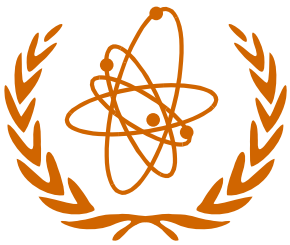
ISSN 0142-193X

TSETSE AND TRYPANOSOMIASIS INFORMATION QUARTERLY

Volume 23
Part 1, 2000
Numbers 11199–11334



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SECTION A – NEWS

PROGRAMME AGAINST AFRICAN TRYPANOSOMIASIS

PAAT Chairman's Report

In my last report I reviewed the progress that PAAT has made and the plans developed for the future at the PAAT Advisory Group Co-ordinators Meeting in Mombasa immediately before the highly successful ISCTRC Conference. Since then the full Programme Committee of PAAT has held its annual meeting (see below). I am pleased to report that the recommendations from both the PAAT Advisory Group Meeting and the ISCTRC Conference were endorsed by the Programme Committee and it is hoped that they can be implemented as rapidly as possible.

At this point in the last days of the twentieth century it is sobering to look back on how much has been achieved since the cause of trypanosomiasis was identified just over one hundred years ago. We now have a vast amount of information on trypanosomes, their vectors and how they might be controlled, yet in the past hundred years we have eradicated the vector from less than 10% of the affected area. There therefore remains an enormous challenge to us all to achieve so much more in the coming century and finally eradicate this scourge of sub-Saharan Africa. I am convinced that with adequate resourcing and co-ordination of control programmes this is now possible and I believe that the international co-operation which PAAT has helped to foster will play a key role in these endeavours.

My warmest best wishes for the New Year and New Century.

Peter Holmes, Chairman PAAT

Fifth Meeting of PAAT Programme Committee

The fifth meeting of the PAAT Committee was held in Rome on 22-23 November 1999 and was attended by representatives of international organisations, donors, scientists and technicians as well as the affected countries in Africa.

Great concern was expressed over the increasing incidence of sleeping sickness now affecting many areas of the continent, particularly Uganda, Democratic Republic of Congo and Angola. In many foci the disease situation has returned to the epidemic levels last recorded in the 1930s with over half of local populations infected. At the same time the animal form of the disease severely constrains food production and is now accepted as a major cause of poverty and suffering in many of the poorest rural regions on the continent. In discussing this issue the committee recognised the need to raise the awareness of both African Governments and the international agencies to this deteriorating situation. The Committee urged that the PAAT Secretariat explore ways to improve the dissemination of information at all levels in order to secure the appropriate long-term commitments needed to address the problem.

The meeting adopted the minutes of the PAAT Advisory Group Coordinators Meeting and unreservedly endorsed of the recommendations of the 25th ISCTRC Meeting, both held in Mombasa in September 1999.

Each of the members of the Secretariat reported highlights and activities of the past year in relation to PAAT. The progress of ongoing and planned projects and regional programmes was also reported; these included FITCA, RTTCP (possible bridging fund to extend the project until December 2000), West and Central Africa and the Southern Rift Valley of Ethiopia (see also separate items below).

The PAAT Committee recognised the need for, and thus approved, the formation of a ***Support Group*** to strengthen the capacity of the Secretariat to respond to the growing demands of the Programme (see also below).

A comprehensive report was presented on the progress of the ***Concerted Action on Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV)*** which is funded by the EU and supports the Research and Development module of PAAT. During its first year ICPTV organised three workshops on: (a) Improved diagnosis of trypanosomosis, Entebbe, Uganda, October 1998; (b) Drug delivery and resistance in the context of integrated disease management, ILRI, Nairobi, Kenya, May 1999; and (c) Data management and decision support systems, including risk assessment and disease impact evaluation, Harare, Zimbabwe, June 1999. The conclusions and recommendations of the three workshops were sent to all stakeholders and details were put on PAAT-L and published in the ICPTV Newsletter. Four more workshops will be organised in 2000-2001, the next being in March 2000 at ITC, The Gambia.

At present five ***PAAT working groups*** have been or are being created: HAT treatment and drug resistance; Quality control of animal trypanocidal drugs; Implications of privatisation for tsetse control; Capacity building and training requirements; Advisory group to assist the reformulation of the project for West and Central Africa.

An overview of ***PAAT position papers*** was presented and it was recommended that those outstanding should be moved forward to publication in the *PAAT Technical and Scientific Series* as soon as possible. Two papers on sleeping sickness (Drugs and treatment in human African trypanosomosis; Refractoriness to treatment and parasite resistance in human African trypanosomosis) should be completed as soon as possible for review over the PAAT-L and eventual publication. A new position paper on the implications of privatisation for tsetse and trypanosomiasis control requires to be commissioned.

A special session was devoted to a DFID-funded study on the ***economic returns of tsetse control***. Five detailed case studies had been carried out by British experts and the complete portfolio had been reviewed by three independent international experts who were nevertheless unable to make value-for-money judgements. Mr L. Budd was therefore commissioned to carry out an in-depth economic analysis of the costs and returns to the British investment in R & D over the previous 18 years. He briefed the meeting on the economic methodologies applied and the major assumptions made. He estimated that a total investment of about $20 billion in tsetse control/eradication over a 20 year period would result in returns accruing from increased agricultural and livestock productivity and improved human health and welfare of over $50 billion in the same period, giving a cost:benefit ratio of 1:2.5, and that returns to investment in research would be in the ratio of 1:75 to 1:175. Since the analysis had been based on other people's figures, it was recommended that smaller but definitive case studies should be initiated to verify the conclusions and to more precisely quantify the scale of costs and benefits involved.

Other main outputs from the meeting included: the more equitable distribution of Secretariat responsibilities between the international agencies, in particular the strengthening of OAU/IBAR as the focal organisation for policy, planning and implementation of control programmes; and agreement on the next stage of action required to ensure the successful implementation of the PAAT Plan of Action as endorsed in principle by the Advisory Group meeting in Maputo two years ago.

The full report of the Rome meeting is now available both in hard copy and through the PAAT-L by e-mail and the PAAT website (http://www.fao.org/paat/default.html).

PAAT Support Group

The specific purpose of this team will be to facilitate the effective uptake of recommendations and conclusions emanating from the Technical Advisory Groups and the Committee. Particular attention will be paid to the strengthening of communications, publications, co-ordination at the policy level, and the transfer of responsibilities for matters relating to policy, planning and project implementation to the OAU/IBAR office in Nairobi. The group will consist of the following senior-level, part-time advisers:

Policy Development: Dr G. Freeland
e-mail guy.freeland@tinyonline.co.uk

Field Programme Support and Quality Control: Prof. A.A. Ilemobade
e-mail peace@infoweb.abs.net

Communications and Publications: Mr B. Hursey
e-mail brian@bhursey.freeserve.co.uk

This group will complement the activities of the PAAT Secretariat, particularly in those areas not directly covered by the respective mandates of the agencies composing the PAAT Secretariat but considered essential to facilitate timely action at the field level.

Regional action in West Africa

One of the recommendations made by the regional meeting of FAO Liaison Officers, and subsequently approved by the PAAT Advisory Group, at their meetings in Mombasa in September 1999, expressed concern over the deteriorating trypanosomiasis situation in West Africa and urged the PAAT community to assist in the development and funding of a regional control programme to combat the problem. The PAAT Committee Meeting endorsed this recommendation and directed that immediate action be taken to prepare a programme proposal for submission to the EC. A Working Group consisting of Prof. A.A. Ilemobade, Dr O. Diall and Dr V. Codjia will, under the direction of OAU/IBAR and in close collaboration with FAO, prepare a preliminary proposal for submission to the EC by 31 March 2000. The FAO Regional Office in Accra has sent all concerned National Liaison Officers a questionnaire to gather and collate the information required as the basis for programme formulation.

For further information, please contact: Solomon Haile Mariam (parcibar@ africaonline.co.ke) or George Chizyuka (george.chizyuka@fao.org).

Ethiopian Project

The project strategy of the Southern Rift Valley of Ethiopia Tsetse Fly Eradication Project is based on phased, area-wide eradication following an integrated, participatory approach involving the sterile insect technique (SIT). Coordination centres and field operation teams have been installed. In addition, a well equipped system for baseline data collection, a GIS unit, two insectaries and a project steering committee have been established. A collaborative scheme has been worked out with the Addis Ababa University, the Ministry of Agriculture and other institutions. The collection of parasitological data is on-going and a facility for breeding tsetse flies, similar to the one in Tanga, will soon be established. There is a possibility of cooperation with the FITCA project as soon as the Ethiopian component becomes operational.

WORLD HEALTH ORGANISATION

WHO Sleeping Sickness Treatment and Drug Resistance Network

The sleeping sickness situation has changed dramatically over the last five years with a marked resurgence being recorded, particularly in central Africa. This is exacerbated by a marked increase in the number of relapses in patients treated with melarsoprol and the declining availability of the five drugs used routinely for treatment.

Solving these growing problems requires the close collaboration of all experts involved, including the public and private sectors, non-governmental aid agencies and drug producers. To effect this, WHO's Department of Communicable Diseases and Surveillance has established the 'Sleeping Sickness Treatment and Drug Resistance Network', the first Steering Committee meeting of which was held in Geneva in April 1999 under the chairmanship of Dr Reto Brun. Working groups have subsequently been formed to address the following issues: (i) Research, (ii) Surveillance, (iii) Drugs and (iv) Information systems. The last is run by the WHO Secretariat, whilst all groups interact with the Steering Committee and with all other relevant organisations and institutes concerned. The overall objective is 'to monitor drug resistance and to recommend solutions to the problems facing the treatment of sleeping sickness'.

The Research Group will establish documented specimen banks, both in Europe and in Africa. It will also compile an inventory of treatment and research centres and identify research priorities in trypanosomiasis for distribution to donors.

The Surveillance Group will develop systems for data collection, management and analysis which will first be pilot tested before being disseminated through training and the production of a manual.

The Drugs Group, which will include representatives from NGOs as well as the public and private sectors, will define the treatment needs over the next five years. It will also be tasked with ensuring the availability of the drugs and of the funds required to ensure their procurement. These vital activities will be co-ordinated by Dr J.P. Helenport under the specific title of 'Sustainable production of trypanocidal drugs'. The first meetings with pharmaceutical companies took place in December 1999.

The incorporation of this new WHO Sleeping Sickness Network into PAAT as an Advisory Group will significantly strengthen the overall expertise available to the Programme. The network will also publish two PAAT position papers on these issues.

Contact: Dr Reto Brun, Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland (e-mail Reto.Brun@unibas.ch).

Production and distribution of eflornithine

After collaborating for twenty years in the development of eflornithine to treat human African trypanosomiasis, Hoechst Marion Roussel and WHO signed a Licence Agreement at WHO headquarters in Geneva in December 1999 which allows WHO, in collaboration with other partners, to arrange for the production and distribution of the drug.

WHO and its partners will actively seek the means to ensure the continued availability of eflornithine. WHO has already established a network (see above) to monitor drug resistance and find and recommend solutions for the treatment of sleeping sickness. The network's Drugs Group is chaired by the NGO Médecins sans Frontières (MSF) and part of its brief is to 'ensure the production, commercialisation and registration of eflornithine in Africa and Europe'.

Technology transfer from Hoechst Marion Roussel will take place once WHO has found a new partner in the private sector, capable of producing eflornithine. In the meantime, MSF and WHO have been in contact with the international donor community to finance the purchase of adequate drug supplies. Since the vast majority of people with sleeping sickness will not be able to afford to pay for the drug, international financing will be needed. Securing procurement funds in advance will facilitate the search for a producer.

New WHO office in Yaoundé to strengthen sleeping sickness surveillance

In order to strengthen sleeping sickness surveillance activities in Central Africa the WHO Department of Surveillance and Action for HAT has opened a network focal point in Yaoundé, Cameroon. This office will help to establish and strengthen links with all national control programmes and NGOs in order to compile a comprehensive geographical database for trypanosomiasis foci; to monitor and ensure the quality of the epidemiological data; to strengthen national surveillance capacities by provision of training and technical assistance; and to ensure effective liaison with PAAT, particularly in the activities of information systems development.

The contact person is Mr Pierre Lucas, OMS, B.P. 155, Yaoundé, Cameroon (tel. 00237 30 15 79; e-mail OCEAC@camnet.cm (enter in the subject box 'To Pierre Lucas')).

CALL FOR COLLABORATORS IN MOLECULAR GENETICS TO ANALYSE TSETSE POPULATION DYNAMICS

Molecular genetic tools based on PCR can now be used to analyse in detail the tsetse genome and that of its symbionts. This analytical approach will enable us to develop a better understanding of intra- and inter-species characteristics of population structure, including isolation, and will ultimately also provide additional epidemiological information, e.g. on vectorial capacity.

Symbiotic micro-organisms inhabiting the gut and reproductive tissue in tsetse flies are intimately associated with the fly but very little is known about their genetic variation and distribution within the same or different species of the insect. For instance, the role of *Wolbachia* in intra-population or inter-species sterility is unknown.

There is a need to develop appropriate genetic tools with which to analyse tsetse population dynamics and determine gene-flow between neighbouring fly populations.

The recent PAAT Committee Meeting welcomed the relevance of such new technologies for (a) the identification of isolated tsetse populations and of confinable 'peninsulas' and (b) a better understanding of the paths that tsetse populations once took or are taking to invade new areas.

Information on the genomes of the tsetse and its symbionts in the field will help in the development of relevant dendrograms, in the construction of tsetse population-genetic maps and in the design of better strategies for tsetse control/eradication in the future.

IAEA intends to facilitate the establishment of a network of scientists in Africa and their collaborators elsewhere in the world who are doing research on the genetic analysis of tsetse populations and their symbionts and can make their results available for use in the development of tsetse population-genetic maps.

This initiative does not aim at supporting general research on tsetse molecular genetics but is specifically targeted at the design of dendrograms and population genetic maps, initially focusing on identified priority areas for population-wide tsetse intervention in East and West Africa. Additional intervention areas in other sub-regions will be covered in a subsequent phase.

Scientists, particularly in Africa, are invited to indicate their interest in participating in the establishment of such a network by contacting Udo Feldmann at IAEA (e-mail U.Feldmann@iaea.org; fax +43 1 2600 7), briefly outlining their relevant experience in the field or in the laboratory.

