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TSETSE AND TRYPANOSOMIASIS INFORMATION

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Austria

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TSETSE AND TRYPANOSOMIASIS INFORMATION

The Tsetse and Trypanosomiasis Information periodical 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), the British Government's Department for International Development (DFID) and the Institute of Tropical Medicine (ITM), Antwerp.

The half-yearly periodical 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 two 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 James Dargie, Brunnstubengasse 43, 2102 Bisamberg, Austria (tel. +43 2262 61735; e-mail j.dargie@aon.at).

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

Distribution dates and copy deadlines

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

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

ABBREVIATIONS USED IN *TTI*

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 Organisation
WHO	World Health Organization

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SECTION A – NEWS

THE 29TH INTERNATIONAL SCIENTIFIC COUNCIL FOR TRYPANOSOMIASIS RESEARCH AND CONTROL (ISCTRC) CONFERENCE

The International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) is a statutory organ of the Africa Union and with its Secretariat located in the International Bureau for Animal Resources (IBAR) in Nairobi. The Council was established in 1948 to control tsetse transmitted African human and animal trypanosomiasis by coordinating research, capacity building and timely dissemination of necessary information.

The Council organized the first ISCTRC biennial Conference a year after it was established and continues to date, bringing together stakeholders that are responsible for management of tsetse and trypanosomiasis problem in the continent, international organizations and private sector to facilitate development of joint strategies. The 29th Conference promises to be a watershed Conference in the history of the Council. The Conference convenes for the first time in Luanda, Angola, from 1 to 5 October 2007 where 100 oral and poster presentations will be made to over 300 participants that work in the tsetse-trypanosomiasis research and development domain in Africa. The themes for the 29th Conference will focus on activities of Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), Human Trypanosomiasis, Animal Trypanosomiasis, Capacity Building, Networking and Related Land Use and Environment. Each theme will be preceded by keynote speech in specialised area of knowledge that together with presentations will be discussed to develop specific time bound recommendations to improve the disease management approaches.

The Conference will be attended by representatives of African member states, research organizations, non governmental organisations, international organizations and private sector that jointly implement the recommendations of the scientific Conference.

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BOOK PUBLICATIONS

1. **Shaw, A., Hendrickx, G., Gilbert, M., Mattioli, R., Codjia, V., Dao, B., Diall, O., Mahama, C., Sidibé, I. & Wint, W., 2006.** *Mapping the benefits: a new decision tool for tsetse and trypanosomiasis interventions.* Research Report. Department for International Development, Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK, and Programme Against Animal Trypanosomiasis, Food and Agriculture Organization of the United Nations, Rome, Italy.

Trypanosomiasis is one of the greatest constraints to animal health in sub-Saharan Africa. It also affects human health, agricultural output and land use. However, despite its importance, decisions relating to trypanosomiasis control are often made on the basis of very limited information, which may lead to some extremely costly errors. The authors of this study address the decision-making process by innovatively combining the analytical potential of geographic information systems with production systems analysis and economics. The study presents economic variables in a way that is accessible to both decision-makers and those concerned with trypanosomiasis control in the field, and which should also provide insights into aspects of the control of other animal and crop health problems.

This study is a joint publication: as one of the "blue series" of Research Reports from the Department for International Development–Animal Health Programme (DFID–AHP), and as a Position Paper from the Programme Against African Trypanosomiasis (PAAT). This joint publication also reflects the shared funding of the work by the Food and Agriculture Organization of the United Nations (FAO), the International Atomic Energy Agency (IAEA) and DFID–AHP. It complements the other FAO publications in the highly successful PAAT Technical and Scientific Series as well as the three related publications in the AHP's blue series. It is also the first publication in any of these series to appear in both English and French.

The purpose of this study was to investigate the feasibility of linking quantitative economic variables to a geographical information system (GIS) spatial framework in order to provide new insights and reinforce the decision-making process for tsetse and trypanosomiasis (T&T) interventions. Hitherto, GIS studies have mapped a series of ecological, demographic and socio-economic indicators, but have stopped short of mapping a derived measure quantified in monetary units. Furthermore, the economic aspects of T&T control have historically been dealt with separately from their other effects, with results usually expressed in terms of benefit–cost ratios or extra income per head of livestock. Even when they have been expressed in terms of US dollars per square kilometre (US$/km^2) these results have not been mapped; instead they have been used as inputs for benefit–cost type analyses. In contrast, the approach developed here combines – for the first time – economic herd models with mapping of both breed/production systems and the expansion of livestock populations under various scenarios.

The first phase of the work tackled Benin, Ghana and Togo. The second phase extended the work to cover parts of Burkina Faso and Mali. A range of standardised livestock population, production and price data were collected at country, province and district level from each of these five countries, together with the most recent livestock population, cropping and disease data. These were amalgamated with the corresponding data layers derived and adapted from the Programme Against African Trypanosomiasis Information System (PAAT-IS). At the mapping stage, the data were extrapolated to cover the areas

around the five countries, notably including Côte d'Ivoire for which considerable data already existed in the authors' archives and databases.

Four breed/production systems were defined and mapped: a predominantly taurine system with minimal use of animal traction; a crossbred taurine×zebu system with moderate use of animal traction; a crossbred zebu×taurine system with very high use of animal traction; and a zebu system with moderate animal traction use. By combining these definitions with the new data and the PAAT-IS data layers, a new distribution map was produced that linked trypanotolerant and susceptible cattle breeds to production systems.

Existing information on the disease's impact on cattle production parameters was incorporated in a series of deterministic herd models, which projected the cattle populations and calculated the income derived from them over a period of 20 years. These modelled the situation both with and without the presence of trypanosomiasis in the "core" population area, (where cattle populations are currently located) and in the 'export' areas (into which cattle populations are likely to expand over the period analysed). Thus 2 × 2 or four interrelated models were produced for each cattle breed/production system. For the purposes of the study, each herd model had two main outputs: an estimate of cattle population growth and an estimate of income. Income from cattle was calculated as the value of meat, milk, animal traction, and herd growth less production costs. By comparing income in the absence and presence of trypanosomiasis, the potential benefits of T&T interventions could be estimated for the different cattle breed/production systems over the 20-year period. These were then discounted to their present value and converted to a single US$ amount, expressed as benefits per head of cattle present at the end of the time period and split between those generated by cattle remaining in the core area and those arising from cattle populations that had expanded into export areas.

The final part of the study mapped livestock population distributions. By applying the estimates of the cattle population growth rates provided by the herd models to maps of the current distribution of cattle, it was possible to map the estimated distribution of livestock in 20 years' time. This future population was compared to the land's estimated carrying capacity to identify those areas where cattle numbers exceeded resources available to sustain them. For these situations, a step-wise spatial expansion model was applied to show how 'excess' cattle populations might spread into nearby areas where grazing was available. The cattle populations that remained in their original locations were those modelled as the core population; the cattle that spread to new areas were defined as the export herd. This spatial expansion model made it possible to quantify the potential benefits of the removal of trypanosomiasis from areas into which new cattle populations would migrate. The need to find ways to estimate the benefits from this type of expansion of livestock production has been a major unresolved issue in analysing the T&T problem.

The results of the work are depicted in a series of maps throughout the text, culminating in the map shown as the frontispiece to this report. This map illustrates the geographical distribution of the potential US$ benefits from the removal of trypanosomiasis throughout the zone studied. As with all modelling and mapping exercises, care must be taken not to interpret the figures as absolute values providing exact answers, but to keep in mind that combining a number of estimates in this way will always generate results that include a greater or lesser margin of error. That said, the resulting maps very clearly illustrate that combining economic and biophysical variables adds a dimension beyond that which has previously been mapped. The summary map highlights the enormous potential benefits to be gained over the 20-year timeframe from those areas where there is already a high reliance on

draught power: in the northern fringes of the tsetse distribution. It also shows that, within this time period, significant benefits from the removal of trypanosomiasis are unlikely to be gained in land to the south of this area for two reasons. Firstly, the cattle numbers are too low, even after expansion of cattle populations into new areas has been accounted for and, secondly, this area makes limited use of animal traction, even taking into account the potential for a significant increase in its use if the constraint of trypanosomiasis were removed.

The complexity of the analysis imposed a number of limitations that point to areas where either the modelling approach or the quality of the data could be improved. In particular, it was impractical to model more than the four production systems considered; these in themselves required 16 herd models, with the resulting US$ values mapped for 12 categories of cattle. The data on the effects of the disease on cattle production parameters are mostly based on in-depth studies conducted in relatively small localities. This inevitably adds more uncertainties about their extrapolation to large areas and slightly different production systems. Another tricky aspect to the study was in determining the level of tsetse challenge and the prevalence of trypanosomiasis in the cattle populations. In particular, the levels of challenge in the areas that are on the limits of tsetse distribution need more study. These aspects were factored into the calculations indirectly, as general effects of the disease within each production system. Finally, the economic models are also highly sensitive to the use made and the value of animal traction, and more fieldwork on these aspects would make the calculations more precise. Nevertheless, the results are in line with those found in other studies and modelling exercises.

From the point of view of decision-making within the field of T&T interventions, having mapped the benefits the obvious next step is to consider mapping the costs. This would, however, first require undertaking a similar exercise to the current one to combine cost models with spatial data. The regions that show benefits that exceed the costs calculated for different interventions could then be mapped, as could the benefit–cost ratios for the various control options.

Thus, this report provides "proof of concept" that mapping economic benefits in this way does add an extra dimension and new insights to the existing range of mapped variables. It goes beyond simply mapping cattle and tsetse distributions and makes it possible to calibrate the effects of the disease in relation to the key components of livestock incomes and to place a value on income generated in new areas into which livestock populations could expand. By combining a demographic variable with projections of economic benefits for a range of production system layers, and taking account of expansion into new areas, this approach could have wide applicability in the analysis of other production constraints affecting agricultural expansion and productivity.

2. **IAEA-TECDOC 1559.** *Developing methodologies for the use of polymerase chain reaction (PCR) in the diagnosis and monitoring of Trypanosomosis. Final Results of an FAO/IAEA Coordinated Research Project. 2001-2005*

The Animal Production and Health Section of the Joint FAO/IAEA Division has promoted the use of modern nuclear based techniques in diagnosis and control of livestock diseases for the past 20 years. Support for methods exploiting the polymerase chain reaction (PCR) began in 1997 with a Coordinated Research Project (CRP) to develop PCR methods to study and diagnose a range of transboundary diseases affecting livestock. Trypanosomes produce a variety of diseases affecting both animals and man. The agents for the disease and immunology of the pathogen/host relationship are

very complex. Diagnosis of the disease has relied on more conventional methods such as direct assessment of organisms through a microscope and serological tests, such as complement fixation and haemagglutination, looking at the serum from animals to detect antibodies. The sensitivity and specificity of the tests have never proven ideal to allow either sufficiently low levels of organisms to be identified or to determine exactly which strain of trypanosome were causing the disease.

The work described in this publication examines many features of the use of PCR in the detection of trypanosomes. These include drug treatment of animals and man where it is imperative to understand whether an individual is infected at all, and to what extent that infection has progressed. The PCR offers a solution to the detection of organisms since theoretically it has an incredible sensitivity, since minute amounts of nucleic acid in samples can be amplified. The specificity of PCR also resides in the identification of absolutely specific parts of a genome and their detection. Theoretically the PCR offers the maximum diagnostic sensitivity and specificity profile. In practice there are many factors that affect the theoretical limits of the PCR. Sample taking, handling, extraction and processing all affect the sensitivity from field samples and reduce the diagnostic potential. The high specificity inherent in using specific probes means that the test is expensive where many probes have to be used for ultimate identification of a trypanosome. Working protocols for the use of specific probes have been determined. The handling and extraction of samples has been optimized. The use of universal primers for the detection of all trypanosomes has been examined with promising results. Validation of methods is paramount and this has been addressed. One of the key benefits from this CRP, as in all others, has been the cooperation generated between scientists from many countries. The links between more established laboratories with expertise and those just starting were forged and enabled accelerated development. The quality of work in all the laboratories has increased generally through the CRP. Such PCR-based tests will allow an unequivocal estimation of the effect of interventions in the eradication of trypanosomosis, such as those involving the sterile insect technology (SIT) in the tsetse control programmes. The officer responsible for compiling this publication was J.R. Crowther of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

Papers

Molecular detection of *Trypanosoma* infection: PCR and PCR-ELISA techniques, *M. F.W. Te pas*

New diagnostics for the detection of animal trypanosomoses, *A.G. Luckins*

Molecular markers for the different (sub)-species of the Trypanozoon subgenus, *F. Claes and P. Büscher*

Application of PCR/CSF for stage determination and therapeutic decision in human African trypanosomosis in Côte d'Ivoire, *V. Jamonneau, P. Solano, A. Garcia, V. Lejon, N. Djé, T.W. Miezan, P. N'Guessan, G. Cuny, P. Büscher*

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Detection of PCR products via oligochromatography (dipsticks), *F. Claes*

UPCOMING CONTRIBUTIONS TO THE PAAT TECHNICAL AND SCIENTIFIC SERIES

1. **Cecchi, G., Mattioli, R.C., Slingenbergh, J., de la Rocque, S. & Feldmann, U., 2007.** *Standardizing land cover mapping for tsetse and trypanosomiasis decision making*

In this paper the Land Cover Classification System (LCCS), developed by the Food and Agriculture Organization (FAO) and the United Nations Environment Programme (UNEP), is proposed as a tool to harmonize land cover mapping exercises carried out in the context of tsetse and trypanosomiasis (T&T) research and control. The potential of land cover maps to describe and predict tsetse habitat at different resolutions is also explored.

In chapter one, the LCCS-compliant Global land cover of Africa of the year 2000 and the predicted areas of suitability for tsetse provided by the PAAT - Information System (PAAT-IS) are matched to study the broad patterns of the association between land cover and the three groups of tsetse flies (i.e. *fusca, palpalis* and *morsitans*).

In chapter two, a standardized legend for land cover mapping in T&T decision making is proposed, which is based on the products and methodology developed by the FAO-Africover project. The 26-class legend derives from thematic aggregation of more than 500 land cover classes present in the original multi-purpose Africover maps of eight T&T affected countries (i.e. Burundi, Democratic Republic of the Congo, Kenya, Rwanda, Somalia, Sudan, Uganda and United Republic of Tanzania). The legend is used to describe tsetse habitat across several countries in a harmonised and coherent manner. A review of the literature allowed matching of standardized land cover classes and suitability for tsetse. The practical and conceptual difficulties posed by the validation of the estimated classes of suitability are discussed; in this regard, one method linking land cover datasets at different resolutions gave positive results.

In chapter three one case study, namely Uganda, illustrates how country maps compliant with LCCS can be analysed in more detail and customised to better meet the requirements of tsetse habitat mapping. In this section, a detailed description of the land cover classes is provided, including key factors for estimating tsetse habitat suitability.
Finally, the standardized description of the land cover classes according to LCCS and the tables of class aggregation are provided as annexes.
Standardization of land cover mapping is an important step towards the harmonization of the Information Systems (IS) and of the GIS-based Decision Support Systems (DSS) for trypanosomiasis intervention. The adoption of LCCS within T&T control programmes will greatly benefit regional cooperation and facilitate the use of existing and upcoming land cover maps.

The high resolution of the datasets discussed in the report (within a range of scales from 1 : 200 000 to 1 : 50 000) will make possible the production of a new generation of risk maps, based on a deeper understanding of the landscape and environmental dynamics that drive the distribution of tsetse in Africa. Habitat modifications are increasingly induced by human actions, either at global scale, as in the case of the climatic change, or at local scale, like in the processes of urbanization and agricultural expansion. The challenges posed in the future by trypanosomiasis are likely to be shaped by those drivers to the extent that no appropriate intervention can possibly be contemplated without considering them.

2. *Spatial datasets for the management of the trypanosomiasis problem: an environmental approach*

The present note describes work in progress within the PAAT Information System for the identification and dissemination of the best global GIS datasets available in the public domain. This activity aims at improving and harmonising planning, implementation and evaluation of tsetse and trypanosomiasis (T&T) interventions.

There is an increasing amount of spatially explicit information freely accessible through internet that has proved to be very useful in assisting all aspects of the T&T decision making process. Still, the potential of such a wide and dynamic source of information has yet to be fully exploited. Field project managers/planners, though recognizing the importance of GIS for targeting and streamlining operations, may not be aware of the existence of datasets

that could greatly contribute to cost-saving, a more efficient use of financial resources and eventually leading to more effective interventions. In other instances there may be a lack of adequate capacity for handling and integrating the GIS techniques into the project cycle.

During a first phase, a thorough review of public domain global geospatial datasets was carried out. Data were selected in consideration of their relevance for T&T decision making, spatial resolution (i.e. scale), accuracy and update. The major products identified concern tsetse distributions, human and livestock population densities, agro-ecological zones, protected areas, digital elevation models (DEM) and satellite imagery. For each data layer a short description is provided, followed by information on data access and related web sites. Preliminary collated information, in form of a draft document that includes the review of GIS datasets, was shared with PAAT partners during PAAT statutory meetings and via e-mail.

In the ongoing second phase, the selected datasets are processed and analysed to support an informed T&T decision making. This activity would greatly benefit from a closer collaboration with PAAT partners and their feedback on implementation of T&T field operations. T&T intervention operators should provide suggestions to orient the analysis according to projects' requirements.

The selected datasets were presented in greater detail during an Interactive Training Workshop held at FAO-HQ in Rome from 27 November - 8 December 2006. The workshop addressed the issue of harmonization of GIS-based Decision Support Systems (DSSs) and Information Systems (ISs) in T&T intervention and was attended by approximately 20 participants, including key GIS specialists from tsetse affected countries, FAO staff from different divisions and external experts. Participants acknowledged the importance of the initiative and recommended PAAT to continue supporting partners in the affected countries in the fields of information management, spatial analysis and harmonization of methodologies.

Ideally, the review of global datasets should be followed by a section devoted to national and local datasets. A call for short contributions to be published as case-studies within the PAAT Technical and Scientific Series was opened to PAAT partners and GIS specialists who are active in planning and implementation of T&T intervention projects. Particularly needed are notes addressing the following issues:

- review, collation, harmonisation and new inside analysis of available/historical entomological and parasitological datasets using GIS/Database applications;
- planning and implementation of baseline entomological/parasitological surveys to complement/improve historical knowledge;
- establishment of national Information Systems for managing T&T related information.

THE WHO/TDR PROGRAMME

1. **Foundation for Innovative New Diagnostics and WHO collaborate to improve diagnosis of sleeping sickness with a Gates Foundation grant.**

The Foundation for Innovative New Diagnostics (FIND) and the World Health Organization (WHO), with a grant from the Bill & Melinda Gates Foundation, announced that they will begin work on the development and evaluation of new diagnostic tests for human African trypanosomiasis, also known as sleeping sickness. African sleeping sickness, a major public

health threat in sub-Saharan Africa, spreads among people bitten by the tsetse fly and is fatal unless treated. Because early-stage infection produces few symptoms, it is thought that only 10 percent of patients with the disease are accurately diagnosed. FIND and the World Health Organization will collaborate in seeking to identify, test and implement diagnostics that will increase the likelihood of early detection of HAT and the opportunity for treatment.

"The spread of human African trypanosomiasis has reached epidemic proportions in regions of Africa. There is clearly a great need for a simple, accurate and cost-effective way to diagnose this disease so that it can be better treated and controlled," said Dr Giorgio Roscigno, CEO of FIND. "FIND is committed to identifying and implementing diagnostics for infectious diseases, and we look forward to securing partnerships and initiating field testing."

"Existing diagnostics for sleeping sickness are difficult to implement in remote, impoverished settings," said Dr Jean Jannin and Dr Pere Simarro, from the Neglected Tropical Diseases Control Department of the World Health Organization. "We look forward to working with FIND to advance new diagnostic tests that could revolutionize human African trypanosomiasis control."

"Developing point-of-care tests to direct sleeping sickness treatment will greatly simplify patient care, allowing for early case detection, simpler and safer treatment, and higher rates of cure that will improve disease management and could lead to the elimination of the disease as a public health problem," said Thomas Brewer, M.D., senior program officer, Infectious Diseases division, Global Health Program, at the Gates Foundation.

Currently, diagnosis of sleeping sickness is made by serologic examinations followed by microscopy, which is laborious, insensitive and costly. FIND's and WHO's efforts will be focused on developing tools that will be simple to use and effective in the remote field conditions that exist where it is most prevalent. In addition to developing appropriate diagnostic technologies, the objectives of the programme include establishing field research sites for clinical studies and evaluating prototype products.

2. **Revised Fact Sheet on African trypanosomiasis (sleeping sickness)**

Definition of the Disease

Human African Trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease. The parasites concerned are protozoa belonging to the *Trypanosoma* Genus. They are transmitted to humans by tsetse fly (*Glossina* Genus) bites which have acquired their infection from human beings or from animals harbouring the human pathogenic parasites.

Tsetse flies are found in sub-Saharan Africa. Only certain species transmit the disease. Different species have different habitats. They are mainly found in vegetation by rivers and lakes, in gallery-forests and in vast stretches of wooded savannah.

- Sleeping sickness occurs only in sub-Saharan Africa in regions where there are tsetse flies that can transmit the disease. For reasons that are so far unexplained, there are many regions where tsetse flies are found, but sleeping sickness is not.
- The rural populations living in regions where transmission occurs and which depend on agriculture, fishing, animal husbandry or hunting are the most exposed to the bite of the tsetse fly and therefore to the disease.

- Sleeping sickness generally occurs in remote rural areas where health systems are weak or non-existent. The disease spreads in poor settings. Displacement of populations, war and poverty are important factors leading to increased transmission.
- The disease develops in areas whose size can range from a village to an entire region. Within a given area, the intensity of the disease can vary from one village to the next.

Human African Trypanosomiasis takes two forms, depending on the parasite involved:

- *Trypanosoma brucei gambiense* (*T. b. g.*) is found in west and central Africa. This form represents more than 90 percent of reported cases of sleeping sickness and causes a chronic infection. A person can be infected for months or even years without major signs or symptoms of the disease. When symptoms do emerge, the patient is often already in an advanced disease stage when the central nervous system is affected.
- *Trypanosoma brucei rhodesiense* (*T. b. r.*) is found in eastern and southern Africa. This form represents less than 10 percent of reported cases and causes an acute infection. First signs and symptoms are observed after a few months or weeks. The disease develops rapidly and invades the central nervous system.

Another form of trypanosomiasis occurs in 15 Central and South American countries. It is known as American trypanosomiasis or Chagas disease. The causal organism is a different species from those causing the African form of the disease.

Animal Trypanosomiasis

Other parasite species and sub-species of the *Trypanosoma* Genus are pathogenic to animals and cause animal trypanosomiasis in many wild and domestic animal species (in cattle the disease is called Nagana, a Zulu word meaning “to be depressed”). Animals can host the human pathogen parasites, especially *T. b. rhodesiense*; thus domestic and wild animals are an important parasite reservoir. Animals can also be infected with *T. b. gambiense*, however the precise epidemiological role of this reservoir is not yet well known. This disease kills animals.

The disease in domestic animals and particularly cattle is a major obstacle to the economic development of the rural areas affected.

Major Human Epidemics

There have been several epidemics in Africa over the last century: one between 1896 and 1906, mostly in Uganda and the Congo Basin, one in 1920 in a number of African countries and the most recent one beginning in 1970. The 1920 epidemic was controlled thanks to mobile teams who organized the screening of millions of people at risk. By the mid 1960s, the disease had almost disappeared. After that success, surveillance was relaxed, and the disease reappeared in several areas over the last thirty years. Recent WHO efforts and those of national control programmes and non-governmental organizations (NGOs) have stopped and begun to reverse the upward trend of new cases.

Geographical Distribution of the Disease

Sleeping sickness threatens millions of people in 36 countries of sub-Saharan Africa. However, only a small fraction of them are under surveillance with regular examination, have access to a health centre that can provide diagnostic facilities, or are protected by vector control interventions.

- In 1986, a panel of experts convened by WHO estimated that some 70 million people lived in areas where disease transmission could take place.
- In 1998, almost 40 000 cases were reported, but this number did not reflect the true situation. It was estimated that between 300 000 and 500 000 more cases remained undiagnosed and therefore untreated.
- During recent epidemic periods, in several villages in the Democratic Republic of Congo, Angola and Southern Sudan, prevalence has reached 50 percent. Sleeping sickness was considered the first or second greatest cause of mortality, even ahead of HIV/AIDS, in those communities.
- By 2005, surveillance had been reinforced and the number of new cases reported throughout the continent had substantially reduced; between 1998 and 2004 the figures for both forms of the disease together fell from 37 991 to 17 616. The estimated number of cases is currently between 50 000 and 70 000

Progress in Disease Control

- In 2000, WHO established a public-private partnership with Aventis Pharma (now sanofi-aventis) which has enabled the creation of a WHO surveillance team, providing support to endemic countries in their control activities and the supply of drugs free of charge for the treatment of patients.
- In 2006, success in curbing the number of sleeping sickness cases has encouraged a number of private partners to sustain WHO's initial effort towards the elimination of the disease as a public health problem.

Current Situation in Endemic Countries

The prevalence of the disease differs from one country to another as well as in different parts of a single country. In 2005, major outbreaks have been observed in Angola, the Democratic Republic of Congo and Sudan. In Central African Republic, Chad, Congo, Côte d'Ivoire, Guinea, Malawi, Uganda and United Republic of Tanzania sleeping sickness remains an important public health problem. Countries such as Burkina Faso, Cameroon, Equatorial Guinea, Gabon, Kenya, Mozambique, Nigeria, Rwanda, Zambia and Zimbabwe are reporting fewer than 50 new cases per year. In countries such as Benin, Botswana, Burundi, Ethiopia, Gambia, Ghana, Guinea Bissau, Liberia, Mali, Namibia, Niger, Senegal, Sierra Leone Swaziland and Togo transmission seems to have stopped and no new cases have been reported for several decades. Nonetheless, it is difficult to assess the current situation in a number of endemic countries because of a lack of surveillance and diagnostic expertise.

Infection and Symptoms

The disease is transmitted through the bite of an infected tsetse fly. At first the trypanosomes multiply in subcutaneous tissues, blood and lymph. In time, the parasites cross the blood-brain barrier to infect the central nervous system. The process can take years with *T.b. gambiense*.

- Mother-to-child infection: the trypanosome can cross the placenta and infect the fetus.
- Mechanical transmission is possible. However, it is difficult to assess the epidemiological impact of transmission through other blood-sucking insects.
- Accidental infections have occurred in laboratories due to pricks from contaminated needles.

The first stage of the disease, known as a haemolymphatic phase, entails bouts of fever, headaches, joint pains and itching. The second stage, known as the neurological phase, begins when the parasite crosses the blood-brain barrier and invades the central nervous system. In general this is when the signs and symptoms of the disease appear: confusion, sensory disturbances and poor coordination. Disturbance of the sleep cycle, which gives the disease its name, is an important feature of the second stage of the disease. Without treatment, sleeping sickness is fatal.

Disease Management

Disease management is performed in three steps:

- Screening for potential infection. This involves the use of serological tests and/or checking for clinical signs - generally swollen cervical glands.
- Diagnosis shows whether the parasite is present.
- Staging to determine the state of progression of the disease entails examination of cerebro-spinal fluid obtained by lumbar puncture and is used to determine the course of treatment.

Diagnosis must be made as early as possible and before the neurological stage in order to avoid complicated, difficult and risky treatment procedures.

The long, asymptomatic first stage of *T.b. gambiense* sleeping sickness is one of the factors that requires the use of exhaustive active screening of the population at risk in order to identify patients at an early stage and reduce transmission. Exhaustive screening of exposed populations requires a major investment in human and material resources. In Africa such resources are often scarce, particularly in remote areas where the disease is mostly found. As a result, many infected individuals may die before they can ever be diagnosed and treated.

Treatment

The type of treatment depends on the stage of the disease. The drugs used in the first stage of the disease are less toxic, easier to administer and more effective. The earlier the identification of the disease, the better the prospect of a cure. Treatment success in the second stage depends on a drug that can cross the blood-brain barrier to reach the parasite. Such drugs are quite toxic and complicated to administer. Four drugs are registered for the treatment of sleeping sickness and provided free of charge to endemic countries through a WHO private partnership with sanofi-aventis (pentamidine, melarsoprol and eflornithine) and Bayer AG (suramin).

First stage treatments

- Pentamidine: discovered in 1941, used for the treatment of the first stage of *T. b. gambiense* sleeping sickness. Despite a few undesirable effects, it is well tolerated by patients.
- Suramin: discovered in 1921, used for the treatment of the first stage of *T. b. rhodesiense*. It provokes certain undesirable effects, in the urinary tract and allergic reactions.

Second stage treatments

- Melarsoprol: discovered in 1949, it is used in both forms of infection. It derives from arsenic and has many undesired side effects. The most dramatic being a reactive encephalopathy (encephalopathic syndrome) which can be fatal (3 percent to 10 percent). An increase of resistance to the drug has been observed in several foci particularly in central Africa.
- Eflornithine: this molecule, less toxic than melarsoprol, was registered in 1990. It is only effective against *T. b. gambiense*. It is an alternative to melarsoprol treatment. The regimen is strict and difficult to apply.

The resurgence of sleeping sickness since the 1970s led WHO to reinforce its Human African Trypanosomiasis programme. The objective is to coordinate activities in endemic countries and mobilize a wide range of partners.

The WHO Programme provides support and technical assistance to national control programmes. A network has been established including donor countries, private foundations, NGOs, regional institutions, research centres and universities to participate in surveillance and control, and to undertake research projects for the development of new drugs and diagnostic tools.

The objectives of the WHO Programme are to:

- Strengthen and coordinate control measures and ensure field activities are sustained;
- Strengthen existing surveillance systems;
- Support monitoring of treatment and drug resistance through the network;
- Develop information database and implement training activities.

- Promote inter-agency collaboration with the Food and Agriculture Organisation (FAO) and the International Atomic Energy Agency (IAEA). This agency is dealing with vector control through flies males made sterile by radiation. In addition there is a joint Programme Against African Trypanosomiasis (PAAT) including WHO (human health), FAO (animal health) and IAEA (vector control).

