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TSETSE AND TRYPANOSOMIASIS INFORMATION

FAO

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, 00153 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

CONTENTS

SECTION A – NEWS

FROM THE EDITOR

Dear Reader,
As mentioned in the introduction to the previous edition of TTI, it was not possible to include in Volume 29 (1) the abstract of any paper dealing with either Experimental Trypanosomiasis or Trypanosome Research. This volume now addresses this "shortfall" in that it includes both abstracts of peer reviewed papers published over the past 12 months falling within the scope of these two categories, and abstracts of papers published over the past 6 months under the headings of "General" and "Tsetse Biology, Tsetse Control, Human and Animal Trypanosomiasis". The result is a volume which is substantially longer than normal despite a shorter News Section than has often been the case before. This, in turn, has prompted further thought about how to keep costs within budget (recall that each volume has to be prepared and printed in English and French) while both maintaining the focus of TTI and meeting the needs of the vast majority of its readers.

During the process of conducting the literature searches for both this and the previous volume of TTI, it has become very clear that papers covering the subjects of "*T. cruzi"*, "Chemotherapy" and "Molecular Methods" dominated. However, in an effort to keep the focus of TTI on tsetse flies and the trypanosomiases in Africa while at the same time covering preferentially abstracts dealing with more applied or "downstream" type of field and research developments, the number of abstracts covering the American trypanosomes were drastically reduced, while many of those dealing with chemotherapy and applications of molecular techniques were increasingly dealt with by omitting abstracts and publishing only titles and authors' names and addresses. Since all of these steps will have be more strictly adhered to in future volumes of TTI, any reader wishing the abstract of a paper referred to in this or future volumes of TTI by title can receive this simply by making a request to me by e-mail.

With best wishes,

James Dargie

PROGRAMME AGAINST AFRICAN TRYPANOSOMIASIS: 10TH MEETING OF THE PROGRAMME COMMITTEE

Foreword

This meeting was convened at the Istituto Agronomico per l'Oltremare (IAO, Overseas Agronomic Institute), Florence, Italy, 26-27 April 2006. The meeting focused on (i) achievements of PAAT mandated organizations (i.e. FAO, IAEA, WHO, AU-IBAR) and AU-PATTEC, (ii) implementation of AfDB-PATTEC supported T&T intervention in six sub-Saharan countries (Burkina Faso, Ghana, Mali in West Africa and Ethiopia, Kenya, Uganda in East Africa), (iii) activities in Tsetse and Trypanosomiasis (T&T) Research and Development by PAAT research partners (CIRAD, ICIPE, ILRI, ITM), and (iv) new and potential PAAT partnerships (IFAH, UNIDO, World Bank, FAO/IGAD-LPI)

The meeting was officially opened by Dr A. Perlini, IAO Director General, who on behalf of IAO warmly welcomed the participants to Florence to work on the problems posed by T&T which affect both human and livestock health and sustainable agricultural development in a substantial portion of Africa. Mr A. Scappini, Livestock Officer, presented an overview of IAO activities focusing on livestock agriculture projects.

An introduction to PAAT and the objectives of the meeting were presented in the opening address of the PAAT chairman, Prof. A. Ilemobade. He reminded participants that in 2006 PAAT celebrates the 10th anniversary of its founding when the idea of a global, international alliance aiming at clarifying the problem of tsetse and trypanosomiasis was mooted at a conference in Brussels, Belgium. The progress made by PAAT in ten years was impressive and internationally acknowledged. Also, the support that PAAT continuously provides to the African countries affected by the T&T problem and to the PATTEC initiative was recalled.

Mr R. Mattioli welcomed the group on behalf of the FAO/PAAT Secretariat and thanked the Italian Government and the IAO for hosting the meeting. He also welcomed the renewed interest in PAAT shown by other FAO initiatives/projects such as the Inter-Governmental Authority on Development-Livestock Policy Initiative (IGAD-LPI) and the Pro-Poor Livestock Policy Initiative (PPLPI), and more generally by the United Nations system, with UNIDO and IFAD participating in the meeting and actively supporting PAAT actions. The contribution and participation of the private sector through the International Federation for Animal Health (IFAH), to PAAT events and activities and the financial assistance of the Japanese Government to the joint Ethiopian Government/IAEA/FAO project on T&T intervention in the Southern Rift Valley of Ethiopia were further recognition of the work of PAAT. The advanced PAAT-PATTEC harmonisation and the fact that T&T intervention is now placed in the broader context of SARD were considered instrumental in creating a positive, collaborative and attractive environment for donors and other potential stakeholders.

The meeting was chaired by Prof. A.A. Ilemobade and FAO provided secretarial assistance.

Minutes of the Previous Meeting

The report and recommendations of the 9th PAAT-PC meeting were revised and adopted.

Reports from UN and Regional Organizations and Institutions

***FAO/PAAT - R.C. Mattioli*:** Activities and progress on the implementation of recommendations since the 9th PAAT-PC meeting were presented.

The recommendation to apply the PAAT-PATTEC criteria in the selection of intervention areas was re-emphasised at the PAAT Advisory Group Coordinators meeting held in Addis Ababa in September 2005, and at a workshop on "Improving decision support for T&T intervention in Uganda". FAO/IAEA/WHO/PAAT developed a document which included terms of reference for an "Assistance Formulation Team" for livestock-agriculture and human health in T&T intervention areas under the current six national AfDB-AU/PATTEC initiatives.

The problem of human resources development was duly addressed by PAAT mandated organizations. WHO organized an international course on African Trypanosomiasis

in Tunisia (10-28 October 2005) and trained staff from Ministries of Health and Veterinary Services Departments on HAT control methods. IAEA convened an FAO/IAEA regional training course on "Standardized Baseline Data Collection for Area-wide Tsetse and Trypanosomiasis Management" in Nairobi from 13 March–7 April 2006. In the period between the 9th and the 10th meetings of the PAAT-PC, i.e. May 2005–April 2006, IAEA funded 54 person-month fellowships for collaborators from seven T&T affected countries. Within the project "Strengthening the PAAT-Information System (IS)" funded by IFAD, FAO/PAAT assessed the training needs and requirements of national technical staff and the development of human resources with respect to Information System management and GIS. In this regard, missions were undertaken in Burkina Faso, Ghana, Mali, Ethiopia, Kenya and Uganda.

In relation to the recommendation to establish standardized guidelines and procedures for field and laboratory operations, the importance of available manuals and technical papers was stressed. Also, work is in progress to produce guidelines for declaring areas free of tsetse flies and transmitted trypanosomiasis, and to develop a new tool for guiding the economic decision making process for field T&T interventions ("Mapping the Benefits"). A paper dealing in particular with SIT was published under the title "Potential Impact of Tsetse Fly Control Involving the Sterile Insect Technique" in "Sterile Insect Technique – Principles and Management in Area-wide Integrated Pest Management" (Eds. V.A. Dyck, J. Hendrichs & A.S. Robinson), Springer, The Netherlands, pp. 701-723. Consultants were recruited to draft "FAO/IAEA Guidelines for Conducting Baseline Tsetse Surveys for Area-wide Integrated Pest Management Programmes".

Regarding the recommendation concerning the involvement of other stakeholders in the management of other diseases and constraints to SARD, the most interesting partnerships concern IFAH, UNIDO and the World Bank (the last, under the umbrella of the African Livestock (ALIVE) initiative. Within FAO, the new collaboration between PAAT and the Inter-Governmental Authority on Development-Livestock Policy Initiative (IGAD-LPI) project was mentioned.

An economic analysis of tsetse suppression techniques, including SAT, was performed and modelled in a paper dealing with the costs of alternative tsetse control approaches in Uganda. The paper was authored by A. Shaw for the FAO/PPLPI project with the assistance of FAO/PAAT. The option to use SAT has also been considered in the joint Ethiopian Government-FAO/IAEA project for T&T intervention and related SARD approved by the Government of Japan and in the FAO/PAAT and FAO/IGAD-LPI project proposal submitted for funding to the Wellcome Trust to develop "A new decision support tool for policy and advocacy: mapping and analysing both estimated costs and potential benefits of T&T control in the Greater Horn of Africa".

IAEA – U. Feldmann: Regarding the recommendation to establish standardized guidelines and procedures for field and laboratory operations, progress was reported on the production of "Guidelines for Declaring Areas Free of Tsetse and Tsetse Transmitted Trypanosomiasis". Further guidelines to member countries for identifying the optimal location of mass-rearing units were produced along with a spreadsheet that assists in defining the room size and associated budget. An international conference on "Area-wide Control of Insect Pests" was held in May 2005 in Vienna. The conference was attended by more than 400 participants. In October 2005, a representative of the Joint FAO/IAEA Division attended the PATTEC regional meeting (Nairobi, Kenya).

An outline was given of the meaning of the "phased approach" to area-wide tsetse control and the need was emphasised for both commitment and a national policy/strategy for T&T intervention in member countries. The need for baseline data was also stressed for deciding on the best approach to deal with tsetse flies, human and animal trypanosomiasis, as was the importance of linking agricultural development to the removal of T&T, capacity building and international assistance. Although SIT remains a major tool for tsetse elimination, it may not be needed in all scenarios. With regard to the production of tsetse sterile males, substantial action and planning are needed to overcome the shortage of sterile flies.

At the meeting for national coordinators in Vienna in December 2005, a questionnaire was used to assess the status and progress of national efforts against the tsetse and trypanosomiasis problem in PATTEC "phase-1" Member States. It was obvious that, along the phased and conditional planning and implementation approach, several aspects/issues remain to be addressed by Member States in the different phases i.e. (i) policy and strategy establishment, (ii) feasibility assessment, (iii) capacity building and pre-operational activities, and (iv) operational intervention. Representatives of the national PATTEC projects were also asked to identify topics on which the three mandated UN agencies (FAO, IAEA and WHO) may provide assistance. The discussions at the meeting revealed that besides the new "FAO/IAEA Guidelines for Standardized Entomological Baseline Data Collection" there are other technical fields where similar manuals and guidelines are needed. A joint effort by the PAAT community and other partners will be needed to develop these manuals and guidelines. In this process the information already available in the existing FAO tsetse control manuals will be instrumental for generating the required updated manuals and guidelines.

***WHO – P. Simarro*:** The support provided to countries involved in the AfDB funded PATTEC initiative, including training activities, was described. Participants were also informed about the contribution of WHO to recent PAAT publications and on the latest update on HAT epidemiology.

WHO is working on better integrating HAT as a component of the AfDB-PATTEC initiative in national projects (e.g. by producing of a plan of action for Ministry of Health (MoH) participation in the AfDB-PATTEC project in Ghana). Screening activities for HAT detection were carried out in several affected areas and capacity building was addressed through service and formal training. In this regard, one of the most important initiatives was the "IV International Course on African Trypanosomoses", held from 10–28 October 2005 in Tunisia and attended by 16 participants from HAT endemic countries. WHO contributed to the upcoming documents on "Mapping the Benefits of Tsetse & Trypanosomiasis Intervention" and to "Linking Sustainable Human and Animal African Trypanosomiasis Control with Rural Development Strategies".

The epidemiology of HAT was outlined with respect to the degree of surveillance at national level and to the average number of cases for the period 1990-2004. Countries have been grouped into five classes according to the number of cases per year: 1 - reporting no cases (no surveillance activities implemented), 2 - reporting no cases (surveillance activities implemented), 3 - reporting less than 50 cases, 4 - reporting between 50 and 1 500 cases, and 5 – reporting more than 1 500 cases. Cases and countries have also been classified on the basis of the pathogenic agent (*Trypanosoma brucei gambiense* or r*hodesiense*). Each class requires a different intervention strategy. The three countries reporting more than 1 500 cases per year (Angola, RDC and Sudan) account for approximately 85 percent of the total number

of registered cases. At continental level, a constant decline in the number of cases has been observed during the last years. This positive trend has been associated with increased disease surveillance and control performed by WHO.

WHO pointed out that both the 11th Meeting of the PAAT Advisory Group Coordinators (PAG) (21-22 September 2005, Addis Ababa, Ethiopia) and the 28th ISCTRC Conference (26–30 September, 2005, Addis Ababa) encouraged WHO to consider HAT as a disease candidate for elimination.

In future, WHO's work will further concentrate on: (i) increasing the awareness among decision makers with a view to removing sleeping sickness from its neglected list, (ii) advocating and developing people's participation programmes (PPP) in order to raise the needed funds, (iii) encouraging and coordinating research for new diagnostic and treatment tools, (iv) providing access to diagnosis and treatment to affected populations, and (v) increasing control activities. Finally, WHO expressed the view that better coordination between AAT - HAT components within national PATTEC initiatives is needed.

***AU/IBAR – S. Haile-Mariam*:** The AU/IBAR representative presented the apologies of the IBAR Director, Mr Modibo Traoré, for not being able to attend. He then summarized the recommendations of the 28th ISCTRC Conference held from 25 - 30 September 2005, in Addis Ababa, Ethiopia.

In the official declaration, the ISCTRC Conference called upon the 37 AU Member States affected by T&T to implement the PATTEC Project by 2015 side by side with the Global Programme for HIV/AIDS and Malaria.

Concerning recommendations, the most relevant were:

- Strengthening the ISCTRC Secretariat through the appointment of a full time secretary, provision of adequate resources and training;
- For national PATTEC projects, each participating country should make feasibility studies, address the problem of capacity building and put in place an efficient management structure before embarking on any field operation;
- With respect to HAT, WHO is encouraged to launch a programme to eliminate sleeping sickness, to introduce control strategies and to support countries in their efforts to update their epidemiological status. R&D groups are urged to look into new diagnostic tools and drugs;
- Further development of standard manuals and field guides (for example on area wide suppression) are needed. Standardized procedure for entomological and veterinary monitoring and subsequent analysis should be formulated and applied at the field level;
- PATTEC should urgently coordinate the assessment of training needs for mid-level and senior staff. PATTEC should also assist the preparation of national and regional action plans for T&T;
- WHO/TDR is encouraged to mobilize resources to address the potential problem of the merger of *T. b. gambiense* and *T. b. rhodesiense* foci in Uganda;
- As regards vector control, gaps should be filled in data collection; the application of integrated technologies needs be pursued. The possible contribution of community participation should be further investigated;

- Environmental issues in T&T interventions should be properly addressed in order to ensure sustainable agriculture and rural development. Standardized methodologies for environmental monitoring and impact assessment should be developed and made available to all countries.

***AU-PATTEC – J. Kabayo*:** The representative of the PATTEC Coordination Office reported on plans and progress in implementing the PATTEC initiative. A brief reminder was given regarding the main features of the PATTEC initiative (decision of the African Heads of State, principles of the "Plan of Action", activities of the "PATTEC Coordination Office", nature of PATTEC projects).

The current status and the roadmap for the activities of the PATTEC initiative were presented. With the support of the AfDB, the first phase of multi-national tsetse eradication projects has already been initiated involving Burkina Faso, Ghana and Mali in West Africa and in Ethiopia, Kenya and Uganda in East Africa. In the tsetse belt of Angola, Botswana, Namibia and Zambia, project implementation is due to start in May 2006. In June 2007 five more multi-national projects should commence (they should tackle transboundary areas in Rwanda and Tanzania, in Benin, Togo, Niger and Nigeria, in Chad, Central African Republic, Cameroon and Nigeria, in Sudan and Ethiopia and in Senegal, Mali and Guinea).

PATTEC indicated that the expected support and assistance to its actions should come from PAAT members and partners. WHO is requested to continue providing surveys, diagnosis and treatment of sleeping sickness in PATTEC project areas; technical support in tsetse mass-rearing, sterilisation and release is expected from the Joint FAO/IAEA Division, while FAO should assure technical support in project development, land cover monitoring and land use planning. Donors are required to provide financial contributions while regional and international research institutions are requested to support operational research, capacity building, project development and evaluation.

AfDB-supported T&T Intervention: Country Reports

Reports on countries benefiting from AfDB support for T&T intervention were presented by representatives of Burkina Faso, Ethiopia, Kenya, Mali and Uganda (representative from Ghana absent with apologies).

Ethiopia – T. Alemu

The Southern Tsetse Eradication Project (STEP) covers an area of around 25 000 km^2 in the Southern Rift Valley in Ethiopia. The project started in 1997 and its impact on livestock, as perceived by the beneficiaries, includes reduced mortality and abortion, improved livestock body condition and increased animal production. The total cost of STEP was estimated to be US$ 43.8 million, but the resources available initially were sufficient for the first phase only (about US$ 8.9 million). Additional funding was provided by the AfDB as part of the regional PATTEC initiative, with the launching workshop scheduled for 16-17 May 2006 in Awassa. Major components of the AfDB project are tsetse suppression and elimination, capacity building, sustainable land management, and project coordination and management.

For the application of the SIT component of STEP, fly mass-rearing and irradiation facilities were established. The fly population of the insectary recently faced problems of

unknown origin, but since January 2006 the colony size has been increasing. Additional funds for STEP are expected from the Japanese Government through the United Nations Trust Fund for Human Security for a total budget of US$ 1.76 million.

Major challenges for the future are: improvement of the tsetse mass production, sterile male release, availability of a skilled expert team to monitor and guide the application of area-wide insect pest management (AW-IPM), including SAT, manpower development, and increased efficiency with respect to the management and use of structure more focused to an operational SIT-based AW programme. An issue for discussion is the presence of inaccessible field sites (approximately 20 percent of project area), in which SAT may be used. Issues also to be addressed by STEP to properly operate are: the establishment of an appropriate data management and information system for prompt day-to-day decision making, and the identification of partner institutions that can provide assistance in land use management and address environmental issues.

In future, STEP will continue tsetse suppression in agreement with the area-wide concept, expand the tsetse colony, extend baseline data collection to the whole project area and establish a structured monitoring system. The project will also strengthen human resource capacity in the fields of GIS, project management and insectary management.

Burkina Faso – I. Sidibe

The AfDB and the Government of Burkina Faso are finalizing the administrative procedures for the initiation of the AfDB supported project in the country and a request for the first disbursement has been submitted. The ongoing activities related to T&T are supported by the country itself.

Four main components are embodied in the AfDB funded project. The "suppression and eradication" component includes community involvement, baseline data collection and processing, tsetse mass-rearing and serial release of sterile males. The "capacity building" component will focus on the creation of an integrated data information system, the rehabilitation of sub-regional training facilities and the reinforcement of national and regional capacities. The "sustainable land management" component concerns land use planning and institutional strengthening, aimed to guide the agricultural intensification and expansion. Last, the "project coordination and management" component will establish a system for information exchange and coordination between the national Project Coordination and Management Units (PCMU), PATTEC "Focal Points" in each country and the AU/PATTEC Office in Addis Ababa, Ethiopia.