MEETING

Third Internet Conference on Salivarian Trypanosomes and Trypanosomatids

The Third Internet Conference on Salivarian Trypanosomes and Trypanosomatids (TICSTT) is currently being organised and will take place from 2 to 18 October 2000. The scope of this conference has been extended this year to include all pathogenic kinetoplastids. The flexibility of the internet format allows every trypanosome researcher with access to the internet to participate, regardless of their current time commitments or budget, and allows interaction between presenters and attendees. In six sessions, TICSST will cover all aspects of trypanosome research including biology and ultrastructure, biochemistry and drug development, immunology and pathology, molecular biology and epidemiology and vectors.

The work presented will initially be held online at Fiocruz but selected full submissions of high quality and abstracts of posters will be published in due course in a special issue of *International Journal for Parasitology* (April 2001).

For invited speakers, the deadline for presentations in html format is 1 September and for presentations in other formats 15 August. Primary authors are requested to restrict themselves to two papers.

For detailed instructions to authors, please visit the TICSTT website (http://www.dbbm.fiocruz.br/trypnews/events/ticstt.html) and/or feel free to contact us for specific questions or clarification: Alberto M.R. Davila, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (davila@gene.dbbm.fiocruz.br) or Kevin Tyler, Northwestern University, Chicago, USA (k-tyler@nwu.edu).

SECTION B – ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

11199 **Annor, S.Y., Garrick, D.J. and Blair, H.T., 1996.** Profitability and efficiency of N'Dama and Zebu cattle in southern Ghana. *Bulletin of Animal Health and Production in Africa*, **44** (4): 243-249.

Animal Science Department, Massey University, Palmerston North, New Zealand.

Local N'Dama (trypanotolerant) and exotic Zebu (trypanosusceptible) cattle were evaluated in the humid climatic zone of southern Ghana to find the profitability and efficiency of raising these breeds. This was done by modelling the life cycle production of a breeding cow and growth performance of her offspring in the two breeds. Profit was defined as the difference between income and expense. Economic efficiency was defined as total returns divided by total enterprise cost. Biological efficiency was defined in two ways: (1) the ratio of product output to feed input, and (2) the amount of body weight of offspring sold, generated by the breeding cow in a year. Profit per cow per year of N'Dama was 17% more than that of the Zebu. Profit per cow per year almost doubled in both breeds when the price of feed was set to zero, but the difference between the two reduced to only 7% in favour of N'Dama. Economic efficiency for N'Dama and Zebu production systems were 31% and 24% respectively. Biological efficiency, defined as the ratio of product output to feed input, was just about 2% for both breeds. N'Dama and Zebu cows were capable of generating only about 0.4 and 0.3, respectively, of their body weight in progeny each year. On the whole, the N'Dama performed better than the Zebu. It is therefore suggested that consideration be given to the use of trypanotolerant breeds of cattle in tsetse-infested areas of Africa.

11200 **Bauer, B., Amsler-Delafosse, S., Kaboré, I. and Kamuanga, M., 1999.** Improvement of cattle productivity through rapid alleviation of African animal trypanosomosis by integrated disease management practices in the agropastoral zone of Yalé, Burkina Faso. *Tropical Animal Health and Production*, **31** (2): 89-102.

Bauer: CIRDES, 01 B.P. 454, Bobo Dioulasso 01, Burkina Faso.

Investigations to identify the causes of high mortalities in cattle in the agropastoral zone (ZAP) of Yalé started in March 1993. African animal trypanosomosis (AAT) (due to *Trypanosoma vivax, T. congolense* and *T. brucei*) was found to be the major constraint,

with incidence rates exceeding 30%, justifying a tsetse control programme, which started in March/April 1994. The treatment of all cattle at bimonthly intervals with deltamethrin 1% pour-on and the deployment of 1500 insecticide-impregnated targets during the 6 months of the dry season each year helped to reduce populations of *Glossina tachinoides* and *G. morsitans submorsitans* by more than 90%. In less than 7 months, the incidence of AAT dropped below 5% and remained there throughout the intervention until June 1996, in spite of an increase to 3 months in the interval between the treatments. Mean PCV values increased significantly from 26.5-30.9% before to 30.7-36.3% during the intervention. The improvement in the overall health resulted in a resumption in fertility and milk production, allowing the sale of dairy products in Léo, thus creating a gross income of about $US 3/day for the Fulani women.

11201 **Hendrickx, G., Napala, A., Dao, B., Batawui, K., Bastiaensen, P., Deken, R. de, Vermeilen, A., Vercruysse, J. and Slingenbergh, J.H.W., 1999.** The area-wide epidemiology of bovine trypanosomosis and its impact on mixed farming in subhumid West Africa; a case study in Togo. *Veterinary Parasitology*, **84** (1-2): 13-31.

Hendrickx: Elsbos 24, B-2650 Edegem, Belgium.

This paper reports on an area-wide study of all major variables determining the expression of trypanosomosis in cattle in the subhumid eco-zone of West Africa, taking Togo as an example. To enable systematic area-wide sampling, the country was divided into 311 grid-squares of 0.125 × 0.125 sides. Cross-sectional surveys were then conducted to generate maps or digital layers on cattle density, herd structure, ownership and breed. These data layers, except for the breed data, were subjected to a cluster analysis in order to define spatial patterns in animal husbandry systems. This analysis revealed two main systems, one oriented towards integration with crop agriculture and the other towards investment in cattle. These two systems could be further characterised by incorporating breed data. Zebu cattle and their crossbreeds are more favoured in the second system. The breed distribution map shows the actual situation but also serves to predict the outcome of progressive crossbreeding. An area-wide trypanosomosis survey allowed the production of prevalence maps for *Trypanosoma congolense*, *T. vivax* and associated PCV values. A simple curvilinear relationship was established between vector density and disease prevalence. The regression between disease prevalence and PCV for taurine and zebu plus crossbreeds separately, revealed that taurine cattle maintain a comparatively high PCV level, particularly in high-prevalence scenarios. The relationship between average herd PCV and cattle density suggests that herd PCV value may provide a mirror for the number of animals not kept because of the prevailing risk. The regression between agricultural intensity and cattle density subsequently in areas with decreasing herd PCV values reveals that the level of integration of cattle in crop production decreases with a decreasing PCV. Thus, despite the presence of taurine animals in Togo, the omnipresence of tsetse, in particular *Glossina tachinoides*, remains a major obstacle to cattle raising and indirectly to mixed farming development and intensification. It is argued that only with the present type of wide-scale, spatial studies does it become possible to clarify all the major variables influencing the expression of trypanosomosis.

Spatial epidemiological studies at a macro level may form the basis for area-wide trypanosomosis control in West Africa.

11202 **Hide, G., 1999.** History of sleeping sickness in East Africa. *Clinical Microbiology Reviews*, **12** (1): 112-125.

Centre for Molecular Epidemiology and Ecology, Department of Biological Sciences, University of Salford, Salford M5 4WT, UK.

The history of human sleeping sickness in East Africa is characterised by the appearance of disease epidemics interspersed by long periods of endemicity. Despite the presence of the tsetse fly in large areas of East Africa, these epidemics tend to occur multiply in specific regions or foci rather than spreading over vast areas. Many theories have been proposed to explain this phenomenon, but recent molecular approaches and detailed analyses of epidemics have highlighted the stability of human-infective trypanosome strains within these foci. The new molecular data, taken alongside the history and biology of human sleeping sickness, are beginning to highlight the important factors involved in the generation of epidemics. Specific, human-infective trypanosome strains may be associated with each focus, which, in the presence of the right conditions, can be responsible for the generation of an epidemic. Changes in agricultural practice favouring the presence of tsetse flies, and the important contribution of domestic animals as a reservoir for the parasites are key factors in the maintenance of such epidemics. This review examines the contribution of molecular and genetic data to our understanding of the epidemiology and history of human sleeping sickness in East Africa.

11203 **Mahmoud, M.M., 1998.** *Trypanosoma evansi* in Sudan: an overview of current research and an evaluation of its impact on Sudan camel wealth and husbandry. *Journal of Protozoology Research*, **8** (3): 182-184.

Al Fashir University, Khartoum, Sudan.

The epidemiology, transmission and economic impact of *T. evansi* infection in dromedary camels in Sudan is briefly reviewed.

11204 **Mikami, T. and Hirumi, H. (eds), 1998.** RCPMI-Obihiro/OIE-Paris International Symposium on Strategies for Research and Control of Surra *Trypanosoma evansi* Infection, 19-22 August 1998, Obihiro University, Japan. Proceedings Parts I and II. *Journal of Protozoology Research*, **8** (3): 90-203; **8** (4): 204-288.

Mikami: Research Center for Protozoan Molecular Immunology, Obihiro University, Obihiro, Hokkaido 080-8555, Japan.

The first part of these proceedings includes a foreword and recommendations, three general papers, 11 papers on epidemiology and four on diagnosis (see **23**: nos. 11203, 11206, 11229, 11240, 11242, 11243, 11246, 11266). The second part includes four papers on molecular biology, two on culture techniques, three on drug therapy and one on vector

control (see **23**: nos. 11222, 11261, 11262, 11264, 11265, 11284, 11291, 11314, 11330, 11331). Abstracts of the 50 papers presented at the symposium are also included.

11205 **Murray, M., 1999.** The parasites, predators, places and people I have known: a great adventure. *Veterinary Parasitology*, **81** (2): 149-158.

University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK.

The author recounts his experiences, during nearly 40 years of research, on the occasion of receiving the WAAVP/Pfizer Award for Excellence in Research in Veterinary Parasitology which was presented to him at the 16th WAAVP Conference in Sun City, South Africa, in August 1997. He highlights the power of pathology and pathogenesis, the application of knowledge, the recognition of genetic resistance and the importance of measurement as key lessons learned while working with research teams to develop better diagnostics, to improve epidemiological understanding as a basis for rational treatment and control, and to extend the understanding of disease processes with a view to developing novel methods of treatment or prevention.

11206 **Touratier, L., 1998.** The O.I.E. ad hoc Group on Non Tsetse Transmitted Animal Trypanosomoses (NTTAT) with special reference to *T. evansi* infection. *Journal of Protozoology Research*, **8** (3): 90-96.

OIE, 12 rue de Prony, 75017 Paris, France. [louis.touratier@club.francetelecom.fr]

The work of the OIE ad hoc Group on NTTAT is described. Its objectives are: (i) to determine the economic impact of *Trypanosoma evansi* infections; (ii) to develop diagnostic tests; (iii) to study variations in pathogenicity of *T. evansi* isolates; (iv) to study the efficacy of trypanocidal drugs; (v) to exchange strains between laboratories; and (vi) to establish new measures for *T. evansi* control. The progress achieved so far is outlined.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

11207 **Borne, F., Petiteau, L., Geoffroy, B., La Rocque, S. de and Cuisance, D., 1999.** Fly Picture Measurement, un nouvel outil informatique pour l'étude des glossines. [Fly Picture Measurement, a new software tool to study tsetse flies.] *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux*, **52** (1): 19-21.

Borne: CIRAD-AMIS, Unité de Modélisation des Plantes, B.P. 5035, 34032 Montpellier Cedex 1, France. [frederic.borne@cirad.fr]

Entomologists have shown interest in biometry, on one hand as a systematics tool, on the other hand as an indicator of the living conditions of insects. Although it traditionally requires classical optical tools, it has now been made more reliable, precise and faster thanks to advances in computer science (data entry and processing). Software that measures the size of particular segments and the mean grey level of the wing has been developed and applied to tsetse fly wings. Its applications for medical and veterinary entomologists are highlighted here.

11208 **Chen, X.-A., Li, S. and Aksoy, S., 1999.** Concordant evolution of a symbiont with its host insect species: molecular phylogeny of genus *Glossina* and its bacteriome-associated endosymbiont, *Wigglesworthia glossinidia*. *Journal of Molecular Evolution*, **48** (1): 49-58.

Aksoy: Department of Epidemiology and Public Health, Yale University School of Medicine, 60 College Street, New Haven, CT 06510, USA.

Many arthropods with restricted diets rely on symbiotic associations for full nutrition and fecundity. Tsetse flies harbour three symbiotic micro-organisms: two reside in different gut cells, while the third is harboured in reproductive tissues and belongs to the genus *Wolbachia*. The primary (P) symbiont, *Wigglesworthia glossinidia*, lives in differentiated epithelial cells (bacteriocytes) which form an organ (bacteriome) in the anterior gut, while the secondary (S) symbionts are present in midgut cells. Here we have characterised the phylogeny of *Wigglesworthia* based on their 16S rDNA sequence analysis from eight *Glossina* species representing the three subgenera: *Austenina* (= *fusca* group), *Nemorhina* (= *palpalis* group) and *Glossina* (= *morsitans* group). Independently, the ribosomal DNA internal transcribed spacer-2 (ITS-2) regions from these species were analysed. The analysis of *Wigglesworthia* indicated that they form a distinct lineage in the γ subdivision of Proteobacteria and display concordance with their host insect species. The trees generated by parsimony confirmed the monophyletic taxonomic placement of *Glossina*, where *fusca* group species formed the deepest branch followed by *morsitans* and *palpalis* groups, respectively. The placement of the species *G. austeni* by both traditional morphological and biochemical criteria has been controversial. Results presented here, based on both the ITS-2 and the symbiont 16S rDNA sequence analysis, suggest that *G. austeni* should be placed in a separate fourth subgenus, *Machadomyia*, which forms a sister-group relationship with the *morsitans* group species.

11209 **Gooding, R.H. and Challoner, C.M., 1999.** Genetics of the tsetse fly *Glossina morsitans submorsitans* Newstead (Diptera: Glossinidae): further mapping of linkage groups I, II, and III. *Canadian Journal of Zoology*, **77** (8): 1309-1313.

Gooding: Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada. [Ron.Gooding@ualberta.ca]

Standard mapping procedures were used to map four loci in linkage group I (the X chromosome), two loci in linkage group II, and two loci in linkage group III of *G. m. submorsitans*. In the presence of the allele Sr^d (the distorter allele favouring production of female offspring), no recombination occurred between any of the following loci: *Pgm*

(phosphoglucomutase), *wht* (white eye colour), *Est-X* (a thoracic esterase) and *Sr* (sex-ratio distortion). However, in the absence of Sr^d (i.e. in females homozygous for Sr^n, the allele that permits males to sire both female and male offspring in approximately equal numbers), the loci *Pgm* and *wht* were separated by 23 ± 4.0% recombination (map distance). These results indicate that our *G. m. submorsitans* strains carry two forms of the X chromosome, designated X^A and X^B. In support of this interpretation, two lines of *G. m. submorsitans* were established: in both lines, males with wild-type eyes sired families that were almost exclusively female, while males with white eyes sired families having males and females in approximately equal numbers. Two loci, *Ao* (aldehyde oxidase) and *Est-1* (a thoracic esterase) were separated by 6.1 ± 2.3% recombination in linkage group II, and two loci, *Mdh* (malate dehydrogenase) and *Pgi* (phosphoglucose isomerase), showed 51.9 ± 4.9% recombination in linkage group III.

