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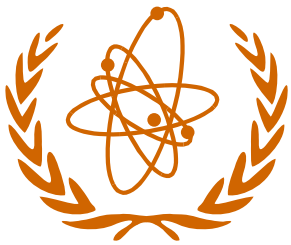

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TSETSE AND TRYPANOSOMIASIS INFORMATION QUARTERLY

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TSETSE AND TRYPANOSOMIASIS INFORMATION QUARTERLY

The Tsetse and Trypanosomiasis Information Quarterly has been established to disseminate current information on all aspects of tsetse and trypanosomiasis research and control to institutions and individuals involved in the problems of African trypanosomiasis. This service forms an integral part of the Programme Against African Trypanosomiasis (PAAT) and is jointly sponsored by the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA), the Inter-African Bureau for Animal Resources of the African Union (AU-IBAR), the World Health Organization (WHO), the Research Department for Livestock Production and Veterinary Medicine of the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD-EMVT) and the British Government's Department for International Development (DFID).

The quarterly is prepared for publication, in both English and French editions, by the Food and Agriculture Organization of the United Nations. Each annual volume consists of four parts and an index. Subscription is free for all recipients engaged in trypanosomiasis research and control, and requests for enrolment may be sent to: Ms Maria Grazia Solari, AGAH, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy (fax +39 06 5705 5749; e-mail MariaGrazia.Solari@fao.org).

Since the value of this information service depends to a great extent on the receipt of relevant material from research workers, campaign planners and organizers and field workers themselves, readers are requested to submit news items and copies of scientific papers and reports to the Editor: Dr John N. Pollock, 25 Palmeira Mansions, Church Road, Hove, East Sussex, BN3 2FA, United Kingdom (tel. +44 1273 326211; e-mail johnnpollock@hotmail.com).

We regret that we are unable to supply photocopies of the papers quoted in the quarterly.

Distribution dates and copy deadlines

	Copy deadline for news items	Distribution (English and French editions)
Part 1	15 January	April/May
Part 2	15 April	July/August
Part 3	15 July	October/November
Part 4	15 October	January/February

The *Index* will be distributed as soon as possible after the completion of each volume.

ABBREVIATIONS USED IN *TTIQ*

a.i.	active ingredient
ACTH	adrenocorticotrophic hormone
ALAT	alanine aminotransaminase
ASAT	aspartic acid aminotransaminase
b.w.	body weight
BIIT	blood incubation infectivity test
CATT	card agglutination test for trypanosomiasis
CD_{50}	median curative dose
CNS	central nervous system
CSF	cerebrospinal fluid
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
HAT	human African trypanosomiasis
HCT	haematocrit centrifugation technique
GIS	geographic information system(s)
GPS	global positioning system(s)
i.m.	intramuscular(ly)
i.p.	intraperitoneal(ly)
i.v.	intravenous(ly)
IFAT	indirect fluorescent antibody test
KIVI	kit for *in vitro* isolation of trypanosomes
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
M	molar
mAEC	miniature anion-exchange centrifugation technique
McAb	monoclonal antibody
MW	molecular weight
NARS	National Agricultural Research Services/Systems
p.i.	post-infection
PCR	polymerase chain reaction
PCV	packed cell volume
ppb	parts per billion (10^9)
ppm	parts per million
r.h.	relative humidity
RNA	ribonucleic acid
SIT	sterile insect technique
sp(p).	species (plural)
ssp(p).	subspecies (plural)
UV	ultra-violet
VAT	variable antigen type
VSG	variant surface glycoprotein
WBC	white blood cell

Organizations

ANDE	Agence Nationale de Développement de l'Elevage
AU	African Union
AU/STRC	African Union/Scientific, Technical and Research Commission
BICOT	Biological Control of Tsetse by the Sterile Insect Technique
CEBV	Communauté Economique du Bétail et de la Viande
CEMV	Centre Universitaire de Formation en Entomologie Médicale et Vétérinaire
CGIAR	Consultative Group on International Agricultural Research
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CIRAD-EMVT	Département d'Elevage et de Médecine Vétérinaire des Pays Tropicaux du CIRAD
CIRDES	Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide
CNERV	Centre National d'Elevage et de Recherches Vétérinaires
CNRS	Centre National de Recherche Scientifique
CREAT	Centre de Recherche et d'Elevage, Avétonou, Togo
CRSSA	Centre de Recherches du Service de Santé des Armées Emile Pardé
CTVM	Centre for Tropical Veterinary Medicine
DFID	Department for International Development (UK)
DSE	German Foundation for International Development
EC/EU	European Community/European Union
EDF	European Development Fund
FAO	Food and Agriculture Organization of the United Nations
FITCA	Farming in Tsetse Control Areas of Eastern Africa

GTZ	Deutsche Gesellschaft für Technische Zusammenarbeit
IAEA	International Atomic Energy Agency
IBAR	Interafrican Bureau for Animal Resources
ICIPE	International Centre of Insect Physiology and Ecology
ICPTV	Integrated Control of Pathogenic Trypanosomes and their Vectors
IFAD	International Fund for Agricultural Development
ILRI	International Livestock Research Institute
INRA	Institut National de Recherche Agronomique
IPR	Institut Pierre Richet
IRD	Institut de Recherche et de Développement (formerly ORSTOM)
ISCTRC	International Scientific Council for Trypanosomiasis Research and Control
ISRA	Institut Sénégalais de Recherches Agricoles
ITC	International Trypanotolerance Centre
KARI	Kenya Agricultural Research Institute
KETRI	Kenya Trypanosomiasis Research Institute
LCV	Laboratoire Central Vétérinaire
LNERV	Laboratoire National de l'Elevage et de Recherches Vétérinaires
LSHTM	London School of Hygiene and Tropical Medicine
MRC	Medical Research Council
MRU	Mano River Union
NITR	Nigerian Institute for Trypanosomiasis Research
NRI	Natural Resources Institute
OCCGE	Organisation de Coopération et de Coordination pour la Lutte contre les Grande Endémies
OCEAC	Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale
OGAPROV	Office Gabonais pour l'Amélioration de la Production de la Viande
OIE	Office International des Epizooties
OMVG	Organisation pour la Mise en Valeur du Fleuve Gambie
PAAT	Programme against African Trypanosomiasis
PATTEC	Pan-African Tsetse and Trypanosomiasis Eradication Campaign
PRCT	Projet de Recherches Cliniques sur la Trypanosomiase
RDI	Rural Development International
RUCA	Rijksuniversitair Centrum Antwerpen
SADC	Southern African Development Community
SIDA	Swedish International Development Authority
SODEPRA	Société pour le Développement des Productions Animales
TDR	UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases
TDRC	Tropical Diseases Research Centre
TPRI	Tropical Pesticides Research Institute
TTRI	Tsetse and Trypanosomiasis Research Institute
UNDP	United Nations Development Programme
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
UTRO	Uganda Trypanosomiasis Research Organization
WHO	World Health Organization

CONTENTS

SECTION A – NEWS

INVITED ESSAY

Genetic diversity and gene flow in *morsitans* group tsetse flies

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The question of how to achieve effective levels of tsetse fly control at financially and environmentally acceptable costs is perennial and contentious. Even though tsetse flies are slow to reproduce, populations seem to recover sooner or later after control measures are relaxed. A great capacity and propensity to disperse is said to be characteristic of tsetse flies, and many experts suggest that area-wide control measures and eradication are unobtainable for this reason alone. Others contend that area-wide methods, including the sterile insect technique, can be used successfully to achieve a high degree of control. Can a study of tsetse fly population genetics add anything to the ongoing debate? I believe it can. Here's why.

While the tsetse fly is traditionally shown on maps as being distributed in broad belts, within these belts tsetse fly populations are patchily distributed. Uninfested regions presumably consist of unsuitable or marginally suitable habitat. Even within infested patches, tsetse flies are aggregated into demes among which there may be varying degrees of isolation. Given application of effective control measures, how large an area must be treated to minimize re-invasion? John Hargrove suggests that very large areas are required, greater than 10 000 square kilometres (Hargrove, J.W. 2000. A theoretical study of the invasion of cleared areas by tsetse flies (Diptera: Glossinidae). *Bulletin of Entomological Research*, **90**, 201-209). Suppose, then, that a tsetse patch has been eliminated. What is the risk of invasion from nearby patches? Frontal advance of *morsitans* group flies has been shown to be of the order of 7 km per year and density-dependent responses might increase that value, but the distances between patches are too great to measure experimentally by mark, release, and recapture methods. Moreover, areas between patches are likely to be unsuitable for tsetse reproduction so that frontal advances fail and long range colonization of cleared areas is necessary.

In principle, we could measure gene frequencies of tsetse flies in two or more patches and derive indices of gene flow within and among them. This is the province of population genetics. Evolutionary insights may also come from such studies, as we shall see. Well developed theory teaches that the exchange of approximately one reproducing fly per generation, on average, is sufficient to prevent fixation of genetic differences between populations. Moreover, this 'critical' migration rate is virtually independent of population size! Whether the 'real' number is 0.5 or 2 is not biologically significant – in principle, numerically little gene flow can overcome local genetic differentiation. In theory, then, we have a powerful tool with which to examine the notion of biologically significant exchange of tsetse flies between patches.

Measurement of gene frequencies nowadays is a fairly routine affair. We can examine genetic diversity at loci that code for enzymes – so called isozymes and allozymes – by using starch, paper, or polyacrylamide electrophoresis, coupled with histochemical

staining to demonstrate enzyme activity and allelic variation. The chief drawback is that of preserving enzyme activity in field-collected samples. The preferred method, using liquid nitrogen, is not always available, and airlines often refuse to accept shipments of this innocuous substance.

Another source of gene diversity is microsatellite loci. Microsatellites are short repetitive nucleotide sequences that vary in number, for example, [CA]n, where the number of repeats n varies. DNA can be extracted from rapidly dried or ethanol preserved flies, thereby making sampling and transport to the laboratory easier. But much energy and skill is required to find microsatellite loci and to design and test the primers necessary to amplify them in the polymerase chain reaction (PCR).

Allozyme, isozyme, and microsatellite loci are present in two copies. Thus they can be used to measure genotypic frequencies. Genotypic frequencies, in turn, allow estimates to be made of departures from random mating within populations. Allele frequencies can be used to test hypotheses about the independence of two or more samples i.e. genetic differentiation.

PCR can be used to measure variation in mitochondrial genes. Mitochondria contain single copy loci and are maternally inherited. Variants do not recombine so the mitochondrial genome is inherited as a unit. Thus mitochondrial loci can be used to measure genetic differentiation of populations and maternal lines of descent because of the clonal pattern of inheritance. Moreover, mitochondrial variation is much more sensitive to population (demographic) events than is nuclear variation because of its inheritance pattern and the fact that its genes are represented by single copies, not double, as is the case for nuclear genes.

The foregoing kinds of genetic variation have been applied to some *morsitans* group tsetse flies. The sampling was carried out by Nigel Griffiths in The Gambia, Kenya, Zambia, Zimbabwe, Mozambique, and Namibia, Reg Allsopp in Botswana, Steve Mihok in Ethiopia, and Marc Vreysen, also in Ethiopia. The laboratory work and analysis was performed in my laboratory at Iowa State University.

Genetic diversity

So, what do the data show? First, let's examine diversities, i.e. magnitudes of genetic variation. Later, I'll deal with gene flow. Diversities at mitochondrial loci estimate the probability that two randomly chosen tsetse flies have different haplotypes. For nuclear genes, diversity can be expressed in terms of the number of variants (alleles) at each locus and as heterozygosities – the proportion of loci with different (non-matching) alleles. Mitochondrial diversities, averaged over populations, were 41 percent in *G. pallidipes*, 35 percent in *G. m. morsitans*, and 43 percent in *G. m. submorsitans*, but only 22 percent in *G. m. centralis*. There were important contrasts in these diversities among regional populations that I'll return to later.

Allozyme data indicate that *morsitans* group flies are heterozygous at about 6 percent of their loci (heterozygosities at polymorphic loci, however, were about 25 percent). The 6 percent value compares with heterozygosities of about 18 percent in house flies and face flies (*Musca domestica* and *M. autumnalis*, respectively). The same methods show similarly high levels of diversity (heterozygosity) in numerous ladybird beetle and leaf beetle species (Chrysomelidae). Microsatellite diversities (heterozygosities) were much greater than the allozyme diversities, largely because they are probably untranscribed, subject to higher mutation rates, and do not respond to natural selection (i.e. they are

'selectively neutral'). Thus, in *G. pallidipes*, the number of alleles per locus was very much greater at polymorphic microsatellite loci (mean, 20.8 per locus) than at polymorphic allozyme loci (mean, 3 per locus). Microsatellite heterozygosities (diversities), averaged over populations and polymorphic loci, were 71 percent in *G. pallidipes*, 73 percent in *G. m. morsitans*, 81 percent in *G. m. submorsitans*, and 70 percent in *G. m. centralis*.

The magnitude of genetic diversity is important from evolutionary, ecological, and historical points of view. For example, theory shows a direct relationship between historical population sizes and diversity. Thus, the comparatively low diversities in tsetse flies are an indication that historical mean tsetse population sizes have been considerably less than those of many Diptera and Coleoptera, and are consistent with tsetse's low reproduction rates.

Low diversities can suggest historical 'bottlenecks' in population size, in which one or more successive generations undergo a great reduction in numbers. Bottlenecks in tsetse populations have been conjectured, as a consequence of rinderpest epizootics in the nineteenth and early twentieth centuries; indeed, such was demonstrated in Zimbabwe and claimed in Uganda (reviews can be found in Ford J., 1971, *The Role of the Trypanosomiases in African Ecology*. Clarendon Press, Oxford, and Leak S.G.A., 1998, *Tsetse Biology and Ecology: Their Role in the Epidemiology and Control of Trypanosomiasis*. ILRI Nairobi/CABI). *Glossina morsitans centralis* in Botswana and Mamili National Park in Namibia showed a remarkable paucity of mitochondrial variation (only a 3 percent chance that two randomly chosen flies would have different mitochondrial haplotypes), populations having recovered from extensive control schemes in the Okavango region. Mitochondrial diversities in *G. m. submorsitans* were much less in The Gambia (26 percent) than in Ethiopia (84 percent); in Zimbabwe, *G. pallidipes* diversities were only 15 percent, but in Kenya and Ethiopia they averaged 54 percent. Microsatellite variation showed no hint of bottlenecks in *G. morsitans* s.l. but was reduced in Zimbabwean *G. pallidipes*. Allozyme diversities, on the other hand, seemed to be totally unaffected by putative bottlenecks. For example, Zimbabwean *G. pallidipes* showed slightly more heterozygosity than Kenyan populations even though mitochondrial and microsatellite variation was very much less. Thus, it seems that tsetse provide an example of 'balancing selection' acting on allozyme heterozygotes, thereby promoting diversity at allozyme loci.

Theory teaches that recovery from bottlenecks requires tens of thousands of generations, far more than the roughly 800 generations since the rinderpest epizootic. Therefore, the tsetse populations that we study today should still exhibit clear evidence of the earlier bottlenecks. The nature of the evidence includes disequilibrium between forces of mutation, migration, and genetic drift. So far, however, we are unable to reject null hypotheses that populations are in mutation-drift equilibrium. Larger sample sizes and the development of more sensitive statistical tests may, in future, allow more definitive investigations.

Gene flow

Three independent lines of genetic evidence show abundant variation in *morsitans* group tsetse flies and this variation can be used to estimate gene flow within and among populations. How can this be done?