Mali – E. Coulibaly

The tsetse infested area in Mali covers 240 000 km^2 (out of a total country area of 1 241 000 km^2). Since 2001, Mali and Burkina Faso have collaborated in joint T&T intervention activities, with financial and technical assistance provided by IAEA. In Mali, the objective of the project is the elimination of tsetse from an area of 40 000 km^2 around Bamako in the northern Niger River basin; the project foresees the use of SIT. Significant success has been achieved in tsetse suppression using the community based approach.

In February 2005, the loan agreement with the AfDB was signed. Within the AfDB project, elimination of flies over an initial project area of 8 000 km^2 will be attempted

through the release of sterile males. After tsetse surveys, suppression will also be expanded on an area of approximately 32 000 km^2.

Kenya – P. Olet

In Kenya there is a close association between the presence of tsetse and high levels of poverty. Eight tsetse species are present in 92 000 km^2. They are spread across three belts: Lake Victoria Basin, Lake Bogoria Basin and Meru-Mwea. Lake Victoria basin is within the first zone that will be targeted; all five districts in this region were also included in the FITCA (Farming in Tsetse Controlled Areas) project, which ended in 2004. Since there is evidence that flies are recovering in the areas previously targeted by FITCA, the new AfDB funded project will take up control activities over those areas in order to guarantee continuity of intervention.

The first disbursement from AfDB was in April 2006. A Project Coordination and Management Unit (PCMU) and members of the National Steering Committee have been identified. Procurement of equipment (e.g. targets, trypanocides, pesticides, GPS receivers and microscopes) has been approved.

For the full implementation of the AfDB funded project, Kenya has requested assistance from PAAT mandated organisations and stakeholders for a range of programme activities: procurement of GIS software and hardware, creation of a centralized database, training on data processing and analysis (GPS/GIS/remote sensing), identification of priority areas of intervention, formulation of a national strategy for T&T intervention. Assistance is also needed in the fields of fly production and release, and for collection of baseline data in the Lambwe Valley.

Uganda – L. Semakula

In February 2006, the AfDB and the Ugandan Government officially launched the project in South East Uganda. The request for an initial advance of the grant was approved in April 2006, while the loan disbursement had to be revised according to specific categories (the request was officially re-submitted in April 2006). A Contracts Committee to undertake procurement of goods and services has been established and is due to start its work in May 2006. In March 2006, the National PATTEC Coordinator participated in a planning workshop in Kigali, Rwanda, to finalize a regional project proposal on eradication of T&T in the Kagera region of Rwanda and Tanzania.

With respect to capacity building, Ugandan staff participated in a course on baseline data collection organized by IAEA in Nairobi in March 2006; further training activities (i.e. GIS) have also been planned for 2006.

Reports from International Centres and Organizations

Activities of the International Livestock Research Institute (ILRI) under PATTEC Initiative - J. Maitima: Possible contributions of the Institute to the implementation of the PATTEC initiative were presented. ILRI developed significant experience in environmental and socio-economic impact assessments of T&T control strategies, sustainable land management and capacity building, in particular in the fields of RS and GIS. ILRI also

offered its support to national institutes and the PATTEC Office for the development of research activities.

With respect to the environmental and socio-economic impacts assessment, it is important to evaluate pressures on the agroecological systems, their status and response. Cause-effect relationships must be established and appropriate indicators identified with a view to scaling the results obtained up and out. Further information on the development of this work is given later.

In the field of Sustainable Land Management (SLM) a proposal to develop an integrated framework for SLM in PATTEC tsetse freed areas is under discussion with UNEP/GEF (Global Environment Facility). Another proposal investigating the ecological and environmental implications of land use/land cover changes in PATTEC tsetse freed areas is under development for submission to NASA.

SLM should build on approaches similar to those used in some already completed projects like LUCID (Land Use Change Impacts and Dynamics) and TOC (Trajectories of Change). Other approaches could include the survey-based expert opinion and indigenous knowledge system analysis, the spatially based land use/land cover modelling and scenario analysis, the identification and scaling out of best land use, land management practices and the policy analysis.

In the field of training, ILRI plans to hold a GIS training course from 8-26 May 2006.

International Centre of Insect Physiology and Ecology (ICIPE) - R. Saini: An overview was provided on the way in which ICIPE operates and on its potential contribution to the PATTEC initiative.

ICIPE works on four major programme areas: human, animal, plant and environmental health. The core activities of the animal health area aim at increasing livestock productivity by effectively managing tsetse flies and ticks. Ongoing projects are:

- Enhancing the diffusion of new tsetse control technologies for improved livestock health and productivity in smallholder indigenous communities in sub-Saharan Africa (Donor: IFAD; Collaborators: KARI-TRC and ILRI);
- Community based tsetse control in the Mwea National Reserve (Donor: Biovision, Switzerland; Collaborators: Kenya Wild Life Services);
- Tsetse control through adaptive management in Ethiopia (Donor: SDC, Biovision & Helvetas; Collaborators: Regional and National Governments).

As regards potential contributions to PATTEC, ICIPE could provide assistance for ecological studies (dispersal/distribution of vectors), vector suppression, barrier development using baits and repellents (push-pull approach), tsetse mass-rearing, backstopping on R&D activities, socioeconomic studies and capacity building.

The capacity building and institutional development programme of the institute was presented, implemented through the "African Regional Postgraduate Programme in Insect Science" (ARPPIS). Within the ARPPIS programme ICIPE trained over 170 PhD students, over 100 MSc students, 600 veterinary services extension staff and IPM specialists and over 14 000 farmers.

***International Federation for Animal Health (IFAH) – F. van Gool*:** IFAH is an international federation representing manufacturers of veterinary drugs, vaccines and other

animal health products in both developed and developing countries across five continents. The representative of IFAH introduced the mission, priorities and members of the federation.

IFAH corporate members' role in T&T interventions is to guarantee the supply of quality drugs to combat tsetse flies and trypanosomiasis in the most efficient and safest way. IFAH is also committed to providing services such as training of veterinarians, technicians and farmers on diseases, diagnosis, food hygiene and good veterinary practice.

An FAO-IFAH cooperation is currently dealing with the problem of quality control of trypanocidal drugs. A considerable amount of dubious quality and fake trypanocidal and other veterinary drugs are found on the African markets. These compounds are characterised by reduced or lack of efficacy, by toxicity and by unknown/unwanted residues in the food chain and by resistance in pathogens. The FAO-IFAH collaboration also foresees the transfer of generated technology to two analytical laboratories (one in West Africa and one in East Africa) for quality control of veterinary drugs available in Africa. The laboratories will use standardised protocols, methodologies and equipment and will provide continuous training to African technicians. Comparative results between original and non-original drugs were presented.

The IFAH representative concluded his presentation by stressing the urgent need for the African veterinarians and farmers to know exactly the quality of veterinary drugs on the local market. The FAO-IFAH cooperation provides a means to assist African partners in controlling the quality of veterinary drugs locally available and in disseminating the results to all involved stakeholder (regulatory authorities, veterinary services, veterinarians, farmers). This effort should support the African market to provide effective and safe drugs, which in turn will enable livestock owners and marketers to significantly increase animal production and contribute to both food safety and food security.

Reports from National Institutes

T&T Research, Development and Training at CIRAD - S. de la Rocque: An overview was presented of CIRAD (French Agricultural Research Centre for International Development), its activities, human resources and ongoing research programmes, with special emphasis on activities related to the T&T problem.

CIRAD-EMVT has a long experience in T&T research activities. Since 2000, EMTV has joined IRD (Research Institute for Development) to establish a unit working on T&T. The main fields of activity concern vectors and pathogens (population genetics; taxonomy; vector competence; drug resistance), interactions between tsetse/trypanosome/host (epidemiology; trypanotolerance; host specificity; competition; symbionts), risk assessment (diagnosis; risk factors and indicators; prevention; aid for decision making), prevention and control (friendly environmental techniques; trapping protocols; vaccine development).

EMTV is currently involved in a project funded by the Wellcome Trust aiming at understanding the impact of habitat fragmentation on tsetse population dynamics. The project uses environmental and remotely sensed datasets to quantify and qualify the fragmentation of riparian and savannah woodland vegetation. The results are expected to contribute to the development of more effective T&T intervention strategies.

CIRAD-EMVT is also deeply involved in training activities, the most important being the "Certificat d'Études Appliquées Vétérinaires", the DESS in Animal Production in Tropical Countries, the International Training Course in Entomology (Pasteur Institute of Paris), Master of Tropical Diseases (Valencia, Spain), Master of Parasitology (University of

Montpellier II), and the "Master International d'Entomologie médicale et vétérinaire" (Cotonou, Benin).

Animal Trypanosomiasis Research at the Institute of Tropical Medicine - S. Geerts: The T&T research objectives for 2006-2007 of the ITM, which is celebrating its 100th anniversary in 2006, were presented.

Focus is on three main subjects: development and validation of molecular techniques for the detection of drug-resistant trypanosomes, study of the animal reservoir of *T. b. gambiense* and molecular epidemiology and control of trypanosomiasis.

Considering that present tests have a numbers of drawbacks, namely cost and labour intensity, the improvement of drug resistance diagnosis is necessary. The novel technology developed foresees that samples are taken on a filter paper and sent by snail mail to a laboratory; results could be obtained within 2 days. Molecular tools for the detection of drug-resistant trypanosomes are expected to be validated by 2007 and then the technology will be transferred to the affected countries.

A brief overview was also provided on distance learning opportunities at the Universities of Pretoria and Utrecht, in collaboration with ITM. Currently available modules target tropical medicine and animal health.

The difficult financial situation of ITC (International Trypanotolerance Centre, The Gambia), a partner centre of ITM was mentioned. Actions taken to improve the situation included reductions in numbers of staff, animals and field stations. The ITC Council has proposed to merge CIRDES and ITC with a view to creating a stronger livestock research centre in West Africa.

Updates on Specific PAAT Activities

The FAO/PAAT Information System - G.Cecchi: Activities carried out within the framework of the IFAD-funded project "Strengthening the Information System of the Programme Against African Trypanosomiasis (PAAT)" were presented.
The on-line accessibility to PAAT information has been increased. The layout of available tsetse distribution maps has been improved and additional maps have been created and made available, GIS datasets and relevant standard metadata have been published on the PAAT web-site and uploaded in the FAO Geospatial portal (GeoNetwork). The PAAT web site has been fully revised and new pages concerning the disease, vectors, parasite, hosts, remote sensing, land use, environment and donors have been created and incorporated into the web site.

Coordination missions have been made to PAAT Secretariat partners (WHO, IAEA, AU-IBAR), PAAT scientific partners (CIRAD, France; CIRDES, Burkina Faso; ICIPE and ILRI, Kenya; ITM, Belgium) and to the six countries (Burkina Faso, Ethiopia, Ghana, Kenya, Mali, Uganda) implementing Phase 1 of the AfDB-PATTEC initiative. The visits contributed to accelerating the process of harmonization of the respective web-sites and Information Systems and the collection of further GIS datasets. In particular, the visits to the T&T affected countries enabled better assessment of the strengths and weaknesses in national GIS and Information Systems capacities, the main constraint presently being the management of entomological data.

With respect to the collection of baseline datasets for planning and implementing T&T control activities, PAAT-IS proposed the adoption of the FAO/UNEP Land Cover

Classification System (LCCS) as a tool to standardise land cover mapping exercises carried out in the context of the alleviation of the African trypanosomiasis problem. These datasets are essential for planning and monitoring T&T interventions but maps available at national level are not necessarily conceived for the needs of the T&T problem and are often produced using heterogeneous classification systems.

To demonstrate the potential of LCCS, present and future land cover maps compliant with LCCS were illustrated. In particular, one initial attempt to customize the Africover map of Uganda was presented. It is believed that the use of LCCS can foster regional coordination and increase the possibility of using standard land cover products within T&T intervention projects.

Future activities within the IFAD-supported project will include additional coordination visits to PAAT scientific partners (CTVM, Edinburgh University and Glasgow University, UK), the production of a CD-ROM with the updated PAAT web-site and GIS resources and the provision of a tool kit (on-line and on CD-ROM) for training of e-conference moderators.

Issues to be tackled through possible future activities were identified as:

- updating predictive maps of tsetse absence/presence and abundance (e.g. by using entomological datasets collected in a consistent manner by countries benefiting from AfDB financial support);
- producing standard land cover maps for T&T (either through adaptation of existing standard multipurpose datasets or producing new ones);
- supporting human resource development through training on GIS, Remote Sensing and DBMS (Data Base Management Systems);
- backstopping efforts at national level to develop environmental monitoring procedures (land use change, biodiversity, etc.), and guidelines for land use planning and natural resources management (at both national or local levels).

FAO/Pro-Poor Livestock Policy Initiative - T. Robinson: The IGAD (Inter-Governmental Authority on Development) Livestock Policy Initiative (LPI) project and its contribution to the planning of trypanosomiasis interventions in the Horn of Africa were presented. This project aims at strengthening capacity in Member States (Djibouti, Eritrea, Ethiopia, Kenya, Somalia, Sudan, Uganda), regional organizations and other stakeholders to formulate and implement livestock sector and related policies that sustainably reduce food insecurity and poverty.

In the context of trypanosomiasis control, the two important policy issues are where to control and how to control. Activities were presented of a project on the "Spatial Targeting of Trypanosomiasis Control", carried out in Uganda in collaboration with COCTU (Coordinating Office for the Control of Trypanosomiasis in Uganda) and ILRI (jointly funded by the Pro-Poor Livestock Policy Initiative [PPLPI] and IFAD). The methodology selected is based on multi-criteria evaluation and it is aimed at generating priority maps for the control of animal trypanosomiasis in the context of poverty alleviation. In a GIS environment, several layers are combined and factors are weighted to identify priority areas for intervention. The input layers used are livestock density, human population density, crop cover, length of vegetation growing period, density of poor livestock keepers and trypanosomiasis risk index. In the future, more layers could be added to the analysis: sleeping sickness data, modelled poverty data, land cover maps, market accessibility and livestock

production systems. Weights are assigned to indexes through pairwise comparisons. In the workshops held in Uganda, the trypanosomiasis risk index, as compared with other indexes, was identified as the single most important criterion for identifying priority areas for intervention, followed by the index of density of poor livestock keepers.

Future steps will concern the development of a multi-objective approach and include a whole set of other determinants, i.e. district-level importance of trypanosomiasis, sleeping sickness risk, willingness to pay (potential benefits), terrain, livestock-wild host ratios, tsetse species associations, degree of isolation of tsetse populations, re-invasion risk, land planning and environmental issues.

***FAO/IGAD LPI - A. Shaw*:** Results were presented of the work carried out under the PPLPI: "Comparable Costing for Alternative Tsetse Control Options: Examples from Uganda".

The core of the presentation concerned the costing of different vector control strategies for Uganda. Of the many possible approaches to dealing with the vector the following were considered: bait technology with insecticide (in this case with traps), bait technology using insecticide-treated cattle (ITC), aerial spraying using fixed wing aircraft and the sequential aerosol technique (SAT) (5 cycles spraying), the use of the sterile insect technique (SIT) after suppression of the fly population by one of the above means. Due to the uncertainties related to the isolation of tsetse populations in Uganda, like in many other countries, both scenarios, i.e. isolated and non-isolated populations, were studied.

In the case of isolated population, the least expensive option to achieve eradication was estimated to be the ITC technique, with an average US$ 250 per km^2 of tsetse infestation; very close values, around US$ 550 per km^2, were estimated for traps and SAT. If SIT is used, either alone or following suppression with others techniques, costs rise up to an average of US$ 1 100/ km^2.

In the case of non-isolated populations, two options for dealing with reinvasion pressure were evaluated:

- intensification of control measures used for clearing tsetse;
- barriers, based on the less expensive technologies (traps in savannah fly areas, alternatively ITC).

In the first scenario, using ITC and traps, the cost per km^2 increases by 11 percent, with the use of SAT it increases by nearly 40 percent, while for SIT the cost grows dramatically and it can be in the magnitude of 500 percent (more than US$ 5 000/ km^2).

In the second and more realistic scenario, barriers can be used to halt reinvasion. In the study presented, barriers are assumed to be kept in place for 3 years and they contribute between 15 percent and 30 percent to the programme cost. In this scenario, the cost of dealing with non-isolated population seems lower. It should be mentioned that the estimated costs consider:

- barriers to be kept in place for 3 years only;
- barriers assumed to be successful;
- re-invasion pressure hypothesized to be exerted only from one side of the area.

The study provides technical options for the elimination of the T&T at various costs and takes also into account sensitivity analysis for contingencies, logistic and field operations.

Further analysis is needed to refine some prices and adjust operational costs (e.g. costs of the use of aircraft, to which SAT and SIT can be very sensitive) and for sensitivity analysis. In addition, it should be considered that the efficacy of tsetse intervention techniques is affected by habitats, fly species and scale of intervention, which in turn impacts the overall cost.

Linking Sustainable Human and Animal African Trypanosomiasis Control with Rural Development Strategies - P. Cattand: The draft document of this PAAT position paper dealing with a logical framework for integrating AAT and HAT in the global effort as delineated in the Millennium Development Goals (MDG) was presented. The paper also integrates T&T in the general context of improved human health, better livelihoods, reduced poverty and increased food security.

Food security in Africa has substantially worsened since 1970. The proportion of malnourished individuals in sub-Saharan Africa has remained in the range of 33–35 percent, but the absolute number of malnourished people has increased substantially with population growth, from 88 million to over 200 million in 2001. UN MDGs stipulate that development should focus on eight points, each goal to be achieved by 2015. T&T control will contribute to many of these goals, among which are:

- to eradicate extreme poverty and hunger;
- to combat HIV/AIDS, malaria and other diseases;
- to develop global partnership for development (e.g. in cooperation with pharmaceutical companies, provide access to affordable essential drugs in developing countries).

With the support of the International Monetary Fund and the World Bank, T&T affected countries produced national Poverty Reduction Strategy Papers (PRSP). With the exception of a few countries, the T&T problem is not included in these documents; hence it is essential to continue to inform national governments on the impact of T&T on the rural development of sub-Saharan Africa and insert actions against trypanosomiasis as a component of the national strategies for poverty reduction.

Guidelines for Declaring Areas Free of Tsetse Flies and Tsetse-transmitted Trypanosomiasis – a new proposed PAAT Technical and Scientific Series Paper – U. Feldmann: No internationally accepted guidelines exist regarding a sequence of agreed veterinary and entomological screening criteria that need to be met for declaring an area free of tsetse flies and trypanosomiasis.