11210 **Hargrove, J.W., 1999.** Nutritional levels of female tsetse *Glossina pallidipes* from artificial refuges. *Medical and Veterinary Entomology*, **13** (2): 150-164.

IPMI Tsetse Research Project, Tsetse Control, Box CY52, Causeway, Harare, Zimbabwe. [jhargrove@rttcp.org.zw]

Female *G. pallidipes* caught in artificial refuges in the Zambezi Valley, Zimbabwe, in October 1993 were subjected to ovarian dissection and analysed for levels of fat, residual dry weight (RDW) and haematin. There were rather small proportions of flies in ovarian categories 0 and 1, in part due to large losses in the immature and teneral stages at the hottest time of year. The distribution of the female catch among pregnancy days was close to uniform. The wet and dry weights and RDW of eggs, larvae and pupae increased by 0.821, 0.303 and 0.204 mg, respectively, with each mm^3 increase in volume. Water accounted for 71.7% of the fat-free WW, and fat for 32.7% of the DW. Between birth and ovulation, fat increased from 2 to 4 mg and RDW from 7 to 11 mg; thoracic RDW increased by 2.5 mg and changed little thereafter. Fat levels increased 3.5 mg by day 6 of pregnancy, but only 0.5 mg thereafter. Over the same periods RDW corrected to zero haematin (CRDW) increased by 1 and 8 mg respectively. Full-term fat and CRDW levels were 8.2 and 19.4 mg respectively. Cumulative haematin frequencies formed a smooth curve with a slope that increased continuously. The raw data were well fitted by a model where feeding rates increased exponentially and capture probability was independent of haematin content. The mean feeding interval was 60 h; feeding probabilities of > 0.9/day were only found in flies that had failed to feed for > 72 h. In early pregnancy, fat levels declined with haematin for flies that had fed > 36 h previously; by days 5-7 fat levels were maintained at a constant high level for 60 h post-feeding. Fat-haematin graphs for female tsetse cannot be used to estimate rates of fat utilisation. Traps sample tsetse with below-average fat and RDW in early and late pregnancy respectively. Refuge samples are less biased than those from traps; they give a better picture of the dynamics of pregnancy in normal flies and facilitate the explanation of existing anomalies.

11211 **Hargrove, J.W., 1999.** Lifetime changes in the nutritional characteristics of female tsetse *Glossina pallidipes* caught in odour-baited traps. *Medical and Veterinary Entomology*, **13** (2): 165-176.

IPMI Tsetse Research Project, Tsetse Control, Box CY52, Causeway, Harare, Zimbabwe. [jhargrove@rttcp.org.zw]

Female *G. pallidipes* were captured in odour-baited traps at Rekomitjie Research Station, Zambezi Valley, Zimbabwe, during February 1994; 2890 were dissected and assigned to their ovarian age category and day of pregnancy using the lengths of the oocytes and uterine content. For 1838 of these flies, the nutritional state of the mother and her uterine content were estimated separately. It was thereby possible to see how, during pregnancy, the females acquired fat and residual dry weight (RDW) and transferred them to the larva. Newly emerged flies contained 1 mg fat and 6 mg RDW, of which 4 mg was in the thorax (TRDW). Fat hardly increased by the first ovulation; RDW increased by 2.5 mg and 1.5 mg of this increase was in TRDW. Mean haematin levels increased from 2 to 8 μg during each pregnancy. Fat increased from 1.2 mg to 4.5-5 mg by day 7 and was then rapidly transferred to the larva. RDW increased by only 1.8 mg by day 7, but larval RDW increased thereafter by > 6 mg. Amino acids from late-pregnancy bloodmeals are incorporated directly, in the uterine gland, into 'milk' that is taken up rapidly by the larva. Capture probability was highest on day 1 of pregnancy, when nutritional levels were lowest, with lesser peaks on days 5 and 8 when the fly was nourishing a rapidly developing larva. On day 1, the peak of the logarithm of the haematin distribution corresponded to flies estimated to have fed ≈ 75 h previously; by day 8 it had shifted to ≈ 60 h post-feeding. A model in which feeding rates and capture probabilities increased exponentially with time since feeding accounted for 97% of the variance in log haematin frequencies. On 4 out of 9 days of pregnancy there was no significant decline in fat with haematin content during the lipolytic phase. The rate of decline is not a satisfactory estimate of the rate of fat usage. Flies in this study had longer wings and higher TRDW than those from refuges in an earlier study, but had lower levels of fat and haematin.

11212 **Hargrove, J.W., 1999.** Reproductive abnormalities in tsetse flies in Zimbabwe. *Entomologia Experimentalis et Applicata*, **92** (1): 89-99.

IPMI Tsetse Research Project, Tsetse Control, Box CY52, Causeway, Zimbabwe. [jhargrove@rttcp.org.zw]

Between November 1988 and July 1995 five technicians carried out ovarian dissections on 16,013 *Glossina morsitans morsitans* and 123,848 *G. pallidipes* captured at Rekomitjie Research Station, Zambezi Valley, Zimbabwe. The ovarian age and uterine content were recorded, as were the lengths of the largest and second largest oocyte, and of any uterine inclusion. Major abnormalities and abnormal spermathecal contents were found in < 0.1% of all flies dissected. Apparent abortion rates varied significantly between dissectors and occurred at frequencies of 0.8-4.5% in *G. m. morsitans* and 0.3-2.8% in *G. pallidipes*. The lowest estimates give the best picture of the field situation. Abortion rates were higher in flies caught on electric nets than in trapped flies where the rate was only 0.15%, indicating that reproductive losses are negligible for most of the year at Rekomitjie. The rates did, however, increase to > 2% when mean temperatures exceeded 27°C and flies were captured in artificial refuges. There was little effect of ovarian age on the abortion rate, but the frequency of empty uteri declined markedly with age – with a suggestion, however, that it might increase again in the oldest flies. A

knowledge of the rates of reproductive loss is important for the construction of realistic models of the dynamics of tsetse populations.

11213 **Krafsur, E.S. and Wohlford, D.L., 1999.** Breeding structure of *Glossina pallidipes* populations evaluated by mitochondrial variation. *Journal of Heredity*, **90** (6): 635-642.

Krafsur: Department of Entomology, Iowa State University, Ames, IA 50011-3222, USA. [ekrafsur@iastate.edu]

Mitochondrial DNA diversity was studied at four loci in six natural populations of *G. pallidipes* from Zimbabwe, Mozambique, Kenya and Ethiopia. Single-locus diversity varied from 0.39 at *12S* to 0.65 at *COII*. A total of 32 haplotypes was found with a mean of 6.4 ± 2.9 per locus. To study breeding structure, diversity at two loci, *COII* and *16S2*, was evaluated in 18 populations sampled from an area of approximately 1,611,000 km^2 and in three laboratory cultures. Twenty-six haplotypes were detected at the two loci and mean haplotype diversity over all natural populations was 0.63. A high degree of population subdivision was detected within and among the Ethiopian and Kenyan populations. The Zimbabwean and Zambian populations showed much less variation and differentiation than the northern populations. A population in Mozambique showed high levels of haplotype variation and affinities closest to populations in eastern Kenya, some 1700 km to the north. Analysis of variance of haplotype frequencies showed that 51.5% of the total lay within populations, 13% among populations within five nested groups, and 35.5% among the five groups. Wright's F_{ST} was 0.485, Nei's G_{ST} was 0.33, and Weir and Cunningham's $\theta = 0.45$. Ecological data show that *G. pallidipes* is highly vagile. The large amount of genetic differentiation may be explained by genetic drift that occurred in scattered, relict populations during the rinderpest panzootic of the late 19th and early 20th centuries.

11214 **Langley, P.A. and Clutton-Brock, T.H., 1998.** Does reproductive investment change with age in tsetse flies, *Glossina morsitans morsitans* (Diptera: Glossinidae)? *Functional Ecology*, **12** (6): 866-870.

Langley: School of Pure and Applied Biology, Cardiff University, P.O. Box 915, Cardiff CF1 3TL, UK.

Viviparous insects such as tsetse provide unusual opportunities to compare age-related changes in the proportion of maternal resources transferred to offspring. In laboratory populations of *G. m. morsitans* the survival of females was high for the first 60 days of adult life but declined rapidly thereafter. Average longevity did not differ significantly between mated and unmated females (93.6 and 90 days, respectively). Nutritional state in terms of fat content and residual dry mass did not decline with adult female age. The fecundity of mated females was constant for the first 60 days of adult life and declined only slightly thereafter. Offspring size did not change towards the end of the adult female lifespan and there was no evidence of an increase in the allocation of resources to reproduction in older females. These results contrast with those obtained recently for vertebrates and may indicate that age-related changes in offspring size in

tsetse are not adaptive, or that so few females reach old age under natural conditions that there is no selection for a strategy of terminal investment.

11215 **Sang, R.C., Jura, W.G.Z.O., Otieno, L.H., Mwangi, R.W. and Ogaja, P., 1999.** The effects of a tsetse DNA virus infection on the functions of the male accessory reproductive gland in the host fly *Glossina morsitans centralis* (Diptera; Glossinidae). *Current Microbiology*, **38** (6): 349-354.

Sang: Virus Research Centre, Kenya Medical Research Institute, P.O. Box 54628, Nairobi, Kenya.

Freshly deposited third-instar *G. m. centralis* larvae were infected with the tsetse DNA virus by microinjection, and at emergence adult males were separated from the females and fed on rabbit blood every second day for 8 days. A control group treated with sterile saline were handled similarly. They were dissected, and comparative observations made on the appearance and size of the accessory reproductive glands (ARG) in infected and control males. Regularly fed 8-day-old males from infected and control groups were mated to 2-day-old normal females obtained from the insectary. After separation from copula, the females were dissected and the uteri examined for the presence and quality of the spermatophore. The spermathecae were also examined for insemination. ARG tissues from the control and virus-infected regularly fed 8-day-old male flies were fixed and processed for electron microscopic studies. The ARGs from control flies were found to be milky in appearance, whereas those from virus-infected flies were transparent in most parts. The ARGs from virus-infected males were significantly smaller in diameter ($F = 42.26$, $P < 0.0001$) and shorter ($F = 200.4$, $P < 0.0001$) than those of the controls. Most of the virus-infected males failed to form a complete spermatophore, whereas almost all the controls formed a complete spermatophore as observed in the uteri of the female mates ($\chi^2 = 111.661$, $P < 0.0001$). The infected males that formed partial spermatophores and those that did not form any at all failed to inseminate their female mates. Histological studies of the ARGs revealed some lesions in the epithelial cells characterised by degeneration of cytoplasmic organelles and detachment of the muscle layer from the basal plasma membrane. However, no virus particles were observed in the affected cells.

11216 **Solano, P., 1998.** *Etude de la variabilité génétique de* Glossina palpalis gambiensis *par le polymorphisme de l'ADN microsatellite. Implications épidémiologiques.* [Study of the genetic variability of *G. p. gambiensis* by microsatellite DNA poly-morphism. Epidemiological implications.] Thesis, Doctorat en parasitologie, Université de Montpellier II. 205 pp.

CIRAD-EMVT, Campus de Baillarguet, B.P. 5035, F-34032 Montpellier Cedex 1, France. [solano@mpl.ird.fr]

In West Africa, *palpalis* group tsetse transmit trypanosomes to cattle, causing severe losses. Little is known about tsetse intraspecific variability and its consequences on the epidemiology of trypanosomosis. In this study, three microsatellite DNA sequences were isolated, cloned and sequenced from a CIRAD/ORSTOM insectarium sample of *G. p. gambiensis*. PCR amplification using primers derived from these microsatellite sequences

showed size polymorphisms and Mendelian inheritance. These loci were subsequently used for genetic studies in natural populations. Significant differences in two microsatellite loci were seen between tsetse populations in Senegal and Burkina Faso, which correlated with wing size. On a smaller geographical scale, *G. p. gambiense* populations from two agropastoral zones of Burkina Faso were compared. At Samorogouan, analysis of tsetse flies caught in both dry and rainy seasons suggested that in this zone individuals breed randomly with each other and movements in the rainy season seem to homogenise the gene flow between groups of individuals. At Sidéradougou, population sampling in 1997 and 1998 along the Koba river network showed variability between the eastern and western ends of the network. A significant difference in mean *Fst* measurements was seen which was equivalent to an exchange of 5-6 individuals per generation. Different infection rates and trypanosome species were also seen in flies in the two parts of the network. High values of *Fis* were seen in the west, notably near a sacred grove near Nyarafo. Genotype analysis showed two genetically different groups, reproducing non-randomly. The way in which these population differences might have come about and their epidemiological implications for the control of trypanosomosis are discussed. This methodology using microsatellite DNA polymorphism to investigate intraspecific variability could be extended to other tsetse species.

11217 **Vreysen, M.J.B. and Vloedt, A.M.V. van der, 1999.** Morphological characterization of the genital armature of male and female hybrids from crosses between *Glossina palpalis palpalis* and *Glossina palpalis gambiensis* (Diptera: Glossinidae). *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux*, **52** (1): 13-18.

Vreysen: IAEA Project RAF/5/040, c/o Ethiopian Science and Technology Commission, P.O. Box 19917, Addis Ababa, Ethiopia. [estc@telecom.net.et]

The subspecies *G. p. palpalis* originating from Nigeria and *G. p. gambiensis* originating from Burkina Faso could be separated morphometrically on the basis of the width of the terminal dilatations of the male inferior claspers. Intermediate values were obtained for male hybrids, but the average size of the head of the parameres was significantly determined by maternal descendance, i.e. the average width of the head of the inferior claspers of male hybrids from the *G. p. gambiensis* × *G. p. palpalis* cross was significantly larger than that of hybrids from the reciprocal cross. Morphological characters of the inferior claspers of the male hybrids differed depending on the cross. The dorsal plates of the genital armature of female *G. p. gambiensis* were significantly longer but less wide than those of female *G. p. palpalis*. Both subspecies could be separated with a minimal overlap (7%) by plotting the length of the dorsal plates against their width. Female hybrids of the *G. p. palpalis* × *G. p. gambiensis* cross had dorsal plates which were significantly longer and wider than those of hybrids from the reciprocal cross.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

[See also **23**: no. 11212.]