If gene flow is unrestricted, gene frequencies among populations will be statistically homogeneous. But what does it mean if they differ significantly? And why should they

differ at all, if they do not respond to natural selection and have a common ancestry? The answer is that differences in gene frequencies arise because the laws of chance operate in the transmission of alleles from one generation to the next; thus, the smaller the breeding population the greater is the variance in gene frequencies. This is termed 'genetic drift' and is a major evolutionary force. The major result of drift is that gene frequencies of populations tend to diverge from each other in proportion to their isolation from each other. The isolation may be spatial, temporal, behavioural, premating, postmating, etc. Opposed to drift is immigration, and, as we have seen, numerically little exchange of reproducing migrants is effective in reversing the effects of drift.

We can do better than a simple test for differences by measuring the magnitudes of departures from random mating. The most commonly used index of departures from random mating is *F*, the so-called inbreeding coefficient. Now *F* may be viewed as a correlation of genes. In theory, *F* can take values from −1 (matings only between unlike) to 1 (like mates only with like: completely inbred). It makes sense that flies in a particular location are more likely to mate with each other than with flies in another location, so there will be a measure of drift that leads to genetic differentiation. The classical estimate of drift (or genetic differentiation) among populations is termed F_{ST}. The null hypothesis is $F_{ST} = 0$. Generally speaking, F_{ST} estimates of ≥ 0.05 are considered to indicate a biologically significant degree of differentiation. Genetic differentiation provides a continuous scale of reproductive isolation, in principle varying from zero to unity.

Gene flow among conspecific tsetse fly populations

Estimates of F_{ST} based on allozyme, mitochondrial, and microsatellite variation were consistent. In *G. m. submorsitans*, $F_{ST} \approx 0.17 – 0.35$ (depending on the method of analysis) among seven populations in The Gambia and Ethiopia, but F_{ST} was only 0.016 among samples within countries. We find that $F_{ST} \approx 0.18$ among six *G. m. morsitans* populations, five of which originated in Zambia and Zimbabwe. More extensive sampling of *G. m. morsitans* is necessary to get an estimate of gene flow among fly belts. Among seven *G. m. centralis* populations extending from Tanzania to Botswana, it was found that $F_{ST} \approx 0.19$. A much greater estimate was obtained at mitochondrial loci, for which $F_{ST} \approx 0.87$ (recall that mitochondrial loci are much more sensitive to demographic upheavals than are nuclear loci).

Glossina pallidipes showed, among 11 populations, a very high degree of differentiation at allozyme loci ($F_{ST} \approx 0.19–0.24$, depending on method of analysis), microsatellite ($F_{ST} \approx 0.29$), and mitochondrial loci ($F_{ST} \approx 0.51$). Study revealed that among northern populations, mitochondrial $F_{ST} \approx 0.52$, whereas $F_{ST} \approx 0.28$ among southern populations. Microsatellite loci showed the same trends but allozyme loci did not. These data indicate that genetic drift at allozyme loci is greatly dampened and provides further evidence of balancing selection acting on allozyme loci (Krafsur, E.S., 2002, Population structure of the tsetse fly *Glossina pallidipes* estimated by allozyme, microsatellite, and mitochondrial gene diversities. *Insect Molecular Biology*, **11**, 37-45).

Estimates of F_{ST} can be converted to hypothetical estimates of the mean number of reproducing organisms exchanged per generation, *Nm*. The mathematical relationship is deceptively simple and entails many assumptions, but *Nm* can provide useful perspective by indicating the amount of gene flow in simple terms. The means, taken over all populations, were exchanges of 1.1 reproductives per generation among *G. m. morsitans*,

0.04–1.1 among *G. m. centralis* (based on microsatellite vs. mitochondrial loci, respectively), 0.5–1.2 among *G. m. submorsitans*, and 0.6–0.8 among *G. pallidipes*. Most of the foregoing estimates do not differ greatly from 1, that is, the 'critical' amount of gene flow below which genetic drift can proceed to fixation of different genotypes in different populations. Most insect species show *Nm* values that vary from five or ten to hundreds and thousands.

The high degree of genetic differentiation among *morsitans* group tsetse in general and *G. pallidipes* in particular is surprising when ecological and experimental data are considered. *Glossina pallidipes* is highly mobile – indeed, it is said to be the most vagile tsetse. I shall return to the contrast between ecological and genetical evidences later.

Gene flow within populations

Genotypic frequencies, in terms of expected and observed heterozygosities, can be used to test hypothesis that matings are random within populations. For example, a deficiency of heterozygotes within a population is evidence that two or more demes of different gene frequencies were sampled; this can happen when samples from different locations are pooled. The chief estimator is F_{IS} (inbreeding F for individuals I in S (sub)populations). Sampling *morsitans* group tsetse indicated that matings were random within populations. Such data indicate no large scale immigration.

What may we conclude?

First, with two important exceptions, the foregoing data on gene diversities do not indicate genetically detectable bottlenecks in population sizes. The exceptions concern *G. m. centralis* in Botswana and *G. pallidipes* in Zimbabwe where the historical record suggests that such bottlenecks occurred in *G. morsitans* s.l. and *G. pallidipes*, due to host animal reduction caused by the rinderpest outbreak. The same rinderpest epizootics swept through East and West Africa, but the genetic data suggest that tsetse populations were not so greatly affected as in southern Africa.

Second, tsetse populations are highly structured by genetic drift, with surprisingly little gene flow among them, a seemingly curious result considering the well known propensity for tsetse flies to disperse. Moreover, comparable work on other Diptera and many economically and medically important insects generally show much higher rates of gene flow than those recorded in tsetse. The literature suggests frontal advances of tsetse populations averaging 5 to 7 km yearly.

How can the foregoing contradiction be resolved? Are the genetic data faulty or, more likely, is their interpretation simply wrong? Genetic analysis is built on Hardy-Weinberg assumptions which are rarely satisfied when dealing with natural populations and are certainly inapplicable to tsetse. If tsetse populations are recovering from earlier severe bottlenecks and disruptive population fragmentations, they would not be at mutation-drift equilibrium, and conclusions based on an assumption of equilibrium could be in error. Statistical tests for equilibrium, however, provide no evidence that the assumption is false.

Natural selection offers another rationalization for the apparent contradiction between ecological and genetical conclusions. In principle, tsetse far removed from their home territories may be at a significant reproductive disadvantage. Adaptation to local environments may be necessary and most immigrants may die without issue.

We also should consider scale. Ecological data pertain to distances in tens of kilometres. The genetic sampling summarized here involved distances ranging from tens to thousands of kilometres. Means taken over samples that vary so greatly in distance are apt to be misleading because the relationship between *Nm* and F_{ST} is nonlinear. But pairwise population estimates show correlations between genetic and geographical distances and they also generally confirm the low rates of gene flow. This is encouraging because low rates of gene flow support the concept of area-wide control, and predict low rates of re-colonization of habitat lost to tsetse. In principle, the sterile insect technique (SIT) involving sterile fly releases by aircraft may well prove to be more effective in reducing natural populations than are traps, targets, and targeted sprays because larger areas can be treated uniformly and efficaciously. And released, sterile males may turn out to be much more effective in finding small, hard-to-reach tsetse foci than are entomologists, rural sociologists, economists, and stakeholders everywhere.

Let's bear in mind that genetic and ecological research measure different things that are not strictly comparable; indeed, the contrast between ecological and genetic dispersals may be less in practice than in theory. Further research now underway should bring into better focus relationships between geographical distances, genetic distances, and, via physiological adaptation, the possibility of natural selection in explaining the breeding structure of tsetse flies.

As for area-wide control, in my opinion experimental sterile fly releases over large areas should first be made in order to learn something of interactions between released and wild flies in terms of sterile mating rates and target population responses to 'birth control'. Confounding treatments designed to maximize sterile to wild ratios should be avoided in such experiments. Later, treatment by SIT of entire patches, as defined by satellite imagery and ground reconnaissance, might provide levels of control that would endure for many, many years.

Acknowledgement. This paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project 6592, was supported by USPHS Grant AI-5245601, Hatch Act, and State of Iowa funds.

REGISTRATION FOR THE 27th MEETING OF THE ISCTRC, PRETORIA

Registration for the 27th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) has opened. The meeting is organized under the auspices of the African Union, and will be held in Pretoria, South Africa, September 29th to October 3rd 2003.

The draft agenda has the following main sections: Review of Research and Control (which will include country reports); Protozoology, Immunology and Diagnosis; Entomology; Human Trypanosomiasis; Animal Trypanosomiasis; *Glossina* control.

The scientific articles for oral presentation should not exceed 3 000 words and should contain an abstract not exceeding 300 words. The abstract should contain: Title, Objective of the Study, Outline of Methodology, Results in Brief and Conclusions.

There will be a Poster Session with brief oral presentation. The abstract and manuscript must conform to the format indicated for oral presentation. Posters will be 1 × 1.25m; the title should be concise, and followed by the author(s) name and affiliations.

Character heights recommended: Title at least 2cm; Subtitles at least 1cm; Text at least 0.25cm. The posters should be legible in comfort from a distance of 1m.

Summaries of scientific articles in duplicate English and French should be sent preferably by e-mail so as to reach the Secretariat not later than 30 April 2003.

The working languages of the meeting will be English and French, and there will be simultaneous translation.

Arrival particulars for participants in Pretoria should be communicated to the ISCTRC Secretary, AU/IBAR, P.O. Box 30786, Nairobi, Kenya. Fax Nos. 254-2-220546/226565. E-mail: jotham.musiime@oau-ibar.org or Solomon.hailemariam@oau-ibar.org, with copy to Dr Rob Bagnall rbagnall@mweb.co.za or bagnallr@allerton.kzntl.gov.za.

ROLE AND IMPORTANCE OF SOCIO-ECONOMIC AND CULTURAL FACTORS IN THE RESEARCH AND CONTROL OF TRYPANOSOMIASIS

The following is a summary of a position paper having the above title, by Dr Mulumba Kamuanga, presented at the Meeting of the Panel of Advisory Group Co-ordinators, Ouagadougou, Burkina Faso, 26-28 September 2001.

Tsetse-transmitted trypanosomiasis still stands as an important constraint to agricultural development in the subhumid (including the wetter areas of the semi-arid zones) and humid zones of Africa. Generally the benefits of tsetse and trypanosomiasis (T&T) control will derive from the reduced risk of contracting the disease, both human and animal. There will also be a diminution in the expenses incurred in prevention and disease treatment. These factors will thus improve human health and the productivity of existing livestock.

The paper is organized around three main topics: (1) an overview of socio-economic factors to account for in the research and control of tsetse and trypanosomiasis; (2) the role of socio-cultural factors affecting community participation in the control activities to ensure sustainability of the benefits derived from T&T control, and (3) the importance of past lessons and experiences in strategic planning incorporating socio-cultural aspects in design, monitoring and evaluation of T&T control programmes. There is a substantial bibliography including 97 items drawn from published and unpublished sources.

A list (with brief comments) of available control techniques is provided. It includes ground and aerial spraying; sterile insect technique (SIT); traps and targets/screens often enhanced with attractant odours; insecticide-treated livestock; husbandry of trypanotolerant livestock; and drug therapy. Items three and four are sometimes bracketed under the term bait technology.

The development of bait technologies has triggered two important shifts in the research and control of trypanosomiasis beyond the issue of costs and returns. The first is the involvement of beneficiaries as partners. The second is the move away from large scale, government-supported schemes to small-scale community-based participation where tsetse control interventions can be regarded as local public goods. Broadly speaking, with the variety of technical options now available, there is consensus that good opportunities and possibilities exist for effective control of the disease.

The technicalities of handling the required concepts of economic analysis are discussed. Most of the failures of development projects have been attributed to the fact that the communities concerned were left out of all the process related to design, formulation

and implementation of policy. Can this weakness be rectified? The present paper advocates community participation and related notions in T&T control programmes, in the context of a more effective approach to sustainable rural development, whether an area-wide or a farmer/community-based approach is envisaged.

What is meant by a community, community participation, and related terms, is defined. Community involvement may range from token participation on the one hand to full participation and empowerment on the other. Theorists also distinguish between "top-down" and "bottom-up" programmes in community participation. The weakness of the top-down approach, initiated and directed by central government or affiliated agencies, is that there is a tendency for a uniform strategy that does not reflect local social, cultural or political conditions. On the other hand, bottom-up strategies are difficult to implement because very often members of the community (farmers, labourers, local opinion leaders) must accept enhanced responsibilities in decision-making actions to fulfil their dreams and aspirations.

Certain case studies of community participation in T&T control are examined in more detail. These include: Community-based T&T control in Burkina Faso, in Côte d'Ivoire; in Busia District, Kenya; and in Lambwe Valley, Kenya.

The major findings are summarized concerning socio-economic and cultural factors that determine when and how it might be appropriate to involve communities and individual livestock farmers in T&T control operations. Information to date is most readily available when targets and traps are the principal techniques being proposed for T&T control. However, experience is slowly emerging with other non-bait technologies, including integrated systems of control involving several approaches to ensure sustainability.

All the research and experiences in sub-Saharan areas suggest that in locations where there is sleeping sickness at present, or where serious outbreaks of human African trypanosomiasis (HAT) have occurred within living memory, there would logically be a major incentive for community action. Outside these locations, it is not easy to identify similar incentives that might mobilise the whole population. Ownership of cattle, and in some instances that of other livestock, have been indicated as a significant factor determining individual willingness to contribute resources to T&T control. Where communities depend on cattle for their livelihood, as is the case in most pastoral societies, it is the costs/benefits calculations of alternative strategies that will influence people in their decisions to participate individually or as a community in T&T control operations. Further related issues are discussed, including whether the community has experienced previous externally initiated research-development action; knowledge by the farming community of the symptoms of animal trypanosomiasis; the amount of time that the community can donate to the project; village and social structure; age of community participants, their level of education, and the distance from the point of action (e.g. the traps to be serviced).

Most of the failures of development projects in general, and tsetse and trypanosomiasis control programmes in particular, have been attributed to the fact that potential and actual beneficiaries were left out of the process related to design, formulation and implementation of policy. There is now an urgent need for the new approach to become demand- rather than supply-driven. There are several lessons to be learnt from the growing disillusionment with both large-scale, government-managed schemes and the questionable sustainability of most small-scale, community-based programmes that will

help to determine when and how it might be appropriate to involve communities and individual livestock owners in T&T control.

GUIDING ECONOMIC PRINCIPLES FOR STRATEGIC PLANNING IN TSETSE AND TRYPANOSOMOSIS CONTROL/ERADICATION IN WEST AFRICA

The following is a draft summary of a position paper having the above title, by Dr Alexandra P.M. Shaw, presented at the Meeting of the Panel of Advisory Group Co-ordinators, Ouagadougou, Burkina Faso, 26-28 September 2001.

This paper seeks to address the issue of how to integrate economic criteria into the strategic planning process for tsetse and trypanosomosis control in West Africa. It was originally prepared for the FAO/IAEA workshop held in Ouagadougou in May 2001, and focuses on the issues raised at that workshop. It takes as its starting point Brent Swallow's PAAT position paper, which reviewed the growing literature on the economic impact of the disease, and complements this with recent references and a rapid overview of the benefit-cost studies undertaken.

Since this has been much debated, and has profound implications for the type of strategy adopted, the methodological issues involved in the economic appraisal of potential projects to control the disease are first discussed in some detail. This discussion is particularly timely in the light of the current Pan-African initiatives, which reveal a need for the wider scientific community and planners to understand the implications for policy and decision-making of the economic techniques used. The literature on the economic appraisal of livestock projects universally advocates putting some value on the use of money over time, reflecting its opportunity cost in terms of resources diverted from other projects and the need to fix some minimum acceptable rate of return on public investments. The use of 'discount' rates is accordingly recommended here, while applying low discount rates in the examples used, so as to reduce the effect of deflating benefits occurring in the distant future as compared to present costs.