A draft of a paper which aims at setting such guidelines and criteria was introduced. The approach consists of a series of parasitological, serological and entomological screening activities used in a phased process (*ante*, *intra* and *post* completion of integrated area-wide intervention measures) to assess the absence of flies and parasites and thus declare the area free. The methodology used follows the general principles developed by OIE for declaring an area free of rinderpest.

T&T intervention can be discontinued when the criteria established for declaring the area "provisional T&T free" are met. This is followed by two post-intervention phases of monitoring of different intensity. If during these two post-intervention phases evidence is obtained on the existence of cyclical trypanosome transmission, or if any adult fly of the target population is trapped, a re-initiation of intervention activities will result. If the veterinary and entomological monitoring activities during both post-intervention phases are conducted properly, and result in no evidence for trypanosome transmission and evidence of tsetse absence, a "declaration of freedom from tsetse flies and the trypanosomosis problem" can be made. The paper provides full details on the techniques and probability models to be used in the different phases of the monitoring and surveillance activities.

A spreadsheet was also presented which guides determining how many traps or how many trapping days are needed to reach a given level of confidence in declaring the area free of tsetse.

General discussion

During the round-table discussion, attention concentrated initially on the comparative costs of tsetse control options. The analysis presented is based on a model, which assumes that all control options compared will eventually result in the complete removal of the target tsetse species. The model aims at illustrating how tsetse elimination can be achieved using various techniques implying variations of costs, with SIT as the most expensive one. Both the necessity of the SIT in all circumstances for tsetse elimination, as well as the applicability of such a model for the complex decision making on practical tsetse control options were questioned.

Kenya was congratulated for taking into consideration the previous FITCA experience in the implementation of the new AfDB project. This approach of learning from past experience was recommended to other countries. A matter of concern was the little attention devoted to biotechnology applied to T&T, tsetse biology, diagnosis and environmental issues. It was recommended to include these activities in the AfDB funded projects.

Another neglected aspect was the development of rural communities and with this, the final objective of assisting rural populations through education and development activities.

Concerning HAT, the participants were reminded that control activities are ongoing in some endemic countries (e.g. Guinea and Côte d'Ivoire) and the important progress made since 1999 in developing control programmes was emphasised.

The meeting expressed its concern on epidemiological changes linked to anthropogenic activities in some areas. In the case of the reintroduction of white rhinoceroses into Matusodona Game Reserve in Zimbabwe, all these animals died of trypanosomiasis due to loss of resistance caused by being bred in captivity without contact with tsetse flies. Another example reported was the cotton belt in West Africa where heavy use of insecticides has had the effect in reducing tsetse populations. The use of insecticides is now being discouraged, due to environmental pollution and the introduction of new varieties of cotton more resistant to disease. This may result in an upsurge of tsetse fly populations.

Discussion on Training Needs of Affected Countries

Participants agreed to evaluate the real impact of training: in this respect, feedback on the recently held training courses should be provided by national PATTEC Coordinators.

Participants were reminded that the United States Department of Agriculture (USDA) is willing to provide 6-months training to young field officers on the principles of tsetse mass rearing.

Training in community participation should also be provided to rural communities to stimulate their active participation in field T&T activities.

Additional training should address:

- GIS technology on three different levels:
  - field level, to collect geo-referenced information and insert it into a database;
  - project office level, to summarise data and produce reports
  - higher level, to design databases and complex data analysis.
- data management (processing and analysis);
- planning of T&T intervention;
- technology transfer and introduction and adoption of new improved technology;
- sustainable capacity building/human resources development.

Recommendations

The following recommendations were formulated.

1. On training and human resource development:

- to develop training modules at various levels for personnel and communities involved in T&T field intervention activities.

Action: PATTEC.

- to improve networking and coordination of training activities among the AfDB beneficiary countries.

Action: PATTEC, beneficiary countries, PAAT, research institutes.

2. On the PAAT Information System:

- to continue and further expand the PAAT-IS resources.

Action: PAAT.

3. On investments to model tsetse populations:

- to enhance GIS applications to facilitate planning, decision making and progress assessment.

Action: Donors, PATTEC, beneficiary countries, PAAT, research institutes.

4. Prior planning of interventions:

While acknowledging the existence of baseline datasets in AfDB benefiting countries and the availability of guidelines and strategies for designing T&T field intervention programmes,

prior to the planning and implementation of standardised baseline data collection the following steps are essential:

- a thorough screening of existing information;
- the efficient use of existing guidelines and strategies for designing T&T field intervention programmes in the AfDB beneficiary countries;
- the transfer to digital format of datasets which are still in paper format

Action: PATTEC, beneficiary countries, assisted by PAAT.

5. On overcoming certain weakness in communication flow:

- all partners make an effort to ensure efficient exchange of information amongst one another, making optimal use of existing dissemination pathways, in particular the PAAT-IS.

Action: all PAAT partners and stakeholders, PATTEC.

6. As follow up to its support for the IGAD Livestock Policy Initiative project:

- to include policy issues related to trypanosomiasis control in the Greater Horn of Africa within the IGAD-LPI project activities.

Action: IGAD-LPI.

7. On HAT Control Activities:

As follow up to the 11th Meeting of the Panel Advisory Group Coordinators of PAAT, held in Addis Ababa, 21-22 September 2005 which encouraged WHO to strengthen control activities to consider HAT as a disease candidate to be eliminated, it is essential:

- to ensure the involvement of MoH in PATTEC project elaboration in order to guarantee that HAT control is included in PATTEC operations.

Action: National coordinators of AfDB beneficiary countries.

8. With reference to the recommendation of the 28th ISCTRC conference in Addis Ababa (September 2005):

- All partners and stakeholders of the ISCTRC should provide feedback to the ISCTRC Secretariat on the progress of the implementation of the 28th ISCTRC recommendation as soon as possible.

Action: beneficiary countries, PAAT, PATTEC, FAO, IAEA, WHO, research institutes.

9. Acknowledging the progress made in producing guidelines in the form of position papers:

- to disseminate to an appropriate audience and thus provide feedback to the two draft PAAT position papers ("Linking Sustainable Human and Animal African Trypanosomiasis Control with Rural Development Strategies", "Guidelines for

Declaring Areas Free of Tsetse Flies and Tsetse-transmitted Trypanosomiasis") and to the PPLPI working paper "Comparable Costings of Alternatives for Dealing with Tsetse: Estimates for Uganda".

- in the case of the paper dealing with declaring areas free of T&T, to complement it with a simple guide for field implementation.

Action: beneficiary countries, PAAT, IAEA.

10. With respect to research activities:

- to adapt the programmes of research institutes to service the requirements of the AfDB beneficiary countries.

Action: CIRAD, ICIPE, ILRI, ITM.

11. Recognizing the importance of applied field research in T&T and related subjects:

- to take advantage of AfDB funded projects to identify and carry out demand driven research.

Action: donors, research institutes, beneficiary countries, PAAT, PATTEC.

12. Acknowledging the support of IFAD and UNIDO to PAAT:

- to further strengthen the inter-Agency (PAAT mandated organisations / IFAD /UNIDO) collaboration;
- to advance implementation of the cooperation with IFAH on quality control/quality assurance of trypanocides and other veterinary drugs;
- to extend partnership with the private sector in the field of both human and animal trypanosomiasis.

Action: PAAT, IFAD, UNIDO, IFAH.

Closing

Ms Alice Perlini, DG of IAO, thanked all participants for attending the meeting. She expressed IAO interest in being informed about the follow-up from the PAAT PC meeting, considering the common goal of alleviating poverty, improving socio-economic conditions in the developing world through technical and scientific collaboration. She declared the meeting closed.

The next PAAT-PC meeting will be held at the WHO Headquarters in Geneva in April or May 2007.

31ST ISCTRC EXECUTIVE COMMITTEE MEETING, ADDIS ABABA, 25-26 SEPTEMBER, 2006

Report and Recommendations

1. On Strengthening the ISCTRC:

The Committee noted with appreciation the good work done by the consultants who were tasked by the AU/IBAR to review the ISCTRC and to recommend actions needed to strengthen it. The Committee noted that the report presented by the consultants contained information necessary for revitalising and reshaping the ISCTRC to respond to the demands of the present and of the future. The Committee adopted the report and recommends to the AU:

- To consolidate this achievement through the initiation of a 3-year Action Plan aimed at strengthening the ISCTRC using the Report as a guide;
- To engage major stakeholders both within and without the African Union in the implementation of the 3-year Action Plan.

2. On the Organization of the 29th ISCTRC Conference:

The Committee noted with appreciation the progress made by the National Organising Committee of Angola for the organization of the 29th ISCTRC conference. The Committee however noted that concerns raised by some observers on issues such as communication, costs of accommodation and problems related to obtaining an Angolan visa needed to be taken seriously. The Committee recommends to the AU and to the NOC:

- To jointly identify the major problems likely to hamper the organization of the conference in Angola and to address them as soon as possible;
- The Committee endorsed the dates chosen for the meeting by the NOC: i.e. 17-21 September 2006.

3. On Capacity Building:

The Committee noted with appreciation the increasing commitment and input being made by the WHO/TDR and WHO/NTD to capacity building for greater efficiency in the control of HAT and recommends:

- That WHO sustains the support given to the capacity building initiatives;
- That countries and international organisations:
    - harmonize all T&T capacity building activities;
    - take stock of available expertise within the region that can contribute to this initiative and this information needs to be availed to those in need; and
    - source funding for these activities.

4. On ISCTRC Proceedings:

The Committee noted with appreciation the timely publication of the proceedings of the 28th ISCTRC conference. It however noted that their production and distribution still pose difficulties. The Committee recommends to the AU:

- To consider increasing circulation of proceedings of the ISCTRC meetings in the form of electronic copies;
- To create a website for the ISCTRC to enable easy access to publications and other information emanating from the Secretariat;

- To form a scientific committee that would restructure the conference and offer advice to potential presenters so as to make the conferences more productive. It was proposed that a committee comprising Dr Grace Murilla, Dr Solomon Haile-Mariam, Dr Modibo Troaré, Mr Francis Oloo, Dr Saini Rajinder and Prof. Josenando should be assigned this responsibility.

On PATTEC:

The Committee noted with appreciation the significant progress made by PATTEC in the last year regarding resource mobilisation, awareness creation and greater participation. The Committee, however, noted with concern that expertise needed to carryout efficient field operations was inadequate. The Committee recommends to PATTEC:

- To carry out training needs assessments for participating countries;
- Initiate actions to address manpower deficiencies in participating countries.

On Private Sector Participation:

The Committee noted the increasing demand for tsetse control/eradication in Africa and realised with concern the budgetary constraints faced by most governments to directly support these operations through state budgets. The Committee recommends to member countries:

- To create an enabling environment that would encourage private sector participation in tsetse and trypanosomiasis control to complement Government effort.
- To put mechanisms in place to monitor and regulate the activities of the private sector to ensure sustainability.
- To take measures to ensure effectiveness, ownership and sustainability of T&T control/eradication.

BOOK PUBLICATIONS

Disease Control Priorities in Developing Countries, 2nd Edition

Editors: Jamison, D.T., Bremen, J.G., Measham, A.R., Alleyne. G., Claeson, M., Evans, D.B., Jha, P., Mills, A. & Musgrove, P., 2006. The International Bank for Reconstruction and Development, The World Bank and Oxford University Press. ISBN: 10 0-8213-0821361791; eISBN: 0-8213-6180-5.

Six hundred public health and policy experts from more than 100 countries contributed to the data sources and methodologies and identified challenges and priorities, resulting in an integrated, comprehensive reference volume of 1450 pages on the state of health in developing countries. Based on careful analysis of health systems and the costs of the burden of disease, the 73 chapters of *DCP2* highlight achievable priorities; measure progress toward providing efficient, equitable care; promote cost-effective interventions to targeted populations; and encourage integrated efforts to optimize health.

The first part of the book provides a summary of cross-cutting themes and includes such topics as: priorities for global research and development of interventions, science and technology for disease control, product development priorities, cost-effectiveness of interventions, and lessons from experience in improving the health of populations. In its subsequent chapters, the book deals in detail with subjects like fiscal policies and financing health systems in the 21st century, ethical issues, cost-effectiveness analysis for priority setting etc., and then moves on to the issue of selecting interventions covering in some detail the principal diseases affecting humans in developing countries ranging from cancer, diabetes and mental health through to malaria, leprosy and vaccine preventable diseases.

Of particular interest to readers of TTI is chapter 23 written by Cattand *et al.,* dealing with Tropical Diseases Lacking Adequate Control Measures: Dengue, Leishmaniasis and African Trypanosomiasis (see Abstract 13605 of this issue).

The book then goes on to deal with risk factors such as water and sanitation, alcohol, tobacco addiction and smoking, and finally over the course of some 20 chapters deals with the need/requirements for strengthening health systems.

In the Editor's view, while there is relatively little direct coverage given to tsetse and trypanosomiasis, this book (cost $125 but available at a discount of 75 percent to most African countries) is a "must read" for anyone involved in the subject at policy, institutional or science and technology levels and whether they be interested in the medical or animal health dimensions of the problem since it contains such a wealth of information and knowledge on so many aspects of disease control in developing countries.

Global Burden of Disease and Risk Factors

Editors: Jamison, D.T., Bremen, J.G., Measham, A.R., Alleyne. G., Claeson, M., Evans, D.B., Jha, P., Mills, A. & Musgrove, P., 2006.. The International Bank for Reconstruction and Development, The World Bank and Oxford University Press. ISBN: 0-8213-6262-3.

This book (6 chapters and 552 pages; price $65) emerges from two separate, but intersecting, strands of work that began in the late 1980s, when the World Bank initiated a review of priorities for the control of specific diseases. The review generated findings about the comparative cost-effectiveness of interventions for most diseases important in developing countries. The purpose of the cost-effectiveness analysis (CEA) was to inform decision making within the health sectors of highly resource-constrained countries. This process resulted in the publication of the first edition of *Disease Control Priorities in Developing Countries (DCP1).*

Also important for informing policy is a consistent, quantitative assessment of the relative magnitudes of diseases, injuries, and their risk factors. *DCP1* included an initial assessment of health status for low- and middle-income countries as measured by deaths from specific causes; importantly, the numbers of cause-specific deaths for each age-sex group were constrained by the total number of deaths as estimated by demographers. This consistency constraint led to downward revision of the estimates of deaths from many diseases. These two strands of work-CEA and burden of disease-were further developed during preparation of the *World Development Report 1993: Investing in Health*. This report drew on both the CEA work in DCP1 and on a growing academic literature on CEA. In addition, the World Bank invested in generating improved estimates of deaths and the disease burden by age, cause, and region for 1990. Over the past six years, the World Health

Organization has undertaken a new assessment of the global burden of disease for 2000-2. The World Health Organization has also invested in improving the conceptual, methodological, and empirical basis of burden of disease assessments and the assessment of the disease and injury burden from major risk factors.

During 1999-2004, the authors of this volume and many collaborators from around the world worked intensively to assemble an updated, comprehensive assessment of the global burden of disease and its causes. The *Global Burden of Disease and Risk Factors* is the definitive, scientific account of these efforts and of the health conditions of the world's population at the beginning of the 21st century. This book includes a full account of methods, the complete results of recent work, and an assessment of trends for total mortality and for major causes of death among children under five. In addition, two chapters cover sensitivity and uncertainty analyses in relation to a broad range of potentially important parameters.

Priorities in Health

Editors: Jamison, D.T., Bremen, J.G., Measham, A.R., Alleyne. G., Claeson, M., Evans, D.B., Jha, P., Mills, A. & Musgrove, P., 2006. The International Bank for Reconstruction and Development, The World Bank and Oxford University Press. ISBN: 0-8213-6260-7.

This 212–page book (the third in the DCP Series and costing $10) begins by dealing with accomplishments, challenges and priorities and then through a series of three chapters deals with cost-effective analysis and strategies for reducing disease burdens. Following chapters on providing interventions and pillars of health systems, it then provides a blueprint for action.

The book as a whole demonstrates that delivering efficacious and inexpensive health interventions leads to dramatic reductions in mortality and disability at modest cost. Globalization has been diffusing the knowledge about what these interventions are and how to deliver them. The pace of this diffusion into a country–more than its level of income–determines the tempo of health improvement in that country.

Also, two overarching themes emerge from the extensive research and analyses: (a) current resources can yield substantial health gains if knowledge of cost-effective interventions were applied more fully; and (b) additional resources are needed in low-income countries to minimize the glaring inequities in health care. Such resources would provide highly-effective interventions, expand research, and extend basic health coverage to more people.

Proceedings of an International Conference on Livestock Agriculture in West and Central Africa

The Proceedings of this 4-day Conference which was held in November 2004 in Banjul; The Gambia, were recently published in electronic (pdf) format. The Conference was jointly organized by the two sub-regional livestock research Centres, the International Trypanotolerance Centre (ITC) in The Gambia and the Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES) in Burkina Faso, in partnership with the Technical Centre for Agricultural and Rural Cooperation (CTA), Wageningen, The Netherlands, and with support from the European Union. It was attended by over 110 participants from 13 West and Central African countries (Benin, Burkina Faso, Cameroon,

Chad, Gambia, Ghana, Guinea, Guinea Bissau, Mali, Niger, Senegal, Sierra Leone, Togo), four European countries (Belgium, France, Germany, UK), from regional and international institutions (CIRDES, CTA, IFPRI, ILRI, ITC) and organisations (CORAF/WECARD, ECOWAS, EU, FAO, FARA, IDRC, UEMOA, UNDP).

The programme was structured in a way that combined the broader past, present and future issues of livestock agriculture with the specific research and development aspects and lessons learnt from the EU-funded regional '*Programme Concerté de Recherche-Développement sur l'Elevage en Afrique de l'Ouest*' (PROCORDEL), as a possible model for future livestock-based R&D for the region.

The main objective was to bring a re-focus to Livestock Agriculture in Sub-Saharan Africa with emphasis on West and Central Africa (WCA) in the context of overall developments in the region. Its specific objectives and intended outputs included:

- Sharing experiences, knowledge and information accrued from various external donor-supported projects and national government efforts in the past 25 years;
- Review and assess the achievements of the latest EU-funded regional Project PROCORDEL executed in 13 countries in West Africa, as a model for future livestock-based R&D for the region;
- Identify new, and strengthen existing communication links among the donor community, other stakeholders and producers, processors and marketers in the region as a means of improving policy dialogue, planning and resource mobilization;
- Identify communication and information tools and resources that will ensure sustainability in dialogue among various actors / stakeholders involved in the development of livestock-based agriculture;
- Concrete policy and biological research and development guidelines as a basis for articulating long term R&D strategies that address the priority needs of stakeholders of the region.

