Land Registration (unit[s]) (Uasin Gishu District, Kenya) |
| LSU rRNA | large subunit ribosomal ribonucleic acid |
| M | molar |
| MAb(s) | monoclonal antibody(ies) |
| MCFs | metacyclic forms (of trypanosomes) |
| MCHC | mean cell haemoglobin concentration |
| MCV | mean cell volume |
| mdr | a transcribed gene whose increased expression is specific to multidrug resistant cell lines |
| MDR | multidrug resistance |
| medRNA | mini-exon-derived ribonucleic acid |
| Mel B | melarsoprol |

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| MEM | modified Eagle's medium |
| 10-mers (etc) | sequences of DNA containing 10 nucleotides etc. |
| mg | milligram (ie $10^{-3}$ of a gram) |
| MHC | major histocompatibility complex |
| min | minute(s) |
| ml | millilitre |
| mM | millimolar |
| MO | monocyte(s) |
| mol | mole |
| Mr | molecular mass |
| mRNA | messenger ribonucleic acid |
| mVAT(s) | metacyclic variable antigen type(s) of trypanosomes |
| MW | molecular weight |
| N-terminal | The amino terminus of a protein or peptide |
| $Na^+$ | sodium ion |
| NAC | non adherent cells |
| NADH | β-nicotinamide-adenine dinucleotide (reduced form) |
| NaOH | sodium hydroxide |
| NDVI | normalized difference vegetation index |
| NEM | N-ethylmaleimide |
| ng | nanogram |
| NIT | neutralization of infectivity test |
| nm | nanometre (ie $10^{-9}$ of a metre) |
| nmole | nanomole |
| NotI | a rare cutting restriction endonuclease |
| OAU | Organization of African Unity (Addis Ababa) |
| OD | optical density (e.g. of the colour reaction in an ELISA) |
| ODA | Overseas Development Administration (UK) |
| OFAGE | orthogonal field alternation gel electrophoresis |
| ORF(s) | open reading frame(s) |
| p67 | a 67-kilodalton protein antigen on the surface of *Theileria parva* sporozoites |
| p85 | an 85-kilodalton protein antigen on the surface of *Theileria parva* sporozoites and schizonts |
| PBL | peripheral blood lymphocyte(s) |
| PBM | peripheral blood monocytes |
| PBMC | peripheral blood mononuclear cell(s) |
| PCR | polymerase chain reaction |
| PCV(s) | packed cell volume(s) (percent) |
| PFR | paraflagellar rod—an organelle in the flagellum of trypanosomes |
| pfu | plaque-forming unit(s) |
| pGEX | an expression vector producing proteins fused to glutathione-S-transferase |
| pH | hydrogen ion concentration |
| pI | isoelectric point |
| pi | post infection |
| PLS | Proleolipid subunit |

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| pMAL | a vector for the expression of introduced genes as proteins |
| pMAL-P | a protein expression system producing the protein expressed to a carrier which can be subsequently cleaved by the protease factor Xa |
| pmole | picomole ($10^{-12}$ of a mole) |
| PNA | peanut agglutinin–a lectin |
| poly(A) | a tract of polyadenylic acid residues |
| poly(A)+RNA | RNA containing poly A tracts—predominantly messenger RNA |
| pSG5 | a commercial eukaryotic expression vector |
| PWM | pokeweed mitogen |
| RAPD | randomly amplified polymorphic DNA |
| RBC | red blood cell(s) |
| RFLP | restriction fragment length polymorphism |
| RH | relative humidity |
| rIL | recombinant interleukin |
| RNA | ribonucleic acid |
| rRNA | ribosomal ribonucleic acid |
| S | Svedberg unit |
| SASD | sulfosuccinimidyl 2-(p-azidosalicylamido) ethyl-1, 3′-dithioproprionate |
| SCF | stem cell factor |
| SCID/Bg | severe combined immunodeficient mice |
| SDS-PAGE | sodium dodecyl sulphate polyacrylamide-gel electrophoresis |
| SEAP | secreted placental alkaline phosphatase |
| SfiI | a rare cutting restriction endonuclease |
| SI | stimulation index |
| SIT | Sterile Insect Technique |
| ss-cDNA | single stranded, complementary deoxyribonucleic acid |
| SSCP | single-strand conformation polymorphism |
| SSU RNA | small subunit RNA |
| SV40 | simian virus 40 |
| T cell | a class of lymphocyte derived from the thymus |
| T-plasmid | a particular type of DNA plasmid useful for cloning polymerase chain reaction products |
| TAO | trypanosome alternative oxidase |
| TcCPP | *Trypanosome congolense* cysteine protease precursor |
| TCGF | T-cell growth factor |
| TCR | T-cell receptor(s) |
| Tf-R | transferrin receptor |
| TFA | trifluoroacetic acid |
| TfR | transferrin receptor |
| TGA | a codon of three nucleotides in DNA signifying the end of the reading frame |
| TNF | tumour necrosis factor—a cytokine |
| TpM | *Theileria parva* stock from Muguga, Kenya |
| Tpr | *Theileria parva* repetitive (sequence) |
| Triton X-100 | a detergent |

| | |
|---|---|
| tRNA | transfer ribonucleic acid |
| UEA | *Ulex europeaus* agglutinin—a lectin |
| UNDP | United Nations Development Program |
| UTR | untranslated region (of a gene sequence) |
| v/v | volume per volume |
| VAT(s) | variable antigen type(s) |
| $V_{max}$ | maximum velocity (of an enzyme/transport reaction) |
| VSG | variable surface glycoprotein(s) (of trypanosomes) |
| VVp104 | a recombinant vaccinia virus |
| WCMC | World Conservation Monitoring Unit |
| WGA | wheat germ agglutinin—a lectin |
| WHO | World Health Organization (Geneva) |
| WK | a strain of *Escherichia coli* |
| WWF | World Wide Fund for Nature |
| Xa | a protease |
| ×g | gravity |
| Xho 1 | a restriction enzyme |
| °C | degrees Celsius |
| α | alpha |
| α2M | alpha-2-macroglobulin, a serum protease inhibitor |
| β | beta |
| δ | delta |
| γ | gamma |
| λgt11 | an engineered bacteriophage for use in cloning DNA sequences |
| $\lambda_{max}$ | maximum wavelength |
| μ | micro ($10^{-6}$) |
| μg | microgram ($10^{-6}$ of a gram) |
| μl | microlitre |
| μM | micromolar |

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