The terms of reference for this work were to produce economic guidelines for planners in the tsetse/trypanosomosis field, accordingly it is argued that in the current institutional context, each individual project or zone should be the subject of a separate benefit-cost analysis, so that it is assessed on its own merits, not on its possible technical contribution to a potential continent-wide programme. This again is part of sound economic practice. The setting out of benefits and costs according to the rules of partial analysis is explained for the case of tsetse and trypanosomosis control. This discussion, in particular, emphasises the importance of incorporating farmers' current strategies to control the disease into the analysis. Studies have shown that in many areas their use of trypanocides is effective; this means that a proportion of disease losses are already being successfully avoided. The benefits from introducing tsetse control in this situation would not be the elimination of all possible losses due to trypanosomosis, but would consist of savings in the use of trypanocides plus a further reduction in the losses due to the disease. A dynamic herd model incorporating animal traction is used to simulate the benefits and costs of tsetse eradication, trypanocide use, and the switch from one to the other. This implies that farmers' current strategy of targeting productive animals brings high returns. Tsetse control becomes more profitable in higher challenge areas if sufficiently large cattle

populations exist to make up the 'benefit units' per square kilometre and where there is evidence of drug resistance.

Secondly, from this discussion on methodology, it is argued that there is a need for planners to adopt a standardised and transparent approach for assessing tsetse and trypanosomosis control schemes. This would aim firstly to be cost-effective and secondly to produce results for different projects that could be compared and used for ranking and priority setting. In this context there is an urgent need for updated and fully comparable costings on the various forms of tsetse control, as they would apply in West Africa and including overheads, so that they can be applied by planners.

Thirdly, the paper tries to complement the GIS work on the spatial distribution of the factors influencing the economics, and the predictions of the likely rates of changes, by looking at the dynamics of benefits and costs over time, especially in relation to the densities of human and cattle populations. A conceptual model shows tsetse control costs falling with rising human populations. Benefits, however, initially rise, then peak when mixed farming is well established but tsetse challenge persists, and lastly, fall when human populations rise to a level where the fly's habitat becomes eroded and/or fewer cattle are kept. This points to the existence of two turning points in the economics of tsetse control: firstly, below a certain cattle or human population density there are insufficient benefit units to make it profitable; and secondly, above a certain level, fly challenge is reduced, losses due to the disease decline, and cattle numbers may also be lower as the amount of grazing land is reduced. This model is used to characterise situations where controlling the disease may or may not be profitable. These situations and the profitability limits or turning points identified coincide to a large extent with those emerging from the GIS priority-setting exercises. The two approaches thus very much complement each other, suggesting that the economic appraisals should focus on those zones that emerge as priority areas from the GIS filtering process.

MORE THAN 100 000 PEOPLE AFFECTED BY SLEEPING SICKNESS IN ANGOLA

Sleeping sickness affects 100 000 people in Angola, the director of the country's Institute for the Eradication of Trypanosomiasis, Teofilo Josenando, has revealed.

Josenando told PANA in Luanda that the country's long civil war was to blame for the alarming spread of the disease, whose prevalence shot up from 0.06 percent [of the population] ten years ago to 10 percent now.

Tsetse flies (*Glossina*) exist in 14 of Angola's 18 provinces; sleeping sickness is a health problem in Bengo, Kuanza-Norte, Kwanza-Sul, Malanje, Uije, Zaire, and Luanda provinces. Some 27 000 cases of sleeping sickness, including 4 000 in Luanda, the capital, have been reported since fighting resumed in 1992, Josenando said, adding that his institute needs at least US $5 million per year to combat the disease. The funds are required to finance the operations of 22 mobile and 43 fixed teams and to buy the required products.

According to Josenando, trypanosomiasis will be eradicated only if there is simultaneous treatment of the disease in Angola, the Democratic Republic of Congo, and Sudan, where it is widespread. Although sleeping sickness has been present in Angola since the 9th century, it was only in 1949 that mobile treatment teams started visiting the

affected regions. Since that era, the situation improved considerably, and the number of cases was reduced from 5 000 to only 3 in 1974. However, the outbreak of civil war after the country's independence in November 1975 severely hampered the Institute's activities. Its infrastructure was destroyed or plundered, while most experts fled abroad. At the end of the civil war in April 2002, the institute decided to extend its operations to all the affected provinces in the country, Josenando said.

JOINT FAO/IAEA DIVISION TECHNICAL CO-OPERATION PROJECTS

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, and the FAO/IAEA Agriculture and Biotechnology Laboratory, Seiberdorf, IAEA, Vienna, have listed the ongoing Technical Co-operation Projects relating to tsetse and trypanosomiasis control, in the Insect Pest Control Newsletter No.59, July 2002. The reader is referred to this Newsletter for fuller details, as the list below does not necessarily cover all the activities of the respective projects.

ETH/5/012: *Integrating SIT for Tsetse Eradication*. This supports the construction of a modern mass-rearing facility for *Glossina pallidipes*.

KEN/5/022: *Integrated Area-Wide Tsetse and Trypanosomosis Management in Lambwe Valley*. High mortality at a local *G. pallidipes* colony is being investigated, to trace the source of the problem.

MLI/5/017: *Integrated Control of Animal Trypanosomosis through creation of a Tsetse Fly Free Zone*. Intensive and extensive surveys for *Glossina palpalis gambiensis* are under way in the La Faya System (of the River Niger basin) in Mali; the results will be used to develop a fly suppression strategy.

RAF/5/051: *SIT for Tsetse and Trypanosomosis Management in Africa*. Technical assistance and equipment are provided for a new tsetse rearing facility at CIRDES, in order to produce flies for use in the Mali project (see above).

SAF/5/005: *Situation Analysis of the Feasibility and Desirability of Tsetse Fly Eradication*. Samples of *Glossina brevipalpis* have been shipped to South Africa to start a colony there (ARC-OVI, Pretoria). Field-collected *G. brevipalpis* and *G. austeni* will also be transferred to this insectary.

URT/5/019: *Support to National Tsetse and Trypanosomosis Management*: Tsetse (*G. brevipalpis*) and trypanosomosis are being surveyed on Mafia Island. Extensions to the existing tsetse rearing facility at Tanga are under way, for this to become regional centre for the rearing of different species of fly.

UGA/5/023: *Integrated Sterile Insect Technique Based Intervention against Tsetse in Buvuma Island*. Insectaries at the Livestock Research Institute, Tororo, are to be upgraded, and a *Glossina fuscipes fuscipes* colony will be established. A strategic programme document has been prepared, outlining a strategy for the creation of a tsetse free zone around the Lake Victoria Shore.

The interested reader may also wish to refer to http://www.iaea.org/programmes/nafa/d4/index.html, and http://www.fao.org/WAICENT/Agricul.htm.

SECTION B - ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

[See also **25**: no. 12417]

12387 **Kabayo, J.P., 2002.** Aiming to eliminate tsetse from Africa. *Trends in Parasitology*, **18** (11): 473–475.

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The problem of tsetse-transmitted trypanosomiasis occurs only in sub-Saharan Africa, where it represents a major constraint to socio-economic development. The East African form of sleeping sickness, caused by *Trypanosoma brucei rhodesiense*, is an acute and fatal disease, whereas the West African form, caused by *Trypanosoma brucei gambiense*, is generally more chronic and debilitating. The African governments have developed a new initiative, known as the Pan African Tsetse and Trypanosomiasis Eradication Campaign, which seeks to employ an area-wide approach and appropriate fly suppression methods to eradicate tsetse from areas of tsetse infestation, progressively, to ultimately create tsetse-free zones.

12388 **Rogers, D.J. & Randolph, S.E., 2002**. A response to the aim of eradicating tsetse from Africa. *Trends in Parasitology*, **18** (12): 534–536.

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An ambitious plan to eradicate tsetse, and therefore tsetse-transmitted trypanosomiases, from Africa was launched at the 36th Organization of African Unity summit meeting (Togo, July 2000) in a bold attempt to re-focus attention on one of Africa's greatest scourges. This plan involves the use of the sterile insect technique to achieve final eradication in areas where the fly is suppressed by more conventional methods (such as traps and targets). In this article the current aims of this project are questioned on historical, ecological, logistical and financial grounds.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

12389 **Akman, L., Yamashita, A., Watanabe, H., Oshima, K., Shiba, T., Hattori, M. & Aksoy, S., 2002.** Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature Genetics*, **32** (3): 402–407.

Aksoy: Department of Epidemiology and Public Health, Section of Vector Biology, Yale University School of Medicine, 60 College Street, 606 LEPH, New Haven, CT 06510 USA.

Many insects that rely on a single food source throughout their developmental cycle harbour beneficial microbes that provide nutrients absent from their restricted diet. Tsetse flies, the vectors of African trypanosomes, feed exclusively on blood and rely on one such intracellular microbe for nutritional provisioning and fecundity. As a result of co-evolution with hosts over millions of years, these mutualists have lost the ability to survive outside the sheltered environment of their host insect cells. We present the complete annotated genome of *Wigglesworthia glossinidia brevipalpis*, which is composed of one chromosome of 697 724 base pairs (bp) and one small plasmid, called pWig1, of 5 200 bp. Genes involved in the biosynthesis of vitamin metabolites, apparently essential for host nutrition and fecundity, have been retained. Unexpectedly, this obligate's genome bears hallmarks of both parasitic and free-living microbes, and the gene encoding the important regulatory protein DnaA is absent.

12390 **Gariou-Papalexiou, A., Yannopoulos, G., Zacharopoulou, A. & Gooding, R.H., 2002.** Photographic polytene chromosome maps for *Glossina morsitans submorsitans* (Diptera : Glossinidae): cytogenetic analysis of a colony with sex-ratio distortion. *Genome*, **45** (5): 871–880.

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

Photographic polytene chromosome maps from trichogen cells of pharate adult *Glossina morsitans submorsitans* were constructed. Using the standard system employed to map polytene chromosomes of *Drosophila*, the characteristic landmarks were described for the X chromosome and the two autosomes (L_1 and L_2). Sex-ratio distortion, which is expressed in male *G. m. submorsitans*, was found to be associated with an X chromosome (X^B) that contains three inversions in each arm. Preliminary data indicate no differences in the fecundity of X^AX^A and X^AX^B females, but there are indications that *G. m. submorsitans* in colonies originating from Burkina Faso and Nigeria have genes on the autosomes and (or) the Y chromosome that suppress expression of sex-ratio distortion.

12391 **Haddow, J.D., Poulis, B., Haines, L.R., Gooding, R.H., Aksoy, S. & Pearson, T.W., 2002.** Identification of major soluble salivary gland proteins in teneral *Glossina morsitans morsitans*. *Insect Biochemistry and Molecular Biology*, **32** (9): 1045–1053.

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Salivary glands of tsetse flies (Diptera: Glossinidae) contain molecules that are involved in preventing blood clotting during feeding as well as molecules thought to be intimately associated with trypanosome development and maturation. Here we present a protein microchemical analysis of the major soluble proteins of the salivary glands of *Glossina morsitans morsitans*, an important vector of African trypanosomes. Differential solubilization of salivary proteins was followed by reverse-phase, high-performance liquid chromatography (HPLC) and analysis of fractions by 1-D gel electrophoresis to reveal four major proteins. Each protein was subjected to amino acid microanalysis and N-terminal microsequencing. A protein chemical approach using high-resolution 2-D gel electrophoresis and mass spectrometry was also used to identify the salivary proteins. Matrix-assisted, laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and quadrupole time-of-flight (Q-TOF) tandem mass spectrometry methods were used for peptide mass mapping and sequencing, respectively. Sequence information and peptide mass maps queried against the NCBI non-redundant database confirmed the identity of the first protein as tsetse salivary gland growth factor-1 (TSGF-1). Two proteins with no known function were identified as tsetse salivary gland protein 1 (Tsal 1) and tsetse salivary gland protein 2 (Tsal 2). The fourth protein was identified as Tsetse antigen-5 (TAg-5), which is a member of a large family of anti-haemostatic proteins. The results show that these four proteins are the most abundant soluble gene products present in salivary glands of teneral *G. m. morsitans*. We discuss the possible functions of these major proteins in cyclical transmission of African trypanosomes.

12392 **Haines, L.R., Haddow, J.D., Aksoy, S., Gooding, R.H. & Pearson, T.W., 2002.** The major protein in the midgut of teneral *Glossina morsitans morsitans* is a molecular chaperone from the endosymbiotic bacterium *Wigglesworthia glossinidia*. *Insect Biochemistry and Molecular Biology*, **32** (11): 1429–1438.

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Molecules in the midgut of the tsetse fly (Diptera: Glossinidae) are thought to play an important role in the life cycle of African trypanosomes by influencing their initial establishment in the midgut and subsequent differentiation events that ultimately affect parasite transmission. It is thus important to determine the molecular composition of the tsetse midgut to aid in understanding disease transmission by these medically important insect vectors. Here, we report that the most abundant protein in the midguts of teneral (unfed) *Glossina morsitans morsitans* is a 60 kDa molecular chaperone of bacterial origin.

Two species of symbiotic bacteria reside in the tsetse midgut, *Sodalis glossinidius* and *Wigglesworthia glossinidia*. To determine the exact origin of the 60 kDa molecule, a protein microchemical approach involving two-dimensional (2-D) gel electrophoresis and mass spectrometry was used. Peptide mass maps were compared with virtual peptide maps predicted for *S. glossinidius* and *W. glossinidia* 60 kDa chaperone sequences. Four signature peptides were identified, revealing that the source of the chaperone was *W. glossinidia*. Comparative 2D gel electrophoresis and immunoblotting further revealed that this protein was localized to the bacteriome and not the distal portion of the tsetse midgut. The possible function of this highly abundant endosymbiont chaperone in the tsetse midgut is discussed.

12393 **Hao, Z.G. & Aksoy, S., 2002.** Proventriculus-specific cDNAs characterized from the tsetse, *Glossina morsitans morsitans*. *Insect Biochemistry and Molecular Biology*, **32** (12): 1663–1671.

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Peritrophic matrix (peritrophic membrane or PM) is an important structure in the gut of most insects at some stage in their development. It is composed of chitin, proteins and proteoglycans. Multiple roles for the PM ranging from partitioning of digestive enzymes and food to protection of gut epithelial cells from viral and parasitic invasion have been proposed. While most adult members of Diptera have a Type I PM synthesized in response to a blood meal, the medically and agriculturally important vector insect, tsetse, has a sleeve-like Type II PM which is constitutively synthesized by cells in the proventriculus (cardia). Using a differential hybridization approach, we have identified three abundant cDNAs from a proventriculus cDNA library of *Glossina morsitans morsitans*: *GmPro1*, *GmPro2* and *GmPro3*. DNA sequence analysis indicates that *GmPro1* and *GmPro2* share similarities with the peritrophin-15 family of larval PM proteins, while *GmPro3* is a member of the serine protease family. Northern analysis indicates that transcripts for all three cDNAs are preferentially expressed in the proventriculus tissue. The expression profile of these genes in response to the presence of trypanosome indicates that transcription of *GmPro1* is increased in the presence of parasites (immune sensitive), while the other two are not affected. Western analysis using antibodies developed against the recombinant GmPro2 shows its primary localization in the gut to be within the peritrophic matrix structure. We discuss the molecular characteristics of these proventriculus specific cDNAs and their products as well as their potential role for vector control studies.

12394 **Pollock, J.N., 2002.** Observations on the biology and anatomy of Curtonotidae (Diptera: Schizophora). [Glossinidae] *Journal of Natural History*, **36**: 1725–1745.

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New information concerning the biology and anatomy of *Cyrtona* spp. and *Curtonotum quinquevittatum* (Curtonotidae, Ephydroidea) is given. During the hot, dry season the latter species leaves its warthog burrow refuges at night. *Cyrtona* spp. rest in densely shaded humid habitats during the same season, dispersing in the cooler seasons. Postabdominal sclerites and internal anatomy of the male abdomen are described for both genera. A suggested ground plan of the Ephydroidea is outlined. The families Gasterophilidae, Glossinidae and Hippoboscidae are regarded as collectively the sister group of Oestridae, and based in part on the comparative anatomy of Curtonotidae, the whole complex is seen as deriving from early ephydroids, not from Calyptratae.