THE FAO/IAEA PROGRAMME

Food and Environmental Protection Section: Work on Quality Control of Trypanocidal Drugs

African trypanosomiasis is a severe disease that is fatal if left untreated. The conventional and most prominent method to combat trypanosomiasis is by chemotherapy. Every year some 35 million doses of trypanocides are administered to domestic ruminants. Several reports indicate the widespread phenomenon of counterfeit and poor quality drugs of isometamidium based trypanocides in sub-Saharan Africa. This has severe implications for both food safety and animal health, posing problems with residues of unspecified, unwanted chemicals and their metabolites in the food chain and the induction of trypanosome resistance, an already widespread phenomenon.

In 2003, the Animal Health Service of the FAO and the International Federation for Animal Health (IFAH) developed a joint concept note on quality assurance/quality control (QA/QC) of trypanocides. The main objective is to pursue internationally and scientifically agreed standards and protocols for QA/QC of trypanocides. The specific objectives include definition of the requirements of analytical quality assurance, establishment of good laboratory practices for chemical analysis, and transfer of the methodologies and technology to laboratories in Africa. Initially, it is proposed to support two regional reference laboratories, one in west Africa and one in the east. Future extensions of this project would hopefully expand the scope to include the development and transfer of methods for QC of other veterinary pharmaceuticals such as anthelmintics, antimicrobials and acaricides/insecticides and for residues of the compounds in animal-derived foods. Discussions are ongoing with the United Nations Industrial Development Organization (UNIDO) and IFAH to secure further funding for the project.

The Agrochemicals Unit of the FAO/IAEA laboratories at Seibersdorf, Austria, and the Department of Pharmaceutical Sciences, Strathclyde Institute for Biomedical Sciences, UK, were selected as partners for the technical aspects of the project. Laboratory work to support this project commenced in 2005. The first technical activity, the validation in Strathclyde and Seibersdorf of an HPLC method for quality control of isometamidium-based trypanocides, has been completed.

Further information on the project can be obtained from the FAO Officer (Raffaele.Mattioli@fao.org) and technical details can be obtained from the FAO/IAEA Agrochemicals Unit (A.Cannavan@iaea.org).

SECTION B - ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

14020. **Delespaux, V. & de Koning, H.P., 2007.** Drugs and drug resistance in African trypanosomiasis. *Drug Resistance Updates*, **10** (1-2): 30-50.

Trypanosomosis Unit, Department of Animal Health, Institute of Tropical Medicine Antwerpen, Nationalestraat 155, B-2000 Antwerp, Belgium, and Institute of Biomedical and Life Sciences, Division of Infection and Immunity, Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, UK. [H.de-Koning@bio.gla.ac.uk].

Despite the many decades of use of most of the current trypanocides, we know little of their mode of action. This may in part be because most of these will act on multiple targets once inside the cell, and they derive their selective action on the parasite from selective accumulation by the pathogen. Loss of this capacity for drug uptake by the trypanosome would thus be a major cause for drug resistance. We here discuss the use of current drugs against human and veterinary African trypanosomiasis, the prevalence, causes and mechanisms of drug resistance and new developments in trypanosomiasis therapy such as the introduction of nifurtimox and DB289.

14021. **Frischknecht, F., 2007.** The skin as interface in the transmission of arthropod-borne pathogens. *Cell Microbiology*. **In press, corrected proof.**

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Animal skin separates the inner world of the body from the largely hostile outside world and is actively involved in the defence against microbes. However, the skin is no perfect defence barrier and many microorganisms have managed to live on or within the skin as harmless passengers or as disease-causing pathogens. Microbes have evolved numerous strategies that allow them to gain access to the layers underneath the epidermis where they either multiply within the dermis or move to distant destinations within the body for replication. A number of viruses, bacteria and parasites use arthropod vectors, like ticks or mosquitoes, to deliver them into the dermis while taking their blood meal. Within the dermis, successful pathogens subvert the function of a variety of skin resident cells or cells of the innate immune system that rush to the site of infection. In this review several interactions with cells of the skin by medically relevant vector-borne pathogens are discussed to highlight the different ways in which these pathogens have come to survive within the skin and to usurp the defence mechanisms of the host for their own ends.

14022. **Garraud, O., Andreu, G., Elghouzzi, M. H., Laperche, S. & Lefrere, J. J., 2007**. Measures to prevent transfusion-associated protozoal infections in non-endemic countries. *Travel Medicine and Infectious Disease*, **5** (2): 110-112.

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To understand the risk of protozoa transmission by blood is critical as: (i) the world has become globalized with extensive travel, and increased immigration; (ii) blood-borne protozoa are common in inter-tropical areas; (iii) protozoa develop biological means to escape hosts' immune systems, together with complicated detection, surveillance, and biological testing; and (iv) life threatening-parasites are inadequately controlled by treatment or prevention. This question is relevant in France, with its non-continental territories, such as French Guiana, located in the Amazon Basin, which is endemic for various *Plasmodium* ssp. responsible for malaria, and for *Trypanosoma cruzi*, which is responsible for Chagas disease. In France, specific questioning of blood donors is haphazard despite the increase in population migration over the last three decades: specific questioning must be emphasized and "at-risk" donors should be identified and subsequently excluded from donation. Donor exclusion alone would only be partially efficient, there is also a need for relevant biological testing of blood donations and in particular for *T. cruzi* through the CE-marked test to organize a coherent prevention policy: precise studies would thus define which blood donations are subjected to this additional qualifying test when available.

14023. **Gourley, S. A., Liu, R. & Wu, J., 2007**. Eradicating vector-borne diseases via age-structured culling. *Journal of Mathematical Biology*, **54** (3): 309-335.

Department of Mathematics, University of Surrey, Guildford, Surrey, GU2 7XH, UK. [s.gourley@surrey.ac.uk].

We derive appropriate mathematical models to assess the effectiveness of culling as a tool to eradicate vector-borne diseases. The model, focused on the culling strategies determined by the stages during the development of the vector, becomes either a system of autonomous delay differential equations with impulses (in the case where the adult vector is subject to culling) or a system of nonautonomous delay differential equations where the time-varying coefficients are determined by the culling times and rates (in the case where only the immature vector is subject to culling). Sufficient conditions are derived to ensure eradication of the disease, and simulations are provided to compare the effectiveness of larvicides and insecticide sprays for the control of West Nile virus. We show that eradication of vector-borne diseases is possible by culling the vector at either the immature or the mature phase, even though the size of the vector is oscillating and above a certain level.

14024. **Huntingford, C., Hemming, D., Gash, J. H. C., Gedney, N. & Nuttall, P. A., 2007.** Impact of climate change on health: what is required of climate modellers? *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **101**(2): 97-103.

Centre for Ecology and Hydrology, Wallingford, Oxfordshire OX10 8BB, UK., The Hadley Centre, Met Office, FitzRoy Road, Exeter, Devon EX1 3PB, UK., Joint Centre for Hydro-Meteorological Research, Met Office, Wallingford, Oxfordshire OX10 8BB, UK., and The Centre for Ecology and Hydrology, Polaris House, North Star Avenue, Swindon, Wiltshire SN2 1EU, UK.

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The potential impacts of climate change on human health are significant, ranging from direct effects such as heat stress and flooding, to indirect influences including changes in disease transmission and malnutrition in response to increased competition for crop and water resources. Development agencies and policy makers tasked with implementing adaptive strategies recognize the need to plan for these impacts. However at present there is little guidance on how to prioritize their funding to best improve the resilience of vulnerable communities. Here we address this issue by arguing that closer collaboration between the climate modelling and health communities is required to provide the focused information necessary to best inform policy makers. The immediate requirement is to create multidisciplinary research teams bringing together skills in both climate and health modelling. This will enable considerable information exchange, and closer collaboration will highlight current uncertainties and hopefully routes to their reduction. We recognize that climate is only one aspect influencing the highly complex behaviour of health and disease issues. However we are optimistic that climate–health model simulations, including uncertainty bounds, will provide much needed estimates of the likely impacts of climate change on human health.

14025. **Jaishankar, R. & Jhonson, C. P., 2006**. Geomatics and public health. *Indian Journal of Public Health*, **50** (1): 24-27.

Indian Institute of Information Technology and Management, Kerala, Thiruvananthapuram, India. [jrnair@iiitmk.ac.in].

Geomatics technology has tremendous potential to address public health issues particularly under the present circumstances of global climate change and climate or technology induced human migration, which result in an increase in the geographical extent and re-emergence of vector-borne diseases. The authors present an overview of the science of geomatics, describe the potential impacts of climate change on vector-borne diseases and review the applications of remote sensing for disease vector surveillance.

14026**. Kennedy P.G., 2007.** Animal models of human African trypanosomiasis-very useful or too far removed? *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **In press, corrected proof**.

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Animal models of human African trypanosomiasis, also known as sleeping sickness, have been used for many years both to investigate disease pathogenesis and to test novel drug therapies. Model systems used have included mice, rats and non-human primates such as monkeys. Whilst such animal models have some definite but unavoidable limitations, it is argued that these are outweighed by their advantages. The latter include the ability to investigate disease pathogenesis mechanistically and the mechanisms of trypanosome traversal of the blood-brain barrier, as well as the identification of new potential drug targets and staging biomarkers, new drug therapies and combinations, and potential drug toxicity.

14027. **Maudlin, I., 2006.** African trypanosomiasis. *Annals of Tropical Medicine and Parasitology*, **100** (8): 679-701.

Centre for Tropical Veterinary Medicine, Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Easter Bush, Roslin EH25 9RG, UK. [ian.maudlin@ed.ac.uk].

Trypanosomiasis remains one of the most serious constraints to economic development in sub-Saharan Africa and, as a consequence, related research has been subject to strong social and political as well as scientific influences. The epidemics of sleeping sickness that occurred at the turn of the 20th Century focussed research efforts on what became known as “the colonial disease”. This focus is thought to have produced 'vertical' health services aimed at this one disease, while neglecting other important health issues. Given the scale of these epidemics, and the fact that the disease is fatal if left untreated, it is unsurprising that sleeping sickness dominated colonial medicine. Indeed, recent evidence indicates that, if anything, the colonial authorities greatly under-estimated the mortality attributable to sleeping sickness. Differences in approach to disease control between Francophone and Anglophone Africa, which in the past have been considered ideological, on examination prove to be logical, reflecting the underlying epidemiological divergence of East and West Africa. These epidemiological differences are ancient in origin, pre-dating the colonial period, and continue to the present day. Recent research has produced control solutions for the African trypanosomiases of humans and livestock that are effective, affordable and sustainable by small-holder farmers. Whether these simple solutions are allowed to fulfil their promise and become fully integrated into agricultural practice remains to be seen. After more than 100 years of effort, trypanosomiasis control remains a controversial topic, subject to the tides of fashion and politics.

14028. **Mihok, S. & Carlson, D. A., 2007.** Performance of painted plywood and cloth Nzi traps relative to Manitoba and greenhead traps for tabanids and stable flies. *Journal of Economic Entomology*, **100** (2): 613-618.

USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, 1600 SW 23rd Dr., Gainesville, FL 32608, USA.

Experiments were conducted to adapt the cloth Nzi trap to a format suitable for fixed applications in biting fly sampling or control. Catches of tabanids [*Tabanus* L., *Chrysops* (Meigen), and *Hybomitra* Enderlein], and stable flies [*Stomoxys calcitrans* (L.)] in painted plywood traps were compared with those in standard phthalogen blue cloth traps, and in similarly painted cloth traps. The Manitoba horse fly trap and the *Tabanus nigrovittatus* Macquart "greenhead" box trap were used as additional standards during one tabanid season. Shiny features of traps reduced catches, e.g., paint on cloth instead of wood, or use of aluminium screening instead of netting. Nevertheless, appropriately painted plywood Nzi traps caught as many biting flies as did standard cloth Nzi traps, if paint finishes were matte, and with the use of phthalogen blue colorants. Nzi traps collected about the same tabanid fauna as the Manitoba and *T. nigrovittatus* traps, but with improved catches of *Chrysops* and *Tabanus*. Recommendations are provided on appropriate colour matching, and selection of readily available materials for trap construction.

14029. **Queyriaux, B., 2007.** Greenhouse effect and climate warming: what impact on vector-borne infectious disease? *Med Trop (Mars)*, **67** (1): 16-17.

Département d'Epidémiologie et de Santé Publique, IMTSSA, Marseille, France.

No abstract available.

14030. **Starchurski, F. & Lancelot, R., 2006.** Footbath acaricide treatment to control cattle infestation by the tick *Amblyomma variegatum*. *Medical and Veterinary Entomology*, **20** (4): 402-412.

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Previous studies have shown that about 90 percent of adult *Amblyomma variegatum Fabricius* (*Ixodidae*) picked up daily by grazing cattle are still attached to the interdigital areas in the evening, when the animals return from pasture. It was therefore postulated that a targeted treatment, designed to kill the ticks attached to the feet, would limit infestation of the predilection sites. Footbaths filled with various pyrethroid formulations were used over 3 years, at the beginning of the rainy season (from mid-May to the end of July), to assess the efficacy of such a control method. It proved efficient in preventing the ticks from attaching to the predilection sites. Although five to 12 *A. variegatum* adults attached to each treated animal daily, and although the tick burden of the predilection sites of control cattle increased each day by four to 10 ticks, the average infestation of the predilection sites of treated cattle that were initially highly infested (over 100 ticks/animal) continuously decreased to reach a level of about 10-30 ticks/animal after 6-8 weeks of treatment. In herds with a lower initial tick burden (40-70 ticks/animal) this level was obtained within 2-3 weeks and the mean infestation subsequently remained consistently low. Footbath treatment carried out every other day during the adult peak infestation period should therefore greatly limit losses due to ticks. This method was appreciated by traditional livestock farmers, essentially because it is not time-consuming and because it requires only c. 200 mL aqueous formulation per animal at each passage. The cost of the acaricide needed to treat one animal during the peak infestation period was assessed at c. Euro 0.20. This control method might also have an impact on some species of tsetse flies and mosquitoes, thereby contributing to trypanosomiasis and malaria control.

14031. **Stingl, P., 2006.** Return of African sleeping sickness. *MMW Fortschritte der Medizin*, **148** (37): 52-53.

Tropenmedizin (DTMH London), Steingaden, Germany.
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At present there is a steady rise in African sleeping sickness (trypanosomiasis) transmitted by the tsetse fly, and which if left untreated, is fatal. Thanks to many years of neglect by research, our therapeutic repertoire is limited to medications with a high level of

toxicity. Both WHO and international aid organizations are pushing hard for the development of new, more efficient drugs that can be readily applied in the field.

14032. **Tibayrenc, M., 2007.** Human genetic diversity and the epidemiology of parasitic and other transmissible diseases. *Advances in Parasitology*, **64**: 377-462.

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This paper aims to review human genetic studies that are generally poorly known by parasitologists and scientists working on other pathogenic agents. The key proposals of this paper are as follows: (i) human susceptibility to transmissible diseases may often have a complex, multigenic background; (ii) recent discoveries indicate that major genomic rearrangements may be involved, possibly more so than DNA sequence; (iii) it is crucial to have a general population genetics framework of the human species based on neutral/historical markers to analyse reliably genetic susceptibility to infectious diseases; and (iv) the population level is a key factor. Ethnic diversity, a highly adaptive genetically driven phenotypic diversity, is possibly a valuable source for exploring human genetic susceptibility to transmissible diseases, since different populations have been exposed to drastically different geographic/climatic environments and different pathogens and vectors for tens of thousands of years. Studies dealing with human genetic susceptibility to transmissible diseases have mostly been based on the hypothesis that this factor is driven by only one or a few genes, and considered the individual more than the population level. Two different approaches have been developed for identifying the genes involved: (i) candidate genes and (ii) blind association studies (linkage analysis), screening the genome with a large number of high-resolution markers. Some loci involved in susceptibility to leishmaniosis, malaria and schistosomosis, for example, have already been identified. South American trypanosomosis (Chagas disease) is reviewed in detail to show the methodological problems of this classical approach. Current knowledge on the general impact of transmissible diseases on human genetic diversity, mainly HLA polymorphism, and the hopes raised by recent major international programmes such as the Human Genome Project (HGP), Human Genome Diversity Project (HGDP), International Human Haplotype Map Project (Hap Map) and extended databases, networks and networks of networks will also be reviewed.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

14033. **Guther, M. L., Lee, S., Tetley, L., Acosta-Serrano, A. & Ferguson, M. A., 2006.** GPI-anchored proteins and free GPI glycolipids of procyclic form *Trypanosoma brucei* are nonessential for growth, are required for colonization of the tsetse fly, and are not the only components of the surface coat. *Molecular Biology of the Cell*, **17** (12): 5265-5274.

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The procyclic form of *Trypanosoma brucei* exists in the midgut of the tsetse fly. The current model of its surface glycocalyx is an array of rod-like procyclin glycoproteins with glycosylphosphatidylinositol (GPI) anchors carrying sialylated poly-N-acetyllactosamine side chains interspersed with smaller sialylated poly-N-acetyllactosamine-containing free GPI glycolipids. Mutants for *Tb*GPI12, deficient in the second step of GPI biosynthesis, were devoid of cell surface procyclins and poly-N-acetyllactosamine-containing free GPI glycolipids. This major disruption to their surface architecture severely impaired their ability to colonize tsetse fly midguts but, surprisingly, had no effect on their morphology and growth characteristics *in vitro*. Transmission electron microscopy showed that the mutants retained a cell surface glycocalyx. This structure, and the viability of the mutants *in vitro*, prompted us to look for non-GPI-anchored parasite molecules and/or the adsorption of serum components. Neither was apparent from cell surface biotinylation experiments but [^{3}H] glucosamine biosynthetic labelling revealed a group of previously unidentified high apparent molecular weight glycoconjugates that might contribute to the surface coat. While characterizing GlcNAc-PI that accumulates in the *Tb*GPI12 mutant, we observed inositolphosphoceramides for the first time in this organism.

14034. **Guz, N., Attardo, G. M., Wu, Y. & Aksoy, S., 2007.** Molecular aspects of transferrin expression in the tsetse fly (*Glossina morsitans morsitans*). *Journal of Insect Physiology*. **In press, corrected proof.**

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Iron is an essential element for metabolic processes intrinsic to life, and yet the properties that make iron a necessity also make it potentially deleterious. To avoid harm, iron homeostasis is achieved via proteins involved in transport and storage of iron, one of which is transferrin. We describe the temporal and spatial aspects of transferrin (*Gmm*Tsf) expression and its transcriptional regulation in tsetse where both the male and female are strictly haematophagous. Using Northern, Western and immunohistochemical analysis, we show that *Gmm*Tsf is abundant in the haemolymph and is expressed in the adult developmental stages of male and female insects. It is preferentially expressed in the female milk gland tubules and its expression appears to be cyclical and possibly regulated in synchrony with the oogenic and/or larvigenic cycle. Although no mRNA is detected, *Gmm*Tsf protein is present in the immature stages of development, apparently being transported into the intrauterine larva from the mother via the milk gland ducts. Transferrin is also detected in the vitellogenic ovary and the adult male testes, further supporting its classification as a vitellogenic protein. Similar to reports in other insects, transferrin mRNA levels increase upon bacterial challenge in tsetse suggesting that transferrin may play an additional role in immunity. Although transferrin expression is induced following bacterial challenge, it is significantly reduced in tsetse carrying midgut trypanosome infections. Analysis of tsetse that have cured the parasite challenge shows normal levels of *Gmm*Tsf. This observation suggests that the parasite in competing for the availability of limited dietary iron may manipulate host gene expression.

14035. **Khachane, A. N., Timmis, K. N. & Martins dos Santos, V. A., 2007.** Dynamics of reductive genome evolution in mitochondria and obligate intracellular microbes. *Molecular Biology and Evolution*, **24** (2): 449-456.

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Reductive evolution in mitochondria and obligate intracellular microbes has led to a significant reduction in their genome size and guanine plus cytosine content (GC). We show that genome shrinkage during reductive evolution in prokaryotes follows an exponential decay pattern and provide a method to predict the extent of this decay on an evolutionary timescale. We validated predictions by comparison with estimated extents of genome reduction known to have occurred in mitochondria and *Buchnera aphidicola*, through comparative genomics and by drawing on available fossil evidences. The model shows how the mitochondrial ancestor would have quickly shed most of its genome, shortly after its incorporation into the protoeukaryotic cell and prior to codivergence subsequent to the split of eukaryotic lineages. It also predicts that the primary rickettsial parasitic event would have occurred between 180 and 425 million years ago (MYA), an event of relatively recent evolutionary origin considering the fact that *Rickettsia* and mitochondria evolved from a common alphaproteobacterial ancestor. This suggests that the symbiotic events of *Rickettsia* and mitochondria originated at different time points. Moreover, our model results predict that the ancestor of *Wigglesworthia glossinidia brevipalpis*, dated around the time of origin of its symbiotic association with the tsetse fly (50-100 MYA), was likely to have been an endosymbiont itself, thus supporting an earlier proposition that *Wigglesworthia*, which is currently a maternally inherited primary endosymbiont, evolved from a secondary endosymbiont.

14036. **Novakova, E. & Hypsa, V., 2007**. A new *Sodalis* lineage from bloodsucking fly *Craterina melbae* (Diptera, *Hippoboscoidea*) originated independently of the tsetse flies symbiont *Sodalis glossinidius*. *FEMS Microbiology Letters*, **269** (1): 131-135.

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Symbiotic bacterium closely related to the secondary symbiont of tsetse flies, *Sodalis glossinidius*, has been described from the bloodsucking fly *Craterina melbae*. Phylogenetic analysis of two genes, 16S rRNA gene and component of type three secretion system, placed the bacterium closer to the *Sitophilus*-derived branch of *Sodalis* than to the tsetse symbionts. This indicates that the *Craterina*-derived lineage of *Sodalis* originated independent of the tsetse flies symbionts and documents the capability of *Sodalis* bacteria either to switch between different host groups or to establish the symbiosis by several independent events

14037**. Strickler-Dinglasan, P. M., Guz, N., Attardo, G. & Aksoy, S., 2006.** Molecular characterization of iron binding proteins from *Glossina morsitans morsitans* (Diptera: *Glossinidae*). *Insect Biochemistry and Molecular Biology*, 36: 921-933.

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The regulation of iron is critical for maintaining homeostasis in the tsetse fly (Diptera: *Glossinidae*), in which both adult sexes are strict blood feeders. We have characterized the cDNAs for two putative iron-binding proteins (IBPs) involved in transport and storage; transferrin (*Gmm*Tsf1) and ferritin from *Glossina morsitans morsitans*. *Gmm*Tsf1 transcripts are detected in the female fat body and in adult reproductive tissues, and only in the adult developmental stage in a bloodmeal independent manner. In contrast, the ferritin heavy chain (*Gmm*Fer1HCH) and light chain (*Gmm*Fer2LCH) transcripts are expressed ubiquitously, suggesting a more general role for these proteins in iron transport and storage. Protein domain predictions for each IBP suggest both the conservation and loss of several motifs present in their vertebrate homologues. In concert with many other described insect transferrins (Tfs), putative secreted *Gmm*Tsf1 maintains 3 of the 5 residues necessary for iron-binding in the N-terminal lobe, but exhibits a loss of this iron-binding ability in the C-terminal lobe as well as a loss of large sequence blocks. Both putative *Gmm*Fer1HCH and *Gmm*Fer2LCH proteins have signal peptides, similar to other insect ferritins. *Gmm*Fer2LCH has lost the 5′UTR iron-responsive element (IRE) and, thus, translation is no longer regulated by cellular iron levels. On the other hand, *Gmm*Fer1HCH maintains both the conserved ferroxidase centre and the 5′UTR IRE; however, transcript variants suggest a more extensive regulatory mechanism for this subunit.

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14038. **Kumar, N. P., Rajavel, A. R., Natarajan, R. & Jambulingam, P., 2007.** DNA barcodes can distinguish species of Indian mosquitoes (Diptera: *Culicidae*). *Journal of Medical Entomology*, **44** (1): 1-7.

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Species identification of mosquitoes (Diptera: *Culicidae*) based on morphological characteristics remains often difficult in field-collected mosquito specimens in vector-borne disease surveillance programs. The use of DNA barcodes has been proposed recently as a tool for identification of the species in many diverse groups of animals. However, the efficacy of this tool for mosquitoes remains unexplored. Hence, a study was undertaken to construct DNA barcodes for several species of mosquitoes prevalent in India, which included major vector species. In total, 111 specimens of mosquitoes belonging to 15 genera, morphologically identified to be 63 species, were used. This number also included multiple specimens for 22 species. The DNA barcode approach based on the DNA sequences of mitochondrial cytochrome oxidase gene could identify 62 species among these, in confirmation with the conventional taxonomy. However, two closely related species, *Ochlerotatus portonovoensis* (Tiwari & Hiriyan) and *Ochlerotatus wardi* (Reinert) could not be identified as separate species based on DNA barcode approach, their lineages indicating negligible genetic divergence (Kimura two-parameter genetic distance = 0.0043).

14039. **Ouma, J. O., Marquez, J. G. & Krafsur, E. S., 2006.** New polymorphic microsatellites in *Glossina pallidipes* (Diptera: *Glossinidae*) and their cross-amplification in other tsetse fly taxa. *Biochemical Genetics*, **44** (9-10): 471-477.

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We report the development and characterization of three new microsatellite markers in the tsetse fly, *Glossina pallidipes* (Diptera: *Glossinidae*). Fifty-eight alleles were scored in 192 individuals representing six natural populations. Allelic diversity ranged from 9 to 28 alleles per locus (mean 19.3 +/- 5.5). Averaged across loci, observed heterozygosity was 0.581 +/- 0.209, and expected heterozygosity was 0.619 +/- 0.181. Cross-species amplifications of the *G. pallidipes* loci in other tsetse fly taxa are reported.

14040. **Terblanche, J. S. & Chown, S. L., 2007.** The effects of temperature, body mass and feeding on metabolic rate in the tsetse fly *Glossina morsitans centralis*. *Physiological Entomology*, **32** (2): 175-180.

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Metabolic rate variation with temperature, body mass, gender and feeding status is documented for *Glossina morsitans centralis*. Metabolic rate [mean +- SE; VCO_2 = 19.78 +- 3.11 µL CO_2 h^{-1} in males (mean mass = 22.72 ± 1.41 mg) and 27.34 ± 3.86 µL CO_2 h^{-1} in females (mean mass = 29.28 ± 1.96 mg) at 24 ^{o}C in fasted individuals] is strongly influenced by temperature, body mass and feeding status, but not by gender once the effects of body mass have been accounted for. A significant interaction between gender and feeding status is seen, similar to patterns of metabolic rate variation documented in *Glossina morsitans morsitans*. Synthesis of metabolic rate-temperature relationships in *G. m. centralis, G. m. morsitans* and *Glossina pallidipes* indicate that biting frequency as well as mortality risks associated with foraging will probably increase with temperature as a consequence of increasing metabolic demands, although there is little evidence for variation among species at present. Furthermore, metabolic rate-body mass relationships appear to be similarly invariant among these species. These data provide important physiological information for bottom-up modelling of tsetse fly population dynamics.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also **30**: 14027, 14031]

14041. **Bouyer, J., Stachurski, F., Kabore, I., Bauer, B. & Lancelot, R., 2007**. Tsetse control in cattle from pyrethroid footbaths. *Preventive Veterinary Medicine*, **78** (3-4): 223-238.

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In Burkina Faso, we assessed the efficacy of treating cattle with a footbath containing aqueous formulations of pyrethroids to control two tsetse-fly species, *Glossina tachinoides* Westwood, 1850 (Diptera, *Glossinidae)* and *Glossina palpalis gambiensis* Vanderplank 1949. Legs were the most targeted parts of the body for tsetse-fly blood meals: 81 percent (95 percent CI: 73, 89) for *G. tachinoides* and 88 percent (81, 95) for *G. palpalis*. The in-stable efficacy of footbath treatments was compared with manual full spraying with a 0.005 percent alphacypermethrin (Dominex, FMC, Philadelphia, USA) formulation (250mL versus 2L). The proportions of knocked-down flies were the same with footbath and full spray but the latter was more protective against fly bites. In field use, the efficacy of both methods should be similar given the recommended treatment frequency: 3 days for footbath versus 7 days for full spray. Among 96 cattle drinking at the same water point in Dafinso (Burkina Faso), 68 (71 percent) were treated with a footbath containing a 0.005 percent deltamethrin formulation (Vectocid, CEVA SA, Libourne, France). We observed the effect of this live-bait technique on the one hand on released cohorts of reared, irradiated flies, and on the other hand on wild tsetse flies. In both cases, the footbath treatment was associated with a reduction of the apparent fly density probably related to an increased mortality.

14042. **Esterhuizen, J. & Van den Bossche, P., 2006**. Protective netting, an additional method for the integrated control of livestock trypanosomosis in KwaZulu-Natal Province, South Africa. *Onderstepoort Journal of Veterinary Research*, **73** (4): 319-321.

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Studies were conducted in KwaZulu-Natal, South Africa, to evaluate the effectiveness of netting in preventing *Glossina austeni* and *Glossina brevipalpis* from entering H-traps. Results indicated that a net of 1.5 m in height was effective in reducing catches of *G. austeni* by 59.6 percent and catches of *G. brevipalpis* by 80.9 percent. Increasing the net height to 2.5 m reduced catches by 96.6 percent and 100 percent for *G. brevipalpis* and *G. austeni*, respectively. Nets of this height also reduced catches of horse flies by 55 percent. Although the potential use of protective netting has limitations in tsetse-infested areas of rural northern KwaZulu-Natal, it is a low-technology method that can be used as part of integrated disease management strategies.

14043. **Mihok, S., Carlson, D. A. & Ndegwa, P. N., 2007**. Tsetse and other biting fly responses to Nzi traps baited with octenol, phenols and acetone. *Medical and Veterinary Entomology*, **21** (1): 70-84.