The Conference also provided an overview on the background and achievements of PROCORDEL in the sub-region, with the possible avenues for out and up scaling the results of the regional project, namely on:

(a) Development of the dairy sector; (b) zoonoses, food safety and public health aspects of livestock production; (c) advances in diagnostics and epidemiological studies; (d) livestock breeds, breeding practices and producer preferences - including the important place of trypanotolerant ruminant livestock; (e) effects of policy reforms and performances of livestock; (f) natural resources management and intensification of agriculture; and (g) communication, training and regional dialogue.

The 150-page document can be accessed and downloaded from the websites of CIRDES, CTA and ITC (e.g.: www.itc.gm/Downloads/BanjulIntConfLivestockWCA.pdf).

DATA RESOURCES FOR TSETSE RESEARCHERS

Considerable raw data are freely available to researchers interested in the performance of fabrics and traps for tsetse and biting flies at http://www.nzitrap.com. Through the link "Resources for Researchers" on the home page, public access has been provided to several major data sets in Excel and/or ACCESS. Although there is a minimum of annotation, several guides to the raw information are provided in WORD documents, e.g. a guide to suitable fabrics with relevant comparisons, a quality assurance guide to standard phthalogen blue fabrics, and a guide to the data collected to date in standardized trials of Nzi traps. A bibliography of over 4 000 references has also been provided in both ENDNOTE and text formats. Of particular interest is the large database of raw reflectance and transmission spectra for fabrics, netting and other materials in EXCEL. Steve Mihok, 388 Church Street, Russell, Ontario, Canada K4R 1A8, smihok@rogers.com .

RECENT RESEARCH AND GUIDELINES DEVELOPED BY ILRI AND PARTNERS

Irungu, P. *, Bett, B., Mbogoh, S.G., Nyamwaro, S.O. and Randolph, T.F. (2006). Utilizing conjoint analysis to evaluate farmers' preference for tsetse repellent attributes in Kenya: An ordered probit application. Published in Proceedings of the 10th KARI Biennial Conference held at the KARI Headquarters, 13-17th November 2006.

Ninety-four pastoral cattle keepers were interviewed in Kajiado and Narok Districts of Kenya between December 2005 and February 2006. The objective of the interview was to evaluate the cattle keepers' preference for the attributes of a new tsetse fly repellent that is in its final stages of development at the International Centre for Insect Physiology and Ecology (ICIPE). This study used the conjoint analysis technique to elicit farmer preferences. An ordered probit model was applied to the data to estimate the choice probabilities. Results indicate that farmers preferred long-acting repellent collars and were willing to pay more relative to improved ease of use. Farmer preferences for repellent attributes were influenced by education level, age and wealth status of the household heads and the District in which they were located. Implications of the results for commercial developers seeking to mass-produce and disseminate the new repellent technology are discussed.

Irungu P.*, Bett, B., Mbogoh, S.G., Nyamwaro, S.O., Murilla, G.A. and Randolph, T.F. (2006). Evidence of extra-label usage of veterinary drugs in cattle in Maasailand, Kenya. Published in Proceedings of the 5th Faculty of Veterinary Medicine Biennial Conference held at the College of Agriculture and Veterinary Sciences, University of Nairobi, 6-8th September 2006.

The extent of farm-level extra-label drug use in Kenya is not well documented in spite of its important implications for food safety, human health and international trade. One hundred thirteen farmers in Kajiado and Narok Districts were interviewed between October 2005 and February 2006 using a pre-tested questionnaire. The aim was to gather information on farmer veterinary drug use practices at the farm level. Descriptive and regression analyses were performed. There was a high level of extra-label usage of veterinary drugs in cattle in the two study areas. Specifically, farmers used the wrong drugs to treat some cattle diseases. They

also used lower than recommended doses of all available trypanocides in all classes of cattle except in adult bulls, which they overdosed with Veriben®, Novidium® and Tryzan®. Adamycin®, the most commonly used antibiotic in the two study sites, was under dosed at all concentrations in all classes of cattle. Except for Novidium® which farmers dissolved correctly, farmers in both study sites used less than the recommended volume of water to prepare trypanocides. Farmers also used less than the recommended strength of acaricides for tick control, except for Dominex®. They also sprayed more cattle at each acaricide strength than the number recommended by the manufacturers. The propensity to use veterinary drugs correctly was positively correlated to farmer's age and District of origin ($p<0.1$), but negatively associated with years of formal education of the household head ($p<0.05$). Policy suggestions are made based on the results.

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Bett B. *, Irungu P., Nyamwaro S., Murilla G., Olet P., Kitala P., Gathuma J., Randolph T.F., & McDermott, J. 2006. Evaluating the effectiveness of a synthetic tsetse repellent technology developed for tsetse and trypanosomosis control in Kenya. Published in Proceedings of the 5th Faculty of Veterinary Medicine Biennial Conference held at the College of Agriculture and Veterinary Sciences, University of Nairobi, 6-8th September 2006. [Paper to be published in Bulletin of Animal Health and Production in Africa].

A field trial was carried out in purposefully selected pastoral areas in Narok and Kajiado districts, Kenya to evaluate the effectiveness of a synthetic tsetse-repellent technology developed for tsetse and trypanosomosis control. The technology is made up of a repellent (guiaicol + carbon) emitted from dispensers attached to a collar worn around the necks of cattle. The technology is applied to all animals in a herd to ensure protection, possibly in conjunction with traps/targets in a strategy referred to as "push-pull" tactic.

The trial used a total of 24 randomly selected pastoral cattle herds. The sample size was estimated assuming an error of 5 percent and intra-herd correlation coefficient of 0.07 and that the repellent technology, if effective, would reduce the incidence of trypanosomosis in treated herds by at least 50 percent. The study was conducted over a period of 12 months preceded by a baseline period of 4 months. All the animals in the recruited herds were screened for trypanosomosis on a monthly basis using the buffy coat technique. Tsetse challenge (measured at the village-level), grazing patterns, status of the repellent dispenser and the amount of trypanocides used per herd by the stock owners were monitored as well. Trypanosomosis incidence was the main outcome indicator of effectiveness.

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Environment and Socio-economic Impacts Assessment of T&T Interventions

Environmental and socio-economic impact assessment on T&T interventions has in the past been conducted on a project by project basis in many countries without a general framework and methodological guidelines for doing so. The methods used to assess have therefore varied both in approaches and methods such that there are inconsistencies in the content of assessment reports. Such results are not comparable between project areas and countries even though the issues being assessed are the same. The PATTEC project being continental wide and applying area-wide approaches across national boundaries, tsetse belts and ecological

zones and being funded centrally will more than before require standardized approaches and methodologies.

The existence of a general framework and a methodological guide would not only standardize the approaches and methods but also inform both the decision makers and project implementers on areas and issues to consider in targeting assessments of the benefits achieved through the expected T&T interventions.

The need to have such a framework and guidelines was made clear in a needs assessment workshop held in ILRI in February 2005 and attended by AU-PATTEC Coordinator, AU-IBAR, United States State Department, Coordinators of country PATTEC projects in Burkina Faso, Ethiopia, Ghana, Kenya, Uganda and Mali as well as the stakeholders involved with research on trypanosomosis control. It was considered necessary to identify these needs from the project practitioners themselves so that the framework and guidelines developed can be articulate and practical. Following this meeting, the following needs were identified:

- A standard methodological guide for environmental and socio-economic impacts assessments that is applicable in the varied ecological and social backgrounds;
- Tools to analyze and demonstrate socio-economic impacts and outcomes of tsetse control interventions that will produce results acceptable to all T&T stakeholders;
- A framework for identifying the environmental and socio-economic changes to serve as an early warning system for short term and long term impacts.

Key observations made by stakeholders in this consultative meeting included:

- That the problems reported by different countries are general enough to be replicated.
- That the indicators (both social and environmental) should look at the tradeoffs in welfare, natural resources, socioeconomics, and livelihoods.

Decisions made at the meeting:

- To synthesize methodologies used in the assessment of environmental and social impacts of tsetse control.
- In consultation with subject specialists develop indicators of environmental, social and economic changes in tsetse eradication areas.

Following this consultative meeting and receipt of financial support from the United States State Department, ILRI completed the development of the framework and the methodological guide for T&T interventions. Initially the guidelines were targeted for the first phase of PATTEC project countries which include: Burkina Faso, Ethiopia, Ghana, Kenya, Uganda, and Mali. However, they can be used in any other tsetse control/ eradication area in sub Saharan Africa.

Drafts of these two documents were discussed at a stakeholders meeting held in ILRI in November 2006. The purpose of the meeting was to share the drafts with the stakeholders to make sure that the contents met their expectations and will be useful to them. The meeting made some very useful suggestions that will make the documents more applicable in impact assessments.

It is anticipated that the final framework and the guidelines will be ready for use by countries by February 2007.

SECTION B – ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

13601. **Bernardi, M., Gommes, R. & Grieser, J., 2006.** Downscaling climate information for local disease mapping. *Parassitologia*, **48** (1-2): 69-72.

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The study of the impacts of climate on human health requires the interdisciplinary efforts of health professionals, climatologists, biologists, and social scientists to analyze the relationships among physical, biological, ecological, and social systems. As the disease dynamics respond to variations in regional and local climate, climate variability affects every region of the world and the diseases are not necessarily limited to specific regions, so that vectors may become endemic in other regions. Climate data at local level are thus essential to evaluate the dynamics of vector-borne disease through health-climate models and most of the times the climatological databases are not adequate. Climate data at high spatial resolution can be derived by statistical downscaling using historical observations but the method is limited by the availability of historical data at local level. Since the 1990s, the statistical interpolation of climate data has been an important priority of the Agrometeorology Group of the Food and Agriculture Organization of the United Nations (FAO), as they are required for agricultural planning and operational activities at the local level. Since 1995, date of the first FAO spatial interpolation software for climate data, more advanced applications have been developed such as SEDI (Satellite Enhanced Data Interpolation) for the downscaling of climate data, LOCCLIM (Local Climate Estimator) and the NEW_LOCCLIM in collaboration with the Deutscher Wetterdienst (German Weather Service) to estimate climatic conditions at locations for which no observations are available. In parallel, an important effort has been made to improve the FAO climate database including at present more than 30,000 stations worldwide and expanding the database from developing countries coverage to global coverage.

13602. **Bowman, D.D., 2006**. Successful and currently ongoing parasite eradication programmes. *Veterinary Parasitology*, **139** (4): 293-307.

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The eradication of parasitic diseases is not a new concept. The most successful programmes of parasite eradication have occurred with species of veterinary importance. The first such program, the eradication of Texas Cattle Fever from the United States, is one of the

great success stories of disease eradication. The American screwworm eradication programme is ongoing and is serving as a guiding impetus for many of the ongoing or proposed vector eradication schemes around the world. The success of these programmes prompted similar successful operations in human health. Although they once led the way, veterinary parasitologists have taken second place in eradication planning. The only three parasitic diseases of veterinary importance that have been targets of recent eradication programs are Hypoderma species in Great Britain and Europe, *Cochliomyia hominivorax* after its introduction into Libya from the Americas, and *Echinococcus granulosus* in Tasmania, Australia. There is also work on the eradication of the tick, Amblyomma variegatum, from the Caribbean Islands. Some animal diseases are targeted under the auspices of the human eradication programs, most notably the eradication of the tsetse fly from parts or all of Africa. This paper reviews some of the past or ongoing successful eradication programs and presents a brief summary of the history of the programmes, the methods used or planned, and potential controversies surrounding their success and implementation.

13603. **Brun, R. & Balmer, O., 2006.** New developments in human African trypanosomiasis. *Current Opinion in Infectious Diseases*, **19** (5): 415-420.

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This paper reviews recent literature on human African trypanosomiasis, focussing on genome sequencing, diagnosis and drug discovery, and typing of trypanosomes. The most important recent development has been the completion of the *Trypanosoma brucei* genome which will greatly facilitate the discovery of new drug targets and genetic markers. Correct staging of the disease is of key importance for treatment. The analysis of sleep patterns is a promising new method to this end and has advanced enough to begin thorough clinical trials. In terms of novel drug candidates, dicationic molecules show the most promise with one oral diamidine in phase 3 clinical trials. New targets and classes of molecules which show *in vitro* trypanocidal activity are also described. Two new methods - MGE-PCR and microsatellites - allow analyses without parasite cultivation, eliminating a major impediment to efficient sampling for population studies. The finding that several wild animal species harbour *T. b. gambiense*, and that parasite transmission is efficient even from very low parasitaemias, sheds a new light on the importance of animal reservoirs. The use of *T. brucei* as a model system for molecular and cell biology is regularly producing new technologies exploitable for diagnosis and new drugs. Drug discovery and development have experienced a revival through new public-private partnerships and initiatives. The challenge remains to translate this progress into improvements for affected people in disease endemic areas.

13604. **Brunet, B., La Ruche, G. & Gastellu-Etchegorry, M., 2006.** Evaluation of the pertinence of international courses on human African trypanosomiasis. *SantéPublique*, **18** (2): 323-332.

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The goal of this study was to evaluate the adequacy and relevance of a training course on Human African Trypanosomiasis, targeted to reach support and coordination staff in charge of activities being carried out in related prevention and control programmes. A questionnaire was e-mailed to the four course organizers and the 65 participants. The response rate among the participants was 41 percent. The training needs expressed covered issues such as treatment, diagnostic and epidemiological techniques, improved knowledge of the disease, and control planning. The lectures given were adapted for participants' professional activities. At the time of the evaluation (one to three years after the course) 67 percent of the participants had begun implementing the knowledge they had acquired and applying it to their practice, particularly in the area of programme planning. The analysis of the questionnaire's results pointed to the sections of the course that would benefit from modifications, such as the need for the development of lessons and modules in the areas of patient management and planning for future training sessions.

13605. **Cattand, P., Desjeux, P., Guzmán, M.G.,Jannin, J., Kroeger, A., Medici, A., Musgrove, P., Nathan, M.B., Shaw, A. & Schofield C.J., 2006.** Tropical diseases lacking adequate control measures: dengue, leishmaniasis, and African trypanosomiasis. In: Jamison, D.T., Bremen, J.G., Measham, A.R., Alleyne. G., Claeson, M., Evans, D.B., Jha, P., Mills, A. & Musgrove, P., (eds.,), *Disease Control Priorities in Developing Countries, 2nd Edition*. International Bank for Reconstruction and Development, The World Bank and Oxford University Press. pp.451-466.

For dengue, leishmaniasis, and African trypanosomiasis, the longstanding problem is the lack of adequate specific treatment. For dengue, no specific treatment is available. For the leishmaniases and African trypanosomiases, specific treatment has long depended on antiquated drugs that would be considered far too toxic for introduction under modern registration systems. Even though progress is being made, especially in relation to the development of new oral drugs for leishmaniasis, in purely pragmatic terms what is currently available will probably represent almost the entire therapeutic arsenal for the coming decades. Even without toxicological problems, the development and registration of a new candidate drug will, given current requirements, take at least a decade. Although basic research will continue, the current challenge is to make better use of what is already available. Dengue can be prevented with available vector control tools and strategies designed to reduce the risk of transmission. This method requires a sustainable surveillance system capable of providing early warning and predictions based on experience of factors predisposing to new epidemic outbreaks. To a large extent, it becomes a management exercise that accepts that some dengue transmission will occur but aims at pre-empting epidemic outbreaks rather than instigating emergency measures after an outbreak is in full crudescence. Moreover, because pre-emptive measures and emergency responses are competing strategies, analyses of their relative cost-effectiveness would be appropriate. Case finding and treatment for the leishmaniases and African trypanosomiases depend on the effectiveness of the diagnostic and treatment packages. Such packages are available, and research is required into the most cost-effective means for large-scale implementation. Again, the management exercise is to accept that some transmission will occur but to be aware that cases can be found and treated with minimal losses to healthy life. As with dengue, predictive surveillance will help focus attention on those areas where outbreaks seem most likely, and

rapid, accurate diagnostics are crucial both to avoid the waste and danger of mistreatment and to minimize delays in administering the specific treatment required. But should such approaches rely on health centres, on mobile teams, or on some combination of the two? To what degree can the specialist diagnosis and treatment teams be integrated into more general approaches to health care? And, most crucially, how is the epidemiological surveillance to be organized: disease and vector notification, geographic information system mapping, analysis, and prediction? For the leishmaniases, vector control seems unlikely to become a major component of disease control except where sandfly distribution overlaps with that of other vectors or where use of personal protection measures can be more widely encouraged. For dengue, vector control is a major component, but unless *Aedes* eradication appears again on the agenda, predicting the levels of control required in specific situations will require much greater understanding of transmission dynamics. Significant resources have been wasted on emergency dengue vector control, which has subsequently been seen to have had little more than a palliative effect, whereas sustained suppression of vector populations may require changes in urban water management and in human behaviour that exceed the usual remit of health specialists. For African trypanosomiasis, however, the prospects for sustainable vector control are more promising. The vector's low reproductive rate, combined with its extreme sensitivity to ultra-low doses of biodegradable insecticides, put tsetse flies among the most promising candidates for large-scale elimination. Campaigns against tsetse flies during the past century were invariably successful until they were discontinued and the controlled areas became reinvaded. Thus, the operational issue is to design large-scale international programs that can successively eliminate tsetse populations and prevent reinvasion of controlled areas, as contemplated by the African Union's Pan African initiative. In essence, all three diseases face parallel needs involving some marginal improvements to existing control techniques, but, most important, they require a management exercise that acknowledges the long-term need for surveillance, adequate reporting, case finding, and treatment. The primary challenges seem to reside less in the domain of new tools and more in the deployment of what is already available.

13606. **Coleman, M., Sharp, B., Seocharan, I. & Hemingway, J., 2006.** Developing an evidence-based decision support system for rational insecticide choice in the control of African malaria vectors. *Journal of Medical Entomology*, **43** (4): 663-668.

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The emergence of *Anopheles* species resistant to insecticides widely used in vector control have the potential to impact directly on the control of malaria. This may have a particularly dramatic effect in Africa, where pyrethroids impregnated onto bed-nets are the dominant insecticides used for vector control. Because the same insecticides are used for crop pests, the extensive use and misuse of insecticides for agriculture has contributed to the resistance problem in some vectors. The potential for resistance to develop in African vectors has been apparent since the 1950s, but the scale of the problem has been poorly documented. A geographical information system-based decision support system for malaria control has recently been established in Africa and used operationally in Mozambique. The system incorporates climate data and disease transmission rates, but to date it has not incorporated

spatial or temporal data on vector abundance or insecticide resistance. As a first step in incorporating this information, available published data on insecticide resistance in Africa has now been collated and incorporated into this decision support system. Data also are incorporated onto the openly available Mapping Malaria Risk in Africa (MARA) Web site (http://www.mara.org.za). New data, from a range of vector population-monitoring initiatives, can now be incorporated into this open access database to allow a spatial understanding of resistance distribution and its potential impact on disease transmission to benefit vector control programs.