12395 **Wren, B.W., 2002.** Deciphering tsetse's secret partner. *Nature Genetics*, **32** (3): 335–336.

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The genome sequence of the bacterial endosymbiont *Wigglesworthia glossinidia* that resides in the gut of the tsetse fly has been determined. Because the tsetse fly relies on this bacterium for fertility and nutrition, this information may be useful in reducing fly populations and halting the spread of the deadly African sleeping disease.

12396 **Yan, J., Cheng, Q., Narashimhan, S., Li, C.-B. & Aksoy, S., 2002.** Cloning and functional expression of a fat body-specific chitinase cDNA from the tsetse fly, *Glossina morsitans morsitans*. *Insect Biochemistry and Molecular Biology*, **32** (9): 979–989.

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A chitinase cDNA, *GChit1* was isolated from *Glossina morsitans morsitans* and shown to be specifically expressed in fat body tissue. GChit1 is encoded by a 1.6 kb mRNA with a putative open reading frame (ORF) of 460 amino acids (predicted pI = 7.5, m.w. = 51kDa) that contains a signal peptide domain and two potential *N*-linked glycosylation sites. The ORF exhibits homology to various chitinases characterized from insects. It has the conserved catalytic site residues and the cysteine-rich 3′-end domain associated with chitin binding although the serine/threonine rich domain is apparently missing. Southern blot data indicate that *GChit1* is present as a single-copy locus in the *Glossina* genome. Northern analysis indicates that transcripts for GChit1 can be detected only from the fat body of adult flies. Similarly, chitinase activity could be detected in fat body but not in the gut or salivary gland tissues. The full-length cDNA was expressed *in vitro* in *Drosophila* S2 cells and the molecule was produced in a soluble form. Polyclonal antibodies raised against recGChit1 could recognize a protein of about 50 kDa in adult fat body extracts. In addition to fat body, chitinase protein was detected by Western analysis from the milk gland tissue of pregnant females as well as from the intrauterine larval and pupal developmental stages. No chitinase specific mRNA transcripts could be observed

however, from larvae and pupae. The intrauterine larva of tsetse may receive the protein from its mother via the milk gland route. The molecular characteristics of *GChit1* and its product and the potential role of this chitinase in tsetse biology are discussed.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

12397 **Evans, W.G. & Gooding, R.H., 2002.** Turbulent plumes of heat, moist heat, and carbon dioxide elicit upwind anemotaxis in tsetse flies *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). *Canadian Journal of Zoology*, **80** (7): 1149–1155.

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The roles and interactions of turbulent plumes of heat, moist heat, and carbon dioxide in mediating upwind flight of adult tsetse flies (*Glossina morsitans morsitans*) were investigated using a wind tunnel in a constant-environment chamber. Heat fluctuations in the plume that were detected by a thermocouple and displayed as oscilloscope traces allowed direct visualization of the structures of the plumes. Significantly more flies flew upwind when exposed to plumes of (i) carbon dioxide (0.0051 percent above background) and air (58 percent relative humidity) compared with air alone; (ii) carbon dioxide and heated air (35 percent relative humidity and temperature fluctuating up to 0.09 ºC above background) compared with carbon dioxide and air; and (iii) carbon dioxide and moist (82 percent relative humidity) heated air (temperature fluctuating up to 0.05 ºC above background) compared with carbon dioxide and heated air. However, there were no significant differences in upwind flight of flies exposed to plumes of (i) air compared with humidified air (65 percent relative humidity); (ii) carbon dioxide and heated air compared with heated air alone; and (iii) carbon dioxide and moist heated air compared with moist heated air alone. Recorded temperature fluctuations in heat plumes transported downwind from a tethered steer in a pasture showed patterns similar to those produced in the wind-tunnel plumes. These results suggest that host emissions of carbon dioxide alone and combined heat and moisture carried downwind by low-velocity winds elicit upwind anemotaxis in tsetse flies, which distinguish these emissions from a background of lower atmospheric levels.

12398 **Krafsur, E.S. & Endsley, M.A., 2002.** Microsatellite diversities and gene flow in the tsetse fly, *Glossina morsitans s.l. Medical and Veterinary Entomology*, **16** (3): 292–300.

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

Tsetse flies occupy discontinuous habitats and gene flow among them needs to be investigated in anticipation of area-wide control programs. Genetic diversities were estimated at six microsatellite loci in seven *Glossina morsitans submorsitans* populations and five microsatellite loci in six *G. m. morsitans* populations. Nei's unbiased diversities were 0.808 and 76 alleles in *G. m. submorsitans* and 0.727 and 55 alleles in *G. m.*

morsitans. Diversities were less in three laboratory cultures. Matings were random within populations. Populations were highly differentiated genetically. Populations were strongly subdivided, as indicated by fixation indices (F (ST)) of 0.18 in *G. m. morsitans* and 0.17 in *G. m. submorsitans*. Thirty-five percent of the genetic variance in *G. m. submorsitans* was attributed to differences between populations from The Gambia and Ethiopia. All available genetic evidence suggests that genetic drift is much greater than gene flow among *G. morsitans s.l.* populations.

12399 **Ruxton, G.D., 2002.** The possible fitness benefits of striped coat coloration for zebra. *Mammal Review*, **32** (4): 237–244.

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The literature addressing evolutionary reasons for the striped patterns of zebra coats is reviewed here. Possible mechanisms, and the evidence for and against them, are discussed. These mechanisms span four general themes: protection from predators; social functions; thermoregulation; and protection from tsetse flies. The last is the only hypothesis that has been tested experimentally, and the results of these tests are inconclusive. Additionally or alternatively, although stripes apparently increase zebra visibility in daylight, it is at least plausible that they provide effective cryptic protection from predators in poor light, although critical testing has not been attempted. Other related evolutionary questions are raised and suggestions made for future research.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also **25**: nos. 12387, 12388, 12421]

12400 **Mamuye H. & Dawit A., 2002.** Pathogenicity of Ethiopian isolates of *Metarhizium anisopliae* and *Beauveria bassiana* against the tsetse fly, *Glossina morsitans morsitans*. *Pest Management Journal of Ethiopia*, **6**: 23–29.

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Entomopathogenic fungi, *Metarhizium anisopliae* EE, *M. anisopliae* MM, *Beauveria bassiana* FF, *B. bassiana* GG and *B. bassiana* AK isolated from different sources in Ethiopia were evaluated against the tsetse fly, *Glossina morsitans morsitans* in the laboratory. *Metarhizium anisopliae* isolates EE and MM caused mortalities of 96.67 percent and 73.33 percent respectively, while *B. bassiana* isolates coded as FF, GG and AK showed percent mortalities of 75.00, 63.33 and 53.33, respectively. *Beauveria bassiana* FF was significantly better than *B. bassiana* AK ($P<0.05$). Spore production of presumably promising isolates, *M. anisopliae* MM and EE, was determined on solid substrates, whole grains of rice, wheat, barley and sorghum. Both isolates grew best on rice giving a yield of 1.42×10^9 spores/g of rice for *M. anisopliae* MM and 1.62×10^9 spores/g of

rice for *M. anisopliae* EE. No relationship was observed between moisture content of grain types and spore yield (P>0.05). The potential of the isolates for the control of tsetse flies is discussed.

12401 **Mihok, S., 2002.** The development of a multipurpose trap (the Nzi) for tsetse and other biting flies. *Bulletin of Entomological Research*, **92** (5): 385–403.

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New trap designs for tsetse (Glossinidae), stable flies (Muscidae: Stomoxyinae), and horse flies (Tabanidae) were tested in Kenya to develop a multipurpose trap for biting flies. Many configurations and colour/fabric combinations were compared with a simplified, blue-black triangular trap to identify features of design and materials that result in equitable catches. New designs were tested against conventional traps, with a focus on *Glossina pallidipes* and *G. longipennis*, *Stomoxys niger*, and *Atylotus agrestis*. A simple design based on minimal blue and black rectangular panels, for attraction and contrast, with a trap body consisting of an innovative configuration of netting, proved best. This 'Nzi' trap (Swahili for fly) caught as many or significantly more tsetse and biting flies than any conventional trap. The Nzi trap represents a major improvement for Stomoxyinae, including the cosmopolitan species *Stomoxys calcitrans*, with up to eight times the catch for key African *Stomoxys* spp. relative to the best trap for this group (the Vavoua). Catches of many genera of Tabanidae, including species almost never caught in traps (*Philoliche*), are excellent, and are similar to those of larger traps designed for this purpose (the Canopy). Improvements in capturing biting flies were achieved without compromising efficiency for the savannah tsetse species *G. pallidipes*. Catches of fusca group tsetse (*G. longipennis* and *G. brevipalpis*) were higher or were the same as catches in good traps for these species (NG2G, Siamese). Altogether, the objective of developing a simple, economical trap with harmonized efficiency was achieved.

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

[See also **25**: nos. 12392, 12421, 12434]

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

12402 **Chappuis, F., Pittet, A., Bovier, P.A., Adams, K., Godineau, V., Hwang, S.Y., Magnus, E. & Büscher, P., 2002.** Field evaluation of the CATT/*Trypanosoma brucei gambiense* on blood-impregnated filter papers for diagnosis of human African trypanosomiasis in southern Sudan. *Tropical Medicine and International Health*, **7**: (11): 942–948.

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Most human African trypanosomiasis (HAT) control programmes in areas endemic for *Trypanosoma brucei gambiense* rely on a strategy of active mass screening with the Card Agglutination Test for Trypanosomiasis (CATT)/*T. b. gambiense*. We evaluated the performance, stability and reproducibility of the CATT/*T. b. gambiense* on blood-impregnated filter papers (CATT-FP) in Kajo-Keji County, South Sudan, where some areas are inaccessible to mobile teams. The CATT-FP was performed with a group of 100 people with a positive CATT on whole blood including 17 confirmed HAT patients and the results were compared with the CATT on plasma (CATT-P). The CATT-FP was repeated on impregnated filter papers stored at ambient and refrigerated temperature for 1, 3, 7 and 14 days. Another 82 patients with HAT, including 78 with a positive parasitology, were tested with the CATT-FP and duplicate filter paper samples were sent to a reference laboratory to assess reproducibility. The CATT-FP was positive in 90 of 99 patients with HAT (sensitivity: 91 percent). It was less sensitive than the CATT-P (mean dilution difference: –2.5). There was no significant loss of sensitivity after storage for up to 14 days both at ambient and cool temperature. Reproducibility of the CATT-FP was found to be excellent (κ: 0.84). The CATT-FP can therefore be recommended as a screening test for HAT in areas where the use of CATT-P is not possible. Further studies on larger population samples in different endemic foci are still needed before the CATT-FP can be recommended for universal use.

12403 **Lejon, V., Legros, D., Richer, M., Ruiz, J.A., Jamonneau, V., Truc, P., Doua, F., Djé, N., N'Siesi, F.X., Bisser, S., Magnus, E., Wouters, I., Konings, J., Vervoort, T., Sultan, F. & Büscher, P., 2002.** IgM quantification in the cerebrospinal fluid of sleeping sickness patients by a latex card agglutination test. *Tropical Medicine and International Health*, **7** (8): 685–692.

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An increased IgM concentration in cerebrospinal fluid (CSF), occurring as a consequence of massive intrathecal IgM synthesis, is a marker of interest for diagnosis of the meningo-encephalitic stage in human African trypanosomiasis. However, in current practice, IgM in CSF is not determined because of the lack of a simple and robust test that is applicable in African rural regions where the disease prevails. We describe the development of a sensitive semiquantitative card agglutination test, LATEX/IgM, for IgM quantification in CSF. The test is simple and fast and the lyophilized reagent remains stable even at 45 ºC. CSF end-titres obtained with LATEX/IgM parallel the IgM concentrations determined by nephelometry and enzyme-linked immunosorbent assay. Detection of intrathecal IgM synthesis is the most sensitive marker for CNS involvement in sleeping sickness. At a cut-off value ≥8, the sensitivity and specificity of LATEX/IgM for intrathecal IgM synthesis are 89.4 and 92.7 percent. As a consequence, patients with LATEX/IgM end-titres ≥8 are likely to have intrathecal IgM synthesis, thus central nervous system involvement and therefore should be treated accordingly. Further studies should concentrate on the relationship between the LATEX/IgM end-titres, presence of

intrathecal IgM synthesis and occurrence of treatment failures in patients treated with pentamidine.

12404 **Radwanska, M., Claes, F., Magez, S., Magnus, E., Perez-Morga, D., Pays, E. & Büscher, P., 2002.** Novel primer sequences for polymerase chain reaction-based detection of *Trypanosoma brucei gambiense*. *American Journal of Tropical Medicine and Hygiene*, **67** (3): 289–295.

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Progress in diagnosis, treatment, and epidemiology of human African trypanosomiasis (sleeping sickness) depends on the existence of specific and sensitive diagnostic tools. Inherent shortcomings of serologic and parasitologic diagnostic methods can be overcome by molecular techniques. Therefore, we have developed a new polymerase chain reaction (PCR) test using primers derived from the recently identified sequence of the *Trypanosoma brucei gambiense*-specific glycoprotein (TgsGP). The specificity of the TgsGP-PCR was evaluated on DNA extracted from 73 different trypanosome populations belonging to diverse taxonomic groups that were isolated from various host species, and from different geographic origins. The TgsG-PCR was shown to be specific for *T. b. gambiense* and was suitable for detection of trypanosome DNA in blood samples of patients with confirmed sleeping sickness.

12405 **Truc, P., Lejon, V., Magnus, E., Jamonneau, V., Nangouma, A., Verloo, D., Penchenier, L. & Büscher, P., 2002**. Evaluation of the micro-CATT, CATT/*Trypanosoma brucei gambiense*, and LATEX/*T. b. gambiense* methods for serodiagnosis and surveillance of human African trypanosomiasis in West and Central Africa. *Bulletin of the World Health Organization*, **80** (11): 882–886.

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The objective of this study was to evaluate the performance of serological tests using dried blood on filter-papers (micro-card agglutination test for trypanosomiasis (micro-CATT)) performed under field and laboratory conditions and using whole blood ((CATT/*T. b. gambiense*) (wb-CATT) and latex agglutination (LATEX/*T. b. gambiense*) (wb-LATEX)) for the serodiagnosis and surveillance of human African trypanosomiasis in West and Central Africa. We evaluated the micro-CATT, wb-CATT and wb-LATEX methods in Côte d'Ivoire and the Central African Republic by screening 940 people. Sensitivity and specificity were calculated for each serological test; only patients with the confirmed presence of trypanosomes in the blood or lymph aspirate were considered true positives. Positive and negative predictive values were also calculated. Each of the tests showed a lower sensitivity in the Central African Republic than in Côte d'Ivoire. The results confirmed the efficiency of the classic wb-CATT to detect sleeping sickness patients. The micro-CATT method can be used for human African trypanosomiasis surveillance if the test is performed on the same day as the blood collection, or if samples are stored at 4 ºC. Otherwise, micro-CATT can be used when absolute sensitivity is not

required. The technique based on wb-LATEX should only be used for high-specificity screening.

(b) PATHOLOGY AND IMMUNOLOGY

(c) TREATMENT

12406 **Blum, J. & Burri, C., 2002.** Treatment of late stage sleeping sickness caused by *T. b. gambiense*: a new approach to the use of an old drug. *Swiss Medical Weekly*, **132** (5–6): 51–56.