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Octenol (1-octen-3-ol), acetone, 4-methylphenol, 3-n-propylphenol, and other potential attractants (human urine, stable fly faeces), as well as guiacol, creosol (potential repellents), were tested as baits for biting flies in North America using standard phthalogen

blue IF3GM cotton Nzi traps, or similar commercial polyester traps. Baits were tested during the summers of 2001-04 at a residence in Canada and during January-August 2001 at a dairy in the USA. Behaviour in the presence of octenol was also studied by intercepting flies approaching a trap through the use of transparent adhesive film. Analogous bait and/or trap comparisons were conducted in natural settings in June 1996 in Kenya and in September-December 1997 in Ethiopia. In Canada, catches of five of six common tabanids (*Tabanus similis* Macquart, *Tabanus quinquevittatus* Wiedemann, *Hybomitra lasiophthalma* [Macquart], *Chrysops univittatus* Macquart, *Chrysops aberrans* Philip) and the stable fly *Stomoxys calcitrans* L. were increased significantly by 1.2-2.1 times with octenol (1.5 mg/h). Catches of *T. quinquevittatus* and *S. calcitrans* were 3.5-3.6 times higher on a sticky enclosure surrounding a trap baited with octenol. No other baits or bait combinations had an effect on trap catches in North America. In Ethiopia, standard Nzi traps baited with a combination of acetone, octenol and cattle urine caught 1.8-9.9 times as many *Stomoxys* as similarly baited epsilon, pyramidal, NG2G, S3, biconical and canopy traps, in order of decreasing catch. When baits were compared, catches in Nzi traps of six stable fly species, including *S. calcitrans*, were not affected by octenol (released at approximately 1 mg/h), or cattle urine (140 mg/h), used alone or in combination with acetone (890 mg/h). Acetone alone, however, significantly increased the catches of common Stomoxys such as *Stomoxys niger niger* Macquart, *Stomoxys taeniatus* Bigot, and *S. calcitrans* by 2.4, 1.6 and 1.9 times, respectively. Catches of *Glossina pallidipes* Austen were increased significantly in traps baited with acetone, urine or octenol, or any combination, relative to those in unbaited traps (1.4-3.6 times). Catches of *Glossina morsitans submorsitans* Newstead were increased significantly by 1.5-1.7 times, but only when baits were used individually. Unlike other studies with East African tsetse, catches of both tsetse species with the complete bait combination (acetone, urine and octenol) did not differ from those in unbaited traps. Experiments with an incomplete ring of electric nets surrounding a Nzi trap, and a new approach using a sticky enclosure made from transparent adhesive film, revealed diverse responses to artificial objects and baits among biting flies. In Kenya, daily trap efficiency estimates for traps baited with either carbon dioxide (6 L/min) or a combination of acetone, cattle urine and octenol were 21-27 percent for *G. pallidipes*, 7-36 percent for *Glossina longipennis* Corti, 27-33 percent for *S. n. niger*, and 19-33 percent for *Stomoxys niger bilineatus* Grunberg, assuming 100 percent electrocution efficiency. Actual trap efficiencies may have been lower, given observed outside: inside electric net catch ratios of 0.6:1.6. Observed ratios averaged 54 percent of expected values, with 10 of 15 possible ratios less than the minimum possible value of 1.0.

14044. **Saini, R. K. & Hassanali, A., 2007**. A 4-alkyl-substituted analogue of guaiacol shows greater repellency to savannah tsetse (*Glossina* spp.). *Journal of Chemical Ecology*, **33**(5): 985-95.

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The responses of *Glossina morsitans morsitans* Westwood to guaiacol (2-methoxyphenol), a mild repellent constituent of bovid odours, and seven analogues comprising 2-methoxyfuran, 2,4-dimethylphenol, 2-methoxy-4-methylphenol (4-methylguaiacol), 4-ethyl-2-methoxyphenol (4-ethylguaiacol), 4-allyl-2-methoxyphenol (4-

allylguaiacol; eugenol), 3,4-methylenedioxytoluene, and 3,4-dimethoxystyrene were compared in a two-choice wind tunnel. The 4-methyl-substituted derivative (2-methoxy-4-methylphenol) was found to elicit stronger repellent responses from the flies compared with guaiacol. None of the other analogues showed significant repellent effects on flies. 4-Methylguaiacol, guaiacol, and eugenol (which were included because of previous reports of its repellency against a number of arthropods) were further evaluated in the field with wild populations of predominantly *Glossina pallidipes* Austen. The presence of guaiacol or eugenol near odour-baited traps caused some nonsignificant reduction in the number of tsetse catches at relatively high release rates (approximately 50 mg/hr). In contrast, the 4-methyl derivative at three different release rates (2.2, 4.5, and 9.0 mg/hr) reduced trap catches of baited traps in a dose-response manner. At 10 mg/hr release rate, it reduced the catches of baited and unbaited traps by approximately 80 and approximately 70 percent, respectively. In addition, the compound not only reduced the number of tsetse attracted to natural ox odour (approximately 80 percent), but also had an effect on their feeding responses, reducing the proportion that fed on an ox by more than 80 percent. Our study shows that the presence of a methyl substituent at the 4-position of guaiacol enhances the repellency of the molecule to savannah tsetse and suggests that 4-methylguaiacol may represent a promising additional tool in the arsenal of techniques in trypanosomiasis control.

14045. **Torr, S. J., Maudlin, I. & Vale, G. A., 2007.** Less is more: restricted application of insecticide to cattle to improve the cost and efficacy of tsetse control. *Medical and Veterinary Entomology*, **21** (1): 53-64.

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Studies were carried out in Zimbabwe of the responses of tsetse to cattle treated with deltamethrin applied to the parts of the body where most tsetse were shown to land. Large proportions of *Glossina pallidipes* Austen (Diptera: *Glossinidae*) landed on the belly (approximately 25 percent) and legs (approximately 70 percent), particularly the front legs (approximately 50 percent). Substantial proportions of *Glossina morsitans morsitans* Westwood landed on the legs (approximately 50 percent) and belly (25 percent), with the remainder landing on the torso, particularly the flanks (approximately 15 percent). Studies were made of the knockdown rate of wild, female *G. pallidipes* exposed to cattle treated with a 1 percent pour-on or 0.005 percent suspension concentrate of deltamethrin applied to the (a) whole body, (b) belly and legs, (c) legs, (d) front legs, (e) middle and lower front legs, or (f) lower front legs. The restricted treatments used 20 percent, 10 percent, 5 percent, 2 percent or 1 percent of the active ingredient applied in the whole-body treatments. There was a marked seasonal effect on the performance of all treatments. With the whole-body treatment, the persistence period (knockdown > 50 percent) ranged from approximately 10 days during the hot, wet season (mean daily temperature > 30 ^{o}C) to approximately 20 days during the cool, dry season (< 22 ^{0} C). Restricting the application of insecticide reduced the seasonal persistence periods to approximately 10-15 days if only the legs and belly were treated, to approximately 5-15 days if only the legs were treated and < 5 days for the more restricted treatments. The restricted application did not affect the landing distribution of tsetse or the duration of landing bouts (mean = 30 s). The results suggest that more cost-effective control of tsetse could be achieved by applying insecticide to the belly and legs of cattle at 2-week

intervals, rather than using the current practice of treating the whole body of each animal at monthly intervals. This would cut the cost of insecticide by 40 percent, improve efficacy by 27 percent and reduce the threats to non-target organisms and the enzootic stability of tick-borne diseases.

14046. **Wamwiri, F. N., Nkwengulila, G. & Clausen, P. H., 2007.** Hosts of *Glossina fuscipes fuscipes* and *G. pallidipes* in areas of western Kenya with endemic sleeping sickness, as determined using an egg-yolk (IgY) ELISA. *Annals of Tropical Medicine and Parasitology*, **101** (3): 225-232.

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Bloodmeal sources of *Glossina fuscipes fuscipes* and *G. pallidipes*, from the western Kenyan foci of human African trypanosomiasis (HAT) on Mageta Island and in Busia district, were identified using an ELISA based on chicken egg-yolk (IgY) antibodies. After absorption with cross-reacting antigens, the antibodies, which were produced against representatives of eight families of vertebrate host, were capable of differentiating serum from the different families. With the ELISA, it was possible to identify the family of host for 100 percent of laboratory-fed flies tested up to 48 h post-bloodmeal but only for 12 percent of such flies tested 96 h post-feed. Subsequently, attempts were made to identify the family of host that was the source of the (most recent) bloodmeal for each of 223 wild-caught flies, and these attempts were successful for 142 (63.7 percent) of the samples. Among the flies with identified bloodmeals, most (81.9 percent) of the *G. f. fuscipes* caught on Mageta Island had last fed on reptiles whereas most of the *G. f. fuscipes* (70.4 percent) and *G. pallidipes* (57.1 percent) caught in Busia had last fed on bovids. Bloodmeals of human origin accounted for <2 percent of the bloodmeals identified, perhaps indicating that, in the presence of alternative hosts, humans are not attractive hosts for tsetse in the study areas. This finding may account for the low reported incidence of HAT, despite the presence of circulating human-infective trypanosomes. In Busia at least, the use of animals, especially cattle, covered in insecticide would probably be an effective method of controlling the tsetse vectors of the trypanosomes that cause human and "animal" trypanosomiases.

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

[See also **30**: 14021, 14023, 14032, 14127, 14145]

14047. **Berrang Ford, L., 2007**. Civil conflict and sleeping sickness in Africa in general and Uganda in particular. *Conflict and Health*, **1**: 6.

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Conflict and war have long been recognized as determinants of infectious disease risk. Re-emergence of epidemic sleeping sickness in sub-Saharan Africa since the 1970s has coincided with extensive civil conflict in affected regions. Sleeping sickness incidence has placed increasing pressure on the health resources of countries already burdened by malaria, HIV/AIDS, and tuberculosis. In areas of Sudan, the Democratic Republic of the Congo, and Angola, sleeping sickness occurs in epidemic proportions, and is the first or second greatest cause of mortality in some areas, ahead of HIV/AIDS. In Uganda, there is evidence of increasing spread and establishment of new foci in central districts. Conflict is an important determinant of sleeping sickness outbreaks, and has contributed to disease resurgence. This paper presents a review and characterization of the processes by which conflict has contributed to the occurrence of sleeping sickness in Africa. Conflict contributes to disease risk by affecting the transmission potential of sleeping sickness via economic impacts, degradation of health systems and services, internal displacement of populations, regional insecurity, and reduced access for humanitarian support. Particular focus is given to the case of sleeping sickness in south-eastern Uganda, where incidence increase is expected to continue. Disease intervention is constrained in regions with high insecurity; in these areas, political stabilization, localized deployment of health resources, increased administrative integration and national capacity are required to mitigate incidence. Conflict-related variables should be explicitly integrated into risk mapping and prioritization of targeted sleeping sickness research and mitigation initiatives.

14048. **Bouyer, J., Pruvot, M., Bengaly, Z., Guerin, P. M. & Lancelot, R., 2007**. Learning influences host choice in tsetse. *Biological Letters*, **3** (2): 113-116.

Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), Département d'élevage et de médecine vétérinaire, 34398 Montpellier Cedex 5, France. [bouyer@cirad.fr].

A learning capacity for feeding is described in many insect species including vectors of diseases, but has never been reported in tsetse flies (Diptera, *Glossinidae*), the cyclic vectors of human (sleeping sickness) and animal trypanosomoses in Africa. Repeated feeding on the same host species by a disease vector is likely to increase the within-species disease-transmission risk, but to decrease it between species. An experiment with cattle and reptiles in a stable provides evidence that the species of host selected for the second blood meal in tsetse flies depends on the host encountered for the first blood meal when the between-meal interval is 2 days. This preference disappears when the between-meal interval is extended to 3 days. The energetic advantages of this acquired preference and its importance in trypanosomoses epidemiology are discussed**.**

14049. **Macleod, E. T., Darby, A. C., Maudlin, I. & Welburn, S. C., 2007**. Factors affecting trypanosome maturation in tsetse flies. *PLoS ONE*, **2**: e239.

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Trypanosoma brucei brucei infections which establish successfully in the tsetse fly midgut may subsequently mature into mammalian infective trypanosomes in the salivary glands. This maturation is not automatic and the control of these events is complex. Utilising direct *in vivo* feeding experiments, we report maturation of *T. b. brucei* infections in tsetse is regulated by antioxidants as well as environmental stimuli. Dissection of the maturation process provides opportunities to develop transmission blocking vaccines for trypanosomiasis. The present work suggests L-cysteine and/or nitric oxide are necessary for the differentiation of trypanosome midgut infections in tsetse.

14050. **Ohaga, S. O., Kokwaro, E. D., Ndiege, I. O., Hassanali, A. & Saini, R. K., 2007.** Livestock farmers' perception and epidemiology of bovine trypanosomosis in Kwale District, Kenya. *Preventive Veterinary Medicine*, **80** (1): 24-33.

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We did cross-sectional surveys in Kwale District, Kenya to determine the epidemiology of bovine trypanosomosis and livestock owners' perceptions of the disease. The surveys involved relative importance of trypanosomosis, examination of the current disease constraints, current control practices and drug-use patterns. Informal meetings were held with farmers and cattle census undertaken. Tsetse-fly densities and trypanosomosis prevalences in cattle were determined. A total of 132 farmers were interviewed. Trypanosomosis, anaplasmosis, East Coast fever, and foot-and-mouth disease were reported to be the major constraints to livestock production. Trypanosomosis was the most important compared to other diseases. Chemotherapy was the most widely used method of controlling the disease. Farmer-based tsetse-control technologies were poorly adopted. Respondents were quite knowledgeable on the symptoms, causes and treatment of trypanosomosis. *Glossina austeni*, *G. brevipalpis* and *G. pallidipes* were found in the area; the latter was the most common (0.2–738 flies/trap). *Trypanosoma congolense* and *T. vivax* were found in cattle with the former more prevalent. Infection prevalences in cattle varied between 0 and 25 percent (median: 22 percent).

14051. **Poinsignon, A., Cornelie, S., Remoue, F., Grebaut, P., Courtin, D., Garcia, A. & Simondon, F., 2007**. Human/vector relationships during human African trypanosomiasis: initial screening of immunogenic salivary proteins of *Glossina* species. *American Journal of Tropical Medicine and Hygiene*, **76** (2): 327-333.

Epidémiologie et Prévention, et Interactions Hôtes-Vecteurs-Parasites dans les Trypanosomoses Unité de Recherche, Institut de Recherche pour le Développement, Montpellier, France. [anne.poinsignon@mpl.ird.fr].

The morbidity and mortality of vector-borne diseases are closely linked to exposure of the human host to vectors. Qualitative and quantitative evaluation of individual exposure to arthropod bites by investigation of the specific immune response to vector saliva would make

it possible to monitor individuals at risk of vectorial transmission of pathogens. The objective of this study was to evaluate and compare the antibody (IgG) response to saliva from uninfected *Glossina* species, vectors, or non-vectors of *Trypanosoma brucei gambiense* by detecting immunogenic proteins in humans residing in an area endemic for human African trypanosomiasis in the Democratic Republic of Congo. Our results suggest that the immunogenic profiles observed seemed specific to the *Glossina* species (vector or non-vector species) and to the infectious status of exposed individuals (infected or not infected). This preliminary work tends to support the feasibility of development of an epidemiologic tool based on this antibody response to salivary proteins.

14052**. Simo, G., Mansinsa Diabakana, P., Kande Betu Ku Mesu, V., Manzambi, E. Z., Ollivier, G., Asonganyi, T., Cuny, G. & Grebaut, P., 2006**. Human African trypanosomiasis transmission, Kinshasa, Democratic Republic of Congo. *Emerging Infectious Diseases*, **12** (12): 1968-1970.

Institute of Medical Research and Study of Medicinal Plants, Yaoundé, Cameroon. [gustavsca@yahoo.fr].

To investigate the epidemiology of human African trypanosomiasis (sleeping sickness) in Kinshasa, Democratic Republic of Congo, two entomologic surveys were conducted in 2005. *Trypanosoma brucei gambiense* and human-blood meals were found in tsetse fly midguts, which suggested active disease transmission. Vector control should be used to improve human African trypanosomiasis control efforts.

14053. **Simukoko, H., Marcotty, T., Phiri, I., Geysen, D., Vercruysse, J. & Van den Bossche, P., 2007.** The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomiasis on the plateau of eastern Zambia. *Veterinary Parasitology*. **In press, corrected proof.**

University of Zambia, School of Veterinary Medicine, Zambia.

To determine and compare the prevalence of trypanosome infections in different livestock species (cattle, pigs and goats) in areas where game animals are scarce and livestock constitute the main food source of tsetse, a survey was conducted on the plateau of the Eastern Province of Zambia in Katete and Petauke districts where *Glossina morsitans morsitans* is the only tsetse species present. Blood was collected from a total of 734 cattle, 333 goats and 324 pigs originating from 59 villages in both districts and was examined using the buffy coat method and the PCR-RFLP as diagnostic tools. The prevalence of trypanosome infections differed substantially between livestock species. Using microscopic diagnostic methods, trypanosome infections were detected in 13.5 percent of the cattle and 0.9 percent of the pigs. All goats were parasitologically negative. The PCR-RFLP analyses increased the trypanosomiasis prevalence to 33.5, 6.5 and 3.3 percent in cattle, pigs and goats respectively. The majority of the infections (91.2 percent) were due to *Trypanosoma congolense*. The presence of a trypanosome infection in cattle and pigs resulted in a significant decline in the packed cell volume. The outcome of the study clearly shows that despite the availability of goats and pigs, cattle seem to be the major livestock species affected by the disease in

trypanosomiasis endemic areas. The high proportion of infections in cattle could be partly attributed to their higher availability and attractiveness to tsetse.

14054. **Wendel, S., 2006**. Transfusion-transmitted American and African trypanosomiasis (Chagas disease and sleeping sickness): neglected or reality? *ISBT Science Series*, **1** (1): 140-151.

Hospital Sírio Libanes, São Paulo, Brazil.

No abstract available.

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

[See also **30**: 14054]

14055. **Parris, G. E., 2007**. How did the ancestral HIV-1 group M retrovirus get to Leopoldville from southeastern Cameroon? *Medical Hypotheses*. **In press, corrected proof**.

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In previous papers in this journal, I have described and elaborated a hypothesis for the origin and evolution of a strain of HIV that has produced a lethal pandemic. Here I address the provocative question of how the ancestral HIV-1 group M retrovirus got to Leopoldville (Kinshasa, where the pandemic clearly spawned) from south-eastern Cameroon (where the HIV-1 strains all seemed to originate from transfer of SIV (cpz) to humans). Consistent with the phylogenetic history of HIV-1 group M (e.g., by Korber *et al.*), I place the critical relocation of the ancestral HIV-1 in the timeframe 1920-1927. However, unlike other hypotheses, I believe that the ancestral retrovirus was already well adapted to humans and can be identified as HIV-1 Group M subtype A (pre)-1927. Based on documents from that time period (1920-1928), it can be shown that it was not unusual for native Africans to be brought as far as 500 miles for treatment at the Leopoldville clinic (national borders were no issue because health agencies had mandate to work throughout Cameroon and Congo-Brazzaville). Specifically, sleeping sickness (trypanosomiasis) was one of the diseases of most concern at the Leopoldville clinic; in the period 1926-1928 there was an outbreak of sleeping sickness in Cameroon; and one of the native African children in the pamaquine (plasmoquineTM) study that I believe was selected for the major HIV-1 group M subgroups had trypanosomiasis. Thus, this child (or other patients/relatives from Cameroon) could have brought the ancestral HIV-1 group M retrovirus to the Leopoldville laboratory and spread it among the group of children who were undergoing treatment for malaria between February and August 1927. The diagnosis and monitoring of these protozoan diseases (trypanosomiasis and malaria) involved repetitive sampling of blood, which provides many opportunities for spreading the ancestral HIV-1 infection.

14056. **Shegokar, V. R., Powar, R. M., Joshi, P. P., Bhargava, A., Dani, V. S., Katti, R., Zare, V. R., Khanande, V. D., Jannin, J. & Truc, P., 2006.** Short report: Human trypanosomiasis caused by *Trypanosoma evansi* in a village in India: preliminary serologic survey of the local population. *American Journal of Tropical Medicine and Hygiene*, **75** (5): 869-870.

Department of Microbiology, Government Medical College, Nagpur, India.

After discovery of the first recorded case of human infection with *Trypanosoma evansi*, serologic screening of 1,806 persons from the village of origin of the patient in India was performed using the card agglutination test for trypanosomiasis and *T. evansi*. A total of 410 (22.7 percent) people were positive by whole blood, but only 81 were confirmed positive by serum. However, no trypanosomes were detected in the blood of 60 people who were positive at a high serum dilution. The results probably indicate frequent exposure of the human population to *T. evansi* in the study area, which suggests frequent vector transmission of parasites to humans. Although *T. evansi* is not infective for humans, a follow-up of seropositive persons is required to observe the evolution of human infection with this parasite.

14057. **van Coller, R., van Rensburg, E., Schutte, C., Brink, D., Welthagen, G. & Dove, M.G., 2007.** Awaking a sleeping epidemic. *South African Medical Journal,* **97** (4): 250-1.

Department of Neurology, University of Pretoria, South Africa. [rvcoller@gmail.com].

No abstract available.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **30**: 14026, 14095, 14105]

14058. **Croft, A. M., Jackson, C. J., Friend, H. M. & Minton, E. J., 2006**. African trypanosomiasis in a British soldier. *Journal of the Royal Army Medical Corps*, **152** (3): 156-160.

Headquarters Fifth Division, Copthorne Barracks, Shrewsbury, Shropshire SY3 8LZ, UK. [ashley.croft810@land.mod.uk].

Human African trypanosomiasis (sleeping sickness) is a parasitic infection transmitted by day-biting tsetse flies. The diagnostic gold standard is microscopy of blood, lymph node aspirates or CSF. The disease is invariably fatal, if not treated. There are over 300 000 new cases of sleeping sickness each year, and approximately 100,000 deaths. We

describe a British soldier who acquired trypanosomiasis in Malawi. He gave no history of a painful insect bite but presented with classical early signs of sleeping sickness (a primary chancre, regional lymphadenopathy, circinate erythema and a cyclical fever pattern). His condition worsened in the next week and trypanosomes were observed in a blood sample. He was aeromedically evacuated to Johannesburg, where Stage One *Trypanosoma brucei rhodesiense* infection was confirmed; he also had renal and liver failure, pancytopenia and heart block. He was treated with intravenous suramin. He recovered fully over the next 5 months. It is recommended that medical officers deploying to eastern and southeastern Africa must be familiar with the common presenting signs and symptoms of *T. b. rhodesiense* sleeping sickness, and should have access to a reliable local microscopy service at all times. Confirmed sleeping sickness requires immediate transfer to a tertiary diagnostic and treatment centre, where suramin (for *T. b. rhodesiense* infection) or pentamidine (for *T. b. gambiense*) and also melarsoprol (for Stage Two disease) must be immediately available.

14059. **Ezzedine, K., Darie, H., Le Bras, M. & Malvy, D., 2007.** Skin features accompanying imported human African trypanosomiasis: hemolymphatic *Trypanosoma gambiense* infection among two French expatriates with dermatologic manifestations. *Journal of Travel Medicine,* **14** (3): 192-6.

Travel Clinics and Tropical Disease Unit, Department of Internal Medicine, Infectious Diseases and Tropical Medicine, University Hospital Centre, Bordeaux, France. [kezzedin@ulb.ac.be].

No abstract available.

14060. **Pelloux, H., Aznar, C. & Bouteille, B., 2006**. Trypanosomiases. *La Revue du Praticien*, **56** (20): 2209-2216.

Services de parasitologie-mycologie, CHU Albert-Michallon, BP 217, 38043 Grenoble. [hpelloux@chu-grenoble.fr].

Trypanosomiases are imported and rare parasitosis on the French metropolitan territory. They are re-emerging in some endemic areas, and their mode of transmission can lead to an increase of imported cases in a near future. They can be responsible for serious disease. In this paper, we describe the basic data concerning epidemiology, clinical features, diagnosis, treatment and prevention of sleeping sickness (Africa) and Chagas disease (Latin America).

14061. **Uslan, D.Z., Jacobson, K. M., Kumar, N., Berbari, E. F. & Orenstein, R., 2006**. A woman with fever and rash after African safari. *Clinical Infectious Diseases*, **43** (5): 609, 661-602.

Division of Infectious Diseases, Department of Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota 55905, USA. [uslan.daniel@mayo.edu].

A 61-year-old white woman presented to our institution complaining of intermittent fevers, chills, and rash. Three months before presentation, she travelled to Kenya and

Tanzania on a photographic safari. Her pretravel care included vaccinations for hepatitis A, yellow fever, tetanus-diphtheria, and polio and prescription for mefloquine. The patient initially noted a small papule on her left foot that began 2 days before her departure from Africa. The fevers, which lacked periodicity, began 1 month after her return home. Her symptoms persisted despite a course of quinine and several antibacterials (levofloxacin, azithromycin, metronidazole, doxycycline, and vancomycin) that were administered at her local hospital. She had no fresh water or direct animal contact on her trip, and all of her meals were prepared in hotel kitchens. She denied insect bites, although her husband was bitten by flies on several occasions. On physical examination, she had a temperature of 39°C, and her skin revealed a large, annular, minimally elevated plaque with central clearing from the left groin ascending to the trunk and with multiple satellite patches Laboratory investigations revealed an elevated erythrocyte sedimentation rate but no other haematological abnormalities. Lumbar puncture revealed an elevated WBC count of 164 cells/µL (normal range, 0–4 cells/µL), with 78 percent lymphocytes, 21 percent monocytes, and 1 percent neutrophils, an elevated protein level of 191 mg/dL (normal range, 14–45 mg/dL), and a normal glucose level of 41 mg/dL. Cultures of CSF samples showed no growth, and Gram stain results were normal. A peripheral blood smear was obtained at admission to our institution.

Diagnosis of human African trypanosomiasis was made on the basis of physical examination findings and the presence of *T. brucei rhodesiense* noted on a peripheral blood screen obtained at admission. Our patient was given 1 dose of intravenous pentamidine and was subsequently given 1 dose of suramin procured from the Centers for Disease Control and Prevention. Because of an elevated WBC count, an elevated total protein level, and an elevated IgM level in CSF samples, CNS involvement was presumed, although the patient had no focal findings or neurologic symptoms. She was treated with intravenous melarsoprol (trivalent organic arsenic) together with prednisone to prevent post-treatment reactive encephalopathy. During week 1 of treatment, the patient received 108 mg of melarsoprol daily for 3 days; during week 2, she received 144 mg of melarsoprol daily for 3 days; and during week 3, she received 216 mg of melarsoprol daily for 3 days. Clinical improvement was associated with resolution of fever, a progressive decrease in the WBC count in CSF samples, and the clearance of parasitaemia on serial blood smears. The patient was subsequently discharged from the hospital with resolution of her fevers and rash. Serial lumbar punctures have been performed every 6 months since discharge from the hospital to evaluate for disease recurrence; to date, results have been negative. The patient's total protein level and WBC count in CSF samples have been normal at follow-up visits.

14062. **Vanhollebeke, B., Nielsen, M. J., Watanabe, Y., Truc, P., Vanhamme, L., Nakajima, K., Moestrup, S. K. & Pays, E., 2007**. Distinct roles of haptoglobin-related protein and apolipoprotein L-I in trypanolysis by human serum. *Proceedings of the National Academy of Sciences USA,* **104** (10): 4118-4123.

Laboratory of Molecular Parasitology, Institut de Biologie et de Médecine Moléculaires, Université Libre de Bruxelles, 12 Rue des Profs Jeener et Brachet, B6041 Gosselies, Belgium.

Apolipoprotein L-I (apoL-I) is a human high-density lipoprotein (HDL) component able to kill *Trypanosoma brucei brucei* by forming anion-selective pores in the lysosomal

membrane of the parasite. Another HDL component, haptoglobin-related protein (Hpr), has been suggested as an additional toxin required for full trypanolytic activity of normal human serum. We recently reported the case of a human lacking apoL-I (apoL-I(-/-)HS) as the result of frameshift mutations in both apoL-I alleles. Here, we show that this serum, devoid of any trypanolytic activity, exhibits normal concentrations of HDL-bound Hpr. Conversely, the serum of individuals with normal HDL-bound apoL-I but who lack Hpr and haptoglobin [Hp(r)(-/-) HS] as the result of gene deletion (anhaptoglobinemia) exhibited phenotypically normal but delayed trypanolytic activity. The trypanolytic properties of Hp(r) (-/-) HS were mimicked by free recombinant apoL-I, whereas recombinant Hpr did not affect trypanosomes. The lysis delay observed with either Hp(r) (-/-) HS or recombinant apoL-I could entirely be attributed to a defect in the uptake of the lytic components. Thus, apoL-I is responsible for the trypanolytic activity of normal human serum, whereas Hpr allows fast uptake of the carrier HDL particles, presumably through their binding to an Hp/Hpr surface receptor of the parasite.

14063. **Vanhollebeke, B., Truc, P., Poelvoorde, P., Pays, A., Joshi, P. P., Katti, R., Jannin, J. G. & Pays, E., 2006.** Human *Trypanosoma evansi* infection linked to a lack of apolipoprotein L-I. *New England Journal of Medicine*, **355** (26): 2752-2756.

Laboratory of Molecular Parasitology, Institut de Biologie et de Médecine Moléculaires, Université Libre de Bruxelles, Gosselies, Belgium.

Humans have innate immunity against *Trypanosoma brucei brucei* that is known to involve apolipoprotein L-I (APOL1). Recently, a case of *T. evansi* infection in a human was identified in India. We investigated whether the APOL1 pathway was involved in this occurrence. The serum of the infected patient was found to have no trypanolytic activity, and the finding was linked to the lack of APOL1, which was due to frameshift mutations in both APOL1 alleles. Trypanolytic activity was restored by the addition of recombinant APOL1. The lack of APOL1 explained the patient's infection with *T. evansi*.

(c) TREATMENT

[See also 30: 14026, 14031, 14146, 14155, 14156, 14159, 14166]

14064. **Balasegaram, M., Harris, S., Checchi, F., Ghorashian, S., Hamel, C. & Karunakara, U., 2006.** Melarsoprol versus eflornithine for treating late-stage Gambian trypanosomiasis in the Republic of the Congo. *Bulletin of the World Health Organization*, **84** (10): 783-791.

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[manica.balasegaram@london.msf.org].