11218 **Hendrickx, G., Napala, A., Dao, B., Batawui, D., Deken, R. de, Vermeilen, A. and Slingenbergh, J.H.W., 1999.** A systematic approach to area-wide tsetse distribution and abundance maps. *Bulletin of Entomological Research*, **89** (3): 231-244.

Hendrickx: FAO Trypanosomosis Project, GCP-RAF-347-BEL, B.P. 2034, Bobo Dioulasso, Burkina Faso. [hendrickx.vangorp@fasonet.bf]

A raster or grid-based GIS with data on tsetse, trypanosomosis, animal production, agriculture and land use has recently been developed in Togo. This paper describes the generation of area-wide digital tsetse distribution and abundance maps and how these accord with the local climatic and agro-ecological setting. Results include: (i) a spatial demarcation of ecologically distinct areas, producing a seasonal cluster map based on seasonal weather data and temporal series of satellite-derived National Oceanic and Atmospheric Administration (NOAA) AVHRR and METEOSAT variables; (ii) tsetse distribution maps of *Glossina tachinoides*, *G. palpalis palpalis*, *G. morsitans submorsitans*, *G. longipalpis*, *G. medicorum* and *G. fusca fusca*; and (iii) tsetse abundance or 'risk' maps, corrected for within database seasonal fluctuations, for *G. tachinoides* and *G. p. palpalis*. It is concluded that grid-based sampling is the ideal method for rapid assessment of the current vector and disease situation within any country or region, and that remote sensing has an important role to play in planning such a sampling system.

11219 **Mohamed-Ahmed, M.M. and Mihok, S., 1999.** Responses of *Glossina fuscipes fuscipes* (Diptera: Glossinidae) and other Diptera to carbon dioxide in linear and dense forests. *Bulletin of Entomological Research*, **89** (2): 177-184.

Mihok: Box 24031, Lethbridge, AB T1H 6H1, Canada. [smihok@telusplanet.net]

The responses of *G. f. fuscipes* and other Diptera to carbon dioxide were studied in linear and dense forests along the shores of Lake Victoria, Kenya. Flies were caught in biconical traps and were intercepted with electric nets while in flight near traps. Carbon dioxide dispensed at a high rate (5 l/min) in linear forest failed to increase the numbers of tsetse attracted to or caught in traps. In contrast, catches of non-biting Muscidae, Stomoxyinae and Tabanidae were improved by up to 11 times. Inside dense forest, carbon dioxide released at half this rate increased both the numbers of female tsetse attracted to a trap and the catches in a trap by about 2-3 times. Catches of male tsetse were, however, not affected. Striking improvements for other Diptera were also realised (up to 102 times). Under a variety of conditions, unbaited biconical traps attracted many Diptera to the vicinity of a trap, but caught few flies due to low capture efficiencies (typically less than 10%). In contrast, efficiency estimates for *G. f. fuscipes* were good, varying from 37 to 82% in different habitats and seasons. These results are discussed in relation to the search for practical odour attractants for riverine tsetse.

11220 **Ndegwa, P.N. and Mihok, S., 1999.** Development of odour-baited traps for *Glossina swynnertoni* (Diptera: Glossinidae). *Bulletin of Entomological Research*, **89** (3): 255-261.

Ndegwa: ICIPE, P.O. Box 30772, Nairobi, Kenya.

Three new prototype traps, S1-S3, were developed during studies of the behavioural ecology of *G. swynnertoni* in Kenya and Tanzania. The traps were compared in latin square experiments relative to the regular biconical trap as a standard and a selection of other conventional tsetse traps. Observations were also made on fly behaviour in the vicinity of traps using electric nets and sticky materials. When baited with acetone and 1-octen-3-ol, the S1 trap was 3.5 times as effective in catching *G. swynnertoni* in Kenya as the biconical trap. In Tanzania, the relative performance of the S1 and biconical traps differed; also, both traps were found to be inferior to an all-black, sticky 1 m^2 target. A second prototype, S2, performed slightly better than the biconical trap, but was still inferior to the black target. The final prototype, S3, was 2.9 times as effective as the biconical trap and performed as well as the black target. The potential for further improvement of traps for capturing *G. swynnertoni* and flies of the *morsitans* group is discussed.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE-EFFECTS)

[See also **23**: nos. 11200, 11215, 11219, 11220.]

11221 **Bossche, P. van den and Mudenge, D., 1999.** The effect of short-interval deltamethrin applications to control tsetse on the seroprevalence of babesiosis in cattle. *Tropical Animal Health and Production*, **31** (4): 215-222.

Bossche: RTTCP, P.O. Box A560, Harare, Zimbabwe. [petervdb@rttcp.org.zw]

For the past decade, treatment of cattle with 0.00375% deltamethrin (Decatix) at 2-weekly intervals has been part of an integrated approach to counteract continuous invasion of Zimbabwe by tsetse (*Glossina morsitans morsitans* and *G. pallidipes*) from the Mozambique fly-belt. To determine the effect of these regular deltamethrin treatments on the epidemiology of babesiosis, a survey was conducted to estimate the prevalence of antibodies against *Babesia bigemina* in adult communal cattle. The seroprevalence of antibodies against *B. bigemina* in adjacent areas, where cattle are treated with short-residual acaricides, was also determined for comparison. The prevalence of antibodies to *B. bigemina* was much higher in areas where dipping with a non-pyrethroid acaricide was conducted. This was attributed to the successful control of *Boophilus* spp., and hence a very low level of *B. bigemina* transmission, in the 'deltamethrin treatment zone'. This low level of disease transmission was confirmed by the low prevalence of antibodies against *B. bigemina* in sentinel cattle that were introduced to the deltamethrin treatment zone. The potential adverse effects of severely reducing the tick population should be taken into

consideration at the onset of tsetse control operations in which cattle are to be treated with deltamethrin at short treatment intervals.

11222 **Djiteye, A., Diarra, M., Ouattara, I. and Traoré, D., 1998.** Comparison of the efficacy of different traps and attractants for Tabanidae and *Stomoxys* in Mali. *Journal of Protozoology Research*, **8** (4): 263-273.

Laboratoire Central Vétérinaire, B.P. 2295, Bamako, Mali.

The relative attractiveness of biconical, cubical (F3), pyramidal and Vavoua traps used with or without attractants, to Tabanidae and *Stomoxys* was investigated in Mali. In woody savanna, F3, Vavoua and pyramidal traps attracted more Tabanidae than biconical traps, while Vavoua traps were most efficient against *Stomoxys*. In gallery forest, F3 traps were most efficient for Tabanidae but Vavoua traps were almost as efficient, catching 3-fold more than biconical traps, and were also most efficient for *Stomoxys*. Tabanidae and *Stomoxys* varied in their response to different combinations of attractants. Since Vavoua traps are also very attractive to tsetse flies, their use is recommended for control of all trypanosomiasis vectors.

11223 **Osir, E.O. and Vundla, W.R.M., 1999.** Characterization of the δ-endotoxin of a *Bacillus thuringiensis* isolate active against tsetse, *Glossina morsitans*, and a stem borer, *Chilo partellus*. *Biocontrol Science and Technology*, **9** (2): 247-258.

Osir: ICIPE, P.O. Box 30772, Nairobi, Kenya.

The δ-endotoxin crystals of a *B. thuringiensis* isolate active against *G. morsitans* were isolated from a nutrient broth culture by low speed centrifugation. Analysis of these crystals by denaturing gel electrophoresis revealed that the major component of the crystal δ-endotoxin was a protein of MW ~ 120,000. Upon solubilisation under alkaline pH and reducing conditions, the crystal yielded a toxin of MW ~ 64,000. Treatment of the toxin with bovine trypsin resulted in a shift in the MW to a toxin of ~ 62,000, while treatment with bovine chymotrypsin gave a toxin of ~ 60,000. Methyl green staining revealed that the endotoxin was phosphorylated, while staining with periodic acid Schiff reagent showed that it was glycosylated. The carbohydrate moiety was of the high mannose type as shown by staining with fluorescein isothiocyanate conjugated to concanavalin A. Following gel permeation chromatography on a Superose 12 column, the solubilised toxin resolved into six main protein peaks, two of which had trypsin-like activity. The δ-endotoxin caused mortalities in *G. m. morsitans* (LC_{50} of 42.4 μg/ml) and 4th instar *C. partellus* larvae (LC_{50} of 53.8 μg/ml), but had no effect on 3rd instar *Aedes aegypti* larvae.

11224 **Warnes, M.L., Bossche, P. van den, Chihiya, J., Mudenge, D., Robinson, T.P., Shereni, W. and Chadenga, V., 1999.** Evaluation of insecticide-treated cattle as a barrier to re-invasion of tsetse to cleared areas in northeastern Zimbabwe. *Medical and Veterinary Entomology*, **13** (2): 177-184.

Warnes: Pestwatch (Bristol), 8 Merrywood Close, Bristol BS3 1EA, UK. [martinwarnes@compuserve.com]

A field trial in Zimbabwe investigated the efficacy of insecticide-treated cattle as a barrier to prevent the re-invasion of tsetse, *Glossina morsitans* and *G. pallidipes*, into cleared areas. The original tsetse barrier consisted of insecticide-treated odour-baited targets, at an operational density of four to five targets per km^2, supported by insecticide treatments of cattle with either deltamethrin dip (Decatix) at 2-weekly intervals, or deltamethrin pour-on (Spoton) at monthly intervals, in a band ≈ 20 km wide from the reinvasion front. Tsetse catch, and trypanosomiasis incidence in nine sentinel herds, were recorded for 7-8 months, respectively, before the targets were removed, leaving only the insecticide treatment of the local cattle to stem the re-invasion of tsetse. After the removal of the target barrier, the tsetse readily invaded the trial area and the incidence of trypanosomiasis in sentinel herds increased, while their PCVs decreased. After 7 months without the targets in place, trypanosomiasis prevalence in the local stock had reached alarmingly high levels; the trial was terminated prematurely and the target barrier re-deployed. Immediately after the re-deployment of the target barrier, the tsetse catch in the trial area reverted to acceptable levels along the re-invasion front, and trypanosomiasis incidence in the sentinel cattle decreased. It is concluded that, under the conditions of the field trial, the insecticidal treatment of local cattle did not in itself form an effective barrier to tsetse re-invasion. By contrast, the target barrier performed as was predicted by mathematical and experimental analysis, and readily cleared the tsetse infestation and reduced trypanosomiasis incidence in the trial area.

4. EPIDEMIOLOGY: VECTOR-HOST AND VECTOR-PARASITE INTERACTIONS

[See also **23**: nos. 11202, 11216, 11233.]

11225 **Abbeele, J. van den, Claes, Y., Bockstaele, D. van, Le Ray, D. and Coosemans, M., 1999.** *Trypanosoma brucei* spp. development in the tsetse fly: characterization of the post-mesocyclic stages in the foregut and proboscis. *Parasitology*, **118** (5): 469-478.

Abbeele: Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. [jvdabb@entom.itg.be]

Post-mesocyclic development of *T. brucei* in *Glossina morsitans morsitans*, in particular its migration from midgut to salivary glands, was investigated by sequential microdissection, morphometry and DNA-cytofluorometry. This development started by day 6 after the infective feed, with passage of mesocyclic midgut trypomastigotes through proventriculus and upward migration along foregut and proboscis to the salivary gland ducts. Kinetics of salivary gland infection showed that colonisation of the salivary glands by epimastigotes occurred only during the time-limited presence of this developmental phase in the foregut and proboscis. Post-mesocyclic trypanosomes in the foregut and proboscis were pleomorphic, with four morphological stages in various constant proportions

and present all through from proventriculus up to the salivary gland ducts: 67% long trypomastigotes, 27% long epimastigotes, 4% long epimastigotes undergoing asymmetric cell division and 2% short epimastigotes. Measurements of DNA content demonstrated a predominant tetraploidy for 67% of these trypanosomes, the remainder consisting of the homogeneous diploid short epimastigotes and some long epimastigotes. According to the experimental data, the following sequence of trypanosome differentiation in the foregut and proboscis is proposed. Incoming mesocyclic trypomastigotes (2N) from the ectoperitrophic anterior midgut start to replicate DNA to a 4N level, are arrested at this point, and differentiate into long epimastigotes (4N) which give rise, by asymmetric cell division, to two unequal, diploid daughter cells: a long, probably dead-end, epimastigote and a short epimastigote. The latter is responsible for the epimastigote colonisation of the salivary glands if launched at the vicinity of the gland epithelium by the asymmetric dividing epimastigote.

11226 **Boid, R., Jones, T.W. and Munro, A., 1999.** A simple procedure for the extraction of trypanosome DNA and host protein from dried blood meal residues of haematophagous Diptera. *Veterinary Parasitology*, **85** (4): 313-317.

Jones: CTVM, Easter Bush, Roslin, Midlothian EH25 9RG, UK. [t.w.jones@ ed.ac.uk]

A two step elution method is described for the extraction of host serum proteins and trypanosome DNA from a single dried insect gut smear preparation. The first low temperature elution yields material suitable for use in ELISA to determine the host species on which the fly last fed while the results of the second high temperature elution can be used in a PCR assay to detect the presence of trypanosomal DNA. The method can be used to extract material from both fly squashes and blood spots dried onto filter paper and could simplify the collection and processing of samples for epidemiological studies on trypanosomoses and other vector borne pathogens.

11227 **Fournet, F., Traoré, S. and Hervouët, J.P., 1999.** Effects of urbanization on transmission of human African trypanosomiasis in a suburban relict forest area of Daloa, Côte d'Ivoire. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93** (2): 130-132.

Fournet: Département des Sciences Humaines appliquées à la Santé, IPR, B.P. 1500, Bouaké, Côte d'Ivoire. [florence.fournet@ird.ci]

The epidemiological risk of human African trypanosomiasis transmission was evaluated from entomological parameters (apparent trap density, female teneral rates, daily survival rates, proportion of human feeds) of *Glossina palpalis palpalis* populations in and around the town of Daloa, Côte d'Ivoire. High tsetse densities were found in the town outskirts, where the calculated risk of transmission was greatest. Environmental changes brought about by urbanisation did not result in the disappearance of tsetse (which included small numbers of *G. pallicera pallicera* and *G. nigrofusca nigrofusca*), or the interruption of sleeping sickness transmission. The few cases of sleeping sickness detected (32) in the years 1990-1995 indicated that transmission was lower than might be

expected from the tsetse density. Despite this, surveillance should be maintained since conditions still exist which favour urban/peri-urban transmission.