13607. **Dafa'alla, T.H., Condon, G.C., Condon, K.C., Phillips, C.E., Morrison, N.I., Jin, L., Epton, M.J., Fu, G. & Alphey, L., 2006.** Transposon-free insertions for insect genetic engineering. *Nature Biotechnology*, **24** (7): 820-821.

Oxitec Limited, 71 Milton Park, Oxford OX14 4RX, UK.

Methods involving the release of transgenic insects in the field hold great promise for controlling vector-borne diseases and agricultural pests. Insect transformation depends on nonautonomous transposable elements as gene vectors. The resulting insertions are stable in the absence of suitable transposase, however, such absence cannot always be guaranteed. We describe a method for post-integration elimination of all transposon sequences in the pest insect Medfly, *Ceratitis capitata.* The resulting insertions lack transposon sequences and are therefore impervious to transposase activity.

13608. **Faulde, M., 2006**. Emergence of vector-borne diseases during war and conflict. *Deutsche Gesellschaft fur allgemeine und angewandte Entomologie,* **15**: 327-335.

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Throughout history, the deadly comrades of war and disease have accounted for a major proportion of human suffering and death. During conflict, human populations are often suddenly displaced, associated with crude mortality rates over 60 times higher than baseline rates. Risk factors like mass population movements, overcrowding, no access to clean water, poor sanitation, lack of shelter, and poor nutritional status directly result in the rapid increase of infectious diseases, especially measles, respiratory tract infections, diarrhoea and vector-borne diseases. In 26 out of 52 retrospectively analysed wars from 480 B.C. to 2002 A.D., vector-borne diseases like plague, louse-borne typhus, malaria, yellow fever, relapsing fever, scrub typhus and visceral leishmaniasis prevailed, or essentially contributed to, overall mortality. During the last decades, devastating war-related outbreaks of malaria, louse-borne typhus, trench fever, African sleeping sickness, visceral and cutaneous leishmaniasis, and dengue fever have been reported. According to the humanitarian imperative to protect or re-establish the health of the affected population, essential medical entomological expertise has been involved increasingly in complex emergencies in order to analyse the transmission modes and epidemiological impact. Adequate countermeasures, such as personal protection against arthropod vectors and vector control efforts, have to be initiated and implemented subsequently, aiming at rapid and efficient interruption of transmission cycles. Recent

experiences made during emergency situations reveal that more medical entomological expertise and involvement is necessary worldwide to successfully react to future disease threats.

13609. **Fevre, E.M., Picozzi, K., Jannin, J., Welburn, S.C. & Maudlin, I., 2006.** Human African trypanosomiasis: epidemiology and control. *Advances in Parasitology*, **61**: 167-221.

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Human African trypanosomiasis (HAT), or sleeping sickness, describes not one but two discrete diseases: that caused by *Trypanosoma brucei rhodesiense* and that caused by *T. b. gambiense*. The Gambian form is currently a major public health problem over vast areas of central and western Africa, while the zoonotic, Rhodesian form continues to present a serious health risk in eastern and southern Africa. The two parasites cause distinct clinical manifestations, and there are significant differences in the epidemiology of the diseases caused. We discuss the differences between the diseases caused by the two parasites, with an emphasis on disease burden, reservoir hosts, transmission, diagnosis, treatment and control. We analyse how these differences impacted on historical disease control trends and how they can inform contemporary treatment and control options. We consider the optimal ways in which to devise HAT control policies in light of the differing biology and epidemiology of the parasites, and emphasise, in particular, the wider aspects of control policy, outlining the responsibilities of individuals, governments and international organisations in control programmes.

13610. **Hemingway, J., Beaty, B.J., Rowland, M., Scott, T.W. & Sharp, B.L., 2006**. The Innovative Vector Control Consortium: improved control of mosquito-borne diseases. *Trends in Parasitology,* **22** (7): 308-312.

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Few new insecticides have been produced for control of disease vectors for public health in developing countries over the past three decades, owing to market constraints, and the available insecticides are often poorly deployed. The Innovative Vector Control Consortium will address these market failures by developing a portfolio of chemical and technological tools that will be directly and immediately accessible to populations in the developing world. The Bill and Melinda Gates Foundation has supported this new initiative to enable industry and academia to change the vector control paradigm for malaria and dengue and to ensure that vector control, alongside drugs, case management and vaccines, can be better used to reduce disease.

13611. **Kissinger, J.C., 2006**. A tale of three genomes: the kinetoplastids have arrived. *Trends in Parasitology,* **22** (6): 240-243.

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July 2005 marked a milestone in kinetoplastid biology research. A tour de force effort led by the Tri-Trypanosomatidae "Tritryp" genome consortium yielded the publication of three prominent kinetoplastid parasite genome sequences: *Trypanosoma brucei, Trypanosoma cruzi* and *Leishmania major*. The individual and combined comparative analyses of these three genome sequences, combined with proteomic analyses, have yielded insights into topics ranging from genome evolution and horizontal gene transfer to potential new therapeutic and vaccine targets.

13612. **Malone, J.B., Nieto, P. & Tadesse, A., 2006.** Biology-based mapping of vector-borne parasites by geographic information systems and remote sensing. *Parassitologia*, **48** (1-2): 77-79.

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Application of growing degree day-water budget analysis and satellite climatology to vector-borne parasites is reviewed to demonstrate the value of using the unique thermal-hydrological preferences and limits of tolerance of individual parasite-vector systems to define the environmental niche of disease agents in the landscape by modern geospatial analysis methods. Applications of geospatial modelling are illustrated by examples on fascioliasis, malaria, leprosy and leishmaniasis.

13613. **Mejia, J.S., Bishop, J.V. & Titus, R.G., 2006**. Is it possible to develop pan-arthropod vaccines? *Trends in Parasitology*, **22** (8): 367-370.

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Hematophagous arthropods that transmit the etiological agents of arthropod-borne diseases have become the focus of anti-vector vaccines, targeted mainly at components of their saliva and midgut. These efforts have been directed mostly towards developing species-specific vaccines. An alternative is to target cross-reactive epitopes that have been preserved during evolution of the arthropods. The N- and O-linked glycans that are attached to arthropod glycoproteins are one of the potential targets of this pan-arthropod vaccine approach. Here, we discuss how genetically modified *Drosophila melanogaster* cells can be used to synthesize and to deliver these arthropod glycans to vertebrate hosts.

13614. **Mihok, S., Carlson, D.A., Krafsur, E.S. & Foil, L.D., 2006**. Performance of the Nzi and other traps for biting flies in North America. *Bulletin of Entomological Research*, **96** (4): 387-397.

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The performance of Nzi traps for tabanids (*Tabanus similis* Macquart, *T. quinquevittatus* Wiedemann, *Chrysops aberrans* Philip, *C. univittatus* Macquart, *C. cincticornis* Walker, *Hybomitra lasiophthalma* (Macquart)), stable flies (*Stomoxys calcitrans* Linnaeus) (Diptera: *Muscidae*) and mosquitoes (*Aedes)* (Diptera: *Culicidae*) was investigated at various sites in Canada (Ontario, Alberta) and USA (Iowa, Florida, Louisiana). Traps made from selected fabrics, insect nettings and hand-dyed blue cotton were compared to the African design to provide practical recommendations for temperate environments. Comparisons of substituted materials showed that trap performance was optimal only when traps were made from appropriate fabrics in the colours produced by either copper phthalocyanine (phthalogen blue), or its sulphonated forms (turquoise). Fabrics dyed with other blue chromophores were not as effective (anthraquinone, disazo, formazan, indanthrone, triphenodioxazine). An appropriate texture as well as an appropriate colour was critical for optimal performance. Smooth, shiny synthetic fabrics (polyester, nylon) and polyester blends reduced catches. Low catches occurred even for mildly phthalogen blue, but slightly-shiny, polyester fabrics in widespread use for tsetse. The most suitable retail fabric in place of phthalogen blue cotton was Sunbrella Pacific Blue acrylic awning/marine fabric. It was both attractive and durable, and had a matching almost black colour.. Nzi traps caught grossly similar numbers of biting flies as canopy, Vavoua, and Alsynite cylinder traps, but with differences in relative performance among species or locations.

13615. **Nwaka, S. & Hudson, A., 2006**. Innovative lead discovery strategies for tropical diseases. *Nature Reviews Drug Discovery*, **5** (11): 941-955.

Special Programme for Research and Training in Tropical Diseases (TDR), World Health Organization, Geneva, Switzerland.

Lead discovery is currently a key bottleneck in the pipeline for much-needed novel drugs for tropical diseases such as malaria, tuberculosis, African sleeping sickness, leishmaniasis and Chagas disease. Here, we discuss the different approaches to lead discovery for tropical diseases and emphasize a coordination strategy that involves highly integrated partnerships and networks between scientists in academic institutions and industry in both wealthy industrialized countries and disease-endemic countries. This strategy offers the promise of reducing the inherently high attrition rate of the early stages of discovery research, thereby increasing the chances of success and enhancing cost-effectiveness.

13616. **Ohta, N., 2006.** Endemic tropical diseases: comtemporary health problem due to abandoned diseases in the developing world. *Kansenshogaku Zasshi*, **80** (5): 469-474.

Section of Environmental Parasitology, Tokyo Medical and Dental University.

There are two kinds of infectious diseases in the world: diseases being paid attention and neglected diseases. The former diseases include HIV/AIDS, tuberculosis and malaria, the latter group include many parasitic, fungal, bacterial and some viral infections. "Neglected Infectious Diseases", which have been renamed as Endemic Tropical Diseases by WHO, are endemic in the developing world and are not new, having affecting humans for decades. In fact, DALYs for several diseases in this category are huge- more than 300 million for soil-

transmitted helminthiasis, 5 million for lymphatic filariasis, 4-5 million for schistosomiasis and so forth. However, those diseases were not recognized as serious health problems because of socio-economical and/or scientific reasons. Furthermore, these diseases are not fatal in the acute phases and are therefore not given appropriate attention by policy makers in the world. From the view point of basic medical sciences, however, there is no reason to neglect those diseases since no improved diagnostics and therapeutics have been developed in spite of the urgent necessities in endemic areas. Considering this situation, WHO has started to take action for solving these problems and many developed countries are recognizing the imbalanced input of human and financial resources only for 3 major infectious diseases, HIV/AIDS, tuberculosis and malaria. There are now various international schemes for supporting research on Neglected diseases. DNDi, Drugs for Neglected Diseases initiative, is one example and it's scope is only on drug development for Neglected diseases. African trypanosomiasis is one of Neglected diseases, causing serious health problem both for humans and domestic animals in Africa. No safe and effective medicine has been available but a drug with serious side effects is only the drug of choice even nowadays. Under the grant support from DNDi, a Japanese group is developing a new drug, ascofuranone, for African trypanosomiasis without any detectable side effects. Developing new prophylactic drugs for schistosomiasis and new diagnostic tools for lymphatic filariasis are underway under the support of grant for Neglected or Re-emerging infectious diseases in Japan. Considering that issues of "Neglected Infectious Diseases" are in urgent need of solution and also are challenging for modern medicine and medical sciences, researchers in the developed countries including Japan should make efforts to promote more active research in this field.

13617. **Riehle, M.A. & Jacobs-Lorena, M., 2005.** Using bacteria to express and display anti-parasite molecules in mosquitoes: current and future strategies. *Insect Biochemistry and Molecular Biology*, **35** (7): 699-707.

Department of Molecular Microbiology & Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD 21205, USA.

Vector-borne diseases impose enormous health and economical burdens throughout the world. Unfortunately, as insecticide and drug resistance spread, these burdens will increase unless new control measures are developed. Genetically modifying vectors to be incapable of transmitting parasites is one possible control strategy and much progress has been made towards this goal. Numerous effector molecules have been identified that interfere with parasite development in its insect vectors, and techniques for transforming the vectors with genes encoding these molecules have been established. While the ability to generate refractory vectors is close at hand, a mechanism for replacing a wild vector population with a refractory one remains elusive. This review examines the feasibility of using bacteria to deliver the anti-parasitic effector molecules to wild vector populations. The first half briefly examines paratransgenic approaches currently being tested in both the triatomine bug and tsetse fly. The second half explores the possibility of using midgut bacteria to control malaria transmission by *Anopheles* mosquitoes.

13618. **Smith, A., Telfer, S., Burthe, S., Bennett, M. & Begon, M., 2006**. A role for vector-independent transmission in rodent trypanosome infection? *International Journal of Parasitology*, **36** (13): 1359-1366.

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Within host-pathogen systems where vector-borne transmission is the primary route of infection, little or no attention has been paid to the relative importance of secondary or alternative routes of transmission. Here, by contrast, we report the results from a controlled longitudinal field-scale experiment in which the prevalence of fleas (Siphonaptera) was manipulated and the occurrence and distribution of a flea-borne protozoan (*Trypanosoma* (Herpetosoma) *microti*) in a natural field vole (*Microtus agrestis*) population was monitored over a 2-year period. A non-systemic insecticide was applied to individual voles within two treatment grids and the prevalences of fleas and of *T. microti* were monitored on these and on two control grids. Blood samples were taken from all voles and PCR-based methods used to determine infection status. Insecticidal treatment was highly effective at reducing overall flea prevalence and recaptured animals (treated ca. 4 weeks previously) were very rarely infested (ca. 3 percent, compared with 50-70+ percent normally). On the other hand, the probability of trypanosome infection was reduced in treated animals on experimental grids to only around one-third of that normally observed. This suggests that direct, as opposed to flea-borne, transmission may not only occur, it may also be of epidemiological importance. The possibility that the importance of such transmission routes may have been underestimated in 'vector-borne' infections more generally is discussed.

13619. **Steverding, D., 2006.** A new initiative for the development of new diagnostic tests for human African trypanosomiasis. *Kinetoplastid Biology and Disease*, **5**: 1.

Biomedical Research Centre, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich NR4 7TJ, UK. [dsteverding@hotmail.com]

Human African trypanosomiasis is a threat to millions of people living in sub-Saharan countries and is fatal unless treated. At present, the serological and parasitological tests used in the field for diagnosis of sleeping sickness have low specificity and sensitivity. There is clearly an urgent need for accurate tools for both diagnosis and staging of the disease. The Foundation for Innovative New Diagnostics and the World Health Organization has announced that they will collaborate to develop and evaluate new diagnostic tests for human African trypanosomiasis.

13620. **Tatem, A.J., Hay, S.I. & Rogers, D.J., 2006.** Global traffic and disease vector dispersal. *Proceedings of the National Academy of Sciences USA*, **103** (16): 6242-6247.

Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.

The expansion of global air travel and seaborne trade overcomes geographic barriers to insect disease vectors, enabling them to move great distances in short periods of time. Here we apply a coupled human-environment framework to describe the historical spread of *Aedes albopictus*, a competent mosquito vector of 22 arboviruses in the laboratory. We contrast this

dispersal with the relatively unchanged distribution of *Anopheles gambiae* and examine possible future movements of this malaria vector. We use a comprehensive database of international ship and aircraft traffic movements, combined with climatic information, to remap the global transportation network in terms of disease vector suitability and accessibility. The expansion of the range of *Ae. albopictus* proved to be surprisingly predictable using this combination of climate and traffic data. Traffic volumes were more than twice as high on shipping routes running from the historical distribution of *Ae. albopictus* to ports where it has established in comparison with routes to climatically similar ports where it has yet to invade. In contrast, *An. gambiae* has rarely spread from Africa, which we suggest is partly due to the low volume of sea traffic from the continent and, until very recently, a European destination for most flights.

13621. **Taylor, J.E. & Rudenko, G., 2006**. Switching trypanosome coats: what's in the wardrobe? *Trends in Genetics*, **22** (11): 614-620.

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The African trypanosome *Trypanosoma brucei* is best known for its extraordinarily sophisticated antigenic variation of a protective variant surface glycoprotein (VSG) coat. *T. brucei* has >1000 VSG genes and pseudogenes, of which one is transcribed at a time from one of multiple telomeric VSG expression sites. Switching the active VSG gene can involve DNA rearrangements replacing the old VSG with a new one, or alternatively transcriptional control. The astonishing revelation from the *T. brucei* genome sequence is that <7 percent of the sequenced VSGs seem to have fully functional coding regions. This preponderance of pseudogenes in the VSG gene repertoire will necessitate a rethink of how antigenic variation in African trypanosomes operates.

13622. **Welburn, S.C., Coleman, P.G., Maudlin, I., Fevre, E.M., Odiit, M. & Eisler, M.C., 2006.** Crisis, what crisis? Control of Rhodesian sleeping sickness. *Trends in Parasitology*, **22 (**3): 123-128.

Centre for Infectious Diseases, College of Medicine and Veterinary Medicine, University of Edinburgh, Roslin, Midlothian, UK. [sue.welburn@ed.ac.uk]

There is an urgent need for cost-effective strategies for the sustainable control of *Trypanosoma brucei rhodesiense* (Rhodesian) sleeping sickness, which is a fatal zoonotic disease that has caused devastating epidemics during the past century. Sleeping sickness continues to be controlled by crisis management, using active case detection, treatment and vector control - activities that occur only during major epidemics; during the intervening periods, farmers and communities must fend for themselves. There are several methods for assessing the burden of this disease and there is a series of farmer-led methodologies that can be applied to reduce the burden of human and animal trypanosomiases.

13623. **Willadsen, P., 2006.** Tick control: thoughts on a research agenda. *Veterinary Parasitology*, **138** (1-2): 161-168.

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Tick control is critical to the control of tick borne disease, while the direct impact of ticks on livestock productivity is also well known. For livestock, tick control today rests overwhelmingly on the twin approaches of genetics and chemical acaricides, although the disadvantages and limitations of both are recognized. The achievement of the full potential of vaccination, the application of biocontrol agents and the coordinated management of the existing technologies all pose challenging research problems. Progress in many areas has been steady over the last decade, while the acquisition of molecular information has now reached a revolutionary stage. This is likely to have immediate impact on the identification of potential antigens for improved vaccines and novel targets for acaricide action. In many circumstances, the rate limiting step in making scientific progress will remain unchanged, namely the resource constraint on evaluating these appropriately in large animals. For other approaches, such as the use of biocontrol agents, the limitation is likely to be less in the identification of suitable agents than in their delivery in an efficient and cost effective way. Our scientific understanding of the molecular basis for the tick vector-tick borne disease interaction is in its infancy but the area is both challenging and, in the long term, likely to be of great practical importance. What is arguably the most difficult problem of all remains: the translation of laboratory research into the extremely diverse parasite control requirements of farming systems in a way that is practically useful.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

13624. **Abubakar, L.U., Bulimo, W.D., Mulaa, F.J. & Osir, E.O., 2006.** Molecular characterization of a tsetse fly midgut proteolytic lectin that mediates differentiation of African trypanosomes. *Insect Biochemistry and Molecular Biology*, **36** (4): 344-352.