To compare the effectiveness of melarsoprol and eflornithine in treating late-stage Gambian trypanosomiasis in the Republic of the Congo, we analysed the outcomes of death

during treatment and relapse within 1 year of discharge for 288 patients treated with eflornithine, 311 patients treated with the standard melarsoprol regimen and 62 patients treated with a short-course (10-day) melarsoprol regimen between April 2001 and April 2005. A total of 1.7 percent (5/288) of patients treated with eflornithine died compared with 4.8 percent (15/311) of those treated with standard melarsoprol and 6.5 percent (4/62) of those treated with short-course melarsoprol. Patients treated with eflornithine tended to be younger and were more likely to have trypanosomes or higher white blood cell counts in their cerebrospinal fluid. The cumulated incidence of relapse among patients who attended at least one follow-up visit 1 year after discharge was 8.1 percent (11/136) for those treated with eflornithine, 14 percent (36/258) for those treated with standard melarsoprol and 15.5 percent (9/58) for those treated with short course melarsoprol. In a multivariate analysis, when compared with eflornithine, standard melarsoprol was found to be a risk factor for both death (odds ratio (OR) = 2.87; 95 percent confidence interval (CI) = 1.03-8.00) and relapse (hazard ratio (HR) = 2.47; 95 percent CI = 1.22-5.03); when compared with eflornithine, short-course melarsoprol was also found to be a risk factor for death (OR = 3.90; 95 percent CI = 1.02-14.98) and relapse (HR = 6.65; 95 percent CI = 2.61-16.94). It is concluded that the effectiveness of melarsoprol treatment appears to have diminished. Eflornithine seems to be a better first-line therapy for treating late-stage Gambian trypanosomiasis in the Republic of the Congo.

14065. **Bisser, S., N'Siesi, F.X., Lejon, V., Preux, P. M., Van Nieuwenhove, S., Miaka Mia Bilenge, C. & Buscher, P., 2007**. Equivalence trial of melarsoprol and nifurtimox monotherapy and combination therapy for the treatment of second-stage *Trypanosoma brucei gambiense* sleeping sickness. *Journal of Infectious Diseases*, **195** (3): 322-329.

Institute of Tropical Medicine, Department of Parasitology, B-2000 Antwerp, Belgium.

Treatment of second-stage sleeping sickness relies mainly on melarsoprol. Nifurtimox has been successfully used to cure melarsoprol-refractory sleeping sickness caused by *Trypanosoma brucei gambiense* infection. An open, randomized trial was conducted to test for equivalence between the standard melarsoprol regimen and 3 other regimens, as follows: standard melarsoprol therapy (3 series of 3.6 mg/kg/day intravenously [iv] for 3 days, with 7-day breaks between the series); 10-day incremental-dose melarsoprol therapy (0.6 mg/kg iv on day 1, 1.2 mg/kg iv on day 2, and 1.8 mg/kg iv on days 3-10); nifurtimox monotherapy for 14 days (5 mg/kg orally 3 times per day); and consecutive 10-day melarsoprol-nifurtimox combination therapy (0.6 mg/kg iv melarsoprol on day 1, 1.2 mg/kg iv melarsoprol on day 2, and 1.2 mg/kg/day iv melarsoprol combined with oral 7.5 mg/kg nifurtimox twice a day on days 3-10). Primary outcomes were relapse, severe adverse events, and death attributed to treatment. A total of 278 patients were randomized. The frequency of adverse events was similar between the standard melarsoprol regimen and the other regimens. Encephalopathic syndromes occurred in all groups and caused all deaths that were likely due to treatment. Relapses (n=48) were observed only with the 3 monotherapy regimens. It is concluded that a consecutive 10-day low-dose melarsoprol-nifurtimox combination is more effective than the standard melarsoprol regimen.

14066. **Checkley, A. M., Pepin, J., Gibson, W. C., Taylor, M. N., Jager, H. R. & Mabey, D. C., 2007.** Human African trypanosomiasis: diagnosis, relapse and survival after severe melarsoprol-induced encephalopathy. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **101** (5): 523-526.

Hospital for Tropical Diseases, London, UK; University College Hospital, London, UK. [annacheckley@yahoo.com].

We describe a case of human African trypanosomiasis with a number of unusual features. The clinical presentation was subacute, but the infection was shown to be due to *Trypanosoma brucei rhodesiense*. The infection relapsed twice following treatment and the patient developed a melarsoprol-associated encephalopathy. Magnetic resonance imaging (MRI) findings were suggestive of microhaemorrhages, well described in autopsy studies of encephalopathy but never before shown on MRI. The patient survived severe encephalopathy with a locked-in syndrome. Our decision to provide ongoing life support may be useful to physicians treating similar cases in a setting where intensive care facilities are available.

14067. **Eperon, G., Schmid, C., Loutan, L. & Chappuis, F., 2007**. Clinical presentation and treatment outcome of sleeping sickness in Sudanese pre-school children. *Acta Tropica*, **101** (1): 31-39.

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Existing data on human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* among children are limited. Here, we described the demographic, clinical, diagnostic, treatment and outcome characteristics of HAT in pre-school children from Kajo-Keji County, South Sudan in comparison with older patients. We did a retrospective analysis of HAT patients treated at the Kiri Sleeping Sickness Treatment Centre (SSTC), Kajo-Keji County, from June 2000 to December 2002. Of 1,958 HAT patients, 119 (6.1 percent) were pre-school children (<6 years) including 56 (47 percent) in first-stage illness and 63 (53 percent) in second-stage. The proportion of children in second-stage HAT was significantly higher in very young children (<2 years). Walking and speech disturbances were more frequent in second-stage HAT but other neurological symptoms and signs were not associated with disease stage. Pentamidine treatment for first-stage illness was very safe and effective among pre-school children. In contrast, 4.9 percent of pre-school children in second-stage illness died during melarsoprol treatment and 46 percent had > or = 1 severe adverse event(s). Macular rash, jaundice and skin necrosis on injection site were significantly more frequent in this age group ($p<0.05$). Melarsoprol-induced encephalopatic syndrome was less frequent but more severe than in older age groups. It is concluded that the clinical features of *T. b. gambiense* HAT among pre-school children are insufficiently stage-specific. Therefore, laboratory-based staging is mandatory to prevent unnecessary harm to HAT patients caused by the high toxicity of melarsoprol.

14068. **Howie, S., Guy, M., Fleming, L., Bailey, W., Noyes, H., Faye, J. A., Pepin, J., Greenwood, B., Whittle, H., Molyneux, D. & Corrah, T., 2006.** A Gambian

infant with fever and an unexpected blood film. *PLoS Medicine*, **3** (9): e355.

Medical Research Council Laboratories, Banjul, Gambia. [showie@mrc.gm].

No abstract available**.**

14069. **Kabasa, J. D., 2007.** Public-private partnership works to stamp out sleeping sickness in Uganda. *Trends in Parasitology*, **23 (**5): 191-192.

Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda. [stamp-out-sleeping-sickness@googlegroups.com].

No abstract available.

14070. **Lejon, V., Robays, J., N'Siesi F. X., Mumba, D., Hoogstoel, A., Bisser, S., Reiber, H., Boelaert, M. & Buscher, P., 2007**. Treatment failure in hemo-lymphatic stage sleeping sickness patients is related to intrathecal IgM synthesis, cerebrospinal fluid IgM and interleukin-10. *Clinical and Vaccine Immunology*. **In press, corrected proof**.

Institute of Tropical Medicine, Department of Parasitology, Antwerpen, Belgium; Institute of Tropical Medicine, Department of Public Health, Antwerpen, Belgium; CDI-Bwamanda, Bwamanda, Democratic Republic of Congo (DRC) and Programme National de Lutte contre la Trypanosomiase Humaine Africaine (PNLTHA), Kinshasa, DRC; Institut National de Recherche Biomédicale, Avenue de la Démocratie, Kinshasa, DRC; University of Hasselt, Centre for Statistics, Diepenbeek, Belgium, EA3174; Neuroparasitologie et Neuroépidémiologie Tropicale, Faculté de Médecine, Limoges, France; Neurochemisches Labor, Universität Gottingen, Robert-Koch Strasse 40, D-3400 Gottingen, Germany.

Human African trypanosomiasis treatment is stage-dependent, but staging is controversial. Central nervous system involvement and its relationship with suramin treatment failure were assessed in 60 patients with parasitologically confirmed *Trypanosoma brucei* (*T. b.*) *gambiense* infection in haemo-lymphatic stage (white blood cell count </= 5/µL and no trypanosomes in the CSF). The prognostic value of cerebrospinal fluid (CSF) interleukin-10, IgM (by nephelometry and point-of-care LATEX/IgM test), total protein and trypanosome specific antibodies was assessed. IgM and interleukin-10 were measured in serum and the presence of neurological signs, intrathecal IgM synthesis and blood-CSF barrier dysfunction was determined. After suramin treatment, 14 out of 60 patients relapsed (23 percent). Relapses were significantly correlated with intrathecal IgM synthesis (OR 46; 95 percent CI 8 to 260), CSF IgM >/= 1.9 mg/l (OR 11.7; 95 percent CI 2.7 to 50), CSF end titre in LATEX/IgM >/= 2 (OR 10.4; 95 percent CI 2.5 to 44) and CSF interleukin-10 > 10 pg/ml (OR 5; 95 percent CI 1.3 to 20). Sensitivity of these markers for treatment failure was 43 to 79 percent and specificity was 74 to 93 percent. The results show that *T.b. gambiense* patients with signs of neuro-inflammation in CSF, who are treated with haemo-lymphatic stage drugs, are at risk of treatment failure. This highlights the need for development and

evaluation of accurate point-of-care tests for staging of human African trypanosomiasis. The authors declare not to have a commercial or other association that might pose a conflict of interest. Parts of the results were presented as a poster at the 28th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Addis Ababa, Ethiopia.

14071. **Luscher, A., de Koning, H. P. & Maser, P., 2007.** Chemotherapeutic strategies against *Trypanosoma brucei*: drug targets vs. drug targeting. *Current Pharmaceutical Design*, **13** (6): 555-567.

University of Bern, Institute of Cell Biology, Bern, Switzerland.

Trypanosoma brucei rhodesiense and *T. b. gambiense* are the causative agents of sleeping sickness, a fatal disease that affects 36 countries in sub-Saharan Africa. Nevertheless, only a handful of clinically useful drugs are available. These drugs suffer from severe side-effects. The situation is further aggravated by the alarming incidence of treatment failures in several sleeping sickness foci, apparently indicating the occurrence of drug-resistant trypanosomes. Because of these reasons, and since vaccination does not appear to be feasible due to the trypanosomes' ever changing coat of variable surface glycoproteins (VSGs), new drugs are needed urgently. The entry of *Trypanosoma brucei* into the post-genomic age raises hopes for the identification of novel kinds of drug targets and in turn new treatments for sleeping sickness. The pragmatic definition of a drug target is a protein that is essential for the parasite and does not have homologues in the host. Such proteins are identified by comparing the predicted proteomes of *T. brucei* and *Homo sapiens*, then validated by large-scale gene disruption or gene silencing experiments in trypanosomes. Once all proteins that are essential and unique to the parasite are identified, inhibitors may be found by high-throughput screening. However powerful, this functional genomics approach is going to miss a number of attractive targets. Several current, successful parasiticides attack proteins that have close homologues in the human proteome. Drugs like DFMO or pyrimethamine inhibit parasite and host enzymes alike - a therapeutic window is opened only by subtle differences in the regulation of the targets, which cannot be recognized *in silico*. Working against the post-genomic approach is also the fact that essential proteins tend to be more highly conserved between species than non-essential ones. Here we advocate drug targeting, i.e. uptake or activation of a drug via parasite-specific pathways, as a chemotherapeutic strategy to selectively inhibit enzymes that have equally sensitive counterparts in the host. The *T. brucei* purine salvage machinery offers opportunities for both metabolic and transport-based targeting: unusual nucleoside and nucleobase permeases may be exploited for selective import of salvage enzymes for selective activation of purine antimetabolites.

14072. **Lutumba, P**., **Makieya, E**., **Shaw, A**., **Meheus, F**. & **Boelaert, M., 2007**. Human African trypanosomiasis in a rural community, Democratic Republic of Congo. *Emerging Infectious Diseases,* **13**(2): 248-252.

Programme National de Lutte contre la Trypanosomiase Humaine Africaine, Kinshasa, Democratic Republic of Congo.

According to the World Health Organization, human African trypanosomiasis (HAT) (sleeping sickness) caused the loss of approximately 1.5 million disability-adjusted life years

(DALYs) in 2002. We describe the effect of HAT during 2000-2002 in Buma, a rural community near Kinshasa in the Democratic Republic of Congo. We used retrospective questionnaire surveys to estimate HAT-related household costs and DALYs. The HAT outbreak in Buma involved 57 patients and affected 47 (21 percent) households. The cost to each household was equivalent to 5 months' income for that household. The total number of HAT-related DALYs was 2,145, and interventions to control HAT averted 1,408 DALYs. The cost per DALY averted was US $17. Because HAT has a serious economic effect on households and control interventions are cost-effective, considering only global burden of disease rankings for resource allocation could lead to misguided priority setting if applied without caution in HAT-affected countries.

14073. **Pepin, J., 2007.** Combination therapy for sleeping sickness: a wake-up call. *Journal of Infectious Diseases*, **195** (3): 311-313.

CHUS, 3001, 12th Avenue N., Sherbrooke, Quebec, J1H 5N4, Canada [Jacques.Pepin@USherbrooke.ca].

No abstract available.

14074. **Priotto, G., Fogg, C., Balasegaram, M., Erphas, O., Louga, A., Checchi, F., Ghabri, S. & Piola, P., 2006**. Three drug combinations for late-stage *Trypanosoma brucei gambiense* sleeping sickness: A randomized clinical trial in Uganda. *PLoS Clinical Trials*, **1** (8): e39.

Epicentre, Paris, France.

Our objective was to compare the efficacy and safety of three drug combinations for the treatment of late-stage human African trypanosomiasis caused by *Trypanosoma brucei gambiense*. This trial was a randomized, open-label, active control, parallel clinical trial comparing three arms. The study took place at the Sleeping Sickness Treatment Center run by Médecins Sans Frontières at Omugo, Arua District, Uganda. Stage 2 patients diagnosed in Northern Uganda were screened for inclusion and a total of 54 selected. Three drug combinations were given to randomly assigned patients: melarsoprol-nifurtimox (M+N), melarsoprol-eflornithine (M+E), and nifurtimox-eflornithine (N+E). Dosages were uniform: intravenous (iv) melarsoprol 1.8 mg/kg/d, daily for 10 d; iv eflornithine 400 mg/kg/d, every 6 h for 7 d; oral nifurtimox 15 (adults) or 20 (children <15 y) mg/kg/d, every 8 h for 10 d. Patients were followed up for 24 months. Outcomes measured were cure rates and adverse events attributable to treatment. Randomization was performed on 54 patients before enrollment was suspended due to unacceptable toxicity in one of the three arms. Cure rates obtained with the intention to treat analysis were M+N 44.4 percent, M+E 78.9 percent, and N+E 94.1 percent, and were significantly higher with N+E ($p = 0.003$) and M+E ($p = 0.045$) than with M+N. Adverse events were less frequent and less severe with N+E, resulting in fewer treatment interruptions and no fatalities. Four patients died who were taking melarsoprol-nifurtimox and one who was taking melarsoprol-eflornithine. It is concluded that the N+E combination appears to be a promising first-line therapy that may improve treatment of sleeping sickness, although the results from this interrupted study do not permit conclusive

interpretations. Larger studies are needed to continue the evaluation of this drug combination in the treatment of *T. b. gambiense* sleeping sickness.

14075. **Robays, J., Lefevre, P., Lutumba, P., Lubanza, S., Kande Betu Ku Mesu, V., Van der Stuyft, P. & Boelaert, M., 2007**. Drug toxicity and cost as barriers to community participation in HAT control in the Democratic Republic of Congo. *Tropical Medicine and International Health*, **12** (2): 290-298.

Institute of Tropical Medicine, Epidemiology and Disease Control Unit, Antwerp, Belgium. [jrobays@itg.be].

Active case-finding programmes by mobile teams are the cornerstone of West African Human African Trypanosomiasis (HAT) control. Low attendance rates of screening and low uptake of treatment after diagnosis are major problems. The objectives of this survey were to explore community perception of HAT, to assess acceptability of control activities and to identify barriers amenable to intervention. In September 2004, we conducted 33 focus group discussions with beneficiaries of the HAT control programme among various ethnic groups in two ecological settings (savannah and fluvial) of the Democratic Republic of Congo. The population had a very detailed knowledge and understanding of HAT transmission, utility of screening, symptoms and treatment. Melarsoprol treatment was feared for its side effects. The sudden death of previously asymptomatic people during treatment was attributed to witchcraft, to which one becomes more vulnerable when the diagnosis is disclosed in public. Lack of confidentiality was also a problem because HAT carries a stigma as a mental disease. Lumbar punctures, especially when performed in public, were disliked but less feared. Financial barriers were a major obstacle for many patients. In conclusion, less toxic drugs, lowering financial barriers and improving confidentiality would have considerable impact on the participation in population screening for HAT.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

[See also **30**: 14053]

14076. **Kaare, M. T., Picozzi, K., Mlengeya, T., Fevre, E. M., Mellau, L. S., Mtambo, M. M., Cleaveland, S. & Welburn, S. C., 2007.** Sleeping sickness-a re-emerging disease in the Serengeti? *Travel Medicine and Infectious Disease*, **5** (2): 117-124.

Department of Veterinary Medicine and Public Health, Faculty of Veterinary Medicine, Sokoine University of Agriculture, P.O. Box 3021, Morogoro, Tanzania.

Sleeping sickness is a re-emerging disease in the Serengeti ecosystem affecting both local people and tourists. Here we report the results of a survey to assess the prevalence of

trypanosomiasis in both domestic and wild animals from this area. Five hundred and eighteen cattle samples were collected from 12 villages that bordered the Serengeti National Park and 220 samples from 15 different wild animal species were collected from within the park. PCR analysis, directed against the human serum resistance associated gene SRA, identified human infective *Trypanosoma brucei rhodesiense* parasites in both cattle and warthogs.

14077. **Maharjan, M. & Mishra, D. R., 2006**. Trypanosomiasis in domestic animals of Makwanpur district, Nepal. *Annals of the New York Academy of Sciences*, **1081**: 320-321.

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Trypanosomiasis is an infectious emerging haemoprotozoan parasitic disease in domestical animals of Nepal. The disease was found to be in 16 of 240 (6.67 percent) domestic animals of Makawanpur district, out of which 9 of 105 were (8.57 percent) cattle; 5 of 75 (6.67 percent) buffalos, and 2 of 15 (13.3 percent) dogs, while none of the goats and pigs acquired infection. The disease was found to be most prevalent during the rainy season when 9 of 82 (10.98 percent) were infected and its prevalence was higher among cross breeds than in local breeds.

14078. **Racloz, V., Griot, C. & Stark, K. D., 2006.** Sentinel surveillance systems with special focus on vector-borne diseases. *Animal Health Research Reviews*, **7** (1-2): 71-79.

Swiss Federal Veterinary Office, Schwarzenburgstrasse 155, 3003 Bern, Switzerland and Institute of Virology and Immunoprophylaxis, Mittelhäusern, Switzerland.

In the past few decades, vector-borne diseases have been spreading into countries previously free of these agents. It is necessary for a surveillance method to be tailored to the biology of these agents in order to detect their incursion. Using a sentinel herd system, it is possible to target high-risk areas where occurrence is most probably due to vector presence. Since the 1970s, diseases such as Akabane, vesicular stomatitis and Bluetongue disease have successfully been monitored using cattle herds as sentinels in many countries such as Saudi Arabia, Australia, China, Indonesia, Sultanate of Oman and most recently in countries in Western Europe. This paper reviews the strengths and weaknesses of sentinel herd surveillance systems in general. In order to determine their efficacy, the following criteria were found to be essential: the choice of sentinel locations, sentinel animal, seasonality of sampling and diagnostic testing methods. We conclude that due to its ability to focus on a specific disease, sentinel herd systems have been successful in the early detection of the spread of a targeted agent. This review is used as a basis for recommendations for the development of future sentinel herd systems.

14079. **Sehgal, R. N., Valkiunas, G., Iezhova, T. A. & Smith, T. B., 2006.** Blood parasites of chickens in Uganda and Cameroon with molecular descriptions of *Leucocytozoon schoutedeni* and *Trypanosoma gallinarum*. *Journal of*

Parasitology, **92 (**6): 1336-1343.

Department of Biology, San Francisco State University, 1600 Holloway Ave., San Francisco, California 94132, USA. [sehgal@sfsu.edu]

Using microscopy and PCR, we determined the prevalence of blood parasites in village chickens in Uganda and Cameroon. Of 148 individuals tested, 18.3 percent were infected with *Leucocytozoon schoutedeni* (Haemosporida, *Leucocytozoidae*) and 4.1 percent were infected with *Trypanosoma gallinarum* (Kinetoplastida, *Trypanosomatidae*). No other blood parasites were detected. Subsequent phylogenetic analysis of the cytochrome b gene of *L. schoutedeni* identified 2 distinct lineages that were found at all 3 sampling locations in Uganda. The sequence divergence between these 2 lineages is 1.5 percent. One of these lineages was also found in chickens in Cameroon, nearly 2,000 km distant. There are no morphological differences between blood stages of the parasites represented by the 2 different lineages, suggesting that cytochrome b gene sequence divergence can be as high as 1.5 percent within a single well-defined morphospecies of *Leucocytozoon*. We sequenced a portion of the small subunit ribosomal RNA gene (SSU rRNA) of *T. gallinarum*, and redescribe *T. gallinarum* for the first time since its discovery in 1911. These are the first assignments of DNA sequence data to these morphospecies of *Leucocytozoon* and *Trypanosoma* and may represent an example of intraspecific sequence divergence.

14080. **Ul Hasan, M., Muhammad, G., Gutierrez, C., Iqbal, Z., Shakoor, A. & Jabbar, A., 2006.** Prevalence of *Trypanosoma evansi* infection in equines and camels in the Punjab region, Pakistan. *Annals of the New York Academy of Sciences*, **1081**: 322-324.

Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan.

A cross-sectional study has been carried out in order to determine the prevalence of *Trypanosoma evansi* infection in susceptible hosts in the Punjab region (Pakistan). A total of 170 equines and 150 dromedary camels were examined. Five (3.3 percent) and 6 (4 percent) camels were positive at parasitological and serological examination, respectively. None of the equines tested positive at any method. These results seem to indicate that *T. evansi* infection has a relatively low prevalence in the Punjab region. However, efforts must be done in order to establish control measures in affected herds and avoid dissemination of the disease.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **30**: 14100, 14105]

14081**. Dia, M. L., 2006.** Parasites of the camel in Burkina Faso. *Tropical Animal Health and Production,* **38** (1): 17-21.

Laboratoire de Parasitologie, BP 167 Nouakchott, Mauritania. [mldsb@hotmail.com].

No abstract available.

14082. **Gonzales, J. L., Chacon, E., Miranda, M., Loza, A. & Siles, L. M., 2007.** Bovine trypanosomosis in the Bolivian Pantanal. *Veterinary Parasitology*, **146** (1-2): 9-16.

Laboratorio de Investigación y Diagnóstico Veterinario "LIDIVET", Av. Ejercito Nacional 153, P.O. Box 29, Santa Cruz, Bolivia.

Trypanosomosis caused by *Trypanosoma vivax* has been a constraint for cattle production in the Bolivian lowlands, since it was introduced in 1996. Flooded areas like the Bolivian Pantanal have a suitable environment for the presence and transmission of salivarian trypanosomes and farmers from that region often report trypanosomosis-like problems on their farms. The objective of the present study, therefore, was to characterize the epidemiology of bovine trypanosomosis in the Bolivian Pantanal. In order to achieve this objective, 202 cattle from the province of Angel Sandoval and 209 cattle from the province of German Busch were randomly sampled (the Pantanal is located in both provinces). Twenty-nine farms in both provinces were visited, the farmers interviewed, and biologic samples collected from their cattle. Samples were submitted for parasitological and PCR evaluation and the prevalence of bovine trypanosomosis was estimated for each province. Laboratory results were correlated with the sampled animals packed cell volume (PCV) and body condition (BC) scores and the observed *T. vivax* parasites measured for morphometry analysis. Results from this study show differences in morphometric measures between *T. vivax* parasites from each province. Differences between provinces were also observed in the *T. vivax*-related disease situation. While in Angel Sandoval the PCV and BC of *T. vivax*-affected animals were significantly lower than those of the *T. vivax*-negative animals, in German Busch no differences were observed in the PCV and BC of *T. vivax*-positive or negative animals. Animal prevalence of *T. vivax* in Angel Sandoval was 27.79 percent (95 percent CI: 14.52-44.28) and in German Busch was 19.03 percent (95 percent CI: 9.19-30.75). The *T. evansi* animal prevalence in each province was 0.99 percent (95 percent CI: 0.27-2.99) and 5.71 percent (95 percent CI: 2.43-12.19), respectively. Based on questionnaire and laboratory results, it was concluded that trypanosomosis is a primary constraint for cattle production in the Bolivian Pantanal.

14083. **Gutierrez, C., Corbera, J. A., Morales, M. & Buscher, P., 2006**. Trypanosomosis in goats: current status. *Annals of the New York Academy of Sciences*, **1081**: 300-310.

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Trypanosomosis is a major constraint on ruminant livestock production in Africa, Asia, and South America. The principal host species affected varies geographically, but buffalo, cattle, camels, and horses are particularly sensitive. Natural infections with *Trypanosoma congolense, T. vivax, T. brucei,* and *T. evansi* have been described in goats. Trypanosomosis in goats produces acute, subacute, chronic, or subclinical forms, *T. vivax, T. congolense* and *T. evansi* being the most invasive trypanosomes for goats. However, the role of goats in the epidemiology of trypanosomosis is largely discussed and not well understood.

Thus, it has commonly been assumed that trypanosomosis presents a subclinical course and that goats do not play an important role in the epidemiology of the disease. This can partially be due to parasitemia caused by trypanosomes which has been considered low in goats. However, this assumption is currently undergoing a critical reappraisal because of goats may also serve as a reservoir of trypanosome infection for other species, including the human beings in the case of *T. brucei rhodesiense*. The present article describes the current status of trypanosomosis in goats in Africa, Asia, and South America. Pathogenesis, clinical features, diagnosis, and treatment of the different trypanosomes are also described. The possible role of goats in the epidemiology of the disease in the different areas is also discussed.

14084. **Mochabo, M. O., Kitala, P. M., Gathura, P. B., Ogara, W. O., Eregae, E. M., Kaitho, T. D. & Catley, A., 2006**. The socio-economic impact of important camel diseases as perceived by a pastoralist community in Kenya. *Onderstepoort Journal of Veterinary Research*, **73** (4): 269-274.

KARI-Trypanosomiasis Research Centre (KARI-TRC), P.O. Box 362, Kikuyu 00902, Kenya. [kmochabo@hotmail.com].

This paper presents the results of a study conducted in a pastoral community in Kenya using participatory appraisal approaches. The objective of the study was to assess the socio-economic impact of camel trypanosomosis (surra) according to the perceptions of the pastoralists. Four livestock grazing units were conveniently selected and in each of them, three groups of key informants comprising five to eight persons were selected for the participatory exercises. Five camel diseases were listed in order of importance according to their severity and frequency of occurrence including trypanosomosis, mange, non-specific diarrhoea, tick infestations and haemorrhagic septicaemia. The losses listed as incurred due to the five diseases were: losses in milk, meat, blood, fats and hides, dowry payments, and depreciation in sale of animals, losses due to infertility and abortions, and losses due to the cost of treatment. There was good agreement ($P < 0.05$) between the informant groups on the losses incurred as a result of the diseases for all the selected loss indicators. Surra and mange were given high median scores on all the indicators while non-specific diarrhoea, tick infestations, and haemorrhagic septicaemia received moderate median scores. Based on the study findings it is concluded that the camel plays a central role in the lives of Turkana pastoralists and that surra has a devastating social and economic impact. There is a need for veterinary and policy decision-makers to focus more attention on the control of surra in this arid and semi-arid area of Kenya.

14085**. Muhammad, G., Saqib, M., Sajid, M. S. & Naureen, A., 2007.** *Trypanosoma evansi* infections in Himalayan black bears (*Selenarctos thibetanus*). *Journal of Zoo and Wildlife Medicine*, **38**(1): 97-100.

Department of Clinical Medicine and Surgery, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan.

The Asiatic or Himalayan black bear (*Selenarctos thibetanus*) is an endangered species. In South Asian countries, captive tamed Himalayan bears are commonly used by roving bear-charmers to entertain the people in rural and urban areas. In captivity, this species confronts

several psychophysical traumas and communicable diseases, which are prevalent in other domestic species. The present report describes four cases of *Trypanosoma evansi* infection in live Himalayan charming bears, which originated from the Faisalabad and Jhang districts of Pakistan. The condition was characterized by pyrexia, accelerated pulse, tachypnea, depression, anaemic mucous membranes, and ataxia (n = 3). Microscopic examination of peripheral blood films revealed moderate (n = 2) or high (n = 2) numbers of *T. evansi*. All four bears were treated twice at 3-day intervals with suramin sodium by using almost twice the dosage recommended for common domestic animals (10 mg/kg). The treated bears were found aparasitaemic on repeat blood testing on days 5, 7, and 10 post-treatment. No adverse effects were noted and all four cases recovered in 3-7 days after completion of the second round of treatment. One bear died 8 days after the second treatment (day 11). This is the first report of *T. evansi* in bears.

(c) TRYPANOTOLERANCE

14086. **Berthier, D., Chantal, I., Thevenon, S., Marti, J., Piquemal, D. & Maillard, J. C., 2006**. Bovine transcriptome analysis by SAGE technology during an experimental *Trypanosoma congolense* infection. *Annals of the New York Academy of Sciences*, **1081**: 286-299.