11228 **Lord, C.C., Barnard, B., Day, K., Hargrove, J.W., McNamara, J.J., Paul, R.E.L., Trenholme, K. and Woolhouse, M.E.J., 1999.** Aggregation and distribution of strains in microparasites. *Philosophical Transactions of the Royal Society of London (B)*, **354** (1384): 799-807.

Lord: Wellcome Centre for the Epidemiology of Infectious Disease, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.

Recent research has shown that many parasite populations are made up of a number of epidemiologically distinct strains or genotypes. The implications of strain structure or genetic diversity for parasite population dynamics are still uncertain, partly because there is no coherent framework for the interpretation of field data. Here, we present an analysis of four published data sets for vector-borne microparasite infections where strains or genotypes have been distinguished: serotypes of African horse sickness (AHS) in zebra; types of *Nannomonas* trypanosomes in tsetse flies (*Trypanosoma congolense* savanna, riverine forest and Kilifi strains, *T. simiae* and *T. godfreyi* in *Glossina pallidipes* and *G. morsitans morsitans* in the Zambezi Valley, Zimbabwe, June 1993); parasite-induced erythrocyte surface antigen (PIESA) based isolates of *Plasmodium falciparum* malaria in humans; and the merozoite surface protein 2 gene (MSP-2) alleles of *P. falciparum* in humans and in anopheline mosquitoes. For each data set we consider the distribution of strains or types among hosts and any pairwise associations between strains or types. Where host age data are available we also compare age-prevalence relationships and estimates of the force of infection. Multiple infections of hosts are common and for most data sets infections have an aggregated distribution among hosts with a tendency towards positive associations between certain strains or types. These patterns could result from interactions (facilitation) between strains or types, or they could reflect patterns of contact between hosts and vectors. We use a mathematical model to illustrate the impact of host-vector contact patterns, finding that even if contact is random there may still be significant aggregation in parasite distributions. This effect is enhanced if there is non-random contact or other heterogeneities between hosts, vectors or parasites. In practice, different strains or types also have different forces of infection. We anticipate that aggregated distributions and positive associations between microparasite strains or types will be extremely common.

11229 **Luckins, A.G., 1998.** Epidemiology of surra: unanswered questions. *Journal of Protozoology Research*, **8** (3): 106-119.

CTVM, Easter Bush, Roslin, Midlothian EH25 9RG, UK.

Insufficient information on the distribution, prevalence and/or incidence and economic significance of *Trypanosoma evansi* throughout its geographical range, an absence of strategies for effective monitoring and surveillance, and an inability to design and implement cost-effective, appropriate control strategies, have resulted in a negative

influence on the attitude of national governments and international funding organisations to implement control or undertake research. Reliable methods for identifying infected animals, estimating morbidity and assessing the economic impact of surra are essential if the situation is to improve.

11230 **Michel, J.F., Michel, V., La Rocque, S. de, Touré, I. and Richard, D., 1999.** Modélisation de l'occupation de l'espace par les bovins. Applications à l'épidémiologie des trypanosomoses animales. [Modelling cattle land use. Applications to the epidemiology of animal trypanosomoses.] *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux*, **52** (1): 25-33.

J.F. Michel: Le Rieu, 38300 Saint Savin, France. [jf.michel@wanadoo.fr]

In order to study the epidemiology of bovine trypanosomoses in Burkina Faso, the spatial relations between the main hosts (cattle) and tsetse flies were explored. An exhaustive georeferenced census of cattle located within a 1200 km^2 area enabled modelling of cattle movements and land use at the end of the dry season, when contact with tsetse flies is greatest. Using two important points on the animals' path at this period, namely night pens and water sources, cattle distribution maps were established for the study area by the simple manipulation of polygons.

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

11231 **Akol, M.N., Olaho-Mukani, W., Odiit, M., Enyaru, J.C.K., Matovu, E., Magona, J. and Okitoi, N.D., 1999.** Trypanosomosis agglutination card test for *Trypanosoma brucei rhodesiense* sleeping sickness. *East African Medical Journal*, **76** (1): 38-41.

Akol: Livestock Health Research Institute, P.O. Box 96, Tororo, Uganda.

A trypanosomosis agglutination card test (TACT) was developed for the diagnosis in the field of sleeping sickness due to *T. b. rhodesiense*, based on stabilised procyclic forms derived from Utat 4.1. Procyclics were fixed in buffered formalin at 4°C for 24 h and further stabilised in acid/alcohol mixture for 30 min. The fixed antigen was stained with Coomassie blue and suspended in 0.1M PBS/sodium azide buffer pH 7.2 at a concentration of 1×10^8 trypanosomes/ml and kept at room temperature. This antigen was used to screen 100 sera from rabbits infected with *T. b. rhodesiense*, eight from normal rabbits, and 220 human sera, 60 of which were from sleeping sickness patients, 50 from normal persons from a non-endemic area and 110 from patients with other parasitic infections. All sera from infected rabbits and 59 from sleeping sickness patients reacted strongly with the antigen, showing agglutination reaction which ranged from 1:4 to 1:1024 serum dilution. There was minimal cross reaction with other parasitic infections: one of 20 malaria patients, none of 20 hookworm patients, one of 30 schistosomiasis patients, none of 10 amoebiasis patients and one of 20 filariasis patients. Agglutination titres from

all these non-sleeping sickness patients were below 1:16. Based on rabbit positive and negative sera, TACT gave a sensitivity and specificity of 100% and 80% respectively, while for human sera a sensitivity of 98.3 % and specificity of 96% were observed. These preliminary results show that TACT could be a promising screening field test for *T. b. rhodesiense* sleeping sickness.

11232 **Büscher, P., Lejon, V., Magnus, E. and Meirvenne, N. van, 1999.** Improved latex agglutination test for detection of antibodies in serum and cerebrospinal fluid of *Trypanosoma brucei gambiense* infected patients. *Acta Tropica*, **73** (1): 11-20.

Büscher: Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. [pbuscher@itg.be]

A rapid latex agglutination test (LATEX/*T. b. gambiense*) for detection of antibodies in patients infected with *T. b. gambiense* is presented. The reagent is coated with a mixture of three variable surface antigens of bloodstream form trypanosomes. Two hundred and forty sera and 79 CSF samples from patients with parasitologically confirmed trypanosome infection along with 173 sera and 38 CSF samples from non-trypanosomiasis patients were tested. At 1:16 serum dilution, test specificity was 99%, while sensitivity ranged from 83.8 to 100% depending on the geographical origin of the samples. Undiluted CSF samples from non-trypanosomiasis and from first-stage patients scored negative, while 42 out of 66 CSF samples from second-stage patients were positive. Stability and reproducibility of the lyophilised reagent were excellent.

11233 **Laveissière, C. and Meda, A.H., 1999.** Incidence de la maladie du sommeil et densité des campements de culture en forêt de Côte d'Ivoire: possibilité de prédiction des zones à risques pour la mise en place précoce d'un réseau de surveillance. [Incidence of sleeping sickness and settlement density in the forests of Côte d'Ivoire: possibility of predicting high risk areas for the early establishment of a surveillance network.] *Tropical Medicine and International Health*, **4** (3): 199-206.

Laveissière: OCEAC, B.P. 288, Yaoundé, Cameroon. [OCEAC@camnet.cm]

A surveillance network based on primary health care, able to detect cases rapidly and to maintain the prevalence of the disease at an acceptable level, seems to be a good alternative to selective intervention by mobile teams for sleeping sickness control in the forests of Côte d'Ivoire. It is difficult to conceive that a surveillance network could be set up everywhere and it is therefore necessary to identify areas at greatest risk. To do this, it is essential to find an appropriate epidemiological indicator. Study of data from several foci in Côte d'Ivoire shows a striking correlation between epidemiological risk and settlement density per km^2 (r = 0983; $P < 0.05$). Epidemiological risk and disease incidence will increase up to the point when human influence on the environment eradicates the vector and halts disease transmission. This hypothesis is supported by the correlation between settlement density (d) and cumulative incidence: i = 0988 d – 0967 (*r*

= 0.951). The prevalence would thus be 0.5% from a density of 1.5 settlements per km^2, and 1% from a density of 2. The first results of remote sensing indicate that it should be possible to identify rapidly the forest areas where settlement density has reached a critical level, justifying the setting up of surveillance facilities and protocols.

11234 **Moore, A., Richer, M., Enrile, M., Losio, E., Roberts, J. and Levy, D., 1999.** Resurgence of sleeping sickness in Tambura County, Sudan. *American Journal of Tropical Medicine and Hygiene*, **61** (2): 315-318.

Moore: Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop F-22, 4770 Buford Highway, Atlanta, GA 30341-3724, USA.

Endemic foci of human African trypanosomiasis are present in southern Sudan. In 1996 and 1997, trypanosomiasis due to *Trypanosoma brucei gambiense* increased sharply in Tambura County. To define the magnitude and geographical distribution of the outbreak, a prevalence survey using population-based cluster sampling was conducted in 16 villages: 1358 participants answered questions about routine activities and tsetse fly contact and received serological testing. Seroprevalence in the surveyed area was 19.4% (95% confidence interval = 16.9%, 21.8%). Infection was confirmed in 66% of seropositive persons who received one parasitological examination and in 95% of those who had serial examinations of lymph node fluid and blood. Activities related to the civil war, such as temporary migration, were not associated with seropositive status. Since the previous population screening in 1988, the trypanosomiasis prevalence had increased two orders of magnitude, and the proportion of villages affected had increased from 54% to 100%. The results suggest that there may be 5000 cases in Tambura County. The absence of trypanosomiasis control for nearly a decade is a factor in the resurgence of the disease.

(b) PATHOLOGY AND IMMUNOLOGY

11235 **Buguet, A., 1999.** Is sleeping sickness a circadian disorder? The serotonergic hypothesis. *Chronobiology International*, **16** (4): 477-489.

Buguet: CRSSA, B.P. 87, F-38702 La Tronche, France.

Patients with human African trypanosomiasis due to *Trypanosoma brucei gambiense* or *T. b. rhodesiense* are ‘sleepy by day and restless by night’. The first 24 h polysomno-graphic recording (electroencephalogram, electromyogram, electro-oculogram), showing a disappearance of the 24 h rhythmicity of sleep and wakefulness, was performed in 1988. Thereafter, our team recorded 18 patients and six control volunteers at bed rest during 24 h sessions. Blood samples were taken hourly from eight of the patients through a venous catheter and every 10 min from the remaining 10 patients. Plasma cortisol, prolactin, growth hormone, melatonin and plasma renin activity were analysed. No disruptions of the circadian rhythms of sleep and wakefulness were seen in the six healthy African subjects, and there were no disturbances of 24 h hormone profiles. The patients experienced a dysregulation of the circadian rhythmicity of sleep and

wakefulness that was proportional to the severity of the disease. Sleep onset rapid eye movement (REM) episodes were more frequent in the most severely sick patients, who also showed major disruptions in the 24 h plasma hormonal profiles, with intermediate profiles being observed at earlier stages of the sickness. However, the relationship between hormonal secretions and the states of vigilance persisted. Unlike the other hormones, melatonin secretion remained undisturbed. These findings indicate that, at the stage of meningoencephalitis, HAT represents a dysregulation of the sleep-wake cycle and sleep structure, rather than a hypersomnia; this dysregulation is proportional to the degree of severity of the clinical and biological symptoms. It is accompanied by a circadian dysrhythmia of hormonal secretions, although the relationship between hormone pulses and sleep states is preserved. We therefore favour the involvement of the serotonergic raphe nuclei-suprachiasmatic nuclei liaison in the reversible disturbance of the circadian rhythms of the sleep-wake cycle and of hormonal secretions.

11236 **MacLean, L., Odiit, M., Okitoi, D. and Sternberg, J.M., 1999.** Plasma nitrate and interferon-gamma in *Trypanosoma brucei rhodesiense* infections: evidence that nitric oxide production is induced during both early blood-stage and late meningoencephalitic-stage infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93** (2): 169-170.

Sternberg: Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK. [j.sternberg@abdn.ac.uk]

Plasma nitrate and interferon (IFN)-γ levels were studied in patients with *T. b. rhodesiense* infection in Iganga District, Uganda, during April-June 1997. Blood and CSF samples were obtained from 33 parasitologically positive trypanosomiasis patients categorised as early or late stage (WBC counts > 5/mm^3) and 11 uninfected individuals. In both early- and late-stage patients plasma nitrate was significantly elevated (P < 0.0001) and infected patients had significantly raised concentrations of plasma IFN-γ (P < 0.01). Following treatment (suramin for early-stage, melarsoprol for late-stage patients), plasma nitrate levels in both groups returned to uninfected control levels but IFN-γ levels remained significantly elevated (P < 0.05). CSF nitrate was not detectable. It is concluded that both early- and late-stage trypanosomiasis is associated with increased nitric oxide and IFN-γ synthesis.

(c) TREATMENT

[See also **23**: no. 11232.]

11237 **Legros, D., Evans, S., Maiso, F., Enyaru, J.C.K. and Mbulamberi, D., 1999.** Risk factors for treatment failure after melarsoprol for *Trypanosoma brucei gambiense* trypanosomiasis in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93** (4): 439-442.

Legros: Epicentre, P.O. Box 2362, Kampala, Uganda. [epicentre@imul.com]

The treatment failure rate among late-stage trypanosomiasis patients treated with melarsoprol in Arua, northern Uganda, between September 1995 and August 1996 was evaluated, and the risk factors for treatment failure were identified. A retrospective cohort study was conducted in October 1998, and a survival analysis performed. A treatment failure was defined as a late-stage HAT patient fully treated with melarsoprol and classified as an HAT case at any follow-up visit within 2 years after treatment. Among 428 patients treated in the study period, 130 (30.4%) were identified as treatment failures within 2 years from discharge. The multivariate analysis showed that patients who experienced treatment failure after melarsoprol were more likely to have been admitted as a relapsing case (relative hazard, RH = 11.15 [6.34-19.61]), and to have been diagnosed with trypanosomes in the lymph nodes (RH = 3.19 [2.10-4.83]) or in the CSF (RH = 1.66 [1.09-2.53]). The risk of treatment failure also increased with the number of cells in the CSF. The treatment failure rate after melarsoprol observed in Arua is greatly above the expected figures of 3-9%. More research is needed to confirm whether it is related to the variation of melarsoprol pharmacokinetics between individuals, or if it is associated with a reduced susceptibility of the trypanosomes to melarsoprol. The study emphasises the need for second-line drugs to treat patients who have already received one or several full course(s) of melarsoprol.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

[See also **23**: nos. 11200, 11265.]