Blum: Swiss Tropical Institute, Socinstrasse 57, PO Box CH–4002 Basel, Switzerland. [Johannes.Blum@unibas.ch]

Melarsoprol is the standard treatment of late-stage trypanosomiasis. The development of treatment schedules was previously purely empirical. Generally melarsoprol is given in three series of three to four consecutive injections, given every 24 hours, with an interval of about one week between the series. Based on pharmacokinetic analysis, computer simulations and extensive literature research covering all schedules previously used and tested, a new schedule, consisting of ten daily consecutive doses of 2.16 mg/kg of the drug was suggested. The pharmacokinetic model was validated in uninfected vervet monkeys. No unexpected drug accumulation and no systemic toxic effects were observed. In a pilot clinical trial in Congo RDC a small group of *T. b. gambiense* patients (n = 11) was treated successfully with the new schedule. In an open randomized clinical trial conducted in 500 patients in Angola the clinical efficacy and safety of this new concise treatment were compared with those of standard protocol treatment. Parasitological cure 24 hours after treatment was 100 percent in both groups. Statistical analysis yielded no significant differences for adverse events between the two treatment protocols. The new schedule reduces the amount and cost for the drug by about one third, and those for hospitalization by about a half.

12407 **Conway-Klaassen, J.M., Wyrick-Glatzel, J.M., Neyrinck, N. & Belair, P.A., 2002.** African sleeping sickness in a young American tourist. *Laboratory Medicine*, **33** (10): 783–788.

Conway-Klaassen: Clinical Laboratory Sciences Program, University of Nevada, Las Vegas, NV, USA.

An account is given of the symptoms, diagnosis, clinical treatment and recovery of an 18-year old male tourist, who had contracted trypanosomiasis during a tour that included parts of Kenya and Tanzania.

12408 **Jennings, F.W., Rodgers, J., Bradley, B., Gettinby, G., Kennedy, P.G.E. & Murray, M., 2002.** Human African trypanosomiasis: Potential therapeutic benefits of an alternative suramin and melarsoprol regimen. *Parasitology International*, **51** (4): 381–388.

Rodgers: Department of Veterinary Clinical Studies, University of Glasgow Veterinary Clinical Studies, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK. [jean.rodgers@vet.gla.ac.uk]

Treatment of late-stage human African trypanosomiasis is complicated by the presence of trypanosomes within the central nervous system (CNS). The regimen commonly prescribed to treat CNS-stage disease involves the use of the trypanocidal drugs suramin and melarsoprol. Suramin does not cross the blood-brain barrier efficiently and therefore, at normal dosages, will not cure CNS-stage infections. An initial treatment with suramin is given to eliminate the parasites from the peripheral tissues. This is followed by a course of intravenous melarsoprol, which can enter the CNS. However, melarsoprol not only produces severe adverse reactions but also is extremely painful to administer. One possible method to help alleviate these problems is to reduce the total amount of melarsoprol in the treatment regimen. This study indicates a synergism between suramin and melarsoprol and demonstrates that experimental murine CNS-trypanosomiasis can be cured with a single intraperitoneal dose of 20 mg/kg suramin followed almost immediately by 0.05 ml (4.5 μmol) topical melarsoprol. These dosages will not cure the infection when administered as monotherapies. Moreover, the timing of the drug administration appears to be crucial to the successful outcome of the regimen. If the interval between injection of suramin and application of topical melarsoprol is extended from 15 min to three or seven days, the infections are not cured, although extended relapse times occur following these regimens when compared with monotherapy approaches. Thus, there is strong evidence that injected suramin and topical melarsoprol should be given almost simultaneously to achieve the most effective combination of the two drugs.

12409 **Legros, D., Ollivier, G., Gastellu-Etchegorry, M., Paquet, C., Burri, C., Jannin, J. & Büscher, P., 2002.** Treatment of human African trypanosomiasis – present situation and needs for research and development. *Lancet Infectious Diseases*, **2** (7): 437–440.

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Human African trypanosomiasis re-emerged in the 1980s. However, little progress has been made in the treatment of this disease over the past decades. The first-line treatment for second-stage cases is melarsoprol, a toxic drug in use since 1949. High therapeutic failure rates have been reported recently in several foci. The alternative, eflornithine, is better tolerated but difficult to administer. A third drug, nifurtimox, is a cheap, orally administered drug not yet fully validated for use in human African trypanosomiasis. No new drugs for second-stage cases are expected in the near future. Because of resistance and the limited number of current treatments, there may soon be no effective drugs available to treat trypanosomiasis patients, especially second-stage cases. Additional research and development efforts must be made for the development of new compounds, including: testing combinations of current trypanocidal drugs, completing the clinical development of nifurtimox and registering it for trypanosomiasis, completing the clinical development of an oral form of eflornithine, pursuing the development of DB 289 and its derivatives, and advancing the pre-clinical development of megazol, eventually

engaging firmly in its clinical development. Partners from the public and private sector are already engaged in joint initiatives to maintain the production of current drugs. This network should go further and be responsible for assigning selected teams to urgently-needed research projects with funds provided by industry and governments. At the same time, on a long term basis, ambitious research programmes for new compounds must be supported to ensure the sustainable development of new drugs.

12410 **Lejon, V., Lardon, J., Kenis, G., Pinoges, L., Legros, D., Bisser, S., N'Siesi, X., Bosmans, E. & Büscher, P., 2002.** Interleukin (IL)–6, IL–8 and IL–10 in serum and CSF of *Trypanosoma brucei gambiense* sleeping sickness patients before and after treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **96** (3): 329–333.

Lejon: Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B–2000 Antwerpen, Belgium. [vlejon@itg.be]

Serum and cerebrospinal fluid (CSF) concentrations of interleukin (IL)–6, IL–8, IL–10, tumour necrosis factor–α and interferon–γ were determined in 46 *Trypanosoma brucei gambiense* sleeping sickness patients in DR Congo, before and after treatment. According to their CSF cell number before treatment, patients were classified as early-stage (0–5 cells/µl), intermediate-stage (6–20 cells/µl) or late-stage patients (>20 cells/µl). In serum, slightly higher IL–8 concentrations were found in early-stage patients compared with intermediate- or late-stage patients. These high IL–8 levels dropped after treatment. Higher IL–10 concentrations were detected in serum of patients in intermediate- or late-stage compared with early-stage patients. In both intermediate- and late-stage groups, serum IL–10 decreased after treatment. In CSF, elevated concentrations of IL–6, IL–8 and especially of IL–10 were observed in late-stage *T. b. gambiense* patients. After treatment, these concentrations dropped to levels similar to those of the other patients. Tumour necrosis factor–α was detected only in a few serum and CSF samples, which were scattered over the different patient groups. Interferon–γ was detected in serum of five patients and remained undetectable in CSF.

12411 **Mpia, B. & Pépin, J., 2002.** Combination of eflornithine and melarsoprol for melarsoprol-resistant Gambian trypanosomiasis. *Tropical Medicine and International Health*, **7** (9): 775–779.

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The objective was to evaluate the efficacy and toxicity of a combination of eflornithine and melarsoprol among relapsing cases of Gambian trypanosomiasis. Forty-two late-stage *Trypanosoma brucei gambiense* trypanosomiasis patients relapsing after initial treatment with melarsoprol were treated with a sequential combination of intravenous eflornithine (100 mg/kg every six hours for four days) followed by three daily injections of melarsoprol (3.6 mg/kg, up to 180 mg). They were then followed up for 24 months. Two (4.8 percent) patients died during treatment. Of the 40 surviving patients, two had a treatment failure, 13 and 19 months after having received the combination therapy.

By Kaplan–Meier analysis, the two-year probability of cure was 93.3 percent (95 percent confidence interval: 84.3–100 percent). This sequential combination has an efficacy and a toxicity similar to a seven-day course of eflornithine monotherapy, but is easier to administer. Whether such therapeutic success corresponds to synergism between eflornithine and melarsoprol, or merely means that four days of eflornithine monotherapy suffices for such patients, will need to be determined in a comparative trial.

12412 **Ruiz, J.A., Simarro, P.P. & Josenando, T., 2002.** Control of human African trypanosomiasis in the Quiçama focus, Angola. *Bulletin of the World Health Organization*, **80** (9): 738–745.

Simarro: WHO, PO Box 155 Yaoundé, Cameroon. [simarro_who @yahoo.fr]

The objective was to update the epidemiological status of human African trypanosomiasis (HAT), also known as sleeping sickness, in the Quiçama focus, province of Bengo, Angola, and to establish a HAT control programme. In 1997, 8 796 people (the population of 31 villages) were serologically screened for *Trypanosoma brucei gambiense*, the causative agent of HAT. In 1998 and 1999, surveys were carried out in villages where HAT cases had been identified in 1997. Individuals were screened using the card agglutination trypanosomiasis test (CATT), and then examined for the presence of the parasite. CATT-positive individuals in whom the presence of the parasite could not be confirmed were further tested with the CATT using serum dilutions, and those with a positive antibody end titre of 1-in-4 or above were followed up. Patients with ≤10 white cells/µl and no trypanosomes in their cerebrospinal fluid (CSF) were classified as being in the first stage of the disease. Vector control was not considered necessary or feasible. It was found that the main transmission areas were on the Kwanza riverbanks, where 5 042 inhabitants live. In 1997, the HAT prevalence was 1.97 percent, but this decreased to 0.55 percent in 1998 and to 0.33 percent in 1999. The relapse rate was 3 percent in patients treated with pentamidine and 1.5 percent in patients treated with melarsoprol. In patients treated with pentamidine, there was no difference in the relapse rate for patients with initial CSF white cell counts of 0–5 cells/µl or 6–10 cells/µl. The overall mortality rate was 0.6 percent and the rate of reactive arsenical encephalopathy among the melarsoprol-treated patients was 1.7 percent. In conclusion, the epidemiological status of the disease was updated and the transmission areas were defined. The control methods implemented allowed the disease prevalence to be reduced. A map of the study area is given.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

12413 **Kidanemariam, A., Hadgu, K. & Sahle, M., 2002.** Parasitological prevalence of bovine trypanosomosis in Kindo Koisha district, Wollaita zone, south Ethiopia. *Onderstepoort Journal of Veterinary Research*, **69** (2): 107–113.

Kidanemariam: Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Private Box X04 Onderstepoort, 0110 South Africa.

A cross-sectional survey to determine the distribution and prevalence of trypanosomosis was conducted in Kindo Koisha district, in the Wollaita zone in southern Ethiopia. A total of 1 008 adult cattle was examined at eight different localities. Dark field examination of the buffy coat, as well as stained thin blood film examination and packed cell volume (PCV) evaluation were the diagnostic techniques used. The overall prevalence of bovine trypanosomosis was 15 percent. Among the positive animals, 108 (71.1 percent), 43 (28.4 percent) and 1 (0.6 percent) were due to *Trypanosoma vivax*, *Trypanosoma congolense* and mixed infection (*T. vivax* and *T. congolense*), respectively. The infection rate of *T. vivax* and *T. congolense* varied significantly ($P<0.01$). The mean PCV of the positive and negative animals ranged between 18.3–32.1 percent and 26.8–33.4 percent, respectively. The mean PCV of negative animals (28 percent) was significantly higher than the mean PCV of positive animals (22.3 percent) ($P<0.001$). There was an inverse association of PCV with the prevalence of trypanosomosis ($P>0.05$). The herd average PCV values of each site decreased with increasing proportion of the positive herds of that particular site. Of the diagnostic tests employed, the microhaematocrit buffy coat technique is relatively sensitive and it has an added advantage of indicating the general condition of the animal by haematocrit measurement. In view of the risk of trypanosomosis, a control intervention through the strategic application of appropriate trypanocidal drugs is recommended. A tsetse fly control scheme to reduce host–tsetse fly contact is equally as important as chemotherapy and chemoprophylaxis against trypanosomosis.

12414 **Magona, J.W. & Mayende, J.S.P., 2002.** Occurrence of concurrent trypanosomosis, theileriosis, anaplasmosis and helminthosis in Friesian, Zebu and Sahiwal cattle in Uganda. *Onderstepoort Journal of Veterinary Research*, **69** (2): 133–140.

Magona: Livestock Health Research Institute, PO Box 96, Tororo, Uganda.

An epidemiological investigation was conducted on farms in Tororo and Soroti districts of Uganda from January to February 2000 to determine the cause of reported persistent mortality of cattle. Blood and faecal material of 98 cattle comprising 33 Friesians, 58 Zebu and 7 Sahiwal were examined. Results revealed that seven (7.1 percent) cattle had trypanosome infection, mainly due to *Trypanosoma vivax* and *T. brucei*, 17 (17.3 percent) *Fasciola* infection, 28 (28.6 percent) gastrointestinal nematode infection, 33 (33.7 percent) *Theileria* sp. infection and 13 (13.3 percent) *Anaplasma marginale* infection. Mixed infections were detected in 30 percent, 20.6 percent and 43 percent of the Friesian, Zebu and Sahiwal cattle respectively. Anaemia (PCV<25) was detected in 24 percent, 19 percent and 14 percent of the Friesian, Zebu and Sahiwal cattle respectively. Persistent mortality of cattle on these farms could have been due to either single or mixed parasitic infections probably exacerbated by malnutrition.

12415 **Magona, J.W., Mayende, J.S.P. & Walubengo, J., 2002.** Comparative evaluation of the antibody-detection ELISA technique using microplates precoated with denatured crude antigens from *Trypanosoma congolense* or *Trypanosoma vivax*. *Tropical Animal Health and Production*, **34** (4): 295–308.

Magona: Livestock Health Research Institute, PO Box 96, Tororo, Uganda.

Two FAO/IAEA indirect enzyme-linked immunosorbent assays (ELISA), which use microplates precoated with denatured crude *Trypanosoma congolense* or *Trypanosoma vivax* antigen for detecting anti-trypanosomal antibodies in bovine sera, were evaluated for their sensitivity, specificity and positive and negative predictive values, using 320 Ugandan field samples (known negative sera, $n = 80$; known positive sera, $n = 80$; cattle herds where control of tsetse and trypanosomosis was practised, $n = 80$; and cattle herds where there was no such control, $n = 80$). Cut-off points of 30 percent and 25 percent positivity were determined for the *T. congolense* and *T. vivax* assays, respectively, using a modified ROC (receiver operating characteristic) analysis. The *T. congolense* assay had estimated diagnostic sensitivity and specificity of 63.7 percent and 57.5 percent, respectively, while the *T. vivax* assay had estimated diagnostic sensitivity and specificity of 81.3 percent and 81.3 percent, respectively. The two assays conducted in parallel had estimated diagnostic sensitivity and specificity of 82.5 percent and 88.7 percent, respectively. Using the sera from the cattle in the area with control (detected prevalence of trypanosomosis 0 percent), both the *T. congolense* and *T. vivax* assays had negative and positive predictive values of 100 percent and 0 percent, respectively. Using the sera from the cattle in the area without control (detected prevalence of trypanosomosis 15 percent), the *T. congolense* assay had negative and positive predictive values of 91 percent and 33 percent, respectively, and the *T. vivax* assay had negative and positive predictive values of 93 percent and 27 percent, respectively. The *T. congolense* assay was in fair agreement with the buffy coat technique (BCT) ($\kappa = 0.25$), while the *T. vivax* assay was in substantial agreement with the BCT ($\kappa = 0.625$), and both assays conducted in parallel were in substantial agreement with the BCT ($\kappa = 0.708$). Both assays were found to be proficient and suitable for the diagnosis of bovine trypanosomosis, especially when used in parallel.

12416 **Irungu, P., Nyamwaro, S.O. & Masiga, D.K., 2002.** Financial implications of rearing sheep and goats under natural trypanosomosis challenge at Galana ranch, Kenya. *Tropical Animal Health and Production*, **34** (6): 503–513.