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In central and sub-Saharan Africa, trypanosomosis is a tsetse fly-transmitted disease, which is considered as the most important impediment to livestock production in the region. However, several indigenous West African taurine breeds (*Bos taurus*) present remarkable tolerance to the infection. This genetic capability, named trypanotolerance, results from numerous biological mechanisms most probably under multigenic dependences, among which are control of the trypanosome infection by limitation of parasitemia and control of severe anaemia due to the pathogenic effects. Today, some postgenomic biotechnologies, such as transcriptome analyses, allow characterization of the full expressed genes involved in the majority of animal diseases under genetic control. One of them is serial analysis of gene expression (SAGE) technology, which consists of the construction of mRNA transcript libraries for qualitative and quantitative analysis of the entire genes expressed or inactivated at a particular step of cellular activation. We developed four different mRNA transcript libraries from white blood cells on a N'Dama trypanotolerant animal during an experimental *Trypanosoma congolense* (*T. congolense*) infection: one before experimental infection (ND0), one at the parasitaemia peak (NDm), one at the minimal packed cell volume (NDa), and the last one at the end of the experiment after normalization (NDf). Bioinformatic comparisons in bovine genomic databases allowed us to obtain more than 75,000 sequences, among which are several known genes, some others are already described as expressed sequence tags (ESTs), and the last are completely new, but probably functional in trypanotolerance. The knowledge of all identified named or unnamed genes involved in trypanotolerance characteristics will allow us to use them in a field marker-assisted selection strategy and in microarrays prediction sets for bovine trypanotolerance.

14087. **Bosso, N.A., Cissé, M.F., van der Waaij, E.H., Fall, A. & van Arendonk, J.A.M., 2007.** Genetic and phenotypic parameters of body weight in West African Dwarf goat and Djallonké sheep. *Small Ruminant Research*, **67** (2-3): 271-278.

International Trypanotolerance Centre, PMB 14 Banjul, Gambia; Institute of Animal Sciences, P.O. Box 338, 6700 AH Wageningen, The Netherlands; Department of Farm Animal Health, Veterinary Faculty, University of Utrecht, P.O. Box 80151, 3508 TD, Utrecht, The Netherlands, and Institut de Recherche Agronomique de Guinée, B.P. 1523 Conakry Guinea. [nguetta.bosso@wur.nl].

The International Trypanotolerance Centre's small ruminant breeding programme was initiated in 1995. The aim was to increase the efficiency of meat production and the trypanotolerance of the animals (sheep and goat). To achieve that goal, selection was based on estimated breeding values for daily weight gain from 4 to 12 months of age measured on trypanosome challenge. The purpose of this study was to estimate genetic parameters for growth traits and to evaluate genetic trends in West African Dwarf goat and Djallonké sheep resulting from the breeding programme under a low input production environment. Data for West African Dwarf goat and Djallonké sheep included birth weight (BW), weaning weight (W120), yearling weight (W360), pre-weaning (GR0–4) and post-weaning (GR4–12) growth rate. The data were analysed using an animal model that accounted for fixed effects of sex, year of birth, season of birth, parity of the dam, type of birth and the interaction year by season of birth. Estimates of heritability for BW, W120, W360, GR0–4 and GR4–12 were 0.5, 0.43, 0.30, 0.32 and 0.11 for goats and 0.39, 0.54, 0.21, 0.54 and 0.23 for sheep, respectively. The genetic correlation between BW and W120 was high for goats (0.74) and moderate for sheep (0.47). Genetic correlations between W120 and GR4–12 were high (0.92) for goats and moderate (0.49) for sheep. Between GR0–4 and BW the correlation was positive but low for sheep (0.26) and moderate for goats (0.60). Positive trends were found in mean estimated breeding values for animals born in the period 1995–2002 which demonstrated the effectiveness of the implemented breeding programmes.

14088. **Pitchford, W.S., 2007.** Improving accuracy of selection of young bulls by pastoralists. *Livestock Science*, **110** (1-2): 141-147.

International Livestock Research Institute, PO Box 30709 Nairobi, and the University of Adelaide, Roseworthy campus, Roseworthy SA 5371, Australia. [Wayne.Pitchford@adelaide.edu.au].

A key to maximising response to selection in pastoral cattle kept by groups such as the sub-Saharan Maasai is an accurate selection of young bulls. A breeding objective was developed based on weight, reproductive rate (days to calving), temperament, tick resistance and trypanotolerance. Accuracy of selection was defined as the correlation between the breeding objective and various selection indices. Accuracy was evaluated assuming availability of information on a range of traits (those in objective plus scrotal circumference) from individuals, parents, grandparents, half-sibs, progeny and genetic markers. Various scenarios that represent what could occur at the village level were tested. Just selecting on weight alone had an accuracy of 0.538. Additional measurements on the individual (including

repeated measures) had a large effect on accuracy. Records on relatives were less helpful than expected. Genetic markers for traits that are difficult to measure (days to calving and trypanotolerance) were helpful for improving accuracy. However, they are unlikely to be used in the near future because of cost and availability. An additional output from this study is simple selection indices that could be implemented immediately at the village level.

14089. **Thevenon, S., Dayo, G. K., Sylla, S., Sidibe, I., Berthier, D., Legros, H., Boichard, D., Eggen, A. & Gautier, M., 2007.** The extent of linkage disequilibrium in a large cattle population of western Africa and its consequences for association studies. *Animal Genetics*, **38** (3): 277-286.

UMR Trypanosomes, CIRAD, Montpellier, F-34398 France; UMR Trypanosomes, IRD, Montpellier, F-34398, France; and URBIO, CIRDES, Bobo-Dioulasso 01, Burkina Faso.

Several previous studies concluded that linkage disequilibrium (LD) in livestock populations from developed countries originated from the impact of strong selection. Here, we assessed the extent of LD in a cattle population from western Africa that was bred in an extensive farming system. The analyses were performed on 363 individuals in a *Bos indicus* x *Bos taurus* population using 42 microsatellite markers on BTA04, BTA07 and BTA13. A high level of expected heterozygosity (0.71), a high mean number of alleles per locus (9.7) and a mild shift in Hardy-Weinberg equilibrium were found. Linkage disequilibrium extended over shorter distances than what has been observed in cattle from developed countries. Effective population size was assessed using two methods; both methods produced large values: 1388 when considering heterozygosity (assuming a mutation rate of 10-3) and 2344 when considering LD on whole linkage groups (assuming a constant population size over generations). However, analysing the decay of LD as a function of marker spacing indicated a decreasing trend in effective population size over generations. This decrease could be explained by increasing selective pressure and/or by an admixture process. Finally, LD extended over small distances, which suggested that whole-genome scans will require a large number of markers. However, association studies using such populations will be effective.

(d) TREATMENT

[See also **30**: 14146, 14153, 14156, 14159, 14166]

14090. **Grace, D., Himstedt, H., Sidibe, I., Randolph, T. & Clausen, P. H., 2007**. Comparing FAMACHA((c)) eye colour chart and Hemoglobin Color Scale tests for detecting anemia and improving treatment of bovine trypanosomosis in West Africa. *Veterinary Parasitology,* **147** (1-2): 26-39.

Institute for Parasitology and Tropical Veterinary Medicine, Freie Universitaet Berlin, Konigsweg 67, 14163 Berlin, Germany.

African animal trypanosomosis (AAT) is considered the most important cattle disease in sub-Saharan Africa but its diagnosis in the field is difficult, resulting in inappropriate

treatments, excessive delay in treatments and under-treatment. A field study in West Africa investigated the usefulness of anaemia in the diagnosis of trypanosomosis. A total of 20,772 cattle blood samples were taken from 121 villages in 3 countries. The average packed cell volume (PCV) of trypanosomosis positive cattle was 23 percent, versus 28 percent for negative cattle. In a sub-set of animals, other causes of anaemia were investigated showing most of the anaemia burden was attributable to trypanosomosis. Anaemia was a reasonably accurate indicator of trypanosomosis in the study area, with a sensitivity of 56 percent and a specificity of 80 percent and a diagnostic odds ratio of 4.2, the highest of all the signs evaluated (anaemia, emaciation, staring coat, lymphadenopathy, fever, lacrimation and salivary or nasal discharge). Having confirmed the usefulness of anaemia as a predictor of trypanosomosis, two potential pen-side tests for anaemia were evaluated (the first reported trial of their use in cattle): firstly a colour chart developed for anaemia detection in sheep through visual inspection of conjunctival membranes (FAMACHA((c))) and secondly the Haemoglobin Colour Scale (HbCS) developed for assessing haemoglobin levels in human patients by comparing blood drops on filter paper with colour standards. In a population of cattle suspected by their owners to be sick with trypanosomosis (n=898) the sensitivity of the HbCS test was 56 percent and the specificity was 77 percent, while the sensitivity of the FAMACHA((c)) test was 95 percent and the specificity was 22 percent. The higher sensitivity but lower specificity suggest the FAMACHA((c)) may be useful as a screening test and the HbCS as a confirmatory test. The two tests were also evaluated in cattle randomly selected from the village herd. Using cut-off points to optimize test performance, the HbCS test had a sensitivity of 81 percent and a specificity of 62 percent (n=505 cattle), while the FAMACHA((c)) had a sensitivity of 92 percent and a specificity of 30 percent (n=298 cattle). Recommendations are made for the appropriate use of these tests in the West African region.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

14091. **Enyaru, J.C., Matovu, E., Nerima, B., Akol, M. & Sebikali, C., 2006**. Detection of *T. b. rhodesiense* trypanosomes in humans and domestic animals in south east Uganda by amplification of serum resistance-associated gene. *Annals of the New York Academy of Sciences,* **1081**: 311-319.

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The human serum resistance-associated (SRA) gene was identified in 28 (80 percent) of the 35 *T. b. rhodesiense* trypanosomes from parasitologically confirmed sleeping sickness cases, using the primers designed by Radwanska and in 27 (77.1 percent) of the same 35 *T. b. rhodesiense* trypanosomes using the primers designed by Gibson. However, about 20 percent of the 35 *T. b. rhodesiense* trypanosomes could not be detected by SRA-polymerase chain reaction (PCR) even when an aliquot of the first PCR was used in the second PCR, indicating that the gene may be absent in those trypanosomes or the trypanosomes could be having

another variant of SRA not detectable by these primers since three variants of SRA genes have so far been identified or the amount of trypanosomal DNA extracted from infected blood was too low to be detected. The trypanosome isolates that are SRA gene negative may indicate the presence of some *T. b. rhodesiense* trypanosomes with modified or lack SRA genes or simple loss of the SRA gene from the expression site in which it resides during antigenic variation. Analysis of trypanosomes derived from domestic animals showed that 79 (90.8 percent) of the 87 trypanosomes isolated from cattle were positive by *Trypanosoma brucei* (TBR)-PCR, indicating that they were *Trypanozoon* while 8 (9.2 percent) of the trypanosome isolates which were negative by TBR-PCR could be *T. vivax, T. congolense*, or *T. theileri*. When subjected to SRA-PCR, 10 (11.5 percent) of the 87 trypanosomes isolates derived from cattle were positive, indicating that there could be *T. b. rhodesiense* circulating in cattle, which is similar to the percentage of *T. b. rhodesiense* previously obtained in cattle in Serere, Soroti district.

14092. **Madruga, C. R., Araujo, F. R., Cavalcante-Goes, G., Martins, C., Pfeifer, I. B., Ribeiro, L. R., Kessler, R. H., Soares, C. O., Miguita, M., Melo, E. P., Almeida, R. F. & Lima, M. M., Jr., 2006.** The development of an enzyme-linked immunosorbent assay for *Trypanosoma vivax* antibodies and its use in epidemiological surveys. *Memórias do Instituto Oswaldo Cruz*, **101** (7): 801-807.

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There are data indicating that the distribution of *Trypanosoma vivax* in the Brazilian territory is expanding with potential to reach other areas, where the vectors are present. The detection of anti-trypanosomal antibodies in serum provides important information of the trypanosomal status in cattle herds. For this reason, an enzyme-linked immunosorbent assay (*Tv*-ELISA-Ab) with crude antigen from one Brazilian isolate of *T. vivax* was developed and evaluated. The sensitivity and specificity were respectively 97.6 and 96.9 percent. In the evaluation of cross-reactions, three calves inoculated with *T. evansi* trypimastigotes blood forms showed optical densities (OD) under the cut-off during the whole experimental period, except one at 45 days post-inoculation. With relation to *Babesia bovis, B. bigemina*, and *Anaplasma marginale*, which are endemic haemoparasites in the studied area, the cross-reactions were shown to be 5.7, 5.3, and 1.1 percent, respectively. The first serological survey of Pantanal and state of Para showed that *T. vivax* is widespread, although regions within both areas had significantly different prevalences. Therefore, this *Tv*-ELISA-Ab may be a more appropriate test for epidemiological studies in developing countries because the diagnostic laboratories in most countries may be able to perform an ELISA, which is not true for the polymerase chain reaction.

14093. **Monzon, C. M., 2006**. Characterisation of a monoclonal antibody against *Trypanosoma evansi* and its application for detecting circulating antibodies. *Revue Scientifique et Technique*, **25** (3): 1067-1074.

Facultad de Ciencias de la Salud, Universidad Nacional de Formosa, Consejo Nacional de Investigaciones Cientificas y Técnicas, Centro de Diagnóstico e Investigaciones Veterinarias de Formosa, Formosa, Argentina.

Monoclonal antibodies were obtained against *Trypanosoma evansi*. The 2-4F6 IgM monoclonal antibody (Mab) was chosen for the study because of its ability to detect antigens and its specificity (as it did not recognise *T. cruzi, T. equiperdum, Babesia equi* or *B. caballi*). The immunoblot test revealed that the 2-4F6 IgM Mab recognises epitopes in two antigenic bands, one measuring 85 kDa and the other 122 kDa. An immunoassay for antigen detection in serum using polyclonal antibodies for capture, the Mab 2-4F6 as primary antibody and an antimouse IgM as secondary antibody gave positive results in 10 of the 11 *Equidae* infected with *T. evansi*, whereas 20 controls gave negative results. These research results show that the Mab 2-4F6 and the antigen it recognises are useful in identifying *Equidae* infected with *T. evansi*.

14094. **Reyna-Bello, A., Eleizalde, M. C. & Silva, A. M., 2007**. Assessment of chromogen suitability in ELISA for the detection of anaplasmosis and trypanosomosis. *Journal of Immunoassay and Immunochemistry*, **28** (1): 1-11.

Universidad Nacional Experimental Simón Rodriguez-IDECYT, Centro de Estudios Biomedicos y Veterinarios, Laboratorio de Inmunobiologia, Caracas, Venezuela. [areyna@inmunobiologia.com].

Two different ELISAs were routinely performed in our laboratory to detect bovine trypanosomosis and anaplasmosis. The ELISA test for trypanosomosis involved the adsorption of a soluble fraction of parasites as the antigen; and, the ELISA for anaplasmosis was performed with a purified recombinant protein MSP5r adsorbed to the plate. With the purpose of assessing the merit of ABTS and TMB, we compared the absorbance obtained from positive and negative control sera from both assays. The results obtained, suggest that TMB is more adequate for recombinant antigens and that ABTS is preferred when partially purified antigenic extracts are used in the ELISA test.

(b) PATHOLOGY AND IMMUNOLOGY

14095. **Baral, T. N., De Baetselier, P., Brombacher, F. & Magez, S., 2007.** Control of *Trypanosoma evansi* infection is IgM mediated and does not require a type I inflammatory response. *Journal of Infectious Diseases*, **195** (10): 1513-1520.

Department of Cellular and Molecular Interactions, Vlaams Interuniversitair Instituut voor Biotechnologie, Laboratorium voor Cellulaire en Moléculaire Immunologie, Vrije Universiteit Brussel, Brussels, B-1050, Belgium. [tbaral@vub.ac.be].

Very recent reports have documented that *Trypanosoma evansi*, the etiological agent of the livestock disease "surra", can cause human trypanosomiasis. In contrast to trypanosomes causing human African trypanosomiasis, *T. evansi* has a wide geographic distribution and host range, yet information about the immunobiological aspects of *T. evansi* trypanosomiasis is limited. Here, we show that, although *T. evansi* causes the induction of tumour necrosis factor (TNF), interferon-gamma, and nitric oxide during the early stage of infection, none of these molecules are crucial for parasitaemia control and survival of the

infected animal. However, TNF and TNF receptor 2 affect the induction of late-stage anaemia. Using B cell- and immunoglobulin M (IgM)-deficient mice, we identified IgM as being crucial for parasitaemia control and host survival. Collectively, our results show that, compared with other trypanosomes, *T. evansi* displays a distinct host-parasite interaction profile, given that, despite an infection-associated induction of proinflammatory molecules, only IgM antibodies contribute significantly to parasite control.

14096. **Bhasin, K. K., Yu, J. M., Tward, A., Shih, D., Campbell, D. A. & Lusis, A. J., 2006.** *Trypanosoma congolense*: paraoxonase 1 prolongs survival of infected mice. *Experimental Parasitology*, **114** (3): 240-245.

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In vitro studies have suggested that a fraction of human high density lipoprotein (HDL), termed trypanosome lysis factor (TLF), can protect against trypanosome infection. We examined the involvement of two proteins located in the TLF fraction, apolipoprotein A-II (apoA-II) and paraoxonase 1 (PON1), against trypanosome infection. To test whether PON1 is involved in trypanosome resistance, we infected human PON1 transgenic mice, PON1 knockout mice, and wild-type mice with *Trypanosoma congolense*. When challenged with the same dosage of trypanosomes, mice overexpressing PON1 lived significantly longer than wild-type mice, and mice deficient in PON1 lived significantly shorter. In contrast, mice overexpressing another HDL associated protein, apoA-II, had the same survival as wild-type mice. Together, these data suggest that PON1 provides protection against trypanosome infection. *In vitro* studies using *T. brucei brucei* indicated that HDL particles containing PON1 and those depleted of PON1 did not differ in their lysis ability, suggesting that protection by PON1 is indirect. Our data are consistent with an *in vivo* role of HDL protection against trypanosome infection.

14097. **Harris, T. H., Mansfield, J. M. & Paulnock, D. M., 2007.** CpG oligodeoxynucleotide treatment enhances innate resistance and acquired immunity to African trypanosomes. *Infection and Immunity*, 75 (5): 2366-2373.

Department of Medical Microbiology and Immunology, University of Wisconsin-Madison School of Medicine and Public Health, Wisconsin 53706, USA.

Relative resistance to African trypanosomiasis is based on the development of a type I cytokine response, which is partially dependent on innate immune responses generated through MyD88 and Toll-like receptor 9 (TLR9). Therefore, we asked whether enhancement of the immune response by artificial stimulation with CpG oligodeoxynucleotide (ODN), a TLR9 agonist, would result in enhanced protection against trypanosomes. In susceptible BALB/c mice, relative resistance to infection was significantly enhanced by CpG ODN treatment and was associated with decreased parasite burden, increased cytokine production, and elevated parasite-specific B- and T-cell responses. In relatively resistant C57BL/6 mice, survival was not enhanced but early parasitaemia levels were reduced 100-fold and the majority of the parasites were nondividing, short stumpy (SS) forms. CpG ODN treatment of

lymphocyte-deficient C57BL/6-scid and BALB/cByJ-scid mice also enhanced survival and reduced parasitaemia, indicating that innate resistance to trypanosome infection can be enhanced. In C57BL/6-scid and BALB/cByJ-scid mice, the parasites were also predominantly SS forms during the outgrowth of parasitaemia. However, the effect of CpG ODN treatment on parasite morphology was not as marked in gamma interferon (IFN-gamma)-knockout mice, suggesting that downstream effects of IFN-gamma production may play a discrete role in parasite cell differentiation. Overall, these studies provide the first evidence that enhancement of resistance to African trypanosomes can be induced in susceptible animals in a TLR9-dependent manner and that CpG ODN treatment may influence the developmental life cycle of the parasites.

14098. **Li, S. Q., Fung, M. C., Reid, S. A., Inoue, N. & Lun, Z. R., 2007.** Immunization with recombinant beta-tubulin from *Trypanosoma evansi* induced protection against *T. evansi, T. equiperdum* and *T. brucei* infection in mice. *Parasite Immunology*, **29** (4): 191-199.

Centre for Parasitic Organisms, State Key Laboratory of Biocontrol and Key Laboratory for Tropical Diseases Control of the Ministry of Education, School of Life Sciences, Sun Yat-Sen (Zhongshan) University, Guangzhou, P.R. China.

The beta-tubulin gene of *Trypanosoma evansi* (STIB 806) was cloned and expressed in *Escherichia coli*. The predicted amino acid sequence of *T. evansi* beta-tubulin shows 100 percent, 99.8 percent, 99.1 percent, and 98.6 percent homology with *T. equiperdum, T. b. brucei, T. cruzi* and *T. danilewskyi*, respectively, but is diverse from that of *T. cyclops*, showing only 51.6 percent of homology. Recombinant beta-tubulin was expressed as inclusion bodies in *E. coli*. It was purified and renatured for immunological studies. Mice immunized with the renatured recombinant beta-tubulin were protected from lethal challenge with *T. evansi* STIB 806, *T. equiperdum* STIB 818 and *T. b. brucei* STIB 940, showing 83.3 percent, 70 percent and 76.7 percent protection, respectively. Serum collected from the rabbit immunized with recombinant beta-tubulin inhibited the growth of *T. evansi, T. equiperdum* and *T. b. brucei in vitro*. Serum from mice and rabbits immunized with recombinant beta-tubulin recognized only *T. evansi* beta-tubulin and not mouse beta-tubulin. The results of this study demonstrated that the recombinant *T. evansi* beta-tubulin is a potential candidate for the development of a vaccine to prevent animal trypanosomiasis caused by these three trypanosome species.

14099. **Namangala, B., Sugimoto, C. & Inoue, N., 2007.** Effects of exogenous transforming growth factor beta on *Trypanosoma congolense* infection in mice. *Infection and Immunity*, **75** (4): 1878-1885.

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The socioeconomic implications of trypanosomosis in sub-Saharan Africa and the limitations of its current control regimes have stimulated research into alternative control methods. Considering the pro- and anti-inflammatory properties of transforming growth factor beta1 (TGF-beta1) and its potential to enhance immunity against protozoan parasites,

we examined the effects of intraperitoneally delivered TGF-beta1 in C57BL/6 mice infected with *Trypanosoma congolense*, the haemoprotozoan parasite causing nagana in cattle. A triple dose of 10 ng TGF-beta1 significantly reduced the first parasitaemic peak and delayed mortality of infected mice. Furthermore, exogenous TGF-beta1 significantly decreased the development of trypanosome-induced anaemia and splenomegaly. The apparent TGF-beta1-induced antitrypanosome protection, occurring mainly during the early stage of infection, correlated with an enhanced parasite antigen-specific Th1 cell response characterized by a skewed type I cytokine response and a concomitant stronger antitrypanosome immunoglobulin G2a antibody response. Infected TGF-beta1-pretreated mice exhibited a significant reduction in the trypanosome-induced hyperexpansion of B cells. Furthermore, evidence is provided herein that exogenous TGF-beta1 activates macrophages that may contribute to parasite control. Collectively, these data indicate that exogenous TGF-beta1 is immunostimulative, inducing partial protection against *T. congolense* infection, possibly through mechanisms involving innate immune responses.

14100. **Oluyinka, O. O., Mairo, I. H., Ajanusi, J. A., David, O., Sekoni, V. & Nok, A. J., 2007.** Semen sialic acid surge and modulation of alpha-L-fucosidase activity: possible link to loss in reproductive capacity during trypanosomiasis. *Cell Biochemistry and Function.* **In press, corrected proof.**

Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria.

The profiles of semen sialic acid and the enzyme alpha-L-fucosidase were studied in rams undergoing chronic infection by *Trypanosoma congolense*. Our data showed a significant surge in the level of sialic acid with parasitaemia. The pattern followed a polynomial function we had reported for erythrocyte sialic acid in mice undergoing acute infection by *T. congolense*. The activity of the enzyme alpha-fucosidase decreased progressively with approximately 60 percent decrease at the end of the 14 weeks of infection. Representative semen samples from the control and infected rams were subjected to kinetic characterization. While the uninfected semen sample showed two active pH peaks at 4.5-5.5 and at 6.8-7.2, respectively, there was an apparent shift to only a single pH optimum at 4.5-5.5 for the pathological semen. The fucosidases from both sources were optimally active at 35 ^{0}C albeit with contrasting activation energies (E (a)) with values 20.58 and 35 kJ/mol for the control and infected semen, respectively. Kinetic studies using methylumbelliferyl-beta-fucoside (4MU-Fuc) as substrate gave K (M) and V (max) values of 3.25 µM and 14.6 µM min^{-1} mg^{-1}, respectively for the control semen. The values for the infected semen were 18.25 µM and 10.5 µM min^{-1} mg^{-1}, respectively. The significance of these results is discussed as they relate to loss in reproductive capacity in trypanosomosis.

14101. **Omer, O. H., Mousa, H. M. & Al-Wabel, N., 2007**. Study on the antioxidant status of rats experimentally infected with *Trypanosoma evansi*. *Veterinary Parasitology*, **145** (1-2): 142-145.

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The antioxidant status of rats experimentally infected with *Trypanosoma evansi* isolated from a camel was studied using established parasitological, haematological and biochemical methods. The results indicated that infections in all rats resulted in a fulminating parasitaemia. Changes in blood parameters in *T. evansi*-infected rats indicated leukocytosis and a macrocytic hypochromic anaemia. A degree of anisocytosis was also observed. The activities of plasma glucose-6-phosphate dehydrogenase and glutathione peroxidase in whole blood of infected rats were significantly higher ($p<0.05$ and $p<0.001$, respectively) compared with control. No statistically significant difference was observed in the activity of superoxide dismutase in infected and control rats. Results obtained indicated that trypanosomosis caused oxidative stress and induced antioxidant enzymes.

14102. **Shi, M. Q., Wang, C. R., Wei, G. J., Pan, W. L., Appleyard, G. & Tabel, H., 2006.** Experimental African trypanosomiasis: lack of effective CD1d-restricted antigen presentation. *Parasite Immunology*, **28** (12): 643-647.

Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada.

BALB/c mice are highly susceptible to African trypanosomiasis, whereas C57BL/6 mice are relatively resistant. Other investigators have reported that the synthesis of IgG antibodies to purified membrane form of variant surface glycoprotein (mfVSG) of *Trypanosoma brucei* is CD1 restricted. In this study, we examine the role of the CD1d/NKT cell pathway in susceptibility and resistance of mice to infection by African trypanosomes. Administration of anti-CD1d antibodies to *Trypanosoma congolense*-infected BALB/c mice neither affects the parasitemia nor the survival time. Correspondingly, CD1d(-/-) and CD1d(+/+) BALB/c mice infected with *T. congolense* or *T. brucei* show no differences in either parasitaemia or survival time. The course of disease in relative resistant C57BL/6 mice infected with *T. congolense* is also not affected by the absence of CD1d. Parasitaemia, survival time, and plasma levels of IgG2a and IgG3 parasite-specific antibodies in infected CD1d(-/-) C57BL/6 are not different from those of infected CD1d(+/+) C57BL/6 mice. We conclude that CD1d-restricted immune responses do not play an important role in susceptibility/resistance of mice infected with virulent African trypanosomes. We speculate that virulent trypanosomes have an evasion mechanism that prevents the induction of a parasite-specific, CD1d-restricted immune response by the host.

14103. **Shi, M. Q., Wei, G. J. & Tabel, H., 2007.** *Trypanosoma congolense* infections: MHC class II-restricted immune responses mediate either protection or disease, depending on IL-10 function. *Parasite Immunology*, **29** (2): 107-111.

Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada.

BALB/c mice are highly susceptible and C57BL/6 relatively resistant to *Trypanosoma congolense* infections. Here we show that relatively resistant wild-type B6 mice infected with *T. congolense* survive significantly longer (> 200 days) than infected major histocompatibility complex (MHC) class II-deficient B6 mice (approximately 50 days). We

also show that blocking of the interleukin-10 (IL-10) receptor induces early death of wild-type B6 mice infected with *T. congolense* (approximately 10 days), but does not affect the survival of infected MHC class II-deficient B6 mice. We conclude that MHC class II-restricted immune responses mediate protection and, when IL-10 function is impaired, MHC class II-restricted immune responses mediate early mortality in otherwise resistant B6 mice. Thus, in *T. congolense* infections, MHC class II-restricted immune responses mediate either protection or disease, depending on IL-10 function.

14104. **Shiflett, A. M., Faulkner, S. D., Cotlin, L. F., Widener, J., Stephens, N. & Hajduk, S. L., 2007.** African trypanosomes: intracellular trafficking of host defence molecules. *Journal of Eukaryotic Microbiology*, **54** (1): 18-21.

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Trypanosoma brucei brucei is the causative agent of Nagana in cattle and can infect a wide range of mammals but is unable to infect humans because it is susceptible to the innate cytotoxic activity of normal human serum. A minor subfraction of human high-density lipoprotein (HDL), containing apolipoprotein A-I (APOA1), apolipoprotein L-I (APOL1) and haptoglobin-related protein (HPR) provides this innate protection against *T. b. brucei* infection. Both HPR and APOL1 are cytotoxic to *T. b. brucei* but their specific activities for killing increase several hundred-fold when assembled in the same HDL. This HDL is called trypanosome lytic factor (TLF) and kills *T. b. brucei* following receptor binding, endocytosis, and lysosomal localization. Trypanosome lytic factor is activated in the acidic lysosome and facilitates lysosomal membrane disruption. Lysosomal localization is necessary for *T. b. brucei* killing by TLF. *Trypanosoma brucei rhodesiense*, which is indistinguishable from *T. b. brucei*, is resistant to TLF killing and causes human African sleeping sickness. Human infectivity by *T. b. rhodesiense* correlates with the evolution of a human serum resistance associated protein (SRA) that is able to ablate TLF killing. When *T. b. brucei* is transfected with the SRA gene it becomes highly resistant to TLF and human serum. In the SRA transfected cells, intracellular trafficking of TLF is altered and TLF mainly localizes to a subset of SRA containing cytoplasmic vesicles but not to the lysosome. These findings indicate that the cellular distribution of TLF is influenced by SRA expression and may directly determine susceptibility.

14105. **Vincendeau, P. & Bouteille, B., 2006.** Immunology and immunopathology of African trypanosomiasis. *Anais da Academia Brasiliera de Ciências*, **78** (4): 645-665.

Laboratoire de Parasitologie, Université de Bordeaux II, Bordeaux, France. [philippe.vincendeau@parasito.u-bordeaux2.fr].