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Differentiation of bloodstream-form trypanosomes into procyclic (midgut) forms is an important first step in the establishment of an infection within the tsetse fly. This complex process is mediated by a wide variety of factors, including those associated with the vector itself, the trypanosomes and the bloodmeal. As part of an on-going project in our laboratory, we recently isolated and characterized a bloodmeal-induced molecule with both lectin and trypsin activities from midguts of the tsetse fly, *Glossina longipennis*. and purified and characterized a midgut lectin-trypsin complex from the tsetse fly, *Glossina longipennis*. The protein (lectin-trypsin complex) was found to be capable of stimulating differentiation of bloodstream trypanosomes *in vitro*. Using polyclonal antibodies to the complex, we screened

a *G. fuscipes fuscipes* cDNA midgut expression library and identified a putative proteolytic lectin gene. The cDNA encodes a putative mature polypeptide with 274 amino acids (designated *Glossina* proteolytic lectin, Gpl). The deduced amino acid sequence includes a hydrophobic signal peptide and a highly conserved N-terminal sequence motif. The typical features of serine protease trypsin family of proteins found in the sequence include the His/Asp/Ser active site triad with the conserved residues surrounding it, three pairs of cysteine residues for disulfide bridges and an aspartate residue at the specificity pocket. Expression of the gene in a bacterial expression system yielded a protein (MWt approximately 32,500). The recombinant protein (Gpl) bound d(+) glucosamine and agglutinated bloodstream-form trypanosomes and rabbit red blood cells. In addition, the protein was found to be capable of inducing transformation of bloodstream-form trypanosomes into procyclic forms *in vitro*. Antibodies raised against the recombinant protein showed cross-reactivity with the alpha subunit of the lectin-trypsin complex. These results support our earlier hypothesis that this molecule is involved in the establishment of trypanosome infections in tsetse flies.

13625. **Amin, D.N., Kamita, S.G., Muluvi, G.M., Machuka, J., Hammock, B.D. & Osir, E.O., 2006.** *Glossina* proteolytic lectin does not require a carbohydrate moiety for enzymatic or trypanosome-transforming activities. *Journal of Medical Entomology*, **43** (2): 301-308.

International Center of Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya.

The developmental cycle of the cyclically transmitted African trypanosome involves an obligatory passage through the tsetse fly, *Glossina* spp. This intricate relationship requires the presence of molecules within the insect vector, including a midgut lectin, that interact with the trypanosome. Recently, a gene encoding for a proteolytic lectin, with trypanosome-transforming activity, was isolated from a midgut cDNA library of *Glossina fuscipes fuscipes* Austen in our laboratory. Using the same approach, we have identified a similar gene from a midgut cDNA library of *Glossina austeni* (Newstead). The protein encoded by this gene was expressed in bacteria and a baculovirus-based expression system. The baculovirus-expressed lectin was found in the medium of baculovirus-infected Sf-21 cell cultures, indicating that the tsetse fly-derived signal peptide was recognized and cleaved by the Sf-21 cells. The baculovirus-expressed protein also was glycosylated despite the absence of classical O-linked and N-linked sugar attachment motifs. Both the baculovirus- and bacterium-expressed lectin proteins were shown to agglutinate trypanosomes and rabbit red blood cells *in vitro*. This agglutination was strongly inhibited by D-glucosamine. D-glucosamine also inhibited the action of the authentic and recombinant lectins upon the chromogenic substrate Chromozym TRY. Interestingly, both baculovirus- and bacterium-expressed lectins showed no significant differences in terms of these activities, indicating that a sugar moiety is not essential for biological activity. Our results provide an important molecular tool for further characterization of *Glossina* proteolytic lectin.

13626. **Attardo, G.M., Guz, N., Strickler-Dinglasan, P. & Aksoy, S., 2006.** Molecular aspects of viviparous reproductive biology of the tsetse fly (*Glossina morsitans*

morsitans): Regulation of yolk and milk gland protein synthesis. *Journal of Insect Physiology,* **52:** 1128-1136.

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Tsetse fly (Diptera: Glossinidae) viviparous reproductive physiology remains to be explored at the molecular level. Adult females carry their young *in utero* for the duration of embryonic and larval development, all the while supplying their offspring with nutrients in the form of a "milk" substance secreted from a modified accessory gland. Flies give birth to fully developed third instar larvae that pupariate shortly after birth. Here, we describe the spatial and temporal expression dynamics of two reproduction-associated genes and their products synthesized during the first and second gonotrophic cycles. The proteins studied include a putative yolk protein, *Glossina morsitans morsitans* yolk protein 1 (GmmYP1) and the major protein found in tsetse "milk" secretions (*Glossina morsitans morsitans* milk gland protein, GmmMGP). Developmental stage and tissue-specific expression of GmmYP1 show its presence exclusively in the reproductive tract of the fly during oogenesis, suggesting that GmmYP1 acts as a vitellogenic protein. Transcripts for GmmMGP are present only in the milk gland tissue and increase in coordination with the process of larvigenesis. Similarly, GmmMGP can be detected at the onset of larvigenesis in the milk gland, and is present during the full duration of pregnancy. Expression of GmmMGP is restricted to the adult stage and is not detected in the immature developmental stages. These phenomena indicate that the protein is transferred from mother to larvae as nourishment during its development. These results demonstrate that both GmmYP1 and GmmMGP are involved in tsetse reproductive biology, the former associated with the process of oogenesis and the latter with larvigenesis.

13627. **Attardo, G.M., Strickler-Dinglasan, P., Perkin, S.A., Caler, E., Bonaldo, M.F., Soares, M.B., El-Sayeed, N. & Aksoy, S., 2006.** Analysis of fat body transcriptome from the adult tsetse fly, *Glossina morsitans morsitans*. *Insect Molecular Biology*, 15 (4): 411-424.

Department of Epidemiology and Public Health, Section of Vector Biology, Yale University School of Medicine, New Haven, CT 06510, USA.

Tsetse flies (Diptera: Glossinidae*)* are vectors of pathogenic African trypanosomes. To develop a foundation for tsetse physiology, a normalized expressed sequence tag (EST) library was constructed from fat body tissue of immune-stimulated *Glossina morsitans morsitans*. Analysis of 20,257 high-quality ESTs yielded 6,372 unique genes comprised of 3,059 tentative consensus (TC) sequences and 3,313 singletons (available at http://aksoylab.yale.edu). We analysed the putative fat body transcriptome based on homology to other gene products with known functions available in the public domain. In particular, we describe the immune-related products, reproductive function related yolk proteins and milk-gland protein, iron metabolism regulating ferritins and transferrin, and tsetse's major energy source proline biosynthesis. Expression analysis of the three yolk proteins indicates that all are detected in females, while only the yolk protein with similarity to lipases, is expressed in males. Milk gland protein, apparently important for larval nutrition, however, is primarily synthesized by accessory milk gland tissue.

13628. **Darby, A.C., Lagnel, J., Matthew, C.Z., Bourtzis, K., Maudlin, I. & Welburn, S.C., 2005.** Extrachromosomal DNA of the symbiont *Sodalis glossinidius*. *Journal of Bacteriology*, **187** (14): 5003-5007.

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The extrachromosomal DNA of *Sodalis glossinidius* from two tsetse fly species was sequenced and contained four circular elements: three plasmids, pSG1 (82 kb), pSG2 (27 kb), and pSG4 (11 kb), and a bacteriophage-like pSG3 (19 kb) element. The information suggests *S. glossinidius* is evolving towards an obligate association with tsetse flies.

13629. **Gariou-Papalexiou, A., Yannopoulos, G., Robinson, A.S. & Zacharopoulou, A., 2006.** Polytene chromosome maps in four species of tsetse flies *Glossina austeni, G. pallidipes, G. morsitans morsitans* and *G. m. submorsitans* (Diptera: Glossinidae): a comparative analysis. *Genetica.* **In press; corrected proof.**

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Photographic polytene chromosome maps from pupal trichogen cells of four tsetse species, *Glossina austeni, G. pallidipes, G. morsitans morsitans* and *G. m. submorsitans* were constructed and compared. The homology of chromosomal elements between the species was achieved by comparing banding patterns. The telomeric and subtelomeric chromosome regions were found to be identical in all species. The pericentromeric regions were found to be similar in the X chromosome and the left arm of L1 chromosome (L1L) but different in L2 chromosome and the right arm of L1 chromosome (L1R). The L2 chromosome differs by a pericentric inversion that is fixed in the three species, *G. pallidipes, G. morsitans morsitans* and *G. m. submorsitans*. Moreover, the two *morsitans* subspecies appeared to be homosequential and differ only by two paracentric inversions on XL and L2L arm. Although a degree of similarity was observed across the homologous chromosomes in the four species, the relative position of specific chromosome regions was different due to chromosome inversions established during their phylogeny. However, there are regions that show no apparent homology between the species, an observation that may be attributed to the considerable intra-chromosomal rearrangements that have occurred following the species divergence. The results of this comparative analysis support the current phylogenetic relationships of the genus *Glossina*.

13630. **Hu, C. & Aksoy, S., 2006.** Innate immune responses regulate trypanosome parasite infection of the tsetse fly *Glossina morsitans morsitans*. *Molecular Microbiology*, **60** (5): 1194-1204.

Department of Epidemiology and Public Health, Yale University School of Medicine, 60 College St., 606 LEPH, New Haven, CT 06510, USA.

Tsetse flies transmit the protozoan parasite African trypanosomes, the agents of human sleeping sickness in sub-Saharan Africa. Parasite transmission in the insect is restricted by a natural resistance phenomenon (refractoriness). Understanding the mechanism of parasite resistance is important as strengthening fly's response(s) via transgenic approaches can prevent parasite transmission and lead to the development of novel vector control strategies. Here, we investigated the role of one of the two major pathways regulating innate immunity in invertebrates, the immunodeficiency (Imd) pathway, for *Glossina morsitans morsitans*'s natural defence against *Trypanosoma brucei* spp. infections. We determined the molecular structure of the Imd pathway transcriptional activator Relish (GmmRel), which shows high amino acid identity and structural similarity to its *Drosophila* homologue. Through a double-stranded RNA-based interference approach, we showed that the pathogen-induced expression profile of the antimicrobial peptides (AMPs) attacin and cecropin is under the regulation of GmmRel. Unexpectedly, the AMP diptericin appears to be constitutively expressed in tsetse independent of the presence of the Rel factor. Through GmmRel knock-down, we could successfully block the induction of attacin and cecropin expression in the immune responsive tissues, fat body and proventriculus (cardia), following microbial challenge. The midgut and salivary gland trypanosome infection prevalence, as well as the intensity of midgut parasite infections were found to be significantly higher in flies when attacin and relish expression were knocked down. Our results provide the first direct evidence for the involvement of antimicrobial peptides in trypanosome transmission in tsetse.

13631. **Krafsur, E.S. & Endsley, M.A., 2006.** Shared microsatellite loci in *Glossina morsitans sensu lato* (Diptera: *Glossinidae*). *Journal of Medical Entomology,* **43** (3): 640-642.

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Estimation of allelic frequencies at three microsatellite loci among 20 populations of *Glossina morsitans morsitans* Westwood, *Glossina morsitans submorsitans* Newstead, and *Glossina morsitans centralis* Machado indicated only two of 99 alleles were shared between three subspecies and 18 between any two subspecies; 81 alleles were unshared. The conserved flanking regions of each locus were completely shared. Genetic differentiation among subspecies, based on allele size, was RST = 0.87, close to the theoretic maximum value. All evidence suggests longstanding and complete reproductive isolation in nature among the sibling species. They should be elevated to specific rank.

13632. **Rio, R.V., Wu, Y.N., Filardo, G. & Aksoy, S., 2006.** Dynamics of multiple symbiont density regulation during host development: tsetse fly and its microbial flora. *Proceedings in Biological Science*, **273** (1588): 805-814.

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Symbiotic associations often enhance hosts' physiological capabilities, allowing them to expand into restricted terrains, thus leading to biological diversification. Stable maintenance of partners is essential for the overall biological system to succeed. The

viviparous tsetse fly (Diptera: Glossinidae) offers an exceptional system to examine factors that influence the maintenance of multiple symbiotic organisms within a single eukaryotic host. This insect harbours three different symbionts representing diverse associations, coevolutionary histories and transmission modes. The enterics, obligate mutualist *Wigglesworthia* and beneficial *Sodalis*, are maternally transmitted to the intrauterine larvae, while parasitic *Wolbachia* infects the developing oocyte. In this study, the population dynamics of these three symbionts were examined through host development and during potentially disruptive events, including host immune challenge, the presence of third parties (such as African trypanosomes) and environmental perturbations (such as fluctuating humidity levels). While mutualistic partners exhibited well-regulated density profiles over different host developmental stages, parasitic *Wolbachia* infections varied in individual hosts. Host immune status and the presence of trypanosome infections did not impact the steady-state density levels observed for mutualistic microbes in either sex, while these factors resulted in an increase in *Wolbachia* density in males. Interestingly, perturbation of the maternal environment resulted in the deposition of progeny harbouring greater overall symbiont loads. The regulation of symbiont density, arising from coadaptive processes, may be an important mechanism driving inter-specific relations to ensure their competitive survival and to promote specialization of beneficial associations.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

[See also **29**: 13601, 13612, 13620, 14004]

13633. **Abd-Alla, A., Bossin, H., Cousserans, F., Parker, A., Bergoin, M. & Robinson, A., 2006.** Development of a non-destructive PCR method for detection of the salivary gland hypertrophy virus (SGHV) in tsetse flies. *Journal of Virological Methods.* **In press; corrected proof**.

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A PCR based diagnostic method to detect salivary gland hypertrophy virus (SGHV) in tsetse flies is described. Two sets of primers GpSGHV1F/GpSGHV1R and GpSGHV2F/GpSGHV2R were selected from a virus-specific sequence. Both primer sets can detect specifically the virus in individual tsetse flies by generating an amplicon of 401bp. Attempts were made to develop a simple and reliable non-destructive virus detection method in live flies. PCR reactions were performed on either crude or purified tsetse DNA from saliva and legs. While saliva can be an indicator for the presence of the virus in flies, the method is laborious. Crude extract from an excised middle leg resulted in a positive PCR reaction equivalent to crude extract from whole fly. However, sensitivity could be significantly increased when purified DNA was used as the template. In conclusion, PCR using a purified DNA template from a single tsetse leg represents an efficient, non-destructive method for virus diagnosis in live tsetse flies.

13634. **Artzrouni, M. & Gouteux, J.P., 2006**. A parity-structured matrix model for tsetse populations. *Mathematical Biosciences*, **204** (2): 215-31.

Department of Mathematics, University of Pau, 64000 Pau, France.

A matrix model is used to describe the dynamics of a population of female tsetse flies structured by parity (i.e., by the number of larvae laid). For typical parameter values, the intrinsic growth rate of the population is zero when the adult daily survival rate is 0.970, corresponding to an adult life expectancy of 1/0.030=33.3 days. This value is plausible and consistent with results found earlier by others. The intrinsic growth rate is insensitive to the variance of the interlarval period. Temperature being a function of the time of the year, a known relationship between temperature and mean pupal and interlarval times was used to produce a time-varying version of the model which was fitted to temperature and (estimated) population data. With well-chosen parameter values, the modelled population replicated at least roughly the population data. This illustrates dynamically the abiotic effect of temperature on population growth. Given that tsetse flies are the vectors of trypanosomiasis ("sleeping sickness") the model provides a framework within which future transmission models can be developed in order to study the impact of altered temperatures on the spread of this deadly disease.

13635. **Camara, M., Caro-Riano, H., Ravel, S., Dujardin, J.P., Hervouet, J.P., De Meeus, T., Kagbadouno, M.S., Bouyer, J. & Solano, P., 2006.** Genetic and morphometric evidence for population isolation of *Glossina palpalis gambiensis* (Diptera: *Glossinidae*) on the Loos islands, Guinee. *Journal of Medical Entomology*, **43** (5): 853-860.

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Allele frequencies at four microsatellite loci, and morphometric features based on 11 wing landmarks, were compared among three populations of *Glossina palpalis gambiensis* (Diptera: Glossinidae) in Guinea. One population originated from the Loos islands separated from the capital Conakry by 5 km of sea, and the two others originated from the continental mangrove area close to Dubreka, these two groups being separated by approximately 30 km. Microsatellites and wing geometry data both converged to the idea of a separation of the Loos island population from those of the mangrove area. Although occasional contacts cannot be excluded, our results support the hypothesis of the Loos population of tsetse flies being a completely isolated population. This situation will favour a sequenced intervention against human African trypanosomosis and the possibility of an elimination of tsetse from this island.

13636. **Dujardin, J.P., Beard, C.B. & Ryckman, R., 2006.** The relevance of wing geometry in entomological surveillance of *Triatominae*, vectors of Chagas disease. *Infection Genetics and Evolution.* **In press, corrected proof**.

UMR IRD-CNRS 2724, Agropolis, 911, BP 64501, 34394 Montpellier, France; Division of Vector-borne Infectious Diseases, U.S. Centers for Disease Control and Prevention, Fort Collins, CO 80526, USA.

An important epidemiological challenge in controlling the Triatominae (Hemiptera: Reduviidae), vectors of Chagas disease, is identifying the origin of insects re-infesting treated areas, especially when reinfestation occurs during the first 1 or 2 years following insecticide application and in the absence of insecticide resistance. When using strict insect characteristics, the standard approach is to compare reinfesting specimens with those collected prior to treatment. Because of the long generation time of Triatominae, the experimental intent is to reject the hypothesis of a previous population, the one prior to insecticide application, to be the parental population of the reinfesting population. Biometric techniques are based on the hypothesis of more similarity between offspring and parents, and have been tested in the field. Reinfesting specimens are very few when discovered, which might cause sampling problems. The present study used museum material to test the performance of modern morphometrics to assess the origin of a single individual. A configuration of 13 landmarks was used to assign a single wing to its known parental line or relatives. For the 313 wings tested, correct attribution to the parental line was four times higher than expected at random. Moreover, most of the apparently wrong assignments were not random, but driven by lower levels of kinship. These results suggest that the geometry of the wing contains helpful information to identify the possible source of reinfesting specimens.