11238 **Butt, A.A., Muhammad, G., Athar, M., Khan, M.Z. and Anwar, M., 1998.** Evaluation of different tests for the diagnosis of trypanosomiasis and dipetalonemiasis in camels. *Journal of Camel Practice and Research*, **5** (2): 261-266.

Butt: Department of Veterinary Pathology, Metropolitan Corporation, Faisalabad, Pakistan.

In order to find the best field diagnostic tests for trypanosomiasis and dipetalonemiasis in camels, the comparative sensitivity, specificity, accuracy, percent agreement, positive and negative predictive values of five direct tests (fresh blood film, thin and thick Giemsa-stained smears, haematocrit centrifugation technique, miniature anion exchange centrifugation technique and modified Knott's technique (MKT)) and three indirect tests (mercuric chloride test (MCT), thymol turbidity test and formol gel test (FGT)) were determined in 200 dromedary camels. HCT was used as a gold standard which detected six camels positive for trypanosomiasis and 29 for dipetalonemiasis. For trypanosomiasis, all direct tests except mAEC showed sensitivity, specificity and predictive values of 100%. Among indirect tests, sensitivity, specificity, accuracy and predictive values were highest in FGT (100, 70.6, 71.5, 90.5 and 100%, respectively). Compared with HCT, percent agreement (Kappa values) for direct tests was excellent (100%) except for mAEC which failed to detect a single positive case of *Trypanosoma evansi*, while for indirect tests there was poor agreement ranging from 8.4 to 12.6%. For dipetalonemiasis,

sensitivity, specificity, accuracy and predictive values ranged from 79.3 to 100%, the highest being for MKT (82.8, 100, 97.5, 100 and 97.2%, respectively). Among indirect tests, the highest values for sensitivity, specificity, accuracy and predictive values were for FGT (86.2, 77.8, 79.0, 39.7 and 97.1%, respectively). Compared with HCT, percent agreement for direct tests ranged from 86.8 to 100%, while for indirect tests, there was poor to acceptable agreement ranging from 29.5 to 43.0% for MCT and FGT, respectively. These findings suggest that, in the absence of HCT, diagnosis of trypanosomiasis can be made with simpler direct tests, while for dipetalonemiasis, besides direct tests, FGT is also reliable.

11239 **Elamin, E.A., El Bashir, M.O.A. and Saeed, E.M.A., 1998.** Prevalence and infection pattern of *Trypanosoma evansi* in camels in mid-eastern Sudan. *Tropical Animal Health and Production*, **30** (2): 107-114.

Elamin: Department of Microbiology and Parasitology, College of Veterinary Medicine and Animal Resources, King Faisal University, P.O. Box 1757, Al-Ahsa 31982, Saudi Arabia.

The antigen detection enzyme immunoassay (Ag-ELISA) was used in conjunction with parasitological examination of blood to study the prevalence of trypanosomiasis in dromedary camels in mid-eastern Sudan. A one-year survey from November 1989 to October 1990 sampled 1738 camels. A prevalence of 5.4% was seen in pastoral camels based on parasitological examination and 31.3% based on Ag-ELISA. The infection rate was higher during the dry period (November to May) than during the wet season. Young camels had a much lower infection rate as detected by parasitological techniques, but not with Ag-ELISA. A lower prevalence of infection was detected by buffy coat technique in herds of camels raised by nomads compared with those kept by agropastoralists and in camels located in the southern districts of mid-eastern Sudan.

11240 **Elsaid, H.M., Nantulya, V.M. and Hilali, M., 1998.** Diagnosis of *Trypanosoma evansi* infection among Sudanese camels imported to Egypt using card agglutination test (CATT) and antigen detection latex agglutination test (Suratex). *Journal of Protozoology Research*, **8** (3): 194-200.

Elsaid: Department of Medicine and Infectious Disease, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.

A total of 125 imported Sudanese camels were tested for *T. evansi* infection using blood smear examination, CATT for antibody detection and Suratex for antigen detection to determine the prevalence of animals with chronic disease that might act as an exotic source of infection. Blood smear examination detected patent parasitaemia in five camels (4.0%), of which two were CATT-negative. CATT and Suratex detected 36 (28.8%) and 45 (36.0%) positive cases, respectively, of which 31 (24.8%) were positive for both tests. Five (4.0%) CATT reactors were negative by Suratex, while 14 (11.2%) camels that were positive by Suratex, including one case with patent parasitaemia, tested CATT-negative. An indirect haemagglutination test (IHA) with *T. brucei gambiense* antigen was also used. Eleven (78.6%) of the 14 CATT-negative camels had IHA titres ranging from 1/64 to

1/2048 (including the case with parasitaemia), giving concurrence with Suratex results. All five cases with patent parasitaemia were positive for IHA. Detection of circulating *T. evansi* antigens using Suratex was found to be a more sensitive and reliable means of diagnosis of camels with chronic latent infections.

11241 **Komoin-Oka, C., Zinsstag, J., Pandey, V.S., Fofana, F. and N'Depo, A., 1999.** Epidémiologie des parasites des ovins de la zone sud forestière de la Côte d'Ivoire. [Epidemiology of parasites of sheep in the southern forest zone of Côte d'Ivoire.] *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux*, **52** (1): 39-46.

Pandey: Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium. [vpandey@itg.be]

An epidemiological study of the parasites of Djallonké sheep was carried out in the southern forest zone of Côte d'Ivoire, where the climate is tropical. Six sheep were necropsied every month for 2 years, from August 1994 to July 1996, a total of 145 sheep. Nine species of nematodes were found, *Trichostrongylus colubriformis* (89.7%) and *Haemonchus contortus* (84.1%) having the highest prevalences. Three species of cestodes and three of trematodes were found. Other parasites observed were: coccidia, microfilaria, *Babesia ovis, Trypanosoma brucei, T. congolense, T. vivax* and *Oestrus ovis*. Parasite burdens and helminth egg excretion were moderate throughout the year. Infection was highest in animals less than a year old and in males. Prophylactic measures are discussed.

11242 **Nantulya, V.M. and Diall, O., 1998.** *Trypanosoma evansi* infections: towards penside diagnosis. *Journal of Protozoology Research*, **8** (3): 185-189.

Nantulya: Brentec Diagnostics, P.O. Box 42477, Nairobi, Kenya.

Parasitological techniques frequently used for diagnosis of *T. evansi* infections have low sensitivity since most infections in the field are not associated with patent parasitaemia. A simple and rapid indirect latex agglutination test, Suratex, has been developed for detecting circulating trypanosomal antigens in the blood of infected animals. Serum, plasma or whole blood is mixed with the Suratex reagent on a test card which is then rocked manually. In positive reactions, agglutination develops in 2 min. Suratex has been shown to have a specificity of 99%, using sera from horses, camels and cattle from non-endemic areas. Its sensitivity is also high: 93-97% of blood samples from animals with parasitologically confirmed diagnosis gave positive reactions. The test also diagnoses latent infections which cannot be detected by parasitological techniques.

11243 **Olaho-Mukani, W., Kakaire, D., Matovu, E. and Enyaru, J., 1998.** Prevalence of surra in dromedary camels in Uganda. *Journal of Protozoology Research*, **8** (3): 120-125.

Olaho-Mukani: Livestock Health Research Institute (LIRI), P.O. Box 96, Tororo, Uganda.

Three herds of camels, comprising 112 animals, in the Moroto district of north-eastern Uganda, were examined for *Trypanosoma evansi* infection using the micro-haematocrit centrifugation technique (MHCT) for parasitological diagnosis and a latex agglutination test (LAT) and an ELISA for immunodiagnosis. The MHCT showed a parasite prevalence ranging from 0 to 47%, whereas the LAT and ELISA showed positivities ranging from 35 to 65% and 78 to 100%, respectively. Low haematocrit values were associated with parasite- or antigen-positive camels. Microscopic examination of Giemsa-stained bloodstream forms and isoenzyme characterisation of eight trypanosome isolates showed them all to be *T. evansi*.

11244 **Rebeski, D.E., Winger, E.M., Rooij, E.M.A. van, Schöchl, R., Schuller, W., Dwinger, R.H., Crowther, J.R. and Wright, P., 1999.** Pitfalls in the application of enzyme-linked immunoassays for the detection of circulating trypanosomal antigens in serum samples. *Parasitology Research*, **85** (7): 550-556.

Rebeski: Animal Production Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, P.O. Box 100, A-1400 Vienna, Austria. [D.Rebeski@iaea.org]

The experimental infection of two goats with *Trypanosoma vivax* provided samples for analysis using parasitology techniques and antigen-detection ELISAs for *T. vivax*, *T. congolense* and *T. brucei*. Clinical, parasitological and serological findings were monitored during the course of infection to identify problems in the application of these ELISAs. The data clearly showed that the ELISAs examined were entirely unsuitable for the reliable detection of trypanosomal antigen. Consequently, research strategies pertinent to the development of a new generation of both antigen and antibody ELISAs are outlined, considering the problems encountered. These were (i) the reactivity of the reagents; (ii) the specificity of the reagents; (iii) the nature of the test sample, e.g. the compartmentalisation of trypanosomes between plasma, serum and red blood cells; (iv) possible interference with the ELISA through immune complexing; and (v) the biology of the host/trypanosome relationship to gain an understanding of fluctuations in trypanosomes in the systemic circulation.

11245 **Solano, P., Michel, J.F., Lefrançois, T., La Rocque, S. de, Sidibé, I., Zoungrana, A. and Cuisance, D., 1999.** Polymerase chain reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkina Faso. *Veterinary Parasitology*, **86** (2): 95-103.

Solano: CIRAD-EMVT, Campus de Baillarguet, B.P. 5035, F-34032 Montpellier Cedex 1, France. [solano@mpl.ird.fr]

During a trypanosomosis survey in November 1997 in the agropastoral zone of Sideradougou, Burkina Faso, 1036 cattle were examined for trypanosomes using microscopy. The PCR was applied on a subset of 260 buffy-coat samples using primers specific for *Trypanosoma congolense* savanna and riverine-forest groups, *T. vivax* and *T. brucei*. Parasitological examination was positive in 55 of the 1036 cattle (prevalence 5.3

± 1.3%) and the PCR was positive in 30 of the subset of 260 (11.5 ± 3.9%). Of these 260, parasitological examination was positive in only 11 (4.2 ± 2.4%). The main difference between the two techniques was in the number of positive cases of *T. congolense* savanna and *T. brucei*, which more than doubled using PCR. In the near future, the PCR is likely to become an efficient tool to estimate the prevalence of African trypanosomoses in affected areas.

11246 **Verloo, D., Tibayrenc, R., Magnus, E., Büscher, P. and Meirvenne, N. van, 1998.** Performance of ecological tests for *Trypanosoma evansi* infections in camels from Niger. *Journal of Protozoology Research*, **8** (3): 190-193.

Verloo: Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.

Card and latex agglutination tests and immune trypanolysis were compared using sera from 24 dromedary camels with parasitologically confirmed *T. evansi* infections and 76 putatively uninfected camels. All serum samples from parasitologically confirmed animals were positive by immune trypanolysis, whereas some were negative in both agglutination tests. Of the 76 parasitologically negative animals, 23 were positive by immune trypano-lysis. The sensitivity and specificity of the different tests are discussed.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **23**: nos. 11200, 11260.]

11247 **Buza, J. and Naessens, J., 1999.** Trypanosome non-specific IgM antibodies detected in serum of *Trypanosoma congolense*-infected cattle are polyreactive. *Veterinary Immunology and Immunopathology*, **69** (1): 1-9.

Naessens: ILRI, P.O. Box 30709, Nairobi, Kenya.

Serum immunoglobulins from six *T. congolense*-infected Boran cattle were affinity-purified using immobilised trypanosome or non-trypanosome antigens (β-galactosidase, cytochrome C and ferritin). The bound and unbound IgG and IgM fractions were collected and tested in ELISA for reactivity to each antigen. The results indicated that the presence of reactivity to non-parasite antigens in serum of infected cattle is due to polyreactive IgM antibodies. However, the IgG fraction only bound to trypanosome antigens and was only present in post-infection sera, indicating that it was induced by the infecting trypanosomes. Since the polyreactive IgM antibodies were also present in preinfection sera, it is probable that they were natural antibodies that were not induced but only amplified by the trypanosome infection.

11248 **Fall, A., Diack, A., Diaité, A., Seye, M. and d'Ieteren, G.D.M., 1999.** Tsetse challenge, trypanosome and helminth infection in relation to productivity of village Ndama cattle in Senegal. *Veterinary Parasitology*, **81** (3): 235-247.

Fall: ISRA, CRZ/Kolda, B.P. 52, Kolda, Senegal. [abdoufal@isra.refer.sn]

Data on tsetse fly, and on village N'Dama cattle collected over a 4-year period (1988-1992) in southern Senegal, were analysed. A total of 431 N'Dama cattle in four herds of three villages in the Upper Casamance area of southern Senegal were monitored monthly. *Glossina morsitans submorsitans* and *G. palpalis gambiensis* are present in the study area. Mean tsetse apparent density was 5.4 flies/trap/day. The trypanosome (*Trypanosoma congolense* and *T. vivax*) infection rate in flies was 2.4 (s.e. 0.37)%. Tsetse challenge index was 17.3 (s.e. 4.18). Mean monthly trypanosome prevalence in cattle was 2.5 (s.e. 0.51)%. Highest trypanosome prevalence occurred during the dry season, and animals less than 1 year old were more frequently infected than older animals. The linear relationship between the log10+1 tsetse challenge and the arcsine of the trypanosome prevalence was significant only when mean monthly values of these variables over the 4-year period were used with tsetse challenge preceding infection rate by 3 months. Mean monthly prevalences of strongyle, *Strongyloides* spp., *Toxocara* spp. and coccidia were 34.4 (s.e. 0.60), 2.1 (s.e. 0.18), 1.2 (s.e. 0.45) and 15.6 (s.e. 0.47)%, respectively. Calf mortality rate at 1, 6 and 12 months of age was 2.1 (s.e. 2.1), 5.2 (s.e. 2.8) and 12.2 (s.e. 3.3)%, respectively. Calving interval (584 s.e. 58 days) was not influenced by trypanosome status of the cow during lactation. Calving interval was shorter by 167 days when the calf died before 1 year of age in comparison to calving intervals for which the calf survived beyond one year. Live weight at birth, 6 and 12 months of age were 15.8 (s.e. 0.54), 48.1 (s.e. 2.56) and 71.1 (s.e. 5.44) kg, respectively. Mean lactation length was 389 (s.e. 16) days, and total and daily milk offtake were 231 (s.e. 15) litres and 0.69 (s.e. 0.037) litres, respectively. Trypanosome infection during lactation did not have a significant effect on the amount of milk extracted for human consumption, nor did trypanosome status affect calf growth.