Major modifications of immune system have been observed in African trypanosomiasis. These immune reactions do not lead to protection and are also involved in immunopathology disorders. The major surface component (variable surface glycoprotein, VSG) is associated with escape to immune reactions, cytokine network dysfunctions and autoantibody production. Most of our knowledge results from experimental trypanosomiasis. Innate resistance elements have been characterised. In infected mice, VSG preferentially

stimulates a Th 1-cell subset. A response of gamma delta and CD8 T cells to trypanosome antigens was observed in trypanotolerant cattle. An increase in CD5 B cells, responsible for most serum IgM and production of autoantibodies has been noted in infected cattle. Macrophages play important roles in trypanosomiasis, in synergy with antibodies (phagocytosis) and by secreting various molecules (radicals, cytokines, prostaglandins). Trypanosomes are highly sensitive to TNF-alpha, reactive oxygen and nitrogen intermediates. TNF-alpha is also involved in cachexia. IFN-gamma acts as a parasite growth factor. These various elements contribute to immunosuppression. Trypanosomes have learnt to use immune mechanisms to their own profit. Recent data show the importance of alternative macrophage activation, including arginase induction. L-ornithine produced by host arginase is essential to parasite growth. All these data reflect the deep insight into the immune response associated with trypanosomes and might suggest interference therapeutic approaches.

14106. **Yang, C., Suo, X., Huang, X., Zhang, G., Jia, Y., Wang, Q. & Shen, J., 2007.** Protection of mice against homologous or heterologous infections with antiserum mixture to the predominant variable antigen type repertoire of *Trypanosoma evansi* YNB stock. *Experimental Parasitology*, **116** (1): 53-58.

Parasitology Laboratory, College of Veterinary Medicine, China Agricultural University, Beijing 100094, China.

The objective of this study was to test a hypothesis that the predominant variable antigen type (VAT) repertoire of a single stock of *Trypanosoma evansi* was limited and small. It was further assumed that six rabbits could produce all antibodies against the predominant VAT repertoire of a stock of *T. evansi* and the antiserum mixture from the six rabbits containing all the antibodies could completely protect mice against any homologous stock infections and partially protect mice against some heterologous stock infections. Mice were each intraperitoneally infected with 100 parasites of clone-derived and non-clone-derived populations of the YNB stock, Kazakhstan strain or Vietnam strain of *T. evansi*, and treated with the antiserum mixture when trypanosomes had been detected in the blood. All of the 10 mice infected with either non-clone-derived or clone-derived populations of the YNB stock survived, and some (4/10) of mice infected with the heterologous Kazakhstan strain survived, while all those (10/10) infected with the heterologous Vietnam strain died. These results support the hypothesis that the predominant VAT repertoire of a single stock of *T. evansi* was limited and small, and have important implications in the consideration of treating human trypanosomosis due to drug resistant strains with antiserum mixture.

(c) CHEMOTHERAPEUTICS

14107. **Boibessot, I., Tettey, J. N. A., Skellern, G. G., Watson, D. G. & Grant, M. H., 2006**. Metabolism of isometamidium in hepatocytes isolated from control and inducer-treated rats. *Journal of Veterinary Pharmacology and Therapeutics*, **29** (6): 547-553.

Bioengineering Unit, University of Strathclyde, Glasgow, UK.

Little is known about the metabolism and mechanism of action of the trypanocide, isometamidium (ISM), the major drug used for prophylaxis of trypanosomiasis. We have investigated its metabolism and distribution in isolated rat hepatocytes using liquid chromatography-mass spectrometry and confocal laser scanning microscopy (CLSM). Two putative metabolites were formed, which were proposed to be a mono-acetyl derivative and an oxidized metabolite (SII). This is the first demonstration of the hepatic metabolism of ISM, as previous *in vivo* studies were hampered by dose-limiting toxicity and insensitive analytical methods. The intrinsic fluorescence of the drug enabled its intracellular uptake to be followed by CLSM. It is taken up rapidly into the nucleolus, nuclear membrane and endoplasmic reticulum within 5 min., and retained in the nucleus for at least 24 h. Persistent binding of ISM to cellular macromolecules may contribute to its prophylactic effect in vivo. Pretreatment of rats with 3-methylcholanthrene, phenobarbitone (PB) or the widely used pyrethroid pesticide, deltamethrin, resulted in an increase in metabolism of ISM to the proposed SII after 1 h incubation with hepatocytes. 3-methylcholanthrene was the most potent inducer, causing a maximal 19.5-fold induction of SII formation after exposure of hepatocytes to ISM for 1 h compared with formation by control hepatocytes. In comparison, at the 1 h timepoint deltamethrin pre-treatment caused a 10.2-fold induction, and PB only 8.2 fold.

14108. **Fijolek, A., Hofer, A. & Thelander, L., 2007.** Expression, purification, characterization, and *in vivo* targeting of trypanosome CTP synthetase for treatment of African sleeping sickness. *Journal of Biological Chemistry*, **282** (16): 11858-11865.

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African sleeping sickness is a fatal disease caused by two parasite subspecies: *Trypanosoma brucei gambiense* and *T. b. rhodesiense*. We previously reported that trypanosomes have extraordinary low CTP pools compared with mammalian cells. Trypanosomes also lack salvage of cytidine/cytosine making the parasite CTP synthetase a potential target for treatment of the disease. In this study, we have expressed and purified recombinant *T. brucei* CTP synthetase. The enzyme has a higher K (m) value for UTP than the mammalian CTP synthetase, which in combination with a lower UTP pool may account for the low CTP pool in trypanosomes. The activity of the trypanosome CTP synthetase is irreversibly inhibited by the glutamine analogue acivicin, a drug extensively tested as an antitumor agent. There is a rapid uptake of acivicin in mice both given intraperitoneally and orally by gavage. Daily injection of acivicin in trypanosome-infected mice suppressed the infection up to one month without any significant loss of weight. Experiments with cultured bloodstream *T. brucei* showed that acivicin is trypanocidal if present at 1 mμM concentration for at least 4 days. Therefore, acivicin may qualify as a drug with "desirable" properties, i.e. cure within 7 days, according to the current Target Product Profiles of WHO and DNDi.

14109**. Gudin, S., Quashie, N. B., Candlish, D., Al-Salabi, M. I., Jarvis, S. M., Ranford-Cartwright, L. C. & de Koning, H. P., 2006.** *Trypanosoma brucei*: A survey of pyrimidine transport activities. *Experimental Parasitology*, **114** (2): 118-125.

Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Biomedical Research Center, Glasgow G12 8TA, UK., and University of Westminster, 115 New Cavendish Street, London W1W 6UW, UK. [H.de-Koning@bio.gla.ac.uk].

Purine uptake has been studied in many protozoan parasites in the last few years, and several of the purine transporters have been cloned. In contrast, very little is known about the salvage of preformed pyrimidines by protozoa, and no pyrimidine transporters have been cloned, yet chemotherapy based on pyrimidine nucleobases and nucleosides has been as effective as purine antimetabolites in the treatment of infectious and neoplastic disease. Here, we surveyed the presence of pyrimidine transporters in *Trypanosoma brucei brucei*. We could not detect any mediated uptake of thymine, thymidine or cytidine, but identified a very high-affinity transporter for cytosine, designated C1, with a Km value of 0.048 ± 0.009 μM. We also confirmed the presence of the previously reported U1 uracil transporter and found it capable of mediating uridine uptake as well, with a Km of 33 ± 5 μM. A higher-affinity U2 uridine transporter (Km = 4.1 ± 2.1 μM) was also identified, but efficiency of the C1 and U2-mediated transport was low. Pyrimidine antimetabolites were tested as potential trypanocidal agents and only 5-fluorouracil was found to be effective. This drug was efficiently taken up by bloodstream forms of *T. b. brucei*.

14110. **Jaeger, T. & Flohe, L., 2006**. The thiol-based redox networks of pathogens: unexploited targets in the search for new drugs. *Biofactors*, **27** (1-4): 109-120.

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Hydroperoxide metabolism in diverse pathogens is reviewed under consideration of involved enzymes as potential drug targets. The common denominator of the peroxidase systems of *Trypanosoma, Leishmania, Plasmodium*, and *Mycobacterium* species is the use of NAD(P)H to reduce hydroperoxides including peroxynitrite via a flavin-containing disulfide reductase, a thioredoxin (Trx)-related protein and a peroxidase that operates with thiol catalysis. In *Plasmodium falciparum*, thioredoxin- and glutathione dependent systems appear to be linked via glutaredoxin and plasmoredoxin to terminal thioredoxin peroxidases belonging to both, the peroxiredoxin (Prx) and glutathione peroxidase (GPx) family. In *Mycobacterium tuberculosis*, a catalase-type peroxidase is complemented by the typical 2-C-Prx AhpC that, in contrast to most bacteria, is reduced by TrxC, and an atypical 2-C-Prx reduced by TrxB or C. A most complex variation of the scheme is found in trypanosomatids, where the unique redox metabolite trypanothione reduces the thioredoxin-related tryparedoxin, which fuels Prx- and GPx-type peroxidases as well as ribonucleotide reductase. In *Trypanosoma brucei* and *Leishmania donovani* the system has been shown to be essential for viability and virulence by inversed genetics. It is concluded that optimum efficacy can be expected from inhibitors of the most upstream components of the redox cascades. For trypanosomatids attractive validated drug targets are trypanothione reductase and trypanothione synthetase; for mycobacteria thioredoxin reductase appears most appealing, while in *Plasmodium* simultaneous inhibition of both the thioredoxin and the glutathione pathway appears advisable to avoid mutual substitution in co-substrate supply to the peroxidases. Financial and organisational needs to translate the scientific progress into

applicable drugs are discussed under consideration of the socio-economic impact of the addressed diseases.

14111. **Luscher, A., Nerima, B. & Maser, P., 2006**. Combined contribution of TbAT1 and *Tb*MRPA to drug resistance in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*, **150** (2): 364-366.

University of Bern, Institute of Cell Biology, Baltzerstrasse 4, 3012 Bern, Switzerland.

No abstract available.

14112. **Martyn, D. C., Jones, D. C., Fairlamb, A. H. & Clardy, J., 2007.** High-throughput screening affords novel and selective trypanothione reductase inhibitors with anti-trypanosomal activity. *Bioorganic and Medicinal Chemistry Letters,* **17** (5): 1280-1283.

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA.

Trypanothione reductase (TR), an enzyme that buffers oxidative stress in trypanosomatid parasites, was screened against commercial libraries containing approximately 134,500 compounds. After secondary screening, four chemotypes were identified as screening positives with selectivity for TR over human glutathione reductase. Thirteen compounds from these four chemotypes were purchased, and their *in vitro* activity against TR and *Trypanosoma brucei* is described.

14113. **Mathis, A. M**., **Bridges, A. S**., **Ismail, M. A**., **Kumar, A**., **Francesconi, I**., **Anbazhagan, M**., **Hu, Q**., **Tanious, F. A**., **Wenzler, T**., **Saulter, J**., **Wilson, W. D**., **Brun, R**., **Boykin, D. W**., **Tidwell, R. R**. & **Hall, J. E**., **2007**. Diphenyl furans and aza analogues: Effects of structural modification on *in vitro* activity, DNA binding, and accumulation and distribution in trypanosomes. *Antimicrobial Agents and Chemotherapy.* **In press, corrected proof**.

Division of Molecular Pharmaceutics, School of Pharmacy, University of North Carolina Chapel Hill, Chapel Hill, NC; Department of Pathology and Laboratory Medicine, UNC School of Medicine, University of North Carolina, Chapel Hill, NC; Department of Chemistry, Georgia State University, Atlanta, GA., USA; Swiss Tropical Institute, Basel, Switzerland.

Human African trypanosomiasis is a devastating disease with only a few treatment options, including pentamidine. Diamidine compounds such as pentamidine, DB75, and DB820 are potent antitrypanosomal compounds. Previous investigations have shown that diamidines accumulate to high concentrations in trypanosomes. However, the mechanism of action of this class of compounds remains unknown. A long-hypothesized mechanism of action has been binding to DNA and interference with DNA-associated enzymes. The fluorescent diamidines, DB75 and DB820, have been shown to localize not only in the DNA

containing nucleus and kinetoplast of trypanosomes, but also to the acidocalcisomes. Here we investigate two series of analogues of DB75 and DB820 with varying *in vitro* antitrypanosomal activity to determine whether any correlation exists between trypanosome accumulation, distribution and *in vitro* activity. Despite wide ranges of *in vitro* antitrypanosomal activity, all of the compounds investigated accumulated to mM concentrations in trypanosomes over 8 h. Interestingly, some of the less potent compounds accumulated to concentrations much higher than more potent compounds. All of the compounds were localized to one or both of the DNA containing nucleus or kinetoplast, and many were also found in the acidocalcisomes. Accumulation in the nucleus and kinetoplast should be important to the mechanism of action of these compounds. The acidocalcisomes also may play a role in the mechanism of action of these compounds. This investigation suggests that the extent of accumulation alone is not responsible for killing trypanosomes and that organelle specific accumulation may not predict *in vitro* activity.

14114. **Mbaya, A. W., Nwosu, C. O. & Onyeyili, P. A., 2007.** Toxicity and anti-trypanosomal effects of ethanolic extract of *Butyrospermum paradoxum* (*Sapotaceae*) stem bark in rats infected with *Trypanosoma brucei* and *Trypanosoma congolense*. *Journal of Ethnopharmacology*, **111** (3): 526-530.

Department of Veterinary Microbiology and Parasitology, University of Maiduguri, P.M.B. 1069 Maiduguri, Borno State, Nigeria.

The ethanolic extract of *Butyrospermum paradoxum* stem bark, commonly used in the traditional treatment of various diseases including animal and human trypanosomosis in north-eastern Nigeria, was tested for toxicity and anti-trypanosomal efficacy in rats infected with *Trypanosoma congolense* and *Trypanosoma brucei*. Following intra-peritoneal administration, the extract induced behavioural changes, morbidity and mortality in the rats. The symptoms observed included anorexia, dehydration, depression, prostration, coma and death. These symptoms were noted at high doses (>800mg/kg) only. At necropsy, the pathological lesions were mainly congestion and oedema of the lungs, bronchi, bronchioles and kidneys, hepatomegally and focal necrosis of the liver cells. The severity of the symptoms and lesions were dose related. The intra-peritoneal LD (50) of the extract was 820mg/kg. The extract produced anti-trypanosomal effect through the complete suppression or delay in parasite establishment with reduction in the level of parasitaemia and the severity of the attendant disease as well as enhanced survival of the rats infected with *Trypanosoma congolense* and *Trypanosoma brucei*. The results suggest that the folkloric medicinal application of the extracts of *Butyrospermum paradoxum* has a pharmacological basis.

14115. **Midgley, I., Fitzpatrick, K., Taylor, L. M., Houchen, T. L., Henderson, S. J., Wright, S. J., Cybulski, Z. R., John, B. A., McBurney, A., Boykin, D. W. & Trendler, K. L., 2007.** Pharmacokinetics and metabolism of the prodrug DB289 (2,5-bis[4-(N-methoxyamidino)phenyl]furan monomaleate) in rat and monkey and its conversion to the antiprotozoal/antifungal drug DB75 (2,5-bis(4-guanylphenyl)furan dihydrochloride). *Drug Metabolism and Disposition*, **35** (6): 955-967.

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DB289 (pafuramidine maleate; 2,5-bis[4-(N-methoxyamidino)phenyl]furan monomaleate) is a prodrug of DB75 (furamidine dihydrochloride; 2,5-bis(4-guanylphenyl)furan dihydrochloride), an aromatic dication related to pentamidine that has demonstrated good efficacy against African trypanosomiasis, *Pneumocystis carinii* pneumonia, and malaria, but lacks adequate oral availability. The pharmacokinetics and metabolism of ^{14}C-DB289 have been investigated in rat and monkey after oral and intravenous administration. Oral doses were well absorbed (approximately 50-70 percent) and effectively converted to DB75 in both species but subject to first-pass metabolism and hepatic retention, limiting its systemic bioavailability to 10 to 20 percent. Clearance of DB289 approximated the liver plasma flow and its large volume of distribution was consistent with extensive tissue binding. Plasma protein binding of DB289 was 97 to 99 percent in four animal species and humans, but that of DB75 was noticeably less and more species- and concentration-dependent. Together, prodrug and active metabolite accounted for less than 20 percent of the plasma radioactivity after an oral dose, but DB75 was the major radiochemical component in key organs such as brain and liver and was largely responsible for the persistence of ^{14}C in the body. The predominant route of excretion of radioactivity was via the feces, although biliary secretion was not particularly extensive. High-performance liquid chromatography and liquid chromatography-mass spectrometry investigations showed that the formation of DB75 from the prodrug involved the sequential loss of the two N-methoxy groups, either directly or by O-demethylation followed by reduction of the resulting oxime to the amidine. It was estimated that almost half of an oral dose of DB289 to rats and about one-third of that to monkeys was metabolized to DB75.

14116. **Muth, M., Hoerr, V., Glaser, M., Ponte-Sucre, A., Moll, H., Stich, A. & Holzgrabe, U., 2007.** Antitrypanosomal activity of quaternary naphthalimide derivatives. *Bioorganic and Medicinal Chemistry Letters*, **17** (6): 1590-1593.

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Sleeping sickness caused by *Trypanosoma brucei gambiense* and *rhodesiense* is fatal if left untreated. Due to the toxicity of drugs currently used and the emerging resistance against these drugs new lead compounds are urgently needed. Within the frame of a broad screening programme for drugs with antitrypanosomal activity, some highly potent tertiary and quaternary mono- and bisnaphthalimides being active in the lower micromolar and nanomolar range of concentration have been identified. These compounds are easily available via a two- or three-step microwave-driven synthesis with high yield.

14117. **Nakata, H., 2007**. Mitogen-activated protein kinase signaling is involved in suramin-induced neurite outgrowth in a neuronal cell line. *Biochemical and Biophysical Research Communications*, **355** (3): 842-848.

Department of Molecular Cell Signaling, Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo 183-8526, Japan. [nakata@tmin.ac.jp].

Suramin is a well-known antitrypanosomal drug and a novel experimental agent for the treatment of several cancers. Previous study showed that suramin is an activator of extracellular signal-regulated kinase (ERK1/2) signaling in several cell lines including Chinese hamster ovary cells, although the physiological relevance of this activation remains uncertain. Here, it was shown that suramin enhances neurite outgrowth concomitant with activation of ERK1/2 in Neuro-2a cells, a neuronal cell line. These neurite outgrowth and ERK1/2 activation were significantly inhibited by PD98059, an inhibitor of mitogen-activated protein kinase, as well as by activation of endogenous adenosine A2A receptors. The suramin-induced phosphorylation of ERK1/2 was also inhibited by inhibitors of Src family kinases. This attenuation of ERK1/2 activity was accompanied by a significant decrease in suramin-induced neurite outgrowth. These results suggest that suramin activates the Src/ERK1/2 signaling pathway that induces neurite outgrowth, both of which are negatively regulated by cAMP produced in response to activation of endogenous adenosine A2A receptors.

14118. **Ndjakou Lenta, B., Vonthron-Senecheau, C., Fongang Soh, R., Tantangmo, F., Ngouela, S., Kaiser, M., Tsamo, E., Anton, R. & Weniger, B., 2007**. *In vitro* antiprotozoal activities and cytotoxicity of some selected Cameroonian medicinal plants. *Journal of Ethnopharmacology*, **111** (1): 8-12.

Department of Chemistry, Higher Teachers' Training College, University of Yaoundé 1, Yaoundé, Cameroon.

Eight extracts from seven selected Cameroonian medicinal plants, traditionally used to treat malaria and other protozoal diseases, were tested *in vitro* for their antiprotozoal activities against *Plasmodium falciparum* K1 chloroquine-resistant strain, *Leishmania donovani, Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense*, protozoa responsible for malaria, visceral leishmaniasis, Chagas disease and African trypanosomiasis, respectively. The most active extract against *Plasmodium falciparum* K1 strain and *Trypanosoma brucei rhodesiense* was the methanolic extract of *Albizia zygia* (*Fabaceae*) stem bark with IC(50) values of 1.0 µg/ml and 0.2 µg/ml, respectively. Five extracts showed IC (50) values below 5mµg/ml against *Leishmania donovani*, with the methanolic seed extract of *Harungana madagascarensis* showing the highest activity, but only the methanolic extract of *Albizia zygia* showed activity against *Trypanosoma cruzi.* Cytotoxicity and selectivity indexes were estimated for the most active extracts. The best ratio of cytotoxicity to antiplasmodial activity (SI(a)=14) was established for the methanolic leaf extract of *Symphonia globulifera* (*Clusiaceae*), while the methanolic stem bark extract of *Albizia zygia* showed the best ratio of cytotoxicity to antitrypanosomal activity (SI(b)=22.5).

14119. **Ogbadoyi, E. O., Abdulganiy, A. O., Adama, T. Z. & Okogun, J. I., 2007**. *In vivo* trypanocidal activity of *Annona senegalensis* Pers. leaf extract against *Trypanosoma brucei brucei*. *Journal of Ethnopharmacology*, **112** (1): 85-89.

Department of Biochemistry, Federal University of Technology, Bosso Road, Minna, Nigeria.

Chemotherapy of African trypanosomiasis still remains far from being satisfactory. There is the urgent need for therapeutic agents that are effective, affordable and accessible to the rural poor in Africa who bear most of the disease burden. Root preparations of *Annona senegalensis* Pers. is claimed by traditional medicine practitioners to be effective in the treatment of sleeping sickness. Validation of this claim, evaluation of the therapeutic effects of other parts of the plant, and standardization of the preparations are necessary in order to fully exploit the chemotherapeutic potentials of this plant. We have evaluated the chemotherapeutic effects of extracts of the leaves, whole root, root and stem bark of the plant in experimental trypanosomiasis. Crude and partially purified aqueous extracts of the leaves, at a dose of 200mg/kg body weight per day completely cured experimental *Trypanosoma brucei brucei* infection in mice. Sub-inoculation of blood and cerebrospinal fluid drawn from the cured mice into healthy mice failed to produce any infection within 60 days of post-inoculation. Treatment of healthy mice with the crude extract before infection did not prevent establishment of infection. Administration of 5000 mg/kg body weight of the crude extract did not lead to fatality in mice. Preliminary phytochemical screening showed the presence of tannin, phlobatanin, and saponin.

14120. **Rosselli, F. P., Albuquerque, C. N. & Da Silva, A. B., 2006.** A chemometric study of megazol derivatives with activity against *Trypanosoma equiperdum*. *SAR QSAR in Environmental Research*, **17** (6): 533-547.

Departamento de Quimica e Fisica Molecular, Instituto de Quimica de Sao Carlos, Universidade de Sao Paulo, CP 780, 13560-970 Sao Carlos SP, Brazil.

The AM1 semiempirical method was employed to study megazol and 13 of its analogues where their activity against *Trypanosoma equiperdum* was obtained from *in vitro* tests. Several molecular properties (descriptors or variables) were calculated for the 14 compounds studied to be correlated with the biological activity. For a practical analysis of large data sets, it is necessary to reduce the dimensionality and select the most relevant descriptors related to the biological activity under study. For this purpose, the following chemometric methods were employed: principal component analysis (PCA), hierarchical cluster analysis (HCA), K-nearest neighbour (KNN), stepwise discriminant analysis (SDA) and soft independent modelling of class analogy (SIMCA). These methods showed that the descriptors molecular electronic energy (Eelet), charge on the first nitrogen at substituent 2 (qN), volume of substituent at C5 position (V-S5), dihedral angle (D3) and bond length between atom C4 and its substituents (L4) are responsible for the separation between active and inactive compounds against *T. equiperdum*.

14121. **Turnipseed, S. B., Clark, S. B., Andersen, W. C., Karbiwnyk, C. M., Miller, K. E. & Hurlbut, J. A., 2006**. Confirmation of diminazene diaceturate in bovine plasma using electrospray liquid chromatography-mass spectrometry. *Journal of Chromatography*, **844** (1): 127-133.

Animal Drugs Research Center, Food and Drug Administration, Denver Federal Center, P.O. Box 25087, Denver, CO 80225, USA. [sherri.turnipseed@fda.hhs.gov].

Diminazene diaceturate is used as a trypanocide for cattle in tropical regions. This paper describes a LC-MS (n) method to confirm the presence of diminazene in bovine plasma. Bound diminazene in plasma samples was freed with dilute phosphoric acid, then concentrated on a bonded C (18) SPE cartridge. The LC-MS (n) method utilized electrospray ionization coupled with an ion trap mass spectrometer. Ions observed in MS (2) and MS (3) product ion spectra, as well as those from the MS (1) spectrum, were monitored. The method was validated with plasma samples fortified with diminazene diaceturate (4-100 ng/mL). Diminazene was confirmed in samples fortified with diminazene diaceturate at levels of 6.4 ng/mL or higher.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

[See also **30**: 14138]

14122. **Karlsbakk, E. & Nylund, A., 2006.** Trypanosomes infecting cod *Gadus morhua* L. in the North Atlantic: a resurrection of *Trypanosoma pleuronectidium* Robertson, 1906 and delimitation of *T. murmanense* Nikitin, 1927 (emend.), with a review of other trypanosomes from North Atlantic and Mediterranean teleosts. *Systematic Parasitology*, **65** (3): 175-203.

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Trypanosomes were isolated from Atlantic cod *Gadus morhua* L. collected from several fjords in western Norway. Morphological studies showed that the 12 infections studied represented a single species, identified as *Trypanosoma pleuronectidium* Robertson, 1906 which is resurrected and redescribed. This species is characterised by its body length (57.9 +/- 5.4 microm), nearly central nucleus (NI = 1.05 +/- 0.12) and relatively short post-kinetoplastic (PK) region (3.2 +/- 0.8 microm). *T. pleuronectidium* is transmitted by the leech *Calliobdella nodulifera* (Malm). *T. murmanense* Nikitin, 1927 (emend.) is delimited to a species transmitted by the leech *Johanssonia arctica* (Johansson). This species is separated from *T. pleuronectidium* by its attained body length, more anterior nucleus, presence of cytoplasmic refractive granules, adnuclear vacuoles and by a longer PK region. Partial SSU rDNA sequences of *T. pleuronectidium* and *T. murmanense* from Norway (1980 nt) diverged by 1.9 percent. The nominal North Atlantic and Mediterranean trypanosome species are reviewed, and *T. flesi* Lebailly, 1904, *T. bothi* Lebailly, 1905 and *T. limandae* Brumpt & Lebailly, 1904 are considered synonyms of *T. platessae* Lebailly, 1904. *T. triglae*

senegalensis Ranque, 1973 is not considered conspecific with *T. triglae* Neumann, 1909, and consequently raised to species status as *T. senegalense* Ranque, 1973. Some other likely synonymies are discussed. In addition to *T. pleuronectidium* and *T. murmanense*, the following marine teleost trypanosomes are provisionally listed as valid species pending further study: *T. callionymi* Brumpt & Lebailly, 1904; *T. cotti* Brumpt & Lebailly, 1904; *T. delagei* Brumpt & Lebailly, 1904; *T. dorhni* Yakimov, 1911; *T. gobii* Brumpt & Lebailly, 1904; *T. laternae* Lebailly, 1904; *T. myoxocephali* Fantham, Porter & Richardson, 1942; *T. platessae* Lebailly, 1904; *T. scorpaenae* Neumann, 1909; *T. soleae* Laveran & Mesnil, 1901; *T. triglae* Neumann, 1909; and *T. yakimovi* Yakimov, 1911.

14123. **Lepesheva, G. I., Hargrove, T. Y., Ott, R. D., Nes, W. D. & Waterman, M. R., 2006.** Biodiversity of CYP51 in trypanosomes. *Biochemical Society Transactions*, **34** (6): 1161-1164.

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Sterol 14alpha-demethylases (CYP51) are metabolic cytochromes P450, found in each biological kingdom. They catalyse a single three-step reaction included in all sterol biosynthetic pathways. Plant CYP51s have strict preference towards their physiological substrate O (obtusifoliol), which is C-4-monomethylated. Natural substrates of animal/fungal CYP51 (lanosterol, 24, 25-dihydrolanosterol or 24-methylenelanosterol) are C-4-dimethylated. CYP51 from the pathogenic protozoa TB (*Trypanosoma brucei*) is the first example of O-specific sterol 14alpha-demethylase in non-photosynthetic organisms. Surprisingly, at 83 percent amino acid identity to the TB orthologue, CYP51 from TC (*Trypanosoma cruzi*) clearly prefers C-4-dimethylated sterols. Replacement of animal/fungi-like Ile (105) in the B' helix of TC CYP51 with phenylalanine, the residue found in this position in all plant and other trypanosome CYP51s, dramatically increases the ability of the enzyme to metabolize O, converting it into a more plant-like sterol 14alpha-demethylase. A more than 100-fold increase in binding and turnover is observed for the 24-desmethyl analogue of O [N (norlanosterol)], which is found *in vivo* in procyclic forms of TB and is a good TB CYP51 substrate *in vitro*. We believe that (i) N is a non-conventional CYP51 substrate, preferred in TB and perhaps other *Trypanosomatidae* and (ii) functional similarity of TC CYP51 to animal/fungal orthologues is a result of evolutionary convergence (including F105I mutation), leading to different pathways for sterol production in TC versus TB.

14124. **Maina, N. W., Oberle, M., Otieno, C., Kunz, C., Maeser, P., Ndung'u, J. M. & Brun, R., 2007.** Isolation and propagation of *Trypanosoma brucei gambiense* from sleeping sickness patients in south Sudan. *Transactions of the Royal Societ of Tropical Medicine and Hygiene*, **101** (6): 540-546.

Trypanosomiasis Research Centre (TRC) of KARI, P.O. Box 362, Kikuyu, Kenya, and Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, P.O. Box, CH-4002 Basel, Switzerland.