Masiga: Molecular Biology and Biotechnology Unit, ICIPE, PO Box 30772, Nairobi, Kenya. [dmasiga@icipe.org]

A study to compare the profitability of rearing sheep and goats under natural trypanosomosis challenge was carried out on Galana ranch in south-eastern Kenya between July 1996 and October 1997. Seventy-nine male weaner sheep and 79 male weaner goats were monitored monthly for weight changes and fortnightly for trypanosomosis. The animals of each species were divided into two groups. Group 1 was an untreated control, while group 2 was treated with isometamidium chloride (Samorin) at 0.5 mg/kg body weight every three months. In both groups, trypanosome infections were detected by microscopy and treated with diminazene aceturate (Veriben), at 3.5 mg/kg body weight, when the packed cell volume reached 17 percent or below. The profitability of each drug regime was expressed as the marginal revenue over the cost of trypanosomosis (MOT). There were greater losses occasioned by trypanosomosis in sheep than in goats. Animals of both species on chemoprophylaxis gave higher MOT values than those that received chemotherapy on diagnosis. However, the MOT values for the chemoprophylactic regime

were higher for sheep than for goats, suggesting that the greater weight gain by sheep more than compensated for the higher cost of maintaining them under high trypanosomosis challenge. Thus, a Galana rancher would be better off keeping sheep rather than goats, other things being equal. The marginal revenue per dose of Samorin was lower than that of Veriben for both species, suggesting that strategic use of Samorin timed to precede the peak incidence of trypanosomosis might be a better option to raise the overall profitability in sheep and goats.

12417 **Masiga, R.C. & Nyang'ao, J.M.N., 2001.** Identification of trypanosome species from camel using polymerase chain reaction and procyclic transformation test. *Journal of Camel Practice and Research*, **8** (1): 17–22.

Masiga: KETRI, PO Box 362, Kikuyu, Kenya.

The identification of trypanosome species in field infections of camels is important if appropriate decisions on treatment and control are to be made. The development of specific DNA probes has greatly improved the accuracy of identification of trypanosomes. The polymerase chain reaction (PCR) is a more sensitive technique that requires as little as a single parasite for identification. This is particularly important in field infections where parasitaemia may be low. The objective of this study was to characterize trypanosomes from field infections of camels using PCR and the Procyclic Transformation Test (PTT). Due to the absence of a *Trypanosoma brucei* specific probe, the *in vitro* transformation was used to distinguish between *T. brucei* and *T. evansi*. Parasites were passaged in mice and DNA extracted from trypanosomes isolated from mouse blood. DNA primers specific to *T. evansi* type A, *T. congolense*, *T. vivax* and a satellite DNA sequence specific for the subgenus *Trypanozoon* were used to screen a total of 80 samples. *Trypanosoma evansi* was detected in 76 percent of the isolates confirming it to be the most important species causing trypanosomosis in camels in Kenya. Five per cent of the samples showed *T. brucei* alone and 15 percent evidenced mixed infections of *T. congolense* and *T. brucei* respectively. Of the 80 samples, 26 were tested for *T. congolense*, 8 (31 percent) samples had *T. congolense* either as a single or mixed infections.

12418 **Michel, J.F., Dray, S., de La Rocque, S., Desquesnes, M., Solano, P., De Wispelaere, G. & Cuisance, D., 2002.** Modelling bovine trypanosomosis spatial distribution by GIS in an agro-pastoral zone of Burkina Faso. *Preventive Veterinary Medicine*, **56** (1): 5–18.

Michel: CIRDES/CIRAD-EMVT, Bobo Dioulasso, Burkina Faso. [jean-francois.michel@cirad.fr]

Modelling of the spatial distribution of bovine trypanosomosis prevalence in Sideradougou district Burkina Faso was performed by using a combination of spatial and statistical analysis. Based on a comprehensive and geographically representative census of herds and farms in the area, more than 2 000 cattle were randomly chosen and their blood sampled during field survey. Data on livestock farming practices were recorded for each farm. All data were mapped within a GIS to generate new information on spatial constraints in the area. Surveys results were analysed and serological prevalence data were

modelled using logistic regression. The model allowed identification and quantification of risk factors. In a second step the statistical model was used predictively on the entire farm population in the area. This method was successful in predicting the serological prevalence for each individual herd in the sample, from their livestock management patterns and spatial location. Predicted prevalences were represented within the GIS, taking daily movements of animals into account. Spatial distribution of prevalence would illustrate specific locations at risk from an epidemiological viewpoint. It gives evidence that the hydrological network and land occupation patterns in the savanna-type countryside are playing an important part when structuring a so-called "trypanosomosis space".

12419 **Ogunsanmi, A., Taiwo, V. & Ohore, G., 2000.** Application of antigen-detection enzyme immunoassay for the diagnosis of porcine *Trypanosoma brucei* infection. *Veterinarski Archiv,* **70** (5): 231–238.

Taiwo: Department of Wildlife and Fisheries Management, University of Ibadan, Ibadan, Nigeria.

The prevalence rate of *Trypanosoma brucei* infection in pigs was appraised by a monoclonal antibody-based antigen-detection enzyme immunoassay (antigen-ELISA). Blood samples were collected in the abattoir from pigs reared in the rain forest and derived savannah region of Nigeria under the traditional extensive management system. Blood samples were also collected from 50 exotic pigs reared on a commercial farm with fly-proof pens. These blood samples were analyzed for presence of trypanosomes and antigens in peripheral blood. Of 189 porcine blood samples 51 (27.0 percent) were positive for circulating antigens, whereas only four (2.1 percent) had demonstrable trypanosomes as revealed by the haematocrit centrifugation/buffy coat technique. When the 51 blood samples collected in EDTA tube corresponding to those sera that were positive for *T. brucei* antigens were subinoculated into mice, 46 (90.1 percent) of the mice became infected. Demonstration of trypanosomes in the infected mice is supportive proof that the parasites were residing in the infected hosts. Samples collected from 50 exotic pigs in fly-proof pens were all antigen-ELISA negative. In addition, none of the corresponding 50 control blood samples had demonstrable trypanosomes by the buffy coat method, nor do they show detectable parasites after subinoculation into mice. Thus, antigen-ELISA appeared to be a better and more useful tool for mass sero-epidemiological survey of porcine *T. brucei* infection as compared with the buffy coat technique.

12420 **Robinson, T.P., Harris, R.S., Hopkins, J.S. & Williams, B.G., 2002.** An example of decision support for trypanoso miasis control using a geographical information system in eastern Zambia. *International Journal of Geographical Information Science*, **16** (4): 345–360.

Robinson: ILRI, PO Box 30709, Nairobi, Kenya.

In many African countries where both Government resources and donor aid for the control of tsetse-transmitted trypanosomiasis are declining, there is an increasing need to identify areas where intervention is most likely to be technically, economically, socially and environmentally sustainable. Activities then can be focused so that the maximum

benefits are obtained from limited resources. We describe a decision-support framework based on a geographical information system to identify areas of high priority for the control of tsetse and trypanosomiasis in the common fly belt of eastern Zambia. Digital coverages were generated for six environmental variables: (1) cattle density, (2) human density, (3) land designation, (4) relative arable potential, (5) crop-use intensity and (6) proximity to existing control operations. The distribution of tsetse in the area was predicted using a multivariate (maximum likelihood) analysis of areas of known presence and absence and a series of environmental data. Experienced Zambian veterinarians and biologists working in the region established criteria weights for the input variables and the data were integrated in a geographical information system (GIS), using weighted linear combinations to prioritize areas for trypanosomiasis control. The results of this exercise and estimates of the errors involved are discussed.

12421 **Sharma, S.P., Losho, T.C., Malau, M., Mangate, K.G., Linchwe, K.B., Amanfu, W. & Motsu, T.K., 2001.** The resurgence of trypanosomosis in Botswana. *Journal of the South African Veterinary Association*, **72** (4): 232–234.

Sharma: National Veterinary Laboratory, Private Bag 0035, Gaberone, Botswana.

In view of the occurrence of several confirmed clinical cases of nagana and reports of heavy bovine mortality, a parasitological survey was conducted to determine the prevalence of trypanosome infection in cattle in Maun and Shakawe areas of Ngamiland district. Wet blood films, buffy coat and Giernsa-stained thick and thin blood smears were used to detect trypanosomes in animals. Overall, trypanosome infection rate was 15.98 percent, with 5.94 percent and 27.29 percent in Maun and Shakawe respectively. The urgent need to combat trypanosomosis in Ngamiland, particularly in the Shakawe area, is highlighted, and a three-phase integrated tsetse control strategy for this disease problem is discussed.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **25**: no. 12426]

12422 **Bengaly, Z., Sidibe, I., Ganaba, R., Desquesnes, M., Boly, H. & Sawadogo, L., 2002.** Comparative pathogenicity of three genetically distinct types of *Trypanosoma congolense* in cattle: clinical observations and haematological changes. *Veterinary Parasitology*, **108** (1): 1–19.

Bengaly: CIRDES, 01 BP 454, Bobo-Dioulasso 01, Burkina Faso. [cirdes@ird.bf]

The pathology of African bovine trypanosomosis was compared in Zebu cattle subcutaneously inoculated with three clones of trypanosomes corresponding to the three genetically distinct types of *Trypanosoma congolense*; savanna-type, west African riverine/forest-type and kilifi-type. All inoculated animals became parasitaemic between

seven and eleven days post-infection (dpi). The savanna-type showed consistently higher levels of parasitaemia, and lower packed red cell volume percentages and leukocyte counts than the other two types. The syndrome was also more severe in the savanna-type and led inexorably to death between 29 and 54 dpi while animals with the forest or the kilifi-types recovered from earlier symptoms and haematological alterations after three months of infection. By the end of the experiment, the animals self-cured from the forest-type infection and the kilifi-type passed under control. The results of the present study indicated clear difference in pathogenicity between the three types of *T. congolense*; the savanna-type was virulent while the forest-type was of low pathogenicity and the kilifi-type was non-pathogenic.

12423 **Masiga, D.K., Okech, G., Irungu, P., Ouma, J., Wekesa, S., Ouma, B., Guya, S.O. & Ndung'u, J.M., 2002.** Growth and mortality in sheep and goats under high tsetse challenge in Kenya. *Tropical Animal Health and Production*, **34** (6): 489–501.

Masiga: Molecular Biology and Biotechnology Unit, ICIPE, PO Box 30772, Nairobi, Kenya. [dmasiga@icipe.org]

Trypanosomosis is a major impediment to livestock production and economic development in those areas of Africa where it is endemic. Although small ruminants appear to perform better than cattle in various agro-ecological zones, the importance of trypanosomosis has not been extensively investigated in these livestock. This study was designed to investigate the prevalence of trypanosomosis in sheep and goats in an endemic area and to evaluate the performance of different breeds under high tsetse challenge and the potential role of chemoprophylaxis in the control of the disease. The results showed that tsetse flies feed readily on small ruminants, and that these animals are susceptible to trypanosomosis. The Small East African goats acquired fewer infections than the Black Head Persian and Dorper sheep used in the study. In both sheep and goats, chemoprophylaxis with isometamidium chloride (Samorin, Rhone Merieux, Annecy, France) was protective, resulting in fewer infections and higher body weight gain. Trypanosomosis caused anaemia in both sheep and goats, and animals whose PCV fell below 15 percent rarely recovered, even with trypanocidal drug treatment. The peak transmission period was between one and three months after the peak tsetse fly density, which raises the possibility of effective strategic prophylaxis.

12424 **Mattioli, R.C. & Mehlitz, D., 2001.** Vector-borne haemoparasitic complexes: a major potential disease risk factor impairing cattle health and production in tsetse-infested areas of West Africa. *Journal of Agriculture and Environment for International Development*, **95** (2–3): 237–244.

Animal Production and Health Division, Food and Agriculture Organization of the United Nations, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy.

Repeated trypanosome infections render both trypanosusceptible and trypanotolerant cattle more susceptible to tick attack and, consequently, to increased challenges of tick-

borne micro-organism species and respective infective inoculated doses. In such a situation, enzootic stability to tick-transmitted infections may vanish. Recently, three pathological syndromes, responsible for high mortality, have been reported in N'Dama cattle populations living in sub-humid and humid zones of Guinea and Senegal infested by tsetse fly and ticks. These syndromes are termed, in vernacular language, Red and Black Woula and Dasso in Guinea and Senegal, respectively. "Red" and "Black" denominations of the Woula syndrome appear to be related, respectively, to haemoglobinuria (Red) and cyanotic aspect (Black) of apparent mucous membranes in sick animals. Woula *per se* signifies, in local language, "unpopulated remote area located far off in the savannah". Dasso designates a not well-defined animal pathological status. We note that, in the case of Red and Black Woula, the description of a delineated geographic area is included by the indigenous people in the definition of these two syndromes. This would indicate that the pathogens involved are confined to defined zones. Preliminary information suggests these syndromes are caused by a common infective haemoparasitic complex composed of trypanosomes and tick-borne anaplasmosis and/or babesiosis. Perspective investigations are needed to obtain epidemiological data to identify the micro-organisms involved, density and seasonality of potential vectors and to assess the impact of the pathological challenge in order to set up, if necessary, adequate and economically profitable control measures.

12425 **Ndoutamia, G., Mbakasse, R.N., Brahim, A. & Khadidja, A., 2002.** Influence de la trypanosomose à *T. congolense* sur les paramètres hématologiques, minéraux et protéo-énergétiques chez les chèvres sahéliennes du Tchad. [Influence of *Trypanosoma congolense* infection on some haematological and serum biochemical parameters in sahelian goats.] *Revue de Médecine Vétérinaire*, **153** (6): 395–400.

Ndoutamia: Laboratoire de Recherches Vétérinaires et Zootechniques de Farcha, B.P. 433, Ndjaména, Tchad. [ndouta@intnet.td]

Forty Sahelian goats whose haemoglobin types had been determined, were experimentally infected, each one with 10^6 trypanosomes (*Trypanosoma congolense* IL 1180 stock savanna type). Clinical signs, body weight, haematological and serum biochemical parameters were recorded over six months. The Sahelian goats were highly susceptible to the infection. They developed an acute disease which was lethal within four weeks. The prepatent period was approximately seven days. During the acute phase of the disease, the animals displayed lack of appetite, pale ocular membranes, watering eyes, staggering movements and occasional diarrhoea. The haematocrit dropped from 38.68 percent to 25.72 percent and often reached the critical point of 15 percent. At that threshold the animals were unable to stand up and died unless a trypanocidal treatment was applied. The *T. congolense* trypanosomiasis evolution was associated at different levels with significant changes in haematological and serum biochemical parameters. The disease was mainly characterized by the decrease of the red blood cells, haemoglobin, albumin, cholesterol, glycaemia and the rise in level of the white blood cells, serum iron, calcium, bilirubines and urea. The most vulnerable enzymes were the transaminases the activity of which varied considerably throughout the experimental period.

12426 **Taiwo, V.O., Adejinmi, J.O. & Oluwaniyi, J.O., 2002.** Non-immune control of trypanosomosis: In vitro oxidative burst of PMA- and trypanosome-stimulated neutrophils of Boran and N'Dama cattle. *Onderstepoort Journal of Veterinary Research*, **69** (2): 155–161.

Taiwo: Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria.