11249 **Goossens, B., Osaer, S., Ndao, M., Winghem, J. van and Geerts, S., 1999.** The susceptibility of Djallonké and Djallonké-Sahelian crossbred sheep to *Trypanosoma congolense* and helminth infection under different diet levels. *Veterinary Parasitology*, **85** (1): 25-41.

Goossens: ITC, P.M.B. 14, Banjul, Gambia. [bart.sabine@commit.gm]

Forty-two Djallonké and 27 Djallonké-Sahelian crossbred sheep were compared during 34 weeks for their disease resistance and productivity in a multifactorial experiment including *T. congolense* infection, helminth infections and dietary level. Eight treatment combinations were formed in which the two breeds were balanced. Pyrexia was observed following trypanosome infection and was not different between the two breeds. However, a significantly higher parasitaemia level, a shorter prepatent period and a lower antibody response in the crossbreds following infection indicated a significant reduction of the trypanotolerance and confirmed the genetic origin of the trait. Neither helminth infection nor dietary level influenced the onset and level of parasitaemia or the level of antibody response following trypanosome infection. Trypanosome infection, helminth infection and low supplementary feeding caused independently significant reductions in PCV level and weight gain but these declines were not worse in crossbreds than in Djallonké. Indepen-dently of the studied factors, crossbreds were generally heavier than

Djallonké and also grew faster, especially during the second phase of the study. Crossbreds had significantly higher mean nematode egg output (epg) compared to Djallonké sheep but reduction of epg following deworming was similar in both breeds. The lower epg in the Djallonké breed indicated an innate resistance to helminths and/or more efficient immune response. Trypanosome infection tended to increase epg, confirming its immuno-suppressive effect. The higher body temperature in the Djallonké compared to crossbreds suggested a better heat tolerance in the former breed. From this study it was concluded that Djallonké-Sahelian crossbred sheep, in spite of a reduced trypanotolerance and lower resistance to helminth infection, possess a higher potential to intensify mutton production as compared to the pure Djallonké. However, appropriate measures should be taken to limit disease and stress factors in order to optimise production environment for this crossbred sheep.

11250 **Katunguka-Rwakishaya, E., Murray, M. and Holmes, P.H., 1999.** The influence of energy intake on some blood biochemical parameters in Scottish Blackface sheep infected with *Trypanosoma congolense*. *Veterinary Parasitology*, **84** (1-2): 1-11.

Katunguka-Rwakishaya: Department of Veterinary Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda. [vetdean@imul.com]

The intensity of parasitaemia, degree of anaemia, live body weight gains and blood biochemical changes were measured in two groups of Scottish Blackface sheep infected experimentally with *T. congolense* and allowed either a high (9.9 MJ metabolisable energy (ME) per day) or a low (6.1 MJ ME per day) energy intake. Infected animals on the low energy intake had a longer mean prepatent period, but following patency they developed more severe anaemia and greater growth retardation than those on the high energy intake. Both infected groups exhibited significant reductions in serum total lipids, phospholipids, plasma cholesterol and albumin but these changes were more severe in the animals on the low energy intake. It was concluded that adequate energy nutrition enhances the ability of infected animals to withstand the adverse effects of *T. congolense* infection by promoting body weight gains and moderating the severity of the pathophysiological changes.

11251 **Mattioli, R.C., Faye, J.A. and Büscher, P., 1999.** Susceptibility of N'Dama cattle to experimental challenge and cross-species superchallenges with bloodstream forms of *Trypanosoma congolense* and *T. vivax*. *Veterinary Parasitology*, **86** (2): 83-94.

Mattioli: ITC, P.M.B. 14, Banjul, Gambia. [raf.mattioli@commit.gm]

Susceptibility to *T. congolense* and *T. vivax* challenge and cross-species super-challenges, and related effects on health and productivity, were assessed in N'Dama cattle. The experimental herd of 25 bulls aged 3-4 years, previously primed with trypanosome infections through natural tsetse exposure over more than one year, was divided into five groups, each composed of five randomly selected animals. Group 1 was challenged with *T. congolense*, group 2 with *T. vivax*, group 3 was inoculated with *T. congolense* followed by a cross-superchallenge with *T. vivax* and group 4 was inoculated with *T. vivax* followed

by *T. congolense* cross-superchallenge. Animals in group 5 were used as controls. Both *T. vivax* and *T. congolense* cross-superchallenges were carried out on day 14 subsequent to respective initial *T. congolense* and *T. vivax* inoculations. All challenges were performed by intradermal needle inoculation of stocks of trypanosome bloodstream forms. In challenged animals (groups 1-4), parasitaemia profiles and PCV were measured for 4 months. Weight changes were recorded monthly and daily weight gain (DWG) computed. All cattle challenged with *T. congolense* became parasitaemic. Conversely, one animal in group 2 and two in group 3 never displayed patent *T. vivax* parasitaemia. Cattle showed higher percentages of positive blood samples and higher parasitaemia levels following *T. congolense* single (group 1), initial (group 3) and cross-superchallenge (group 4) inoculations than following *T. vivax* single (group 2), initial (group 4) and cross-superchallenge (group 3) inoculations ($P < 0.04$ or greater). Throughout the pre-challenge period, PCV values and DWGs were nearly identical in the five groups. Conversely, over the post-challenge period, cattle singly, initially and cross-superinoculated with *T. congolense* (groups 1, 3 and 4) displayed lower PCV values and DWGs in comparison with both control animals (group 5) and singly *T. vivax* challenged cattle (group 2) ($P < 0.05$ or greater). No difference in mean PCV levels and DWGs was found between animals in group 2 and those in group 5. It is concluded that trypanotolerant N'Dama cattle suffered more from *T. congolense* and mixed *T. congolense*/*T. vivax* infections, while pure *T. vivax* infection did not produce appreciable negative effects on their health and productivity. It is recommended that species-specific trypanosome prevalence and relative impact are assessed in various cattle populations and breeds differing in trypanosome susceptibility before advising any trypanosomosis control intervention. Moreover, virulence and related effects of *T. congolense* and *T. vivax* endemic stocks on health and productivity in local cattle populations should also be estimated in order to counsel appropriate economic protection measures, i.e. vector control and/or strategic use of trypanocidal drugs.

11252 **Mertens, B., Taylor, K., Muriuki, C. and Rocchi, M., 1999.** Cytokine mRNA profiles in trypanotolerant and trypanosusceptible cattle infected with the protozoan parasite *Trypanosoma congolense*: protective role for interleukin-4? *Journal of Interferon and Cytokine Research*, **19** (1): 59-65.

Mertens: ILRI, Old Naivasha Road, P.O. Box 30709, Nairobi, Kenya.

The association of cytokine responses with disease susceptibility in *T. congolense*-infected cattle was investigated. Changes in interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-12 p40, tumor necrosis factor (TFN)-α, CD40L, and transforming growth factor (TGF)-β gene expression were compared in peripheral blood mononuclear cells of infected trypanotolerant N'Dama (*Bos taurus*) and trypanosusceptible Boran (*B. indicus*) cattle. Results revealed that IL-2 transcription was decreased in both breeds of cattle at 21 days p.i. IL-12 p40 mRNA expression was increased in N'Dama cattle at 21 days p.i. and at a later time in Boran cattle. The highest IL-4 mRNA expression was observed at 32 days p.i. in N'Dama cattle. IL-6 mRNA expression increased in Boran cattle at 11 days p.i. and was elevated at 21 and 32 days p.i. in both breeds. Transcripts for IL-5 were barely detectable throughout the experimental period in both Boran and N'Dama cattle. Expression of TNF-α, IL-1β, and TGF-β mRNA did not change notably during the course

of infection. In summary, differences in the expression of IL-4 and IL-6 mRNA were identified between the two breeds of cattle during infection with *T. congolense,* suggesting a possible protective role for IL-4 and a disease-promoting role for IL-6 in bovine trypanosomosis.

11253 **Obasi, O.L., Ogwu, D., Mohammed, G. and Okon, E.D., 1999.** Reduced ovulatory and oestrous activity in zebu heifers following *Trypanosoma vivax* infection. *Tropical Animal Health and Production,* **31** (1): 55-62.

Obasi: Faculty of Agiculture, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria.

This paper describes a study on the oestrous and ovarian activity and responses to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) administration and artificial insemination in zebu heifers. Four cycling heifers were artificially infected with 5×10^6 *T. vivax* organisms. Two heifers served as controls. Two injections of $PGF_{2\alpha}$ were given 11 days apart, commencing at the peak of parasitaemia in the infected animals, followed by artificial insemination 72 and 96 h after the second administration of $PGF_{2\alpha}$. Sera were analysed for progesterone by radioimmunoassay, while ovarian activity and oestrus were determined by rectal palpation and visual observation, respectively. All the infected heifers developed the clinical disease. All control and infected heifers had progesterone profiles consistent with luteolysis and the occurrence of oestrus following the second administration of $PGF_{2\alpha.}$ Progesterone levels did not return to normal luteal values in infected animals, however, whilst they did so in control animals. No control or infected heifers became pregnant. The findings suggest that $PGF_{2\alpha}$ will induce a non-fertile oestrus in zebu heifers acutely infected with *T. vivax*. Re-ovulation is also inhibited within 22 days in a majority of infected animals.

11254 **Onah, D.N., Hopkins, J. and Luckins, A.G., 1999.** Changes in peripheral blood lymphocyte subpopulations and parasite-specific antibody responses in *Trypano-soma evansi* infection of sheep. *Parasitology Research,* **85** (4): 263-269.

Onah: Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Changes in peripheral blood lymphocyte (PBL) composition in sheep infected with *T. evansi* were studied, and parasite-specific IgG_1 and IgM antibody responses were monitored using a double-sandwich ELISA. Eight sheep were infected with 2×10^6 *T. evansi* TREU 2143. The infection was characterised by chronicity and ended in self-cure in two of the sheep which were designated group A, while the other six sheep, which remained parasitaemic until treated, were designated group B. Analysis of the PBLs by indirect immunofluorescence staining and flow cytometry revealed significant alterations in the numbers of T- and B-cell subsets in all infected sheep. In group A, whereas the numbers of $CD8^+$ cells decreased, $CD4^+$ cells showed marginal decreases, remaining at or above pre-infection figures and resulting in an increase in the CD4:CD8 ratio. In group B,

$CD8^+$ cells showed few marginal decreases, being at or above pre-infection figures most of the time, whereas $CD4^+$ cells decreased significantly from day 26 p.i. such that the CD4:CD8 ratio decreased. Infection also resulted in significant increases ($P < 0.001$) from day 26 p.i. in circulating B-cells in group B as shown by the numbers of sIg^+, $CD45R^+$, $CD1^+$ and major histocompatibility complex (MHC) II^+ cells. The increases, however, were moderate and biphasic in group A. *T. evansi*-specific IgM and IgG_1 antibody isotypes were detected in all infected sheep, but their levels were significantly higher in group A than in group B (IgM $P < 0.05$; IgG_1 $P < 0.01$). In addition, although an initially higher level of IgM response was subsequently replaced by a higher level of IgG_1 response in group A, this was never the case in group B until after drug treatment.

11255 **Osaer, S., Goossens, B., Kora, S., Gaye, M. and Darboe, L., 1999.** Health and productivity of traditionally managed Djallonké sheep and West African dwarf goats under high and moderate trypanosomosis risk. *Veterinary Parasitology*, **82** (2): 101-119.

Osaer: ITC, P.M.B. 14, Banjul, Gambia.

Trypanosome infections, PCV levels, body weight and nematode faecal egg counts of village-based Djallonké sheep ($n = 500$) and West African dwarf goats ($n = 650$) were monitored in two areas in The Gambia with either moderate or high trypanosomosis risk for 24 and 30 months respectively. Outflows from the flock and new-born animals were recorded and data on housing and management were compiled. Reported mortality rates were higher in goats than in sheep, but for both species were highest in the moderate risk area. The peak of trypanosome infections lagged behind the peak of tsetse densities by 1-3 months in both areas. *Trypanosoma vivax* was the predominant species found in the infected animals, followed by *T. congolense*. Trypanosome prevalence was, in general, higher in sheep than in goats but only significantly higher during the first year in the moderate-risk area. PCV levels were significantly reduced by trypanosome infection and were also significantly lower during the rains. Trypanosome infection significantly depressed weight gain in both species at periods when infection rates were highest, and in both species considerably lower weight gains were observed during the rainy season. Abortion rates were higher in goats than in sheep in both study sites, and highest in the high-risk site. Infection in ewes in the high-risk area increased lamb mortality significantly but had no effect on birth weights, nor on growth rates up to 4 months. Offspring mortality up to 4 months was generally high at both sites. Trypanosome infection in the dam between 3.5 and 7 months post parturition significantly increased parturition interval in both species. Peak faecal egg output occurred at the end of the rainy season and was highest for both species in the moderate-risk site. Poor grazing management was responsible for a seasonal nutritional constraint. Based on these results, these breeds of sheep and goats can be considered as trypanotolerant since they are able to remain productive under high and moderate levels of trypanosome challenge. Nevertheless, trypanosomosis affected their health and production level. In addition, during the rains, helminth infections and poor management leading to nutritional constraints also reduced the innate resilience to trypanosomosis of these indigenous breeds. Adaptations in management could have as great an impact as certain disease

control measures in improving biological and economical returns from small ruminants in tsetse-infested rural areas.

11256 **Ouma, J.O., Olaho-Mukani, W., Mutani, A., Wishitemi, B.E.L. and Guya, S.O., 1998.** Dromedary complement (C3): purification, characterisation and quantitation of its levels during experimental *Trypanosoma evansi* infection in camels. *Journal of Camel Practice and Research*, **5** (2): 213-218.

Ouma: Division of Biochemistry and Immunology, KETRI, P.O. Box 362, Kikuyu, Kenya.