This study aimed at isolating *Trypanosoma brucei gambiense* from human African trypanosomiasis (HAT) patients from south Sudan. Fifty HAT patients identified during

active screening surveys were recruited, most of whom (49/50) were in second-stage disease. Blood and cerebrospinal fluid samples collected from the patients were cryopreserved using Triladyl ((R)) as the cryomedium. The samples were stored at -150 degrees C in liquid nitrogen vapour in a dry shipper. Eighteen patient stabilates could be propagated in immunosuppressed *Mastomys natalensis* and/or SCID mice. Parasitaemia was highest in SCID mice. Further subpassages in *M. natalensis* increased the virulence of the trypanosomes and all 18 isolates recovered from *M. natalensis* or SCID mice became infective to other immunosuppressed mouse breeds. A comparison of immunosuppressed *M. natalensis* and Swiss White, C57/BL and BALB/c mice demonstrated that all rodent breeds were susceptible after the second subpassage and developed a parasitaemia $>10^6$/ml by Day 5 post infection. The highest parasitaemias were achieved in C57/BL and BALB/c mice. These results indicate that propagation of *T. b. gambiense* isolates after initial isolation in immunosuppressed *M. natalensis* or SCID mice can be done in a range of immunosuppressed rodents.

14125. **Njiru Z. K., Constantine C. C., Gitonga P. K., Thompson, R. C. A. & Reid, S. A., 2007.** Genetic variability of *Trypanosoma evansi* isolates detected by inter-simple sequence repeat anchored-PCR and microsatellite. *Veterinary Parasitology*, **147**(1-2): 51-60.

School of Nursing – Peel Campus, Murdoch University, Carleton Place, 15-17 Mandurah, WA 6150, Australia, Trypanosomiasis Research Centre, Kenya Agricultural Research Institute, P.O. Box 362, Kikuyu 00902, Kenya, Centre for MEGA Epidemiology, School of Population Health, the University of Melbourne, level 2, 723 Swanston Street, Carlton, Vic. 3053, Australia, and School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Murdoch, WA 6150, Australia. [zknjiru@hotmail.com].

Studies on genetic variability in *Trypanosoma evansi* have been limited by a lack of high-resolution techniques. In this study, we have investigated the use of inter-simple sequence repeats (ISSR) and microsatellites in revealing polymorphism among *T. evansi* isolates. Twelve ISSR primers and five microsatellite loci were used to generate polymorphic bands and alleles, respectively, to investigate the genetic variability among *T. evansi* isolates from Africa and Asia. Seven of the twelve ISSR primers showed variability between isolates with a total of 71 fragments of which 49(69 percent) were polymorphic. Microsatellite analysis revealed a total of 60 alleles. On average the ISSR markers revealed a higher genetic diversity (23 percent) than microsatellites (21.1 percent). The two techniques showed a strong agreement of $r = 0.95$ for Dice and $r = 0.91$ for Jaccard indices in estimating the genetic distances between isolates. The distance UPGMA tree revealed two major clusters of *T. evansi* which correlate with the minicircle classification of subtype A and B. The cophenetic correlation coefficient between Dice and Jaccard based matrices were $r = 0.79$ for microsatellites and $r=0.73$ for ISSR indicating a strong agreement between dendrograms. The results suggest that both ISSR and microsatellites markers are useful in detecting genetic variability within *T. evansi*.

14126. **Shahada, F., Clausen, P-H., Tietjen, U., Chuma, T. & Okamoto, K., 2007.** Absence of correlation between karyotype profiles of *Trypanosoma congolense*

and resistance to isometamidium chloride. *Veterinary Parasitology.* **In press, corrected proof.**

Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Konigsweg 67, D-14163 Berlin, Germany, and Laboratory of Veterinary Public Health, Department of Veterinary Medicine, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan. [shahada@ms.kagoshima-u.ac.jp].

Chromosome profiles of 10 *Trypanosoma (T.) congolense* populations with different isometamidium sensitivities were compared using the pulsed field gel electrophoresis technique. The aim was to elucidate whether there was a karyotype pattern specific to eight isometamidium resistant phenotypes. Analysis of the profiles indicated that all populations displayed several discrete bands at the region of small, intermediate and large chromosomes. The highest similarity was observed between two isolates originating from Burkina Faso, indicating that they had the same genetic origin. Other eight strains exhibited different patterns in terms of chromosome size and numbers such that there was no characteristic karyotype pattern that was established specifically to identify resistant populations and discriminate them from the sensitive ones. This study has revealed that isometamidium resistance is not correlated to karyotype profile in *T. congolense.*

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

14127. **Adams, E. R., Malele, I. I, Msangi, A. R. & Gibson, W C., 2006.** Trypanosome identification in wild tsetse populations in Tanzania using generic primers to amplify the ribosomal RNA ITS-1 region. *Acta Tropica*, **100** (1-2): 103-109.

School of Biological Sciences, University of Bristol, Bristol, UK.

Tsetse flies transmit many species of trypanosomes in Africa, some of which are human and livestock pathogens of major medical and socio-economic impact. Identification of trypanosomes is essential to assess the disease risk posed by particular tsetse populations. We have developed a single generic PCR test to replace the multiple species-specific PCR tests used previously to identify the trypanosome species carried by individual tsetse flies. In the generic PCR test, inter-species size variation in the PCR product of the internal transcribed spacer (ITS-1) region of the ribosomal RNA repeat region enables species identification. The test was applied to identify trypanosomes in midgut samples stored on FTA cards from wild-caught flies in two regions of Tanzania. Identifications were verified by sequencing the amplified ITS-1 region and/or species-specific PCR tests. The method facilitated the identification of large numbers of field samples quickly and accurately. Whereas species-specific tests are incapable of recognising previously unknown species, the generic test enabled a new species to be identified by the unique size of the amplified product. Thus, even without access to any isolate of this new species, we could collect data on its distribution, prevalence and co-occurrence with other trypanosomes. The combined molecular and ecological profiles should facilitate the isolation and full biological characterization of this species in the future.

14128. **Al-Salabi, M. I., Wallace, L. J., Luscher, A., Maser, P., Candlish, D., Rodenko, B., Gould, M. K., Jabeen, I., Ajith, S. N. & de Koning, H. P., 2007**. Molecular interactions underlying the unusually high adenosine affinity of a novel *Trypanosoma brucei* nucleoside transporter. *Molecular Pharmacology*, **71** (3): 921-929.

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Trypanosoma brucei encodes a relatively high number of genes of the equilibrative nucleoside transporter (ENT) family. We report here the cloning and in-depth characterization of one *T. brucei brucei* ENT member, TbNT9/AT-D. This transporter was expressed in *Saccharomyces cerevisiae* and displayed a uniquely high affinity for adenosine (Km = 0.068 +/- 0.013 µM), as well as broader selectivity for other purine nucleosides in the low µM range, but was not inhibited by nucleobases or pyrimidines. This selectivity profile is consistent with the P1 transport activity observed previously in procyclic and long-slender bloodstream *T. brucei*, apart from the 40-fold higher affinity for adenosine than for inosine. We found that, like the previously investigated P1 activity of long/slender bloodstream trypanosomes, the 3'-hydroxy, 5'-hydroxy, N3, and N7 functional groups contribute to transporter binding. In addition, we show that the 6-position amine group of adenosine, but not the inosine 6-keto group, makes a major contribution to binding (DeltaG0 = 12 kJ/mol), explaining the different Km values of the purine nucleosides. We further found that P1 activity in procyclic and long-slender trypanosomes is pharmacologically distinct, and we identified the main gene encoding this activity in procyclic cells as NT10/AT-B. The presence of multiple P1-type nucleoside transport activities in *T. brucei brucei* facilitates the development of nucleoside-based treatments for African trypanosomiasis and would delay the onset of uptake-related drug resistance to such therapy. We show that both TbNT9/AT-D and NT10/AT-B transport a range of potentially therapeutic nucleoside analogues.

14129. **Alsford, S., Kawahara, T., Isamah, C. & Horn, D., 2007**. A sirtuin in the African trypanosome is involved in both DNA repair and telomeric gene silencing but is not required for antigenic variation. *Molecular Microbiology*, **63** (3): 724-736.

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Silent information regulator 2 (Sir2)-related proteins or sirtuins function as NAD^+-dependent deacetylases or ADP ribosylases that target a range of substrates, thereby influencing chromatin structure and a diverse range of other biological functions. Genes encoding three Sir2-related proteins (SIR2rp1-3) have been identified in the parasitic trypanosomatids, early branching protozoa with no previously reported transcriptional silencing machinery. Here we show that, in the mammalian-infective bloodstream-stage of the African trypanosome, *Trypanosoma brucei*, SIR2rp1 localizes to the nucleus while SIR2rp2 and SIR2rp3 are both mitochondrial proteins. The nuclear protein, SIR2rp1, controls DNA repair and repression of RNA polymerase I-mediated expression immediately adjacent to telomeres. Antigenic variation, however, which involves the silencing and Pol I-mediated transcriptional switching of subtelomeric variant surface glycoprotein genes, continues to operate independent of SIR2rp1.

14130. **Babbarwal, V. K., Fleck, M., Ernst, N. L., Schnaufer, A. & Stuart, K., 2007**. An essential role of KREPB4 in RNA editing and structural integrity of the editosome in *Trypanosoma brucei*. *RNA*, **13 (**5): 737-744.

Seattle Biomedical Research Institute, Seattle, Washington 98109, USA.

RNA editing in the sleeping sickness parasite *Trypanosoma brucei* remodels mitochondrial transcripts by the addition and deletion of uridylates as specified by guide RNAs. Editing is catalyzed by at least three distinct approximately 20S multiprotein editosomes, all of which contain KREPB4, a protein with RNase III and Pumilio motifs. RNAi repression of KREPB4 expression in procyclic forms (PFs) strongly inhibited growth and *in vivo* RNA editing, greatly diminished the abundance of 20S editosomes, reduced cellular levels of editosome proteins, and generated approximately 5-10S editosome subcomplexes. Editing TUTase, exoUase, and RNA ligase activities were largely shifted from approximately 20S to approximately 5-10S fractions of cellular lysates. Insertion and deletion endonuclease activities in approximately 20S fractions were strongly reduced upon KREPB4 repression but were not detected in the 5-10S subcomplex fraction. Abundance of MRP1 and RBP16 proteins, which appear to be involved in RNA processing but are not components of the 20S editosome, was unaltered upon KREPB4 repression. These data suggest that KREPB4 is important for the structural integrity of approximately 20S editosomes, editing endonuclease activity, and the viability of PF *T. brucei* cells.

14131. **Barnes, R. L. & McCulloch, R., 2007**. *Trypanosoma brucei* homologous recombination is dependent on substrate length and homology, though displays a differential dependence on mismatch repair as substrate length decreases. *Nucleic Acids Research.* **In press, corrected proof.**

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Homologous recombination functions universally in the maintenance of genome stability through the repair of DNA breaks and in ensuring the completion of replication. In some organisms, homologous recombination can perform more specific functions. One example of this is in antigenic variation, a widely conserved mechanism for the evasion of host immunity. *Trypanosoma brucei*, the causative agent of sleeping sickness in Africa, undergoes antigenic variation by periodic changes in its variant surface glycoprotein (VSG) coat. VSG switches involve the activation of VSG genes, from an enormous silent archive, by recombination into specialized expression sites. These reactions involve homologous recombination, though they are characterized by an unusually high rate of switching and by atypical substrate requirements. Here, we have examined the substrate parameters of *T. brucei* homologous recombination. We show, first, that the reaction is strictly dependent on substrate length and that it is impeded by base mismatches, features shared by homologous recombination in all organisms characterized. Second, we identify a pathway of homologous recombination that acts preferentially on short substrates and is impeded to a lesser extent by

base mismatches and the mismatch repair machinery. Finally, we show that mismatches during *T. brucei* recombination may be repaired by short-patch mismatch repair.

14132. **Baron, D. M., Ralston, K. S., Kabututu, Z. P. & Hill, K. L., 2007**. Functional genomics in *Trypanosoma brucei* identifies evolutionarily conserved components of motile flagella. *Journal of Cell Science*, **120** (3): 478-491.

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Cilia and flagella are highly conserved, complex organelles involved in a variety of important functions. Flagella are required for motility of several human pathogens and ciliary defects lead to a variety of fatal and debilitating human diseases. Many of the major structural components of cilia and flagella are known, but little is known about regulation of flagellar beat. *Trypanosoma brucei*, the causative agent of African sleeping sickness, provides an excellent model for studying flagellar motility. We have used comparative genomics to identify a core group of 50 genes unique to organisms with motile flagella. These genes, referred to as *T. brucei* components of motile flagella (TbCMF) include 30 novel genes, and human homologues of many of the TbCMF genes map to loci associated with human ciliary diseases. To characterize TbCMF protein function we used RNA interference to target 41 TbCMF genes. Sedimentation assays and direct observation demonstrated clear motility defects in a majority of these knockdown mutants. Epitope tagging, fluorescence localization and biochemical fractionation demonstrated flagellar localization for several TbCMF proteins. Finally, ultrastructural analysis identified a family of novel TbCMF proteins that function to maintain connections between outer doublet microtubules, suggesting that they are the first identified components of nexin links. Overall, our results provide insights into the workings of the eukaryotic flagellum, identify several novel human disease gene candidates, reveal unique aspects of the trypanosome flagellum and underscore the value of *T. brucei* as an experimental system for studying flagellar biology.

14133. **Cifuentes-Rojas, C., Pavia, P., Hernandez, A., Osterwisch, D., Puerta, C. & Cruz-Reyes, J., 2007.** Substrate determinants for RNA editing and editing complex interactions at a site for full-round U insertion. *Journal of Biological Chemistry*, **282** (7): 4265-4276.

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Multisubunit RNA editing complexes catalyze uridylate insertion/deletion RNA editing directed by complementary guide RNAs (gRNAs). Editing in trypanosome mitochondria is transcript-specific and developmentally controlled, but the molecular mechanisms of substrate specificity remain unknown. Here we used a minimal A6 pre-mRNA/gRNA substrate to define functional determinants for full-round insertion and editing complex interactions at the editing site 2 (ES2). Editing begins with pre-mRNA cleavage within an internal loop flanked by upstream and downstream duplexes with gRNA. We found that substrate recognition around the internal loop is sequence-independent and that completely artificial duplexes spanning a single helical turn are functional. Furthermore, after

our report of cross-linking interactions at the deletion ES1 (35), we show for the first time editing complex contacts at an insertion ES. Our studies using site-specific ribose 2' substitutions defined 2'-hydroxyls within the (a) gRNA loop region and (b) flanking helixes that markedly stimulate both pre-mRNA cleavage and editing complex interactions at ES2. Modification of the downstream helix affected scissile bond specificity. Notably, a single 2'-hydroxyl at ES2 is essential for cleavage but dispensable for editing complex cross-linking. This study provides new insights on substrate recognition during full-round editing, including the relevance of secondary structure and the first functional association of specific (pre-mRNA and gRNA) riboses with both endonuclease cleavage and cross-linking activities of editing complexes at an ES. Importantly, most observed cross-linking interactions are both conserved and relatively stable at ES2 and ES1 in hybrid substrates. However, they were also detected as transient low-stability contacts in a non-edited transcript.

14134. **de Souza Leite, M., Thomaz, R., Fonseca, F. V., Panizzutti, R., Vercesi, A. E. & Meyer-Fernandes, J. R., 2007.** *Trypanosoma brucei brucei*: biochemical characterization of ecto-nucleoside triphosphate diphosphohydrolase activities. *Experimental Parasitology*, **115** (4): 315-323.

Instituto de Bioquimica Medica, CCS, Universidade Federal do Rio de Janeiro, Cidade Universitaria, Rio de Janeiro, RJ, Brazil.

In this work we describe the ability of living cells of *Trypanosoma brucei brucei* to hydrolyze extracellular ATP. In these intact parasites there was a low level of ATP hydrolysis in the absence of any divalent metal (4.72+/-0.51 nmol Pi x 10^{-7} cells x h^{-1}). The ATP hydrolysis was stimulated by $MgCl_2$ and the Mg-dependent ecto-ATPase activity was 27.15+/-2.91 nmol Pi x 10^{-7} cells x h^{-1}. This stimulatory activity was also observed when $MgCl_2$ was replaced by $MnCl_2$. $CaCl_2$ and $ZnCl_2$ were also able to stimulate the ATPase activity, although less than $MgCl_2$. The apparent K (m) for ATP was 0.61 mM. This ecto-ATPase activity was insensitive to inhibitors of other ATPase and phosphatase activities. To confirm that this Mg-dependent ATPase activity is an ecto-ATPase activity, we used an impermeable inhibitor, DIDS (4, 4'-diisothiocyanostylbene 2'-2'-disulphonic acid), as well as suramin, an antagonist of P(2) purinoreceptors and inhibitor of some ecto-ATPases. These two reagents inhibited the Mg^{2+}-dependent ATPase activity in a dose-dependent manner. Living cells sequentially hydrolyzed the ATP molecule generating ADP, AMP and adenosine, and supplementation of the culture medium with ATP was able to sustain the proliferation of *T. brucei brucei* as well as adenosine supplementation. Furthermore, the E-NTPDase activity of *T. brucei brucei* is modulated by the availability of purines in the medium. These results indicate that this surface enzyme may play a role in the salvage of purines from the extracellular medium in *T. brucei brucei*.

14135. **Denninger, V., Figarella, K., Schonfeld, C., Brems, S., Busold, C., Lang, F., Hoheisel, J. & Duszenko, M., 2007.** Troglitazone induces differentiation in *Trypanosoma brucei*. *Experimental Cell Research*, **313** (9): 1805-1819.

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Trypanosoma brucei, a protozoan parasite causing sleeping sickness, is transmitted by the tsetse fly and undergoes a complex lifecycle including several defined stages within the insect vector and its mammalian host. In the latter, differentiation from the long slender to the short stumpy form is induced by a yet unknown factor of trypanosomal origin. Here we describe that some thiazolidinediones are also able to induce differentiation. In higher eukaryotes, thiazolidinediones are involved in metabolism and differentiation processes mainly by binding to intracellular receptors. Our studies focus on the effects of troglitazone on bloodstream form trypanosomes. Differentiation was monitored using mitochondrial markers (membrane potential, succinate dehydrogenase activity, inhibition of oxygen uptake by KCN, amount of cytochrome transcripts), morphological changes (transmission EM and light microscopy), and transformation experiments (loss of the variant surface glycoprotein coat and increase of dihydroliponamide dehydrogenase activity). To further investigate the mechanisms responsible for these changes, microarray analyses were performed, showing an upregulation of expression site associated gene 8 (ESAG8), a potential differentiation regulator.

14136. **Folgueira, C. & Requena, J. M., 2007**. A postgenomic view of the heat shock proteins in kinetoplastids. *FEMS Microbiology Revi*ews. **In press, corrected proof.**

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The kinetoplastids *Leishmania major, Trypanosoma brucei* and *Trypanosoma cruzi* are causative agents of a diverse spectrum of human diseases: leishmaniasis, sleeping sickness and Chagas' disease, respectively. These protozoa possess digenetic life cycles that involve development in mammalian and insect hosts. It is generally accepted that temperature is a triggering factor of the developmental programme allowing the adaptation of the parasite to the mammalian conditions. The heat shock response is a general homeostatic mechanism that protects cells from the deleterious effects of environmental stresses, such as heat. This response is universal and includes the synthesis of the heat-shock proteins (HSPs). In this review, we summarize the salient features of the different HSP families and describe their main cellular functions. In parallel, we analyse the composition of these families in kinetoplastids according to literature data and our understanding of genome sequence data. The genome sequences of these parasites have been recently completed. The HSP families described here are: HSP110, HSP104, group I chaperonins, HSP90, HSP70, HSP40 and small HSPs. All these families are widely represented in these parasites. In particular, kinetoplastids possess an unprecedented number of members of the HSP70, HSP60 and HSP40 families, suggesting key roles for these HSPs in their biology.

14137. **Galland, N., Demeure, F., Hannaert, V., Verplaetse, E., Vertommen, D., Van der Smissen, P., Courtoy, P. J. & Michels, P. A., 2007.** Characterization of the role of the receptors PEX5 and PEX7 in the import of proteins into glycosomes of *Trypanosoma brucei*. *Biochimica and Biophysica Acta*, **1773** (4): 521-535.

Research Unit for Tropical Diseases, Christian de Duve Institute of Cellular Pathology and Laboratory of Biochemistry, Université catholique de Louvain, Brussels, Belgium.

Peroxins 5 and 7 are receptors for protein import into the peroxisomal matrix. We studied the involvement of these peroxins in the biogenesis of glycosomes in the protozoan parasite *Trypanosoma brucei*. Glycosomes are peroxisome-like organelles in which a major part of the glycolytic pathway is sequestered. We here report the characterization of the *T. brucei* homologue of PEX7 and provide several data strongly suggesting that it can bind to PEX5. Depletion of PEX5 or PEX7 by RNA interference had a severe effect on the growth of both the bloodstream-form of the parasite, that relies entirely on glycolysis for its ATP supply, and the procyclic form representative of the parasite living in the tsetse-fly midgut and in which also other metabolic pathways play a prominent role. The role of the two receptors in import of glycosomal matrix proteins with different types of peroxisome/glycosome-targeting signals (PTS) was analyzed by immunofluorescence and subcellular fractionation studies. Knocking down the expression of either receptor gene resulted, in procyclic cells, in the mislocalization of proteins with both a type 1 or 2 targeting motif (PTS1, PTS2) located at the C- and N-termini, respectively, and proteins with a sequence-internal signal (I-PTS) to the cytosol. Electron microscopy confirmed the apparent integrity of glycosomes in these procyclic cells. In bloodstream-form trypanosomes, PEX7 depletion seemed to affect only the subcellular distribution of PTS2-proteins. Western blot analysis suggested that, in both life-cycle stages of the trypanosome, the levels of both receptors are controlled in a coordinated fashion, by a mechanism that remains to be determined. The observation that both PEX5 and PEX7 are essential for the viability of the parasite indicates that the respective branches of the glycosome-import pathway in which each receptor acts might be interesting drug targets.

14138. **Gibson, W., 2007**. Resolution of the species problem in African trypanosomes. *International Journal of Parasitology*, **37** (8-9): 829-838.

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There is a general assumption that eukaryote species are demarcated by morphological or genetic discontinuities. This stems from the idea that species are defined by the ability of individuals to mate and produce viable progeny. At the microscopic level, where organisms often proliferate more by asexual than sexual reproduction, this tidy classification system breaks down and species definition becomes messy and problematic. The dearth of morphological characters to distinguish microbial species has led to the widespread application of molecular methods for identification. As well as providing molecular markers for species identification, gene sequencing has generated the data for accurate estimation of relatedness between different populations of microbes. This has led to recognition of conflicts between current taxonomic designations and phylogenetic placement. In the case of microbial pathogens, the extent to which taxonomy has been driven by utilitarian rather than biological considerations has been made explicit by molecular phylogenetic analysis. These issues are discussed with reference to the taxonomy of the African trypanosomes, where pathogenicity, host range and distribution have been influential in the designation of species and subspecies.

Effectively, the taxonomic units recognised are those that are meaningful in terms of human or animal disease. The underlying genetic differences separating the currently recognised trypanosome taxa are not consistent, ranging from genome-wide divergence to presence/absence of a single gene. Nevertheless, if even a minor genetic difference reflects adaptation to a particular parasitic niche, for example, in *Trypanosoma brucei rhodesiense*, the presence of a single gene conferring the ability to infect humans, then it can prove useful as an identification tag for the taxon occupying that niche. Thus, the species problem can be resolved by bringing together considerations of utility, genetic difference and adaptation.

14139. **Glover, L., Alsford, S., Beattie, C. & Horn, D., 2007.** Deletion of a trypanosome telomere leads to loss of silencing and progressive loss of terminal DNA in the absence of cell cycle arrest. *Nucleic Acids Research*, **35** (3): 872-880.

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Eukaryotic chromosomes are capped with telomeres which allow complete chromosome replication and prevent the ends from being recognized by the repair machinery. The African trypanosome, *Trypanosoma brucei*, is a protozoan parasite where antigenic variation requires reversible silencing of a repository of telomere-adjacent variant surface glycoprotein (VSG) genes. We have investigated the role of the telomere adjacent to a repressed VSG. In cells lacking telomerase, the rate of telomere-repeat loss appeared to be inversely proportional to telomere length. We therefore constructed strains in which a single telomere could be immediately removed by conditional I-SceI meganuclease cleavage. Following telomere deletion, cells maintain and segregate the damaged chromosome without repairing it. These cells continue to proliferate at the normal rate but progressively lose terminal DNA at the broken end. Although sirtuin-dependent repression is lost along with the telomere, VSG-silencing is preserved. The results provide direct evidence for telomere-dependent repression but suggest a telomere-independent mode of VSG-silencing. They also indicate the absence of a telomere-loss checkpoint in *T. brucei*.

14140. **Goulah, C. C. & Read, L. K., 2007.** Differential effects of arginine methylation on RBP16 mRNA binding, guide RNA (gRNA) binding, and gRNA-containing ribonucleoprotein complex (gRNP) formation. *Journal of Biological Chemistry*, **282** (10): 7181-7190.

Department of Microbiology and Immunology and Witebsky Center for Microbial Pathogenesis and Immunology, SUNY Buffalo School of Medicine, Buffalo, New York 14214, USA.

Mitochondrial gene expression in *Trypanosoma brucei* involves the coordination of multiple events including polycistronic transcript cleavage, polyadenylation, RNA stability, and RNA editing. Arg methylation of RNA binding proteins has the potential to influence many of these processes via regulation of protein-protein and protein-RNA interactions. Here we demonstrate that Arg methylation differentially regulates the RNA binding capacity and macromolecular interactions of the mitochondrial gene regulatory protein, RBP16. We show that, in *T. brucei* mitochondria, RBP16 forms two major stable complexes: a 5 S multiprotein

complex and an 11 S complex consisting of the 5 S complex associated with guide RNA (gRNA). Expression of a non-methylatable RBP16 mutant protein demonstrates that Arg methylation of RBP16 is required to maintain the protein-protein interactions necessary for assembly and/or stability of both complexes. Down-regulation of the major trypanosome type 1 protein arginine methyltransferase, *Tb*PRMT1, disrupts formation of both the 5 and 11 S complexes, indicating that *Tb*PRMT1-catalyzed methylation of RBP16 Arg-78 and Arg-85 is critical for complex formation. We also show that Arg methylation decreases the capacity of RBP16 to associate with gRNA. This is not a general effect on RBP16 RNA binding, however, since methylation conversely increases the association of the protein with mRNA. Thus, *Tb*PRMT1-catalyzed Arg methylation has distinct effects on RBP16 gRNA and mRNA association and gRNA-containing ribonucleoprotein complex (gRNP) formation.

14141. **Gourguechon S., Savich, J. M. & Wang, C. C., 2007.** The multiple roles of cyclin E1 in controlling cell cycle progression and cellular morphology of *Trypanosoma brucei*. *Journal of Molecular Biology*, **368** (4): 939-950.

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Regulation of eukaryotic cell cycle progression requires sequential activation and inactivation of cyclin-dependent kinases. Previous RNA interference (RNAi) experiments in *Trypanosoma brucei* indicated that cyclin E1, cdc2-related kinase (CRK)1 and CRK2 are involved in regulating G1/S transition, whereas cyclin B2 and CRK3 play a pivotal role in controlling the G2/M checkpoint. To search for potential interactions between the other cyclins and CRKs that may not have been revealed by the RNAi assays, we used the yeast two-hybrid system and an *in vitro* glutathione-S-transferase pulldown assay and observed interactions between cyclin E1 and CRK1, CRK2 and CRK3. Cyclins E1–E4 are homologues of yeast Pho80 cyclin. But yeast complementation assays indicated that none of them possesses a Pho80-like function. Analysis of cyclin E1 + CRK1 and cyclin E1 + CRK2 double knockdowns in the procyclic form of *T. brucei* indicated that the cells were arrested more extensively in the G1 phase beyond the cumulative effect of individual knockdowns. But BrdU incorporation was impaired significantly only in cyclin E1 + CRK1-depleted cells, whereas a higher percentage of cyclin E1 + CRK2 knockdown cells assumed a grossly elongated posterior end morphology. A double knockdown of cyclin E1 and CRK3 arrested cells in G2/M much more efficiently than if only CRK3 was depleted. Taken together, these data suggest multiple functions of cyclin E1: it forms a complex with CRK1 in promoting G1/S phase transition; it forms a complex with CRK2 in controlling the posterior morphogenesis during G1/S transition; and it forms a complex with CRK3 in promoting passage across the G2/M checkpoint in the trypanosome.

14142. **Hammarton, T. C., 2007.** Cell cycle regulation in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*, **153** (1): 1-8.

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Cell division is regulated by intricate and interconnected signal transduction pathways that precisely coordinate, in time and space, the complex series of events involved in replicating and segregating the component parts of the cell. In *Trypanosoma brucei*, considerable progress has been made over recent years in identifying molecular regulators of the cell cycle and elucidating their functions, although many regulators undoubtedly remain to be identified, and there is still a long way to go with respect to determining signal transduction pathways. However, it is clear that cell cycle regulation in *T. brucei* is unusual in many respects. Analyses of trypanosome orthologues of conserved eukaryotic cell cycle regulators have demonstrated divergence of their function in the parasite, and a number of other key regulators are missing from *T. brucei.* Cell cycle regulation differs in different parasite life cycle stages, and *T. brucei* appears to use different checkpoint control strategies compared to model eukaryotes. It is therefore probable that *T. brucei* has evolved novel pathways to control its cell cycle.

14143. **Hee Lee, S., Stephens, J. L. & Englund, P. T., 2007.** A fatty-acid synthesis mechanism specialized for parasitism. *Nature Reviews, Microbiology*, **5** (4): 287-297.

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Most cells use either a type I or type II synthase to make fatty acids. *Trypanosoma brucei*, the sleeping sickness parasite, provides the first example of a third mechanism for this process. Trypanosomes use microsomal elongases to synthesize fatty acids *de novo*, whereas other cells use elongases to make long-chain fatty acids even longer. The modular nature of the pathway allows synthesis of different fatty-acid end products, which have important roles in trypanosome biology. Indeed, this newly discovered mechanism seems ideally suited for the parasitic lifestyle.

14144. **Kang, X., Gao, G., Rogers, K., Falick, A. M., Zhou, S. & Simpson, L., 2006**. Reconstitution of full-round uridine-deletion RNA editing with three recombinant proteins. *Proceedings of the National Academy of Sciences USA*, **103** (38): 13944-13949.

Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA 90095, USA.