An *in vitro* assay that measures the generation of superoxide anions (0_2^-) was used to assess the level of oxidative burst of phorbol myristate acetate (PMA)- and trypanosome-stimulated neutrophils isolated from healthy Boran and N'Dama cattle, and those infected with *Trypanosoma congolense*. PMA stimulation of healthy bovine neutrophils resulted in between 300–400 percent increase in 0_2^- generation. Neutrophils of Boran cattle exhibited slightly (but not significantly) higher 0_2^- generation capacity than those of the N'Dama breed. *In vitro* stimulation by trypanosomes of neutrophils isolated from *Trypanosoma congolense*-infected cattle caused significant increases in 0_2^- generation, especially on days 14, 28 and 42 post-infection, in both breeds of cattle. No significant differences were observed in 0_2^- generation capacity of the neutrophils of either breed of infected cattle throughout the period of assay. The results of this study have shown that PMA and trypanosomes do cause an enhanced *in vitro* oxidative burst, hence trypanosome phagocytosis and killing activity of neutrophils. Neutrophils have been shown to play very significant roles in parasite clearance, hence reduction of trypanosome parasitaemia. The rates of both *in vitro* generation of 0_2^- and trypanosome phagocytosis over time did not differ significantly between Boran and N'Dama breeds of cattle, even during *T. congolense* infection in this study. Hence, it may be inferred that sustained and higher parasitaemia, more pronounced neutropenia, inadequate bone marrow response and less effective trypanosome-specific immune response, rather than defective neutrophil trypanosome destruction, may be the problem of trypanosusceptible cattle breeds.

(c) TRYPANOTOLERANCE

[See also **25**: no. 12434]

12427 **Faye, D., Osaer, S., Goossens, B., Van Winghem, J., Dorny, P., Lejon, V., Losson, B. & Geerts, S., 2002.** Susceptibility of trypanotolerant West African Dwarf goats and F1 crosses with the susceptible Sahelian breed to experimental *Trypanosoma congolense* infection and interactions with helminth infections and different levels of diet. *Veterinary Parasitology*, **108** (2): 117–136.

Faye: ITC, PMB 14, Banjul, The Gambia. [dethie.faye@itc.gm]

Forty pure West African Dwarf (WAD) goats and 35 of its F1 crosses with the Sahelian breed were used in a multifactorial experimental design to evaluate the effects of an experimental *Trypanosoma congolense* infection and interactions with natural helminth infections and different levels of diet on health and productivity of these two breeds. Trypanosome infection caused a severe drop in packed cell volume (PCV), but this was not significantly affected by breed. Neither deworming nor diet had any effect on the course of

anaemia after trypanosome infection. The mean score of parasitaemia tended to be higher in crossbreeds than in WAD goats although this was not significant ($P > 0.05$). Similarly, the antibody response to trypanosome infection was not significantly different between breeds. Parasitaemia level was significantly influenced by the level of diet with the group under high supplementation having a higher mean parasitaemia score than the group under low supplementation. Weight loss due to trypanosome infection tended to be greater in crossbreeds than in WAD goats ($P > 0.05$). During this study, there was no difference in mean helminth egg output between crossbred and WAD goats. However, between weeks 4 and 10 after trypanosome infection (corresponding to a period of heavy rainfall and highly infective pastures), the mean egg output was higher in the crossbreeds. The immunosuppressive effect of trypanosome infections was revealed by a lower antibody response to *Haemonchus contortus* in infected animals compared with the uninfected controls. Trypanosome infection tended to increase strongyle egg output. This study did not reveal any superior trypanotolerance of WAD goats compared with crossbreeds.

12428 **Ogunsanmi, A., Taiwo, V., Onawumi, B., Mbagwu, H. & Okoronkwo, C., 2001.** Correlation of physiological plasma lipid levels with resistance of cattle to trypanosomosis. *Veterinarski Arhiv*, **70** (5): 251–257.

Taiwo: Department of Wildlife and Fisheries Management, University of Ibadan, Ibadan, Nigeria.

Haematological values and indices as well as plasma lipids (cholesterol and triglyceride) levels were studied in trypanotolerant N'Dama and trypanosusceptible White Fulani (Zebu) cattle raised in the same environment in order to determine the probable role of plasma lipids levels in the phenomenon of trypanotolerance in tropical cattle. The haematological parameters and indices, such as PCV, Hb concentration, RBC, WBC counts, MCV and MCH, showed no significant differences ($P>0.05$) between the two cattle breeds or sex. N'Dama cattle had significantly lower levels of plasma cholesterol ($P<0.05$) and triglycerides ($P<0.01$) than Fulani cattle. Male N'Dama cattle had significantly higher plasma cholesterol levels than females. While no significant gender difference ($P>0.05$) was observed in plasma triglyceride levels in N'Dama cattle, a significantly higher ($P<0.012$) plasma triglyceride level was recorded in female White Fulani cattle than in their male counterparts. The findings in this study suggest a possible correlation between plasma lipid levels and trypanotolerance or susceptibility between N'Dama and White Fulani cattle. The roles of plasma lipids in trypanosome growth and differentiation, as well as in the pathology of the disease in the host, are discussed.

12429 **Tano, K., Kamuanga, M., Faminow, M.D. & Swallow B.M., 2001.** Adoption and demand for trypanotolerant cattle in the sub-humid zone of West Africa. *Journal of Agriculture and Environment for International Development*, **95** (2/3): 213–236.

Tano: Centre Ivoirien de Recherches Economique et Sociales (CIRES), Université d'Abidjan–Cocody, 08 BP 1295 Abidjan 08, Côte d'Ivoire.

Baoulé is one of several breeds of West African Shorthorn cattle (*Bos taurus brachycheros*) that are trypanotolerant and thus can survive and produce in areas of low to moderate tsetse (*Glossina* spp.) challenge without the aid of drugs. In southern Burkina Faso, Baoulé are raised under different management systems along with trypanosusceptible Zebu (*Bos indicus*) and crossbred Méré cattle. A trend among livestock farmers towards large Zebu-type cattle is perceptible, raising concerns about the risk of extinction of Baoulé. A study was undertaken in two areas of southern Burkina Faso (Pays Lobi and Kourouma) to evaluate the main factors affecting adoption and utilization of Baoulé and to determine the prospects for its conservation. Emphasis was placed on the rôle of farmers' subjective perceptions of the traits of Baoulé relative to other breeds. The results of a Logit model indicate that indigenous farmers and those generally involved in subsistence production systems are more likely to keep Baoulé cattle in their herds. In contrast, the probability is very low for migrants and mixed crop-livestock farmers to raise this breed. Farmers' high rating of the overall desirability of Baoulé increases the probability of adoption while a high rating of Zebu's fitness to traction negatively affects the probability of adoption of Baoulé. The strategy for *in situ* breed conservation should be targeted to traditional farmers of Pays Lobi given their high probability of adopting Baoulé and the high discretion they exercise over the choice of this breed as shown by the large proportion of Baoulé cattle purchased and introduced into their herds.

(d) TREATMENT

[See also **25**: no. 12439]

12430 **Delespaux, V., Geerts, S., Brandt, J., Elyn, R. & Eisler, M.C., 2002.** Monitoring the correct use of isometamidium by farmers and veterinary assistants in Eastern Province of Zambia using the isometamidium-ELISA. *Veterinary Parasitology*, **110** (1–2): 117–122.

Delespaux: Institute of Tropical Medicine, Nationalestraat 155, B–2000 Antwerp, Belgium. [vdelespaux@itg.be]

A survey to monitor the use of trypanocidal drugs by cattle breeders was conducted in Zambia. Use was made of a questionnaire and of the isometamidium-ELISA technique. One hundred and twenty two farmers and 50 veterinary assistants were interviewed. The isometamidium-ELISA was used to monitor the isometamidium serum concentration in 72 cattle, one week after unsupervised treatment by 56 farmers and 16 veterinary assistants. Although there was no clear indication of underestimation of the weight of the animals and although farmers had adequate knowledge of the correct usage of isometamidium, the results suggest frequent underdosing when considering isometamidium serum concentrations one week after treatment. In 76 percent of the cases, the expected protection period was equal or shorter than 28 days and equal or shorter than 33 days in 90 percent of the treated cattle.

12431 **Olila, D., McDermott, J.J., Eisler, M.C., Mitema, E.S., Patzelt, R.J., Clausen, P.H., Poetzsch, C.J., Zessin, K.H., Mehlitz, D. & Peregrine, A.S., 2002.** Drug

sensitivity of trypanosome populations from cattle in a peri-urban dairy production system in Uganda. *Acta Tropica*, **84** (1): 19–30.

Peregrine: Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph ON, N1G 2W1, Canada.

Cattle from 50 farms in Mukono County, Uganda, were monitored for trypanosomes every second month over an 18-month period (1995–1996) by mini-anion exchange chromatography and haematocrit centrifugation techniques. Eighteen trypanosome isolates collected from cattle during this period were characterized in cattle, goats and mice for their sensitivity to homidium, isometamidium and diminazene; ten of the isolates were selected randomly, eight were from animals that had the highest serum isometamidium concentrations at the time the isolates were collected. All the isolates contained only *Trypanosoma brucei* and/or *T. vivax*. In naive Boran (*Bos indicus*) cattle the isolates exhibited low pathogenicity and were sensitive to diminazene aceturate at 3.5 mg/kg body weight (bw) and isometamidium chloride at 0.5 mg/kg bw. In goats, five of eight isolates were highly pathogenic, producing clinical signs indicative of central nervous system involvement within 60 days of infection; all such isolates contained *T. brucei*. However, all eight populations were sensitive in goats to diminazene aceturate at 3.5 mg/kg bw. In contrast, four populations were refractory to treatment with isometamidium chloride at 0.5 mg/kg bw in at least one out of three goats each. Furthermore, five populations were refractory to treatment with homidium chloride at 1.0 mg/kg bw in a minimum of two out of three goats each. In mice, the 50 percent curative dose values for 11 Mukono isolates that contained *T. brucei* ranged from 0.30 to 1.89 mg/kg bw for diminazene aceturate, from 0.02 to 0.17 mg/kg bw for isometamidium chloride and from 0.90 to 4.57 mg/kg bw for homidium chloride. Thus, by comparison with reference drug-sensitive populations, all the stabilates were highly sensitive to diminazene and isometamidium, while some expressed low levels of resistance to homidium.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

12432 **Desquesnes, M. & Dávila, A.M.R., 2002**. Applications of PCR-based tools for detection and identification of animal trypanosomes: a review and perspectives. *Veterinary Parasitology*, **109** (3–4): 213–231.

Desquesnes: CIRAD–EMVT/CIRDES, 01 BP, Bobo-Dioulasso, Burkina Faso. [m.desquesnes@fasonet.bf]

This paper aims to review the applications of the polymerase chain reaction (PCR) for the detection and identification of trypanosomes in animals. The diagnosis of trypanosomes, initially based on microscopic observations and the host range of the parasites, has been improved, since the 1980s, by DNA-based identification. These diagnostic techniques evolved successively through DNA probing, PCR associated to DNA probing, and currently to PCR alone. Several DNA sequences have been investigated

as possible targets for diagnosis, especially multi-copy genes such as mini-exon, kinetoplastid mini-circles, etc., but the most favoured target is the nuclear satellite DNA of mini-chromosomes, which presents the advantages, and the drawbacks, of highly repetitive short sequences (120–600bp). Several levels of specificity have been achieved from sub-genus to species, sub-species and even types. Random priming of trypanosome DNA has even allowed "isolate specific" identification. Other work based on microsatellite sequences has provided markers for population genetic studies. For regular diagnosis, the sensitivity of PCR has increased with the advancement of technologies for sample preparation, to reach a level of 1 trypanosome/ml of blood, which has brought to field samples a sensitivity two to three times higher than that obtained from microscopic observation of the buffy coat. Similarly, PCR has allowed an increase in the specificity and sensitivity of diagnosis in vectors such as tsetse flies. However, because of the diversity of *Trypanosoma* species potentially present in a single host, PCR diagnosis carried out on host material requires several PCR reactions; for example, in cattle, up to five reactions per sample may be required. Research is now focusing on a diagnosis based on the amplification of the internal transcribed spacer–1 (ITS–1) of ribosomal DNA which presents the advantages of being a multi-copy locus (100–200), having a small size (300–800 bp), which varies from one taxon to another but is conserved in size in a given taxon. This may lead to the development of a multi-species-specific diagnostic protocol using a single PCR. By reducing the cost of the PCR diagnosis, this technique would allow a greater number of field samples to be tested in epidemiological studies and/or would increase the variety of *Trypanosoma* species that could be detected. Further investigations are required to develop and optimize multi-species-specific diagnostic tools for trypanosomes, which could also serve as a model for such tools in other pathogens.

12433 **Radwanska, M., Magez, S., Perry-O'Keefe, H., Stender, H., Coull, J., Sternberg, J.M., Büscher, P. & Hyldig-Nielsen, J.J., 2002.** Direct detection and identification of African trypanosomes by fluorescence *in situ* hybridization with peptide nucleic acid probes. *Journal of Clinical Microbiology*, **40** (11): 4295–4297.

Radwanska: Department of Immunology, Groote Schuur Hospital, Old Main Building H47, Observatory 7925, Cape Town, South Africa. [mradwans@uctgsh1.uct.ac.za]

We have developed a rapid and easy-to-perform fluorescence *in situ* hybridization test that allows specific identification of trypanosomes from the subgenus *Trypanozoon*, using peptide nucleic acid probes. Probes were designed to target subgenus-specific sequences on the multiple-copy 18S rRNA, greatly facilitating the detection of a single trypanosome.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **25**: no. 12428]

12434 **Noël, W., Gh, G.H., Raes, G., Namangala, B., Daems, I., Brys, L., Brombacher, F., De Baetselier, P. & Beschin, A., 2002.** Infection stage-dependent

modulation of macrophage activation in *Trypanosoma congolense*-resistant and -susceptible mice. *Infection and Immunity*, **70** (11): 6180–6187.

Beschin: Cellular Immunology Unit, Flemish Interuniversity Institute for Biotechnology, VIB–VUB, Paardenstraat 65, B–1640 St–Genesius-Rode, Belgium. [abeschin@vub.ac.be]

The contribution of cytokines and chemokines to resistance and susceptibility to African trypanosomiasis remains controversial. In the present study, the levels of type I and type II cytokines and of the MCP–1 chemokine were compared during the early and late stages of *Trypanosoma congolense* infection in susceptible BALB/c and resistant C57BL/6 mice. Moreover, the status of macrophage activation was compared in these animals by analyzing the inducible nitric oxide synthase-arginase balance, tumor necrosis factor secretion, and expression of the FIZZ1 and YM genes. Data show that changing from a predominant type I cytokine environment in the early stage of infection to a predominant type II cytokine environment and an enhanced MCP–1 secretion in the late stage of infection correlates with resistance to *T. congolense*. Concomitantly, macrophage activation evolves from a classical to a predominant alternative phenotype. We further confirmed that the simultaneous occurrence of type I/type II cytokines in the early stage of infection in susceptible BALB/c mice, reflected by the presence of macrophages exhibiting a mixed classical/alternative activation phenotype, is associated with uncontrolled parasite growth and early death. Interleukin–4 (IL–4) and IL–13 signalling did not influence the susceptibility of BALB/c mice to *T. congolense* infection and interestingly were not the main trigger to alternative macrophage activation. In *T. congolense*-resistant C57BL/6 mice, our results corroborated the induction of FIZZ1 and YM gene expressions with the alternative pathway of macrophage activation. In susceptible BALB/c mice, however, YM but not FIZZ1 induction reflected the emergence of alternatively activated macrophages. Hence, the FIZZ1 and YM genes may be useful markers to discriminate between distinct populations of alternatively activated macrophages.

12435 **Ojok, L., Kaeufer-Weiss, I. & Weiss, E., 2002.** Distribution of *Trypanosoma congolense* in infected multimammate rats (*Mastomys coucha*): light and electron microscopical studies. *Veterinary Parasitology*, **105** (4): 327–336.

Ojok: Department of Veterinary Pathology, Faculty of Veterinary Medicine, Makerere University, PO Box 7062, Kampala, Uganda. [vetpath@ infocom.co.ug]

In an attempt to determine whether *Trypanosoma congolense* occurs both within and outside the blood vessels in an infected animal host, multimammate rats (*Mastomys coucha*) were infected with *T. congolense* and samples from spleen, lymph nodes, bone marrow, liver, kidney, lungs, brain, heart, intestines, ovaries and testes were collected. The tissue samples were fixed and processed for light and electron microscopical examination. In all the tissues examined, trypanosomes were found only within the lumen of large and small blood vessels, capillaries and sinuses. It is concluded that following entry into the blood circulation after intra-peritoneal infection of *M. coucha*, *T. congolense* remains restricted to the bloodstream.