The present study aimed at isolating and partially characterising the third component of the dromedary complement system (C3) and determining its dynamics during infection with *T. evansi*. C3 was isolated from camel serum by polyethylene glycol precipitation and chromatography on DEAE-Sephadex A-50, CM-Sephadex C-50 and Sephacryl S-200. Molecular characterisation on SDS-PAGE showed that the protein has a molecular weight of 185 kDa. Monospecific antiserum prepared in goats produced single precipitin lines with both the purified form of C3 and normal camel serum. Following experimental infection of camels with *T. evansi*, serum C3 levels showed a slight initial increase. The levels later dropped as the infection progressed and correlated negatively with parasitaemia levels. The mean C3 level of infected animals was significantly lower than that of controls ($P < 0.05$) and only recovered to normal values following the elimination of trypanosomes by treatment. The hypocomplementaemia in *T. evansi*-infected camels was attributed to the presence of the parasite. It is concluded that camel C3 is a high MW protein and that its depletion (activation) occurs in trypanosome-infected camels. This may be responsible for the *in vivo* control of parasitaemia and immunosuppression widely reported in animal trypanosomoses.

11257 **Ouma, J.O., Olaho-Mukani, W., Wishitemi, B.E.L. and Guya, S.O., 1998.** Alternative complement pathway activity in experimental surra. *Journal of Camel Practice and Research*, **5** (2): 219-224.

Ouma: Division of Biochemistry and Immunology, KETRI, P.O. Box 362, Kikuyu, Kenya.

Haemolytic complement activity in dromedary camels experimentally infected with *Trypanosoma evansi* was assayed under alternative pathway conditions. Complement fixing antibody titres and circulating trypanosomal antigen levels were also monitored throughout the infection period. A rapid initial increase (47%) in mean alternative pathway haemolytic complement (ACH_{50}) level occurred during the first week of infection. ACH_{50} levels later decreased significantly in infected camels and recovered only after drug treatment was started. The mean ACH_{50} units of uninfected control camels showed only slight variations throughout the study and were significantly higher than those of infected camels ($P < 0.05$). Complement fixing (CF) antibody titres and circulating trypanosomal antigens rose considerably following infection and decreased only when treatment was started. It is concluded that complement depletion occurs in *T. evansi*-infected camels. This is probably due to complement activation and may have

several important implications in both the pathogenesis of trypanosomiasis and pathology in the dromedary camel.

11258 **Stevenson, P., Rossiter, P.B., Munga, L., Ndung'u, E.K. and Dolan, R.B., 1999.** Rinderpest vaccination and the incidence and development of trypano-somosis in cattle. *Tropical Animal Health and Production*, **31** (2): 65-73.

Stevenson: KETRI, P.O. Box 362, Kikuyu, Kenya.

An investigation was made into whether recent vaccination of cattle with tissue culture rinderpest virus would cause immunosuppression and lead to more frequent or more severe infection with trypanosomes in animals grazing in tsetse-infested areas. Herds of cattle on Galana Ranch in Kenya were divided, with approximately half of each herd being vaccinated with tissue culture rinderpest virus strain Kabete 'O', while the rest remained unvaccinated. The herds were then exposed to the risk of natural infection with trypanosomes on the ranch. Three experiments were performed during different seasons. Infections with *Trypanosoma congolense* and *T. vivax* were frequently detected but there was no evidence that vaccinated animals were more likely to acquire trypanosome infections or to show a more severe disease than unvaccinated cattle. It is concluded that tissue culture rinderpest vaccine does not cause immunosuppression and can safely be used in cattle likely to be exposed to tsetse flies and trypanosomosis.

11259 **Suliman, H.B., Logan-Henfrey, L., Majiwa, P.A.O., ole-Moiyoi, O. and Feldman, B.F., 1999.** Analysis of erythropoietin and erythropoietin receptor genes expression in cattle during acute infection with *Trypanosoma congolense*. *Experimental Hematology*, **27** (1): 37-45.

Suliman: Department of Medicine, Duke University Medical Center, Box 2620, Durham, NC 27710, USA.

Acute *T. congolense* infection induced moderate, transient anaemia in N'Dama cattle (trypanotolerant) and severe anaemia in Boran cattle (trypanosusceptible). Erythropoietin receptor (EpoR) was cloned and sequenced from the two breeds of cattle. A single position mutation of Tyr in the Boran to His in the N'Dama predicted amino acid sequence was revealed. The mRNA transcription of erythropoietin (Epo) in kidneys and EpoR in the bone marrow of infected cattle was determined by competitive reverse transcription and the polymerase chain reaction (RT-PCR). Though Epo mRNA transcription increased in the kidneys during infection, the increase was not significantly different ($P > 0.05$) between the two breeds of infected cattle. The level of EpoR transcripts in the bone marrow of infected N'Damas was significantly higher ($P < 0.05$) than that detected in the marrows from infected Boran cattle. While infection seemed to increase levels of transcription of IL-1α and β and TNF-α in kidneys from both Boran and N'Dama cattle, no significant difference was detected in the level of mRNAs of these cytokines in the kidney from the two breeds. The amounts of IFN-γ mRNA transcripts were not changed with infection in N'Dama cattle, but significantly higher levels of IFN-γ were found in kidneys from infected Boran cattle compared to the other groups. Significant ($P < 0.05$) increases in the levels of IL-1α and β and IFN-γ mRNA transcripts

were detected in the marrows of infected Borans compared to infected N'Dama cattle. In this study the increase in the level of TNF-α mRNA did not differ in the marrows of the two infected breeds, implying that there is no negative effect of TNF-α on haematopoiesis during acute infection. These findings suggest that the levels of Epo and EpoR in the infected Boran cattle were inadequate for their degree of anaemia, which might be due in part to high expression of IFN-γ during acute infection with *T. congolense*.

(c) TRYPANOTOLERANCE

[See also **23**: nos. 11199, 11252, 11255, 11259.]

11260 **Wang, Q., Murphy, N. and Black, S.J., 1999.** Infection-associated decline of Cape buffalo blood catalase augments serum trypanocidal activity. *Infection and Immunity*, **67** (6): 2797-2803.

Black: Department of Veterinary and Animal Sciences, Paige Laboratory, University of Massachusetts, Amherst, MA 01003, USA. [sblack@vasci.umass.edu]

Clearance of trypanosomes (*Trypanosoma brucei* and *T. congolense*) from the blood of infected Cape buffalo was associated with the development of two responses: (i) complement-dependent and clone-specific lytic activity, and (ii) complement-independent trypanocidal activity that was not restricted by trypanosome clone or species. This latter activity was mediated by H_2O_2 and required the presence of xanthine oxidase in serum but not the addition of purine substrates. Expression of the xanthine oxidase-dependent trypanocidal activity in Cape buffalo serum was coincident with, and required, a decline in its H_2O_2 catabolic activity. The H_2O_2 catabolic activity of Cape buffalo serum was due solely to catalase and declined by eightfold around the time that trypanosomes were cleared from the blood, accompanied by a fivefold drop in erythrocyte-associated catalase activity. The Cape buffalo did not develop subsequent parasitaemic waves. Clearance of parasitaemia in similarly infected cattle was also associated with development of trypanosome clone-specific lytic activity, but not with the acquisition of H_2O_2-dependent trypanocidal activity in serum, and the cattle supported recurring parasitaemia. The lack of trypanocidal activity in pre- and post-infection cattle sera was due to their low content of xanthine oxidase and sustained catalase activity. These data strongly suggest that an infection-induced serum oxidative response, the efficacy of which is amplified by a decline in blood catalase, contributes to suppression of recurring parasitaemia in Cape buffalo.

(d) TREATMENT

11261 **Akbar, S.J., Munawar, G., Ul-Haq, A., Khan, S.M. and Khan, M.A., 1998.** Efficacy of trypanocidal drugs on experimentally induced trypanosomiasis in racing camels. *Journal of Protozoology Research*, **8** (4): 249-252.

Akbar: Dubai Camel Hospital, P.O. Box 9220, Dubai, United Arab Emirates.

The efficacy of three trypanocidal drugs for the treatment of experimental *Trypanosoma evansi* infection in racing camels was assessed. Sixteen dromedaries were divided into four groups of four animals each. Groups 1, 2 and 3 were treated with 0.25 mg/kg melarsomine (Cymelarsan), 3.5 mg/kg diminazene (Trypan) and 0.025 ml/kg quinapyramine (Triquin), respectively. Group 4 served as non-infected and non-treated controls, while within each of the other groups one animal served as an infected non-treated control. Cure rates of 66.66% (2 of 3), 66.66% (2 of 3) and 33.33% (1 of 3) were achieved for melarsomine, diminazene and quinapyramine, respectively. The mean haematocrit of infected animals dropped from 29 ± 3 to 21.35 ± 6.5 before treatment. The mean values of neutrophils, eosinophils, basophils, lymphocytes and monocytes in infected animals were 32.7, 1.80, 1.06, 62.4 and 1.39%, respectively, while the mean total protein value was 8.92 g/dl, and albumin and globulin values were 2.62 and 6.20 g/dl, respectively.

11262 **Bourdichon, A.J., 1998.** Report on the use of the trypanocidal drug Trypan. *Journal of Protozoology Research*, **8** (4): 258-262.

Department of Research and Development of Pharmaceutical Drugs, Atarost, Glockengieflerwall 26, 20095 Hamburg, Germany.

Trypan is a combination of the trypanocide diminazene aceturate, the antipyretic agent phenyldimethyl pyrazolon (Phenazone) and the synergist procaine hydrochloride. The results of a series of laboratory and field trials using this drug formulation are described and reviewed. A single injection of 15 ml can protect cattle against re-infection with trypanosomes for a period of 3 months.

11263 **Geerts, S., Diarra, B., Eisler, M.C., Brandt, J., Lemmouchi, Y., Kageruka, P., Deken, R. de, Ndao, M., Diall, O., Schacht, E., Berkvens, D., Speybroeck, N. and Holmes, P.H., 1999.** Extension of the prophylactic effect of isometamidium against trypanosome infections in cattle using a biodegradable copolymer. *Acta Tropica*, **73** (1): 49-58.

Geerts: Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. [sgeerts@itg.be]

Two trials were carried out in order to compare the prophylactic effect of a subcutaneously implanted sustained release device (SRD) containing a mixture of a biodegradable copolymer, poly(caprolactone-co-L-lactide), and isometamidium with that obtained after intramuscular injection of the drug. In a first experiment under controlled conditions, two groups of cattle were treated with 0.5 mg/kg isometamidium either as a SRD or i.m., and exposed at monthly intervals to *Glossina morsitans morsitans* infected with *Trypanosoma congolense*. The average protection period was at least 24 months in the SRD-treated against 5.7 months in the i.m.-treated group. Using an isometamidium ELISA, the drug could be detected until 140 days post-treatment in the latter group, whereas in the former group traces of the drug were detectable until 330 days after treatment. Furthermore, a field trial was carried out at the Madina Diassa ranch in Mali involving three groups of N'Dama cattle, each containing 23 or 24 animals. Two groups

were treated with 1 mg/kg isometamidium either as a SRD or i.m. and a third group served as untreated controls. Twelve months after treatment, the cumulative infection rates were 56.5, 87.8 and 91.6% in the SRD-implanted, i.m.-treated and control groups, respectively. The isometamidium concentrations were slightly lower than in the laboratory trial, but the overall pattern of drug disappearance from the sera of the SRD-treated cattle was very similar in both trials. Statistical analysis showed that the incidence of trypanosomiasis was significantly lower in the SRD-treated than in the i.m.-treated group.

11264 **Maina, N.W.N., Otieno, C., Farah, R., Ngatia, P.N., Olaho-Mukani, W., Sutherland, D.V. and Ndung'u, J.M., 1998.** Treatment failure in camel trypanosomosis in Uaso region of Kenya. *Journal of Protozoology Research*, **8** (4): 253-257.

Maina: KETRI, P.O. Box 362, Kikuyu, Kenya.

To investigate treatment failure of dromedary trypanosomiasis in the Uaso area of Kenya, 10 trypanosome isolates from infected animals were characterised by morphology and the procyclic transformation test, and their sensitivity to quinapyramine sulphate and melarsomine determined using an *in vitro* assay. Six isolates were identified as *Trypanosoma evansi*, three as *T. congolense* and one included both *T. congolense* and *T. evansi*. All the *T. congolense* isolates were resistant to melarsomine at 1.2 mg/kg and one was resistant to quinapyramine sulphate at 7.4 mg/kg. One *T. evansi* isolate was resistant to melarsomine at 1.2 mg/kg and three were resistant to quinapyramine sulphate. The efficacy of trypanocidal treatment in the Uaso area therefore appears to be hampered by drug resistance and inappropriate drug use due to lack of veterinary advice.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

[See also **23**: nos. 11277, 11307.]

11265 **Donelson, J.E. and Artama, W.T., 1998.** Diagnosis of *Trypanosoma evansi* by the polymerase chain reaction (PCR). *Journal of Protozoology Research*, **8** (4): 204-213.

Donelson: Department of Biochemistry, University of Iowa, Iowa City, IA 52242, USA.

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[See also **23**: no. 11315.]

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8. TRYPANOSOME RESEARCH

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[See also **23**: no. 11284.]

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(b) TAXONOMY, CHARACTERISATION OF ISOLATES

[See also **23**: nos. 11302, 11307, 11331.]

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This study presents new findings concerning the evolution of the human pathogens, *T. brucei* and *T. cruzi*, which suggest that these parasites have divergent origins and fundamentally different patterns of evolution. Phylogenetic analysis of 18S rRNA sequences places *T. brucei* in a clade comprising exclusively mammalian trypanosomes of African origin, suggesting an evolutionary history confined to Africa. *T. cruzi* (from humans and sylvatic mammals) clusters with trypanosomes specific to Old and New World bats, *T. rangeli* and a trypanosome species isolated from an Australian kangaroo. The origins of parasites within this clade, other than some of those from bats, lie in South America and Australia, suggesting an ancient southern super-continent origin for *T. cruzi*, possibly in marsupials; the only trypanosomes from this clade to have spread to the Old World are those infecting bats, doubtless by virtue of the mobility of their hosts. Viewed

in the context of palaeogeographical evidence, the results date the divergence of *T. brucei* and *T. cruzi* to the mid-Cretaceous, around 100 million years before present, following the separation of Africa, South America and Euramerica. The inclusion in this study of a broad range of trypanosome species from various different hosts has allowed long phylogenetic branches to be resolved, overcoming the limitations of many previous studies. Moreover, *T. brucei* and the other mammalian tsetse-transmitted trypanosomes appear, from these data, to be evolving several times faster than *T. cruzi* and its relatives.

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