13637. **Geiger, A., Cuny, G. & Frutos, R., 2005.** Two tsetse fly species, *Glossina palpalis gambiensis* and *Glossina morsitans morsitans*, carry genetically distinct populations of the secondary symbiont *Sodalis glossinidius*. *Applied Environmental Microbiology*, **71** (12): 8941-8943.

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Genetic diversity among *Sodalis glossinidius* populations was investigated using amplified fragment length polymorphism markers. Strains collected from *Glossina palpalis gambiensis* and *Glossina morsitans morsitans* flies group into separate clusters, being differentially structured. This differential structuring may reflect different host-related selection pressures and may be related to the different vector competences of *Glossina* spp.

13638. **Geiger, A., Ravel, S., Mateille, T., Janelle, J., Patrel, D., Cuny, G. & Frutos, R., 2006.** Vector competence of *Glossina palpalis gambiensis* for *Trypanosoma brucei* s.l. and genetic diversity of the symbiont *Sodalis glossinidius*. *Molecular Biology and Evolution.* **In press; corrected proof.**

UMR 17, IRD-CIRAD, CIRAD TA 207 / G, Campus International de Baillarguet, 34398 Montpellier, Cedex 5, France.

Tsetse flies transmit African trypanosomes, responsible for sleeping sickness in humans and Nagana in animals. This disease affects many people with considerable impact on public health and economy in sub-Saharan Africa, while trypanosomes resistance to drugs is rising. The symbiont *Sodalis glossinidius* is considered to play a role in the ability of the fly to acquire trypanosomes. Different species of *Glossina* were shown to harbour genetically

distinct populations of *S. glossinidius*. We therefore investigated whether vector competence for a given trypanosome species could be linked to the presence of specific genotypes of *S. glossinidius*. *Glossina palpalis gambiensis* individuals were fed on blood infected either with *Trypanosoma brucei gambiense* or *Trypanosoma brucei brucei*. The genetic diversity of *S. glossinidius* strains isolated from infected and non-infected dissected flies was investigated using AFLP markers. Correspondence between occurrence of these markers and parasite establishment was analysed using multivariate analysis. *S. glossinidius* strains isolated from *T. brucei gambiense*-infected flies clustered differently than that isolated from *T. brucei brucei*-infected individuals. The ability of *T. brucei gambiense* and *T. brucei brucei* to establish in *G. palpalis gambiensis* insect midgut is statistically linked to the presence of specific genotypes of *S. glossinidius*. This could explain variations in *Glossina* vector competence in the wild. Then, assessment of the prevalence of specific *S. glossinidius* genotypes could lead to novel risk-management strategies.

13639. **Kubi, C., Van Den Abeele, J., de Deken, R., Marcotty, T., Dorny, P. & Van Den Bossche, P., 2006.** ffect of starvation on the susceptibility of teneral and non-teneral tsetse flies to trypanosome infection. *Medical and Veterinary Entomology,* **206** (4) 388-392.

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Transmission of vector-borne diseases depends largely on the ability of the insect vector to become infected with the parasite. In tsetse flies, newly emerged or teneral flies are considered the most likely to develop a mature, infective trypanosome infection. This was confirmed during experimental infections where laboratory-reared *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae) were infected with *Trypanosoma congolense* or *T. brucei brucei*. The ability of mature adult tsetse flies to become infected with trypanosomes was significantly lower than that of newly emerged flies for both parasites. However, the nutritional status of the tsetse at the time of the infective bloodmeal affected its ability to acquire either a *T. congolense* or *T. b. brucei* infection. Indeed, an extreme period of starvation (3–4 days for teneral flies, 7 days for adult flies) lowers the developmental barrier for a trypanosome infection, especially at the midgut level of the tsetse fly. Adult *G. m. morsitans* became at least as susceptible as newly emerged flies to infection with *T. congolense*. Moreover, the susceptibility of adult flies, starved for 7 days, to an infection with *T. b. brucei* was also significantly increased, but only at the level of maturation of an established midgut infection to a salivary gland infection. The outcome of these experimental infections clearly suggests that, under natural conditions, nutritional stress in adult tsetse flies could contribute substantially to the epidemiology of tsetse-transmitted trypanosomiasis.

13640. **Marquez, J.G., Malele, II, Ouma, J.O. & Krafsur, E.S., 2006.** *Glossina swynnertoni* (Diptera: Glossinidae): effective population size and breeding structure estimated by mitochondrial diversity. *Bulletin of Entomological Research*, **96** (4): 353-360.

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Nucleotide diversity was examined at mitochondrial COI and r16S2 loci in eight *Glossina swynnertoni* Austen collections from northern Tanzania and from a culture maintained by the International Atomic Energy Agency. Eighteen composite haplotypes were observed among 149 flies, two of which were common to all samples and 10 were private. Mean haplotype diversity was 0.59 and nucleotide diversity was 0.0013. There were excess singular haplotypes and mutation-drift disequilibrium suggesting that populations had experienced an earlier bottleneck and subsequent expansion. Factorial correspondence analysis showed that haplotype frequencies varied much more temporally (G ST=0.18) than spatially (G ST=0.04). The estimate of effective population size N in Tarangire was a harmonic mean of approximately 50 reproductive flies, averaged over approximately 47 generations. The mean rate of gene flow was estimated to be approximately 5+/-1 reproducing females per generation but inflated because of mutation-drift disequilibrium arising from likely earlier bottlenecks.

13641. **Ouma, J.O., Marquez, J.G. & Krafsur, E.S., 2006**. Patterns of genetic diversity and differentiation in the tsetse fly *Glossina morsitans morsitans* Westwood populations in East and southern Africa. *Genetica.* **In press; corrected proof**.

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Genetic diversity and differentiation within and among nine *G. morsitans morsitans* populations from East and southern Africa were assessed by examining variation at seven microsatellite loci and a mitochondrial locus, cytochrome oxidase (COI). Mean COI diversity within populations was 0.63 +/- 0.33 and 0.81 taken over all populations. Diversities averaged over microsatellite loci were high (mean number of alleles/locus >/=7.4; mean H (E) >/= 65 percent) in all populations. Diversities averaged across populations were greater in East Africa (mean number of alleles = 22 +/- 2.6; mean h (e) = 0.773 +/- 0.033) than in southern Africa (mean number of alleles = 18.7 +/- 4.0; mean h (e) = 0.713 +/- 0.072). Differentiation among all populations was highly significant (R (ST) = 0.25, F (ST) = 0.132). Nei's G (ij) statistics were 0.09 and 0.19 within regions for microsatellites and mitochondria, respectively; between regions, G (ij) was 0.14 for microsatellites and 0.23 for mitochondria. G (ST) among populations was 0.23 for microsatellite loci and 0.40 for mitochondria. The F, G and R statistics indicate highly restricted gene flow among *G. m. morsitans* populations separated over geographic scales of 12-917 km.

13642. **Ravel, S., de Meeus, T., Dujardin, J.P., Zeze, D.G., Gooding, R.H., Dusfour, I., Sane, B., Cuny, G. & Solano, P., 2006.** The tsetse fly *Glossina palpalis palpalis* is composed of several genetically differentiated small populations in the sleeping sickness focus of Bonon, Côte d'Ivoire. *Infection Genetics and Evolution.* **In press; corrected proof**.

IRD UR 177, Laboratoire de Recherche et de Coordination sur les Trypanosomoses IRD/CIRAD, Campus de Baillarguet, 34398 Montpellier Cedex 5, France.

Glossina palpalis is the main vector of human African trypanosomosis (HAT, or sleeping sickness) that dramatically affects human health in sub-Saharan Africa. Because of the implications of genetic structuring of vector populations for the design and efficacy of control campaigns, *G. palpalis palpalis* in the most active focus of sleeping sickness in Côte d'Ivoire was studied to determine whether this taxon is genetically structured. High and statistically significant levels of within population heterozygote deficiencies were found at each of the five microsatellite loci in two temporally separated samples. Neither null alleles, short allele dominance nor trap locations could fully explain these deviations from random mating, but a clustering within each of the two samples into different genetic sub-populations (Wahlund effect) was strongly suggested. These different genetic groups, which could display differences in infection rates and trypanosome identity, were composed of small numbers of individuals that were captured together, leading to the observed Wahlund effect. Implications of this population structure on tsetse control are discussed.

13643. **Terblanche, J.S. & Chown, S.L., 2006**. The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae). *Journal of Experimental Biology*, **209** (6): 1064-1073.

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Recent reviews of the adaptive hypotheses for animal responses to acclimation have highlighted the importance of distinguishing between developmental and adult (non-developmental) phenotypic plasticity. There has been little work, however, on separating the effects of developmental plasticity from adult acclimation on physiological traits. Therefore, we investigated the relative contributions of these two distinct forms of plasticity to the environmental physiology of adult tsetse flies by exposing developing pupae or adult flies to different temperatures and comparing their responses. We also exposed flies to different temperatures during development and re-exposed them as adults to the same temperatures, to investigate possible cumulative effects. Critical thermal maxima were relatively inflexible in response to acclimation temperatures (21, 25, 29^0 C) with plasticity type accounting for the majority of the variation (49-67 percent, nested ANOVA). By contrast, acclimation had a larger effect on critical thermal minima with treatment temperature accounting for most of the variance (84-92 percent). Surprisingly little of the variance in desiccation rate could be explained by plasticity type (30-47 percent). The only significant effect of acclimation temperature on standard (resting) metabolic rate of adult flies was at 21^0 C, resulting in treatment temperature, rather than plasticity type, accounting for the majority of the variance (30-76 percent). This study demonstrates that the stage at which acclimation takes place has significant, though often different, effects on several adult physiological traits in *G. pallidipes*, and therefore that it is not only important to consider the form of plasticity but also the direction of the response and its significance from a life-history perspective.

13644. **Terblanche, J.S., Klok, C.J., Krafsur, E.S. & Chown, S.L., 2006**. Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse

Glossina pallidipes (Diptera: Glossinidae): implications for distribution modelling. *American Journal of Tropical Medicine and Hygiene*, **74** (5): 786-794.

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Using the tsetse, *Glossina pallidipes*, we show that physiologic plasticity (resulting from temperature acclimation) accounts for among-population variation in thermal tolerance and water loss rates. Critical thermal minimum (CT (min)) was highly variable among populations, seasons, and acclimation treatments, and the full range of variation was 9.3^0 C (maximum value = 3.1 x minimum). Water loss rate showed similar variation (max = 3.7 x min). In contrast, critical thermal maxima (CT (max)) varied least among populations, seasons, and acclimation treatments, and the full range of variation was only approximately 1^0 C. Most of the variation among the four field populations could be accounted for by phenotypic plasticity, which in the case of CT (min), develops within 5 days of temperature exposure and is lost rapidly on return to the original conditions. Limited variation in CT (max) supports bioclimatic models that suggest tsetse are likely to show range contraction with warming from climate change.

13645. **Weiss, B.L., Mouchotte, R., Rio, R.V., Wu, Y.N., Wu, Z., Heddi, A. & Aksoy, S., 2006.** Interspecific transfer of bacterial endosymbionts between tsetse fly species: infection establishment and effect on host fitness. *Applied Environmental Microbiology*, **72** (11): 7013-7021.

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Tsetse flies (*Glossina* spp.) can harbour up to three distinct species of endosymbiotic bacteria that exhibit unique modes of transmission and evolutionary histories with their host. Two mutualist enterics, *Wigglesworthia* and *Sodalis*, are transmitted maternally to tsetse flies' intrauterine larvae. The third symbiont, from the genus *Wolbachia*, parasitizes developing oocytes. In this study, we determined that *Sodalis* isolates from several tsetse fly species are virtually identical based on a phylogenetic analysis of their ftsZ gene sequences. Furthermore, restriction fragment-length polymorphism analysis revealed little variation in the genomes of *Sodalis* isolates from tsetse fly species within different subgenera (*Glossina fuscipes fuscipes* and *Glossina morsitans morsitans*). We also examined the impact on host fitness of transinfecting *G. fuscipes fuscipes* and *G. morsitans morsitans* flies with reciprocal *Sodalis* strains. Tsetse flies cleared of their native *Sodalis* symbionts were successfully repopulated with the *Sodalis* species isolated from a different tsetse fly species. These transinfected flies effectively transmitted the novel symbionts to their offspring and experienced no detrimental fitness effects compared to their wild-type counterparts, as measured by longevity and fecundity. Quantitative PCR analysis revealed that transinfected flies maintained their *Sodalis* populations at densities comparable to those in flies harbouring native symbionts. Our ability to transinfect tsetse flies is indicative of *Sodalis*' recent evolutionary history with its tsetse fly host and demonstrates that this procedure may be used as a means of streamlining future paratransgenesis experiments.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also **29**: 13606, 13607, 13614, 13623, 13651]

13646. **Esterhuizen, J., Kappmeier Green, K., Nevill, E.M. & Van Den Bossche, P., 2006**. Selective use of odour-baited, insecticide-treated targets to control tsetse flies G*lossina austeni* and *G. brevipalpis* in South Africa. *Veterinary Entomology.* **In press; corrected proof.**

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The effectiveness of odour-baited targets treated with 0.8 percent deltamethrin in controlling *Glossina austeni* Newstead and *G. brevipalpis* Newstead (Diptera: Glossinidae) was evaluated in Zululand, South Africa. Targets were initially deployed in the three habitat types (grassland, woodland and forest) of two adjacent areas at a density of four targets per km^2. One area functioned as the treatment block (ca. 35 km^2) and included the focus of the target deployment, and the second area functioned as a barrier block (ca. 40 km^2) against tsetse fly re-invasion from the untreated area to the south. After 8 months, targets were removed from open grassland in both areas and target density in wooded habitats and sand forest was increased to eight per km^2. Twelve months later, all targets were removed from the barrier block and used to increase target density in the wooded and sand forest habitats of the treatment block to 12 per km^2. This target density was maintained for 14 months. In the treatment area, a 99 percent reduction in *G. austeni* females occurred after 13 months at a target density of eight per km^2 in wooded habitat; this was maintained for 22 months. Reduction in *G. brevipalpis* was less marked. The relatively poor reduction in *G. brevipalpis* is attributed to the high mobility of this species and its distribution throughout less wooded and more open habitats.

13647. **Kgori, P.M., Modo, S. & Torr, S.J., 2006.** The use of aerial spraying to eliminate tsetse from the Okavango Delta of Botswana. *Acta Tropica*, **99** (2-3): 184-199.

Tsetse Control Division, Maun, Botswana.

In Botswana, 16,000 km^2 of the Okavango Delta were aerial sprayed five times with deltamethrin, applied at 0.26-0.3g/ha, to control *Glossina morsitans centralis* Machado (Diptera: Glossinidae) over a period of approximately 8 weeks. The northern half of the Delta (7,180 km^2) was sprayed in June-September 2001 and the southern half (8,720 km^2)) in May-August 2002. A barrier (mean width approximately 10 km) of 12,000 deltamethrin-treated targets was deployed at the interface of these two blocks to prevent tsetse from invading from the southern to the northern block. Prior to spraying, the mean catches of tsetse from man fly-rounds were 44.6 flies/day in the northern block and 101 in the southern. Between September 2002 and November 2005, surveys (approximately 820 daily fly-rounds and approximately 2050 trap-days) in the northern and southern blocks failed to detect tsetse. Simulations of tsetse populations suggest that while spraying operations can reduce tsetse populations to

levels that are difficult to detect by standard survey techniques, such populations will recover to densities >100 tsetse/km^2 after 1000 days, at which density there is a very high probability (>0.999) that the survey methods will catch at least one fly. Since none was caught, it is argued that tsetse have been eliminated from the Delta. The particular success of this operation in comparison to the 18 aerial spraying operations conducted in the Delta prior to 2001 is attributed to the application of an adequate dose of insecticide, the use of a GPS-based navigation system to ensure even application of insecticide, and the large size and spatial arrangement of the spray blocks coupled with the use of a barrier of targets which prevented tsetse from re-invading the northern sprayed block before the southern one was treated.

13648. **Mamoudou, A., Zoli, A., Mbahin, N., Tanenbe, C., Bourdanne, Clausen, P.H., Marcotty, T., Van Den Bossche, P. & Geerts, S., 2006**. Prevalence and incidence of bovine trypanosomosis on the Adamaoua plateau in Cameroon 10 years after the tsetse eradication campaign. *Veterinary Parasitology*, **142** (1-2): 16-22.

Université de Dschang, Faculté d'Agronomie et des Sciences Agricoles BP 96, Dschang, Cameroun; Freie Universitat Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Konigsweg 67, D-14163 Berlin, Germany.

Between March 2004 and February 2005, the monthly incidence of trypanosome infections was measured in cattle from nine sentinel herds in the Adamaoua province of Cameroon. Three herds of 20 cattle each were kept on the plateau which has been cleared from tsetse flies about 10 years ago, three other herds were grazing in the tsetse infested valley whereas the last three were herded in the buffer zone. The cross-sectional study showed that the initial trypanosomosis prevalence was 1.8, 5.2 and 2.0 percent on the plateau, in the buffer zone and the valley, respectively. During the longitudinal study, the trypanosomosis incidence was high in the valley (3.7-20 percent) and the buffer zone (1.8-13.4 percent), whereas it was significantly lower (0-2.1 percent) on the plateau. Tsetse flies, mainly *Glossina morsitans submorsitans* and a few *G. tachinoides*, were caught in the valley and the buffer
zone, but none on the plateau. The data indicate a low trypanosomosis risk on the plateau. Further entomological studies, however, are required to clarify the origin of the trypanosome infections on the plateau.

13649. **Symeonakis, E., Robinson, T. & Drake, N., 2006**. GIS and multiple-criteria evaluation for the optimisation of tsetse fly eradication programmes. *Environmental Monitoring and Assessment.* **In press; corrected proof.**

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Tsetse flies are the vectors of trypanosomes, the causal agent of trypanosomiasis, a widespread disease of livestock and people in Africa. Control of tsetse may open vast areas of land to livestock-keeping, with the associated benefits of developing mixed crop-livestock production systems. However, as well as possible positive impacts there are also risks: bush clearing would accelerate and cattle numbers would rise, leading to a reduction of vegetation

cover, and an increase in runoff and erosion; there may also be increased pressure on conserved areas and reductions in biodiversity. The objective of this study is to show how remotely sensed and other environmental data can be combined in a decision support system to help inform tsetse control programmes in a manner that could be used to limit possible detrimental effects of tsetse control. For Zambia, a methodology is developed that combines a tree-based decision-support approach with the use of Multiple-Criteria Evaluation (MCE), within a Geographical Information System (GIS), in order to target areas for tsetse control. The results show clear differentiation of priority areas under a series of hypothetical scenarios, and some areas (e.g. northwest of Petauke in the Eastern Province of Zambia) are consistently flagged as high priority for control. It is also demonstrated that priority areas do not comprise isolated tsetse populations, meaning that disease control using an integrated approach is likely to be more economically viable than local eradication.