Uridine (U)-insertion/deletion RNA editing in trypanosome mitochondria involves an initial cleavage of the preedited mRNA at specific sites determined by the annealing of partially complementary guide RNAs. An involvement of two RNase III-containing core editing complex (L-complex) proteins, MP90 (KREPB1) and MP61 (KREPB3) in, respectively, U-deletion and U-insertion editing, has been suggested, but these putative enzymes have not been characterized or expressed in active form. Recombinant MP90 proteins from *Trypanosoma brucei* and *Leishmania major* were expressed in insect cells and cytosol of *Leishmania tarentolae*, respectively. These proteins were active in specifically cleaving a model U-deletion site and not a U-insertion site. Deletion or mutation of the RNase III motif abolished this activity. Full-round guide RNA (gRNA)-mediated *in vitro* U-

deletion editing was reconstituted by a mixture of recombinant MP90 and recombinant RNA editing exonuclease I from *L. major*, and recombinant RNA editing RNA ligase 1 from *L. tarentolae*. MP90 is designated REN1, for RNA-editing nuclease 1.

14145. **Law, J. A., O'Hearn, S. & Sollner-Webb, B., 2007**. In *Trypanosoma brucei* RNA editing, *Tb*MP18 (band VII) is critical for editosome integrity and for both insertional and deletional cleavages. *Molecular and Cellular Biology*, **27** (2): 777-787.

Department of Biological Chemistry, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA.

In trypanosome RNA editing, uridylate (U) residues are inserted and deleted at numerous sites within mitochondrial pre-mRNAs by an approximately 20S protein complex that catalyzes cycles of cleavage, U addition/U removal, and ligation. We used RNA interference to deplete *Tb*MP18 (band VII), the last unexamined major protein of our purified editing complex, showing it is essential. *Tb*MP18 is critical for the U-deletional and U-insertional cleavages and for integrity of the approximately 20S editing complex, whose other major components, *Tb*MP99, *Tb*MP81, *Tb*MP63, *Tb*MP52, *Tb*MP48, *Tb*MP42 (bands I through VI), and *Tb*MP57, instead sediment as approximately 10S associations. Additionally, *Tb*MP18 augments editing substrate recognition by the *Tb*MP57 terminal U transferase, possibly aiding the recognition component, *Tb*MP81. The other editing activities and their coordination in precleaved editing remain active in the absence of *Tb*MP18. These data are reminiscent of the data on editing subcomplexes reported by A. Schnaufer *et al.* (*Mol. Cell* **12**: 307-319, 2003) and suggest that these subcomplexes are held together in the approximately 20S complex by *Tb*MP18, as was proposed previously. Our data additionally imply that the proteins are less long-lived in these subcomplexes than they are when held in the complete editing complex. The editing endonucleolytic cleavages being lost when the editing complex becomes fragmented, as upon *Tb*MP18 depletion, should be advantageous to the trypanosome, minimizing broken mRNAs.

14146. **Laxman, S., Riechers, A., Sadilek, M., Schwede, F. & Beavo, J. A., 2006.** Hydrolysis products of cAMP analogues cause transformation of *Trypanosoma brucei* from slender to stumpy-like forms. *Proceedings of the National Academy of Sciences USA*, **103** (50): 19194-19199.

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African sleeping sickness is a disease caused by *Trypanosoma brucei*. *T. brucei* proliferates rapidly in the mammalian bloodstream as long, slender forms, but at higher population densities they transform into nondividing, short, stumpy forms. This is thought to be a mechanism adopted by *T. brucei* to establish a stable host-parasite relationship and to allow a transition into the insect stage of its life cycle. Earlier studies have suggested a role for cAMP in mediating this transformation. In this study, using membrane-permeable nucleotide analogues, we show that it is not the cAMP analogues themselves but rather the hydrolyzed products of membrane-permeable cAMP analogues that prevent proliferation of

T. brucei. The metabolic products are more potent than the cAMP analogues, and hydrolysis-resistant cAMP analogues are not antiproliferative. We further show that the antiproliferative effect of these membrane-permeable adenosine analogues is caused by transformation into forms resembling short, stumpy bloodstream forms. These data suggest that the slender-to-stumpy transformation of *T. brucei* may not be mediated directly by cAMP and also raise the possibility of using such adenosine analogues as antitrypanosomal drugs.

14147. **Lecordier, L., Devaux, S., Uzureau, P., Dierick, J. F., Walgraffe, D., Poelvoorde, P., Pays, E. & Vanhamme, L., 2007.** Characterization of a TFIIH homologue from *Trypanosoma brucei*. *Molecular Microbiology*, **64** (5): 1164-1181.

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Trypanosomes are protozoans showing unique transcription characteristics. We describe in *Trypanosoma brucei* a complex homologous to TFIIH, a multisubunit transcription factor involved in the control of transcription by RNA Pol I and RNA Pol II, but also in DNA repair and cell cycle control. Bioinformatics analyses allowed the detection of five genes encoding four putative core TFIIH subunits (*Tb*XPD, *Tb*XPB, *Tb*p44, *Tb*p52), including a novel XPB variant, *Tb*XPBz. In all cases sequences known to be important for TFIIH functions were conserved. We performed a molecular analysis of this core complex, focusing on the two subunits endowed with a known enzymatic (helicase) activity, XPD and XPB. The involvement of these *T. brucei* proteins in a *bona fide* TFIIH core complex was supported by (i) colocalization by immunofluorescence in the nucleus, (ii) direct physical interaction of *Tb*XPD and its interacting regulatory subunit *Tb*p44 as determined by double-hybrid assay and tandem affinity purification of the core TFIIH, (iii) involvement of the core proteins in a high molecular weight complex and (iv) occurrence of transcription, cell cycle and DNA repair phenotypes upon either RNAi knock-down or overexpression of essential subunits.

14148. **Marcello, L. & Barry, J. D., 2007.** From silent genes to noisy populations -dialogue between the genotype and phenotypes of antigenic variation. *Journal of Eukaryotic Microbiology*, **54** (1): 14-17.

Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow Biomedical Research Centre, 120 University Place, Glasgow G12 8TA, UK.

African trypanosomes evade humoral immunity through antigenic variation whereby they switch expression of the variant surface glycoprotein (VSG) gene encoding their glycoprotein surface coat. Switching proceeds by duplication from an archive of silent VSG genes into a transcriptionally active locus, and precedent suggests silent genes can contribute, combinatorially to formation of expressed, functional genes through segmental gene conversion. The genome project has revealed that most of the silent archive consists of hundreds of VSG genes in subtelomeric tandem arrays, and that most of these are not functional genes. The aim of this review is to explore links between the uncovered trypanosome genotype and the phenotype of antigenic variation, stretching from the broad

phenotype-transmission in the field and the overcoming of herd immunity to events within single infections. Highlighting in particular the possible impact of phenotype selection on the evolution of the VSG archive and the mechanisms for its expression leads to a theoretical framework to further our understanding of this complex immune evasion strategy.

14149. **Martinez-Oyanedel, J., McNae, I. W., Nowicki, M. W., Keillor, J. W., Michels, P. A., Fothergill-Gilmore, L. A. & Walkinshaw, M. D., 2007**. The first crystal structure of phosphofructokinase from a eukaryote: *Trypanosoma brucei*. *Journal of Molecular Biology*, **366** (4): 1185-1198.

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The crystal structure of the ATP-dependent phosphofructokinase (PFK) from *Trypanosoma brucei* provides the first detailed description of a eukaryotic PFK, and enables comparisons to be made with the crystal structures of bacterial ATP-dependent and PPi-dependent PFKs. The structure reveals that two insertions (the 17-20 and 329-348 loops) that are characteristic of trypanosomatid PFKs, but absent from bacterial and mammalian ATP-dependent PFKs, are located within and adjacent to the active site, and are in positions to play important roles in the enzyme's mechanism. The 90 residue N-terminal extension forms a novel domain that includes an "embracing arm" across the subunit boundary to the symmetry-related subunit in the tetrameric enzyme. Comparisons with the PPi-dependent PFK from *Borrelia burgdorferi* show that several features thought to be characteristic of PPi-dependent PFKs are present in the trypanosome ATP-dependent PFK. These two enzymes are generally more similar to each other than to the bacterial or mammalian ATP-dependent PFKs. However, there are critical differences at the active site of PPi-dependent PFKs that are sufficient to prevent the binding of ATP. This crystal structure of a eukaryotic PFK has enabled us to propose a detailed model of human muscle PFK that shows active site and other differences that offer opportunities for structure-based drug discovery for the treatment of sleeping sickness and other diseases caused by the trypanosomatid family of protozoan parasites.

14150. **Morrison L. J., McCormack, G., Sweeney L., Likeufack, A. C., Truc, P., Turner, C. M., Tait, A. & Macleod, A., 2007.** Use of multiple displacement amplification to increase the detection and genotyping of *Trypanosoma* species samples immobilized on FTA filters. *American Journal of Tropical Medicine and Hygiene,* **76** (6): 1132-1137.

Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow, UK.; Institut de Recherche pour le Développement, UR 177 Trypanosomoses Africaines, Montpellier, France; Institut de Recherche pour le Développement, UR 177 Trypanosomoses Africaines, Luanda, Angola; and Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK.

Whole genome amplification methods are a recently developed tool for amplifying DNA from limited template. We report its application in trypanosome infections, characterized by low parasitaemias. Multiple displacement amplification (MDA) amplifies

DNA with a simple *in vitro* step and was evaluated on mouse blood samples on FTA filter cards with known numbers of *Trypanosoma brucei* parasites. The data showed a 20-fold increase in the number of PCRs possible per sample, using primers diagnostic for the multicopy ribosomal ITS region or 177-bp repeats, and a 20-fold increase in sensitivity over nested PCR against a single-copy microsatellite. Using MDA for microsatellite genotyping caused allele dropout at low DNA concentrations, which was overcome by pooling multiple MDA reactions. The validity of using MDA was established with samples from Human African Trypanosomiasis patients. The use of MDA allows maximal use of finite DNA samples and may prove a valuable tool in studies where multiple reactions are necessary, such as population genetic analyses.

14151. **Navarro, M., Peñate, X. & Landeira, D., 2007.** Nuclear architecture underlying gene expression in *Trypanosoma brucei*. *Trends in Microbiology*, **15** (6): 263-270.

Instituto de Parasitología y Biomedicina López-Neyra, Consejo Superior de Investigaciones Científicas (Spanish National Research Council), Avda. del Conocimiento s/n, 18100 Granada, Spain. [miguel.navarro@ipb.csic.es].

The influence of nuclear architecture on the regulation of developmental gene expression has recently become evident in many organisms ranging from yeast to humans. During interphase, chromosomes and nuclear structures are in constant motion; therefore, correct temporal association is needed to meet the requirements of gene expression. *Trypanosoma brucei* is an excellent model system in which to analyze nuclear spatial implications in the regulation of gene expression because the two main surface-protein genes (procyclin and VSG) are transcribed by the highly compartmentalized RNA polymerase I and undergo distinct transcriptional activation or downregulation during developmental differentiation. Furthermore, the infective bloodstream form of the parasite undergoes antigenic variation, displaying sequentially different types of VSG by allelic exclusion. Here, we discuss recent advances in understanding the role of chromosomal nuclear positioning in the regulation of gene expression in *T. brucei.*

14152. **Nowicki, C. & Cazzulo, J. J., 2007.** Aromatic amino acid catabolism in trypanosomatids. *Comparative Biochemistry and Physiology*. **In press, corrected proof.**

IQUIFIB/Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junin 956, CP1113, Argentina.

Trypanosomatids cause important human diseases, like sleeping sickness, Chagas disease, and the leishmaniases. Unlike in the mammalian host, the metabolism of aromatic amino acids is a very simple pathway in these parasites. *Trypanosoma brucei* and *Trypanosoma cruzi* transaminate the three aromatic amino acids, the resulting 2-oxo acids being reduced to the corresponding lactate derivatives and excreted. In *T. cruzi*, two enzymes are involved in this process: a tyrosine aminotransferase (TAT), which despite a high sequence similarity with the mammalian enzyme, has a different substrate specificity; and an aromatic L-2-hydroxyacid dehydrogenase (AHADH), which belongs to the subfamily of the cytosolic malate dehydrogenases (MDHs), yet has no MDH activity. In *T. cruzi* AHADH the

substitution of Ala102 for Arg enables AHADH to reduce oxaloacetate. In the members of the 2-hydroxyacid dehydrogenases family, the residue at this position is known to be responsible for substrate specificity. *T. cruzi* does not possess a cytosolic MDH but contains a mitochondrial and a glycosomal MDH; by contrast *T. brucei* and *Leishmania* spp. possess a cytosolic MDH in addition to glycosomal and mitochondrial isozymes. Although *Leishmania mexicana* also transaminates aromatic amino acids through a broad specificity aminotransferase, the latter presents low sequence similarity with TATs, and this parasite does not seem to have an enzyme equivalent to *T. cruzi* AHADH. Therefore, these closely related primitive eukaryotes have developed aromatic amino acid catabolism systems using different enzymes and probably for different metabolic purposes.

14153. **Oberholzer, M., Marti, G., Baresic, M., Kunz, S., Hemphill, A. & Seebeck, T., 2007.** The *Trypanosoma brucei* cAMP phosphodiesterases *Tbr*PDEB1 and *Tbr*PDEB2: flagellar enzymes that are essential for parasite virulence. *FASEB Journal*, **21** (3): 720-731.

Institute of Cell Biology, University of Bern, Switzerland.

Cyclic nucleotide specific phosphodiesterases (PDEs) are pivotal regulators of cellular signalling. They are also important drug targets. Besides catalytic activity and substrate specificity, their subcellular localization and interaction with other cell components are also functionally important. In contrast to the mammalian PDEs, the significance of PDEs in protozoal pathogens remains mostly unknown. The genome of *Trypanosoma brucei,* the causative agent of human sleeping sickness, codes for five different PDEs. Two of these, *Tbr*PDEB1 and *Tbr*PDEB2, are closely similar, cAMP-specific PDEs containing two GAF-domains in their N-terminal regions. Despite their similarity, these two PDEs exhibit different subcellular localizations. *Tbr*PDEB1 is located in the flagellum, whereas *Tbr*PDEB2 is distributed between flagellum and cytoplasm. RNAi against the two mRNAs revealed that the two enzymes can complement each other but that a simultaneous ablation of both leads to cell death in bloodstream form trypanosomes. RNAi against *Tbr*PDEB1 and *Tbr*PDEB2 also functions *in vivo* where it completely prevents infection and eliminates ongoing infections. Our data demonstrate that *Tbr*PDEB1 and *Tbr*PDEB2 are essential for virulence, making them valuable potential targets for new PDE-inhibitor based trypanocidal drugs. Furthermore, they are compatible with the notion that the flagellum of *T. brucei* is an important site of cAMP signalling.

14154. **Ochsenreiter, T. & Hajduk, S. L., 2006**. Alternative editing of cytochrome c oxidase III mRNA in trypanosome mitochondria generates protein diversity. *EMBO Reports*, **7** (11): 1128-1133.

Program in Global Infectious Diseases, Josephine Bay Paul Center, Marine Biological Laboratory, 7 MBL Street, Woods Hole, Massachusetts 02543, USA.

Trypanosomes use RNA editing to produce most functional mitochondrial messenger RNA. Precise insertion and deletion of hundreds of uridines is necessary to make full-length cytochrome c oxidase III (COXIII) mRNA. We show that COXIII mRNA can be alternatively edited by a mechanism using an alternative guide RNA to make a stable mRNA.

This alternatively edited mRNA is translated to produce a unique protein that fractionates with mitochondrial membranes and colocalizes with mitochondrial proteins *in situ*. Alternative RNA editing represents a previously unknown mechanism generating protein diversity and, as such, represents an important function for RNA editing.

14155. **Ott, R., Chibale, K., Anderson, S., Chipeleme, A., Chaudhuri, M., Guerrah, A., Colowick, N. & Hill, G. C., 2006.** Novel inhibitors of the trypanosome alternative oxidase inhibit *Trypanosoma brucei brucei* growth and respiration. *Acta Tropica*, **100** (3): 172-184.

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African trypanosomiasis is a deadly disease for which few chemotherapeutic options are available. The causative agents, *Trypanosoma brucei rhodesiense* and *T. b. gambiense*, utilize a non-cytochrome, alternative oxidase (AOX) for their cellular respiration. The absence of this enzyme in mammalian cells makes it a logical target for therapeutic agents. We designed three novel compounds, ACB41, ACD15, and ACD16, and investigated their effects on trypanosome alternative oxidase (TAO) enzymatic activity, parasite respiration, and parasite growth *in vitro*. All three compounds contain a 2-hydroxybenzoic acid moiety, analogous to that present in SHAM, and a prenyl side chain similar to that found in ubiquinol. ACD15 and ACD16 are further differentiated by the presence of a solubility-enhancing carbohydrate moiety. Kinetic studies with purified TAO show that all three compounds competitively inhibit TAO, and two compounds, ACB41 and ACD15, have inhibition constants five- and three-fold more potent than SHAM, respectively. All three compounds inhibited the respiration and growth of continuously cultured *T. b. brucei* bloodstream cells in a dose-dependent manner. None of the compounds interfered with respiration of rat liver mitochondria, nor did they inhibit the growth of a continuously cultured mammalian cell line. Collectively, the results suggest we have identified a new class of compounds that are inhibitors of TAO, have trypanocidal properties *in vitro*, and warrant further investigation *in vivo*.

14156. **Persson, L., 2007**. Ornithine decarboxylase and S-adenosylmethionine decarboxylase in trypanosomatids. *Biochemical Society Transactions*, **35 (**2): 314-317.

Department of Experimental Medical Science, Lund University, BMC F:13, S-221 84 Lund, Sweden. [lo.persson@med.lu.se].

The production of polyamines has been shown to be an effective target for a drug against the West African form of sleeping sickness caused by *Trypanosoma brucei gambiense*. *T. brucei* belongs to the group of protozoan parasites classed as trypanosomatids. Parasitic species of this group are the causative agents of various tropical diseases besides African sleeping sickness, e.g. Chagas' disease (*Trypanosoma cruzi*), cutaneous (*Leishmania* spp.) and visceral (*Leishmania donovani*) leishmaniasis. The metabolism of polyamines in the parasites is a potential target for the development of new drugs for treatment of these diseases. The key steps in polyamine synthesis are catalysed by ODC (ornithine decarboxylase) and AdoMetDC (S-adenosylmethionine decarboxylase). In the present paper,

some of the available information on ODC and AdoMetDC in trypanosomatids will be described and discussed.

14157. **Richmond, G. S. & Smith, T. K., 2007**. A novel phospholipase from *Trypanosoma brucei*. *Molecular Microbiology*, **63** (4): 1078-1095.

Wellcome Trust Biocentre, Division of Biological Chemistry and Molecular Microbiology, College of Life Sciences, University of Dundee, Scotland, UK.

Phospholipase A (1) activities have been detected in most cells where they have been sought and yet their characterization lags far behind that of the phospholipases A(2), C and D. The study presented here details the first cloning and characterization of a cytosolic PLA (1) that exhibits preference for phosphatidylcholine (GPCho) substrates. *Trypanosoma brucei* phospholipase A (1) (*Tb*PLA(1)) is unique from previously identified eukaryotic PLA(1) because it is evolutionarily related to bacterial secreted PLA(1). A *T. brucei* ancestor most likely acquired the PLA (1) from a horizontal gene transfer of a PLA (1) from *Sodalis glossinidius*, a bacterial endosymbiont of tsetse flies. Nano-electrospray ionization tandem mass spectrometry analysis of *Tb*PLA (1) mutants established that the enzyme functions *in vivo* to synthesize lysoGPCho metabolites containing long-chain mostly polyunsaturated and highly unsaturated fatty acids. Analysis of purified mutated recombinant forms of *Tb*PLA (1) revealed that this enzyme is a serine hydrolase whose catalytic mechanism involves a triad consisting of the amino acid residues Ser-131, His-234 and Asp-183. The *Tb*PLA (1) homozygous null mutants generated here constitute the only PLA (1) double knockouts from any organism.

14158. **Schlecker, T., Comini, M. A., Melchers, J., Ruppert, T. & Krauth-Siegel, R. L., 2007.** Catalytic mechanism of the glutathione peroxidase-type tryparedoxin peroxidase of *Trypanosoma brucei*. *Biochemical Journal*. **In press, corrected proof**.

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Trypanosoma brucei, the causative agent of African sleeping sickness, encodes three nearly identical genes for cysteine-homologues of the selenocysteine-containing glutathione peroxidases. The enzymes - which are essential for the parasites - lack glutathione peroxidase activity but catalyze the trypanothione/tryparedoxin-dependent reduction of hydroperoxides. Cys-47, Gln-82, and Trp-137 correspond to the SeCys, Gln, and Trp catalytic triad of the mammalian selenoenzymes. Site directed mutagenesis revealed that Cys-47 and Gln-82 are essential. A glycine mutant of Trp-137 had 13 percent of wild-type activity which suggests that the aromatic residue may play a structural role but is not directly involved in catalysis. Cys-95, conserved in related yeast and plant proteins but not in the mammalian selenoenzymes, proved to be essential as well. In contrast, replacement of the highly conserved Cys-76 by a serine resulted in a fully active enzyme species and its role remains unknown. Thr-50, proposed to stabilize the thiolate anion at Cys-47, is also not essential for catalysis. Treatment of the C76S/C95S but not of the C47S/C76S double mutant with H_2O_2 induced formation of a sulphinic acid and covalent homodimers in accordance with Cys-47 being the peroxidative active site thiol. In the wild-type peroxidase, these oxidations are

prevented by formation of an intramolecular disulfide bridge between Cys-47 and Cys-95. As shown by mass spectrometry, regeneration of the reduced enzyme by tryparedoxin involves a transient mixed disulfide between Cys-95 of the peroxidase and Cys-40 of tryparedoxin. The catalytic mechanism of the tryparedoxin peroxidase resembles that of atypical 2-Cys-peroxiredoxins but is distinct from that of the selenoenzymes.

14159. **Scory, S., Stierhof, Y. D., Caffrey, C. R. & Steverding, D., 2007.** The cysteine proteinase inhibitor Z-Phe-Ala-CHN2 alters cell morphology and cell division activity of *Trypanosoma brucei* bloodstream forms *in vivo*. *Kinetoplastid Bioloy and Disease*, 6: 2.

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Current chemotherapy of human African trypanosomiasis or sleeping sickness relies on drugs developed decades ago, some of which show toxic side effects. One promising line of research towards the development of novel anti-trypanosomal drugs are small-molecule inhibitors of *Trypanosoma brucei* cysteine proteinases. In this study, we demonstrate that treatment of *T. brucei*-infected mice with the inhibitor, carbobenzoxy-phenylalanyl-alanine-diazomethyl ketone (Z-Phe-Ala-CHN_2), alters parasite morphology and inhibits cell division. Following daily intra-peritoneal administration of 250 mg kg^{-1} of Z-Phe-Ala-CHN_2 on days three and four post infection (p.i.), stumpy-like forms with enlarged lysosomes were evident by day five p.i. In addition, trypanosomes exposed to the inhibitor had a 65 percent greater protein content than those from control mice. Also, in contrast to the normal 16 percent of parasites containing two kinetoplasts - a hallmark of active mitosis, only 4 percent of trypanosomes exposed to the inhibitor were actively dividing, indicating cell cycle-arrest. We suggest that inhibition of endogenous cysteine proteinases by Z-Phe-Ala-CHN_2 depletes the parasite of essential nutrients necessary for DNA synthesis, which in turn, prevents progression of the cell cycle. This arrest then triggers differentiation of the long slender into short-stumpy forms.

14160. **Stephens, J. L., Lee, S. H., Paul, K. S. & Englund, P. T., 2007.** Mitochondrial fatty acid synthesis in *Trypanosoma brucei*. *Journal of Biological Chemistry*, **282** (7): 4427-4436.

Department of Biological Chemistry, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, USA.

Whereas other organisms utilize type I or type II synthases to make fatty acids, trypanosomatid parasites such as *Trypanosoma brucei* are unique in their use of a microsomal elongase pathway (ELO) for *de novo* fatty acid synthesis (FAS). Because of the unusual lipid metabolism of the trypanosome, it was important to study a second FAS pathway predicted by the genome to be a type II synthase. We localized this pathway to the mitochondrion, and RNA interference (RNAi) or genomic deletion of acyl carrier protein (ACP) and beta-ketoacyl-ACP synthase indicated that this pathway is likely essential for bloodstream and procyclic life cycle stages of the parasite. *In vitro* assays show that the largest major fatty

acid product of the pathway is C_{16}, whereas the ELO pathway, utilizing ELOs 1, 2, and 3, synthesizes up to C_{18}. To demonstrate mitochondrial FAS *in vivo*, we radioabelled fatty acids in cultured procyclic parasites with ^{14}C pyruvate or ^{14}C threonine, either of which is catabolized to ^{14}C acetyl-CoA in the mitochondrion. Although some of the ^{14}C acetyl-CoA may be utilized by the ELO pathway, a striking reduction in radiolabelled fatty acids following ACP RNAi confirmed that it is also consumed by mitochondrial FAS. ACP depletion by RNAi or gene knockout also reduces lipoic acid levels and drastically decreases protein lipoylation. Thus, octanoate (C_8), the precursor for lipoic acid synthesis, must also be a product of mitochondrial FAS. Trypanosomes employ two FAS systems: the unconventional ELO pathway that synthesizes bulk fatty acids and a mitochondrial pathway that synthesizes specialized fatty acids that are likely utilized intramitochondrially.

14161. **Urwyler, S., Studer, E., Renggli, C. K. & Roditi, I., 2007.** A family of stage-specific alanine-rich proteins on the surface of epimastigote forms of *Trypanosoma brucei*. *Molecular Microbiology*, **63** (1): 218-228.

Institut für Zellbiologie, Universität Bern, Bern, Switzerland.

A “two coat” model of the life cycle of *Trypanosoma brucei* has prevailed for more than 15 years. Metacyclic forms transmitted by infected tsetse flies and mammalian bloodstream forms are covered by variant surface glycoproteins. All other life cycle stages were believed to have a procyclin coat, until it was shown recently that epimastigote forms in tsetse salivary glands express procyclin mRNAs without translating them. As epimastigote forms cannot be cultured, a procedure was devised to compare the transcriptomes of parasites in different fly tissues. Transcripts encoding a family of glycosylphosphatidyl inositol-anchored proteins, BARPs (previously called bloodstream alanine-rich proteins), were 20-fold more abundant in salivary gland than midgut (procyclic) trypanosomes. Anti-BARP antisera reacted strongly and exclusively with salivary gland parasites and a BARP 3' flanking region directed epimastigote-specific expression of reporter genes in the fly, but inhibited expression in bloodstream and procyclic forms. In contrast to an earlier report, we could not detect BARPs in bloodstream forms. We propose that BARPs form a stage-specific coat for epimastigote forms and suggest renaming them *brucei* alanine-rich proteins.

14162. **Wang, Y., Singh, U. & Mueller, D. M., 2007**. Mitochondrial genome integrity mutations uncouple the yeast *Saccharomyces cerevisiae* ATP synthase. *Journal of Biological Chemistry*, **282** (11): 8228-8236.

Department of Biochemistry and Molecular Biology, Rosalind Franklin University of Medicine and Science, The Chicago Medical School, North Chicago, Illinois 60064, USA.

The mitochondrial ATP synthase is a molecular motor, which couples the flow of protons with phosphorylation of ADP. Rotation of the central stalk within the core of ATP synthase effects conformational changes in the active sites driving the synthesis of ATP. Mitochondrial genome integrity (mgi) mutations have been previously identified in the alpha-, beta-, and gamma-subunits of ATP synthase in yeast *Kluyveromyces lactis* and trypanosome *Trypanosoma brucei*. These mutations reverse the lethality of the loss of mitochondrial DNA

in petite negative strains. Introduction of the homologous mutations in *Saccharomyces cerevisiae* results in yeast strains that lose mitochondrial DNA at a high rate and accompanied decreases in the coupling of the ATP synthase. The structure of yeast F1-ATPase reveals that the mgi residues cluster around the gamma-subunit and selectively around the collar region of F1. These results indicate that residues within the mgi complementation group are necessary for efficient coupling of ATP synthase, possibly acting as a support to fix the axis of rotation of the central stalk.

14163. **Welburn, S. C., Macleod, E., Figarella, K. & Duzensko, M., 2006.** Programmed cell death in African trypanosomes. *Parasitology*, 132 Suppl: S7-S18.

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Until recently it had generally been assumed that apoptosis and other forms of programmed cell death evolved during evolution of the metazoans to regulate growth and development in these multicellular organisms. However, recent research is adding strength to the original phenotypic observations described almost a decade ago which indicated that some parasitic protozoa may have evolved a cell death pathway analogous to the process described as apoptosis in metazoa. Here we explore the implications of a programmed cell death pathway in the African tsetse-transmitted trypanosomes.

14164. **Willert, E. K., Fitzpatrick, R. & Phillips, M. A., 2007**. Allosteric regulation of an essential trypanosome polyamine biosynthetic enzyme by a catalytically dead homologue. *Proceedings of the National Academy of Sciences USA*, **104** (20): 8275-8280.

Department of Pharmacology, University of Texas Southwestern Medical Center, 6001 Forest Park Road, Dallas, TX 75390-9041, USA.

African sleeping sickness is a fatal disease that is caused by the protozoan parasite *Trypanosoma brucei.* Polyamine biosynthesis is an essential pathway in the parasite and is a validated drug target for treatment of the disease. S-adenosylmethionine decarboxylase (AdoMetDC) catalyzes a key step in polyamine biosynthesis. Here, we show that trypanosomatids uniquely contain both a functional AdoMetDC and a paralogue designated prozyme that has lost catalytic activity. The *T. brucei* prozyme forms a high-affinity heterodimer with AdoMetDC that stimulates its activity by 1,200-fold. Both genes are expressed in *T. brucei*, and analysis of AdoMetDC activity in *T. brucei* extracts supports the finding that the heterodimer is the functional enzyme *in vivo*. Thus, prozyme has evolved to be a catalytically dead but allosterically active subunit of AdoMetDC, providing an example of how regulators of multimeric enzymes can evolve through gene duplication and mutational drift. These data identify a distinct mechanism for regulating AdoMetDC in the parasite that suggests new strategies for the development of parasite-specific inhibitors of the polyamine biosynthetic pathway.

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