12436 **Olaniyi, M.O., Taiwo, V.O. & Ogunsanmi, A.O., 2001.** Haematology and dynamics of erythrocyte membrane sialic acid concentration during experimental *Trypanosoma congolense* and *T. brucei* infection of sheep. *Journal of Applied Animal Research*, **20** (1): 57–64.

Taiwo: Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria.

Haematological changes and the dynamics of erythrocyte membrane sialic acid concentration were studied in sheep experimentally infected with *Trypanosoma congolense* (Binchi Bassa strain) and *T. brucei* (Lafia strain). Both species of trypanosomes caused varying degrees of pathogenicity. The anaemia was more severe ($P < 0.05$) in *T. brucei* than in *T. congolense* infected sheep. There was significant ($P < 0.05$) reduction in erythrocyte membrane surface sialic acid concentration with progression of infection in both *T. congolense* and *T. brucei* infected sheep.

(c) CHEMOTHERAPEUTICS

12437 **Ali, H., König, G.M., Khalid, S.A., Wright, A.D. & Kaminsky, R., 2002.** Evaluation of selected Sudanese medicinal plants for their in vitro activity against hemoflagellates, selected bacteria, HIV–1–T and tyrosine kinase inhibitory, and for cytotoxicity. *Journal of Ethnopharmacology*, **83** (3): 219–228.

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Ethnobotanical investigations led to the selection of 19 plant species, used traditionally in Sudan against malaria and other similar tropical diseases, for further studies. *Pamianthe peruviana* (Amaryllidaceae) exhibited significant activity against a chloroquine-resistant *Plasmodium falciparum* strain (K1) and a chloroquine-sensitive strain (NF54) with IC_{50} values of 0.6 and 1.1 µg/ml, respectively. Additionally, *P. peruviana* showed considerable activities against *Trypanosoma brucei rhodesiense* (IC_{50} 1.5 µg/ml) and *T. cruzi* (IC_{50} 11.8 µg/ml). The antiplasmodial activity of the different extracts of *Salvadora persica* (Salvadoraceae) against *P. falciparum* NF54 strain were found to be 0.6 µg/ml (stems) and 0.7 µg/ml (leaves). Extracts of different parts of *Combretum hartmannianum* (Combretaceae) possessed significant activity against the chloroquine-sensitive *P. falciparum* strain (NF54) with IC_{50} values of 0.2 µg/ml (bark), 0.4 µg/ml (stem) and 4.3 µg/ml (leaves). Most interestingly, the extracts of the leaves of *C. hartmannianum* totally inhibited the enzyme HIV–1 reverse transcriptase (HIV–1 RT) at a concentration of 66 µg/ml. A comparably strong activity against $p56^{lck}$ tyrosine kinase was also seen for this extract.

12438 **D'Silva, C. & Daunes, S., 2002.** The therapeutic potential of inhibitors of the trypanothione cycle [*T. brucei*]. *Expert Opinion on Investigational Drugs*, **11** (2): 217–231.

D'Silva: Department of Chemistry and Materials, The Manchester Metropolitan University, John Dalton Building, Chester Street. Manchester M1 5GD, UK.

There is an urgent need for new drugs in the treatment of human African trypanosomiasis, Chagas' disease and leishmaniasis. This article provides an overview of current drugs, their formulations and their deficiencies. Targets for the design of new drugs and a rationale provided for targeting enzymes of the trypanothione cycle are described. Biochemical aspects of the cycle and the currently investigated target trypanothione reductase are discussed as are the several classes of inhibitors and their *in vitro* potencies. Evidence is provided for considering the tryparedoxins as a new target for antiprotozoal chemotherapy and a summary of glutathione-based inhibitors with significant *in vitro* activity is presented.

12439 **Karanja, W.M., Mdachi, R.E. & Murilla, G.A., 2002.** A competitive enzyme-linked immunosorbent assay for diminazene. *Acta Tropica*, **84** (2): 75–81.

Karanja: KETRI, PO Box 362, Kikuyu, Kenya.

Diminazene aceturate has remained a very important therapeutic drug for trypanosomosis in cattle, sheep and goats since its introduction into the market in 1955. Despite its continued use, the methods available for its detection in body fluids are lengthy and inefficient for routine monitoring of drug levels in treated animals. A competitive enzyme linked immunosorbent assay (ELISA) has now been developed and optimized for the detection of diminazene in bovine serum. In the assay, diminazene in the test samples and that in a newly developed diminazene-horseradish peroxidase conjugate compete for antibodies to diminazene raised in rabbits and immobilized on a microtitre plate. Tetramethylbenzidine-hydrogen peroxide (TMB/H_2O_2) is used as chromogen-substrate system. The assay has a detection limit of 0.8 ng/ml of serum with a high specificity for diminazene. Cross-reactivity with either homidium bromide and quinapyramine sulphate/chloride of 0.0004 percent is negligible while that with isometamidium chloride is 0.71 percent. The assay was able to detect diminazene levels in normal Boran steers for at least two weeks after intramuscular injection with the drug at a dose of 3.5 mg/kg bw. The assay will be useful in monitoring diminazene use, and development of resistance in trypanosomosis endemic areas.

12440 **Marasco, C.J. Jr., Kramer, D.L., Miller, J., Porter, C.W., Bacchi, C.J., Rattendi, D., Kucera, L., Iyer, N., Bernacki, R., Pera, P. & Sufrin, J.R., 2002.** Synthesis and evaluation of analogues of 5′-([(Z)-4-amino-2-butenyl]methylamino)-5′-deoxyadenosine as inhibitors of tumor cell growth, trypanosomal growth, and HIV–1 infectivity. [*T. brucei*] *Journal of Medicinal Chemistry*, **45** (23): 5112–5122.

Sufrin: Department of Pharmacology and Therapeutics, Grace Cancer Drug Center, Roswell Park Cancer Institute, Buffalo, New York 14263 USA. [Janice.sufrin@roswellpark.org]

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

12441 **Gibson, W., 2002.** Will the real *Trypanosoma brucei rhodesiense* please step forward? *Trends in Parasitology*, **18** (11): 486–490.

Gibson: School of Biological Sciences, University of Bristol, Bristol, BS8 1UG, UK. [w.gibson@bristol.ac.uk]

The sleeping sickness trypanosomes *Trypanosoma brucei rhodesiense* and *T. brucei gambiense* are morphologically indistinguishable from each other and from *T. brucei brucei*, which does not infect humans. The relationships between these three subspecies have been controversial. Several years ago, the characterization of *T. brucei gambiense* was reviewed in an attempt to clarify and draw together the results, and to put them in the context of the biology of the organism. The discovery of a gene associated with human-serum resistance in *T. brucei rhodesiense* and the consequent reappraisal of the identity of this trypanosome prompt this companion article.

12442 **Momen, H., 2002.** Molecular taxonomy of trypanosomatids: Some problems and pitfalls. *Archives of Medical Research*, **33** (4): 413–415.

Momen: editor, Bulletin of the World Health Organization, EIP/IMD, WHO, 20 Avenue Appia, 1211 Geneva, 27, Switzerland. [momenh@who.int]

Trypanosomatids appear to have attracted the particular attention of taxonomists and a wealth of data from studies using a variety of techniques is available. There are, however, some potential pitfalls in such studies. A general problem in the taxonomy of trypanosomatids is that only a small amount of the true diversity is reflected in the limited number of isolates identified and studied from this family. An associated problem is that of confusion over the identity of the organisms. Other concerns include the problems of long branch attractions and mutational saturation, the loss of phylogenetic signal from the accumulation of overlapping mutations, and the fact that gene phylogeny cannot be equated with organism phylogeny and that organisms are more than just the sum of their genes. Additional complications can occur due to numerous cases of horizontal gene transfer between organisms. The use of a large sample of recent isolates from the field is also important so that the true diversity of these organisms is reflected in these studies, and bias due to selection, contamination, and misidentification when isolates have been maintained for long periods in culture is eliminated.

12443 **Van der Heyden, N. & Docampo, R., 2002.** Significant differences between procyclic and bloodstream forms of *Trypanosoma brucei* in the maintenance of

their plasma membrane potential. *Journal of Eukaryotic Microbiology*, **49** (5): 407–413.

Docampo: Laboratory of Molecular Parasitology, Department of Pathobiology, University of Illinois at Urbana–Champaign, 2001 S. Lincoln Avenue, Urban IL 61802, USA. [rodoc@uiuc.edu]

(c) LIFE CYCLE, MORPHOLOGY, BIOCHEMICAL AND MOLECULAR STUDIES

[See also **25**: no. 12395]

12444 **Boibessot, I., Turner, C.M.R., Watson, D.G., Goldie, E., Connel, G., McIntosh, A., Grant, M.H. & Skellern, G.G., 2002.** Metabolism and distribution of phenanthridine trypanocides in *Trypanosoma brucei*. *Acta Tropica*, **84** (3): 219–228.

Grant: Bioengineering Unit, University of Strathclyde, 106 Rottenrow East, Wolfson Centre, Glasgow G4 0NW, UK. [m.h.grant@strath.ac.uk]

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Rudenko: The Peter Medawar Building for Pathogen Research, University of Oxford, Oxford OX1 3SY, UK. [gloria.rudenko@medawar.ox.ac.uk]

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Schneider: Department of Biology/Zoology, University of Fribourg, Chemin du Musée 10, CH–1700 Fribourg, Switzerland. [andre.schneider@unifr.ch]

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Bringaud: Laboratoire de Parasitologie Moléculaire, Université Victor Segalen Bordeaux II, UMR–5016 CNRS, 33076 Bordeaux cedex, France.

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associated with polymorphic subtelomeric regions of *Trypanosoma brucei* (vol 1, pg 137, 2002). (Correction) *Eukaryotic Cell*, **1** (2): 315.

Bringaud: Laboratoire de Parasitologie Moléculaire, Université Victor Segalen Bordeaux II, UMR–5016 CNRS, 33076 Bordeaux cedex, France.

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Alexandrov: Department of Physical Biochemistry, Max Planck Institute for Molecular Physiology, Otto Hahn Strasse 11, 44227 Dortmund, Germany. [kirill.alexandrov@mpi-dortmund.mpg.de]

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Yokoyama: Department of Chemistry, University of Washington, Seattle, WA 98195, USA. [koheiy@u.washington.edu]

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Camacho: Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, UK.

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Hill: Department of Microbiology, Meharry Medical College, Nashville, Tenessee 37208–3599, USA.

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Colman: Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Victoria 3050, Australia.

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McCulloch: The Wellcome Centre for Molecular Parasitology, The Anderson College, University of Glasgow, 56 Dumbarton Road, Glasgow G11 6NU, UK. [rmc9z@udcf.gla.ac.uk]

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Borst: The Netherlands Cancer Institute, Division of Molecular Biology and Center for Biomedical Genetics, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. [PBorst@nki.nl]

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Brimacombe: School of Life Sciences (Chemistry), Carnelly Building, University of Dundee, Dundee, DD1 4HN, UK. [j.s.brimacombe @dundee.ac.uk]

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Turrens: Department of Biomedical Sciences, University of South Alabama, Mobile, AL, USA. [jturrens@usouthal.edu]

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Hoheisel: Division of Functional Genome Analysis, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 506, 69120 Heidelberg, Germany. [j.hoheisel@dkfz.de]

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Beattie: Department of Biochemistry and Molecular Pharmacology, PO Box 9142, West Virginia University School of Medicine, Morgantown, WV 26506–9142, USA. [dbeattie@hsc.wvu.edu]

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Foster: Section on Functional Neuroanatomy, National Institute of Mental Health, 36 Convent Drive, Building 36, Room 2D15, Bethesda, MD 20892–4070, USA. [jaf@codon.nih.gov]

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Kita: Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, 7–3–1 Hongo, Bunkyo–ku, Tokyo 113–0033, Japan. [kitak@m.u-tokyo.ac.jp]

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García-Salcedo: Laboratory of Molecular Parasitology, ULB – Institute of Molecular Biology and Medicine, 12 Rue des Professeurs Jeener et Brachet, B–6041 Gosselies, Belgium. [jantonio@dbm.ilb.ac.be]

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Stuart: Seattle Biomedical Research Institute, Seattle, Washington 98109, Department of Pathobiology, University of Washington, Seattle, Washington 98199, USA.

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Englund: Department of Biological Chemistry, John Hopkins Medical School, Baltimore, MD 21205, USA. [penglund@jhmi.edu]

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Wang: Department of Pharmaceutical Chemistry, UCSF, 513 Parnassus Avenue, San Francisco, CA 94143–0446, USA. [ccwang@cgl.ucsf.edu]

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Michaeli: Faculty of Life Sciences, Bar–Ilan University, Ramat–Gan 52900, Israel. [michaes@mail.biu.ac.il]

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Magez: Laboratory of Cellular Immunology, Free University of Brussels/Flemish Interuniversity Institute for Biotechnology, Paardenstraat 65, 1640 Sint Genesius Rode, Belgium. [stmagez@vub.ac.be]

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Maslov: Department of Biology, University of California, Riverside, CA 92521, USA. [maslov@ucrac1.ucr.edu]

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Gleeson: Department of Biochemistry and Molecular Biology, University of Melbourne, Victoria 3010, Australia. [pgleeson@unimelb.edu.au]

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Vassella: Institut für Zellbiologie, Universität Bern, CH–3012 Bern, Switzerland. [erik.vassella@izb.unibe.ch]

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Field: Wellcome Trust Laboratories for Molecular Parasitology, Department of Biological Sciences and Centre for Molecular Microbiology and Infection, Imperial College of Science, Technology and Medicine, Exhibition Road, London SW7 2AY, UK.

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Williams: Department of Microbiology, Witebsky Center for Microbial Pathogenesis and Immunology, State University of New York at Buffalo, Buffalo, NY 14214, USA. [nwl@acsu.buffalo.edu]

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Clayton: ZMBH, Im Neuenheimer Feld 282, D–69120 Heidelberg, Germany. [cclayton@zmbh.uni-heidelberg.de]

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Krauth-Siegel: University of Heidelberg, Neuenheimer Feld 506, 69120 Heidelberg, Germany. [krauth-siegel@urz.uni-heidelberg.de]

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Schmidt: Institute für Pharmazeutische Biologie, Heinrich–Heine–Universtät Dusseldorf, Universitätsstrasse 1, Geb.26.23, 40225 Düsseldorf, Germany. [schmidtt@uni-duesseldorf.de]

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Steenkamp: Division of Chemical Pathology, Faculty of Health Sciences, University of Cape Town, Observatory 7935, South Africa. [daan@chempath.uct.ac.za]

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Cross: Laboratory of Molecular Parasitology, The Rockefeller University, Box 185, 1230 York Avenue, New York, NY 10021–6399, USA. [george.cross@rockefeller.edu]

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Ferguson: Division of Biological Chemistry and Molecular Microbiology, The Wellcome Trust Biocentre, University of Dundee, Dundee DD1 6NR, UK. [m.a.j.Ferguson@dundee.ac.uk]

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Matthews: School of Biological Sciences, University of Manchester, Manchester M13 9PT, UK. [keith.matthews@man.ac.uk]

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Borst: Department of Molecular Biology and Centre of Biomedical Genetics, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. [p.borst@nki.nl]

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Englund: Department of Biological Chemistry, John Hopkins Medical School, Baltimore, MD 21205, USA. [penglund@jhmi.edu]

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Gull: Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK. [keith.gull@pathology.ox.ac.uk]

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