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

[See also **29**: 13603, 13618, 13701, 13758, 13836, 13971]

13650. **Caljon, G., Van Den Abbeele, J., Sternberg, J.M., Coosemans, M., De Baetselier, P. & Magez, S. 2006.** Tsetse fly saliva biases the immune response to Th2 and induces anti-vector antibodies that are a useful tool for exposure assessment. *International Journal for Parasitology,* **36** *(9), 1025-1035.*

Unit of Cellular and Molecular Immunology, Flanders Interuniversity Institute for Biotechnology, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium; Unit of Entomology, Prins Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium; School of Biological Sciences, Zoology Building, University of Aberdeen, Aberdeen AB24 2TZ, Scotland, UK.

Tsetse flies (*Glossina* sp.) are blood-feeding dipteran insects that transmit African trypanosomes, parasites that are responsible for human sleeping sickness and veterinary infections. Increasing attention is being paid to the effects of tsetse fly saliva deposited at the feeding site, which enables the blood-feeding process and putatively promotes parasite transmission. Here we demonstrate that saliva induces strong humoral responses against the major 43–45 kDa protein fraction (tsetse salivary gland proteins 1 and 2 – Tsal1 and Tsal2) in mice and humans and suppresses murine T and B cell responses to heterologous antigen. The saliva-induced immune response is associated with a Th2-biased cytokine profile and the production of mainly IgG1 and IgE antibody isotypes. Functionally, the antibodies raised in mice exposed to tsetse fly bites or induced after experimental saliva immunisation do not affect the fly's blood-feeding efficiency nor its survival. We propose that anti-saliva as well as anti-Tsal1/2 antibody responses can be used in epidemiological studies as a tool to analyze human exposure to tsetse flies.

13651**. Grace, D., 2005.** Epidemiology and control of cattle trypanosomosis in villages under risk of trypanocide resistance in West Africa. *Thesis, Freie Universitat Berlin.*195 pp.

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African Animal Trypanosomiasis (AAT) is the most serious cattle disease in sub-Saharan Africa. It is managed through vector control, keeping trypanotolerant cattle, but most importantly, by the use of trypanocidal drugs. Resistance to trypanocidal drugs is emerging and threaten the livelihoods of pastoralists and agropastoralists in sub-Saharan Africa who depend on cattle for traction, manure, milk, meat, savings, insurance, status and cultural obligations. A study was carried out in the cotton zone of west Africa (south west Burkina Faso, south Mali and north east Guinea) to: firstly, characterise trypanosomiasis control and epidemiology in villages with presence or risk of drug resistance; secondly develop, test, and evaluate best-bet strategies for the control of trypanosomiasis in the presence/risk of drug resistance; thirdly, model the dynamics of trypanocide resistance. To understand the situation, Knowledge, Attitude and Practice questionnaires were administered to all cattle-keepers in 65 villages, an Agricultural Knowledge and Information Study on trypanosomiasis management was carried out in eight villages, Participatory Rural Appraisals held in seven villages and 73 animal health service providers interviewed. Entomological studies were carried out in 54 villages, 16,935 cattle were examined parasitologically for trypanosomes, 834 blood samples were checked for haemoparasites and 1,463 coprological samples examined. Three strategies were evaluated for trypanosomiasis management: participatory vector control in eight villages, keeping trypanotolerant cattle in 65 villages and rational drug use (RDU); the latter by informing farmers in 46 villages, establishing/evaluating primary health services in 18 villages and training service providers who covered 235 villages. A dynamic mathematical model was developed to elucidate development and reversal of trypanocide resistance. We found AAT was the most important cattle disease in the area and was managed at community level. Animal health services were dysfunctional, with a large informal sector and low quality in the formal sector. Policy deficits and incoherence impede the management of AAT: most actors were unaware of trypanocide resistance. Modelling suggested resistance is inevitable given agricultural intensification, will worsen without intervention, but can be reversed by vector control. All strategies were effective at managing trypanosomiasis, but rational drug use had the highest benefit-cost ratio. Vector control delivered most benefits, but because of high transaction costs requires continued support. Vector control, funded as a public good, is recommended for the containment of resistance, and RDU for its prevention. Trypanotolerant cattle-keeping is less attractive to farmers but should be retained as a fall-back option. Integrated approaches to AAT management combined with initiatives to promote evidence-based policy are likely to prove the best bet for trypanosomiasis management under risk of resistance in the cotton zone of West Africa.

13652. **Steuber, S., Abdel-Rady, A. & Clausen, P.H., 2005.** PCR-RFLP analysis: a promising technique for host species identification of blood meals from tsetse flies (Diptera: Glossinidae). *Parasitology Research*, **97** (3): 247-254.

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A polymerase chain reaction with the restriction fragment length polymorphism (PCR-RFLP) method using universal primers complementary to the conserved region of the cytochrome b gene (cyt b) of the mitochondrion DNA (mtDNA) of vertebrates was applied to the identification of the origin of blood meals in tsetse flies. Blood samples from ten potential tsetse hosts of the family *Bovidae* (cattle, water buffalo, red buffalo, waterbuck, springbok, goat, sheep, sable antelope, oryx and dik-dik) were included in this study. Sites for appropriate restriction endonucleases cuts were chosen by pairwise alignment of the amplified 359 bp fragments. A flow chart of endonucleases digestion using three restriction enzymes (e.g. TaqI, AluI and HindII) for the unequivocal identification of the respective bovid species was developed. A number of additional non-specific DNA fragments attributed to the co-amplification of cytochrome b pseudogenes were observed in some species (e.g. in red buffalo and dik-dik after digestion with AluI) but did not hamper assignment of bovid species. The detection rate of host DNA in tsetse by PCR-RFLP was 100, 80, 60 and 40 percent at 24, 48, 72 and 96 h after *in vitro* feeding, respectively. Identification of the last blood meal was possible even when tsetse had previously fed on different hosts.

13653. **Van Den Bossche, P., Akoda, K., Djagmah, B., Marcotty, T., De Deken, R., Kubi, C., Parker, A. & Van Den Abbeele, J., 2006.** Effect of isometamidium chloride treatment on susceptibility of tsetse flies (Diptera: Glossinidae) to trypanosome infections. *Journal of Medical Entomology,* **43** (3): 564-567.

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Experiments were conducted to determine the effect of a single isometamidium chloride treatment of teneral tsetse flies, *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae), on the subsequent susceptibility to an infection with *Trypanosoma congolense* or *Trypanosoma brucei brucei*. Flies were offered a first bloodmeal on sterile gamma-irradiated defibrinated bovine blood that contained either 10 or 100 µg of isometamidium chloride/ml. Treated flies were subsequently infected with *T. congolense* IL 1180 or *T. b. brucei* AnTAR1 on day 3, 5, 10, or 20 post-treatment. To determine the effect of a single treatment with isometamidium chloride at 10 µg/ml on the fly's susceptibility to infection with isometamidium chloride-resistant trypanosome strains, treated flies were infected with one of two resistant isogenic *T. congolense* IL 1180 strains 3 d after the first feed. Results showed that a single isometamidium chloride treatment at 10 µg/ml blood sufficed to reduce significantly the fly's subsequent susceptibility to infection. Only 6.8 percent of the flies that were treated with isometamidium chloride developed a mature infection with *T. congolense* in the mouthparts compared with 34.3 percent of the control group. None of the flies that were administered isometamidium chloride and subsequently infected on day 3 or 6 with *T. b. brucei* developed a metacyclic infection in the salivary glands compared with 22.7 percent of the control flies. Likewise for the resistant *T. congolense* strains, a single treatment with isometamidium chloride significantly reduced the subsequent susceptibility to infection (6.5 and 33.5 percent of flies with metacyclic infections for treated and untreated flies, respectively). In practice and with respect to the release of sterile male flies to eradicate an

isolated tsetse fly population, our results show that administering isometamidium chloride during the first bloodmeal (and before release) would significantly reduce the ability of these released males to transmit trypanosomes.

13654. **Waiswa, C., Picozzi, K., Katunguka-Rwakishaya, E., Olaho-Mukani, W., Musoke, R.A. & Welburn, S.C., 2006.** *Glossina fuscipes fuscipes* in the trypanosomiasis endemic areas of south eastern Uganda: apparent density, trypanosome infection rates and host feeding preferences. *Acta Tropica*, **99** (1): 23-29.

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A study was undertaken in three districts in south eastern Uganda endemic for human and animal trypanosomiasis, to investigate the status of the vector tsetse fly population. Apparent density (AD) of tsetse was between 2 and 21 flies/trap/day across the three districts, with *Glossina fuscipes fuscipes* identified as the predominant species. Trypanosomes were observed in *G. f. fuscipes* with an infection rate, as determined by microscopy, of 1.55 percent across the three studied areas. However, trypanosome infections were only identified in female flies giving an infection rate of 2.39 percent for the female tsetse when this sex was considered in isolation; no male flies were found to be infected. Bloodmeal analysis highlighted 3 principal vertebrate hosts, namely cattle, pigs and monitor lizards (*Varanus niloticus*). The implication of this, in relation to the cycle of transmission for human infective trypanosomes between domestic animals and man, is discussed.

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

[See also **29**: 13603, 13605, 13609, 13619, 13622, 13688]

13655. **Berrang-Ford, L., Berke, O., Abdelrahman, L., Waltner-Toews, D. & McDermott, J., 2006.** Spatial analysis of sleeping sickness, southeastern Uganda, 1970-2003. *Emerging Infectious Diseases*, **12** (5): 813-820.

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Sleeping sickness re-emerged in south eastern Uganda in the 1970s and remains a public health problem. It has continued to spread north into new districts, and gaps remain in the understanding of the causes of its spread and distribution. We report the distribution and magnitude of sleeping sickness in south eastern Uganda from 1970 to 2003. Data were collected from records of the Ugandan Ministry of Health, individual sleeping sickness treatment centres, and interviews with public health officials. Data were used to develop incidence maps over time, conduct space-time cluster detection analyses, and develop a velocity vector map to visualize spread of sleeping sickness over time in south eastern

Uganda. Results show rapid propagation of sleeping sickness from its epicentre in southern Iganga District and its spread north into new districts and foci.

13656 **Deborggraeve, S., Claes, F., Laurent, T., Mertens, P., Leclipteux, T., Dujardin, J.C., Herdewijn, P. & Buscher, P., 2006.** Molecular dipstick test for diagnosis of sleeping sickness. *Journal of Clinical Microbiology*, **44** (8): 2884-2889.

Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium. [sdeborggraeve@itg.be]

Human African trypanosomiasis (HAT) or sleeping sickness is a neglected disease that affects poor rural populations across sub-Saharan Africa. Confirmation of diagnosis is based on detection of parasites in either blood or lymph by microscopy. Here we present the development and the first-phase evaluation of a simple and rapid test (HAT-PCR-OC [human African trypanosomiasis-PCR-oligochromatography]) for detection of amplified *Trypanosoma brucei* DNA. PCR products are visualized on a dipstick through hybridization with a gold-conjugated probe (oligochromatography). Visualization is straightforward and takes only 5 min. Controls both for the PCR and for DNA migration are incorporated into the assay. The lower detection limit of the test is 5 fg of pure *T. brucei* DNA. One parasite in 180 µl of blood is still detectable. Sensitivity and specificity for *T. brucei* were calculated at 100 percent when tested on blood samples from 26 confirmed sleeping sickness patients, 18 negative controls (non endemic region), and 50 negative control blood samples from an endemic region. HAT-PCR-OC is a promising new tool for diagnosis of sleeping sickness in laboratory settings, and the diagnostic format described here may have wider application for other infectious diseases.

13657. **Inojosa, W.O., Augusto, I., Bisoffi, Z., Josenado, T., Abel, P.M., Stich, A. & Whitty, C.J., 2006.** Diagnosing human African trypanosomiasis in Angola using a card agglutination test: observational study of active and passive case finding strategies. *British Medical Journal*, **332** (7556): 1479.

CUAMM-Angola and Angotrip Project, Caritas, Luanda, Angola. [inojosawalter@hotmail.com]

A study was made within a control programme in the Negage focus, northern Angola, during a period of civil war to assess the operational feasibility of detecting human African trypanosomiasis by active and passive case-finding using the card agglutination test with serial dilution of serum to guide treatment. It involved 359 patients presenting themselves to health centres with symptoms (passive case finding) and 14,446 people actively screened in villages. Whole blood and serological tests were performed at different dilutions using the card agglutination test, and detection of parasites was by microscopy. Active case finding identified 251 people with a positive card agglutination test result, 10 of whom had confirmed parasites. In those presenting for investigation 34 of 51 with a positive card agglutination test result at the dilution of 1:8 or more used to guide treatment had parasites in blood, lymph node fluid, or cerebrospinal fluid, compared with 10 of 76 in those detected by active case finding: positive predictive values of 67 percent for passive case detection and 13 percent for active case detection. Only at a cut-off dilution more than 1:32 was the positive

predictive value in active case detection reasonable (46 percent) and at this dilution 40 percent of microscopically proved cases were missed. The results suggest that the card agglutination test is useful for initial screening in active detection of cases with human African trypanosomiasis but, given the toxicity of the drugs, serology using the card agglutination test should be not used alone to guide treatment after active case finding. A second confirmatory test is needed.

13658. **Kinde-Gazard, D., Alyko-Chaffa, E., Atchade, P. & Massougbodji, A., 2006**. The re-emergence of the human African trypanosomiasis in Kerou, Benin. *Bulletin de la Société de pathologie exotique*, **99** (3): 191-193.

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Located in the northern part of Bénin, the district of Kerou is an historical HAT focus of the 1960s formerly called the "Atacora focus". This survey was conducted in 2001 to determine the prevalence of HAT in Kerou. The methodology consisted of a cross-sectional survey based on random sampling with two levels of stratification. 3367 persons were included (i=5 percent). After screening based on the CATT test using whole blood, the examination of trypanosomes was performed with QBC on the subjects that had persistent antibodies above a serum dilution of 1/4, followed by lumbar puncture. For the 3,367 surveyed subjects, the CATT seroprevalence was 4.2 percent and it was 2.4 percent using serum diluted at 1/8. The detection of trypanosomes with QBC was positive in 48 patients and the prevalence was 1.4 percent. The community survey conducted among 106 positive persons with CATT test serum at 1/4 dilution revealed that 71 (67 percent) persons never left the area since their birth. HAT was actually emerging in Atacora district in the north of Bénin, especially in Kerou.

13659. **Koffi, M., Solano, P., Denizot, M.., Courtin, D., Garcia A., Lejon, V., Büscher, P., Cuny, G., & Jamonneau, V. 2006.** Aparasitemic serological suspects in *Trypanosoma brucei gambiense* human African trypanosomiasis: A potential human reservoir of parasites? *Acta Tropica*, **98** (2): 183-188.

Institut de Recherche Pour le Développement, Unité de Recherche 177, Programme Santé Animale, TA 207/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France.

The serological and parasitological tests used for *Trypanosoma brucei gambiense* human African trypanosomiasis (HAT) diagnosis have low specificity and sensitivity, respectively, and in the field, control programme teams are faced with subjects with positive serology but negative parasitology who remain untreated. The aim of this work was to explore, using PCR tool, the significance of these aparasitaemic serological suspects. Since discordant PCR results have been observed earlier with different extraction methods, two DNA extraction methods were compared (the Chelex 100® resin and the DNeasy® Tissue kit). The study was conducted on 604 blood samples: 574 from parasitologically confirmed patients, aparasitaemic serological suspects and endemic controls collected in Côte d'Ivoire and 30 from healthy volunteers collected in France. No significant differences were observed

between the PCR results obtained with the two extraction methods. Concerning PCR, problems of reproducibility and discordances with both serological and parasitological test results were observed, mainly for the aparasitaemic serological suspects. In addition to previous results that pointed to the existence of non-virulent or non-pathogenic trypanosome strains and of individual susceptibility leading to long term seropositivity without detectable parasitaemia but positive PCR, the results of this study support the notion of a long lasting human reservoir that may contribute to the maintenance or periodic resurgences of HAT in endemic foci.

13660. **Lejon, V., Jamonneau, V., Solano, P., Atchade, P., Mumba, D., Nkoy, N., Bebronne, N., Kibonja, T., Balharbi, F., Wierckx, A., Boelaert, M. & Buscher, P., 2006.** Detection of trypanosome-specific antibodies in saliva, towards non-invasive serological diagnosis of sleeping sickness. *Tropical Medicine and International Health*, **11** (5): 620-627.

Department of Parasitology, Institute of Tropical Medicine, Antwerp, Belgium. [vlejon@itg.be]

The detection of trypanosome-specific antibodies in saliva is technically feasible, and, if clinically validated, could become an attractive option for non-invasive diagnosis of sleeping sickness. We wanted to optimize the test format of an enzyme-linked immunosorbent assay (ELISA)-based antibody detection system. Different ELISA formats for antibody detection in serum and saliva were developed and standardized. Saliva and serum samples were collected from 78 patients and 128 endemic controls, and sensitivity and specificity of saliva ELISAs, serum ELISAs and the card agglutination test for trypanosomiasis (CATT), were evaluated. All ELISA formats showed sensitivity and specificity above 90 percent. Saliva ELISAs showed a similar test performance as serum ELISAs and the CATT on whole blood or serum. This study confirmed the potential of trypanosome-specific antibody detection in saliva.

13661. **Lutumba, P., Robays, J., Miaka, C., Kande, V., Mumba, D., Buscher, P., Dujardin, B. & Boelaert, M., 2006.** Validity, cost and feasibility of the mAECT and CTC confirmation tests after diagnosis of African sleeping sickness. *Tropical Medicine and International Health*, **11** (4): 470-478.

Programme National de Lutte contre la Trypanosomiase Humaine Africaine, Kinshasa, République Democratique du Congo.

A study was conducted to evaluate the validity, cost and feasibility of two parasitological tests for the confirmation of Human African Trypanosomiasis (HAT): the mini Anion-exchange Centrifugation Technique (mAECT) and Capillary Tube Centrifugation (CTC). During a sleeping sickness screening campaign in 2004, we screened 6,502 people in Kwamouth, DRC. Those with a positive result in the Card Agglutination Test for trypanosomiasis (CATT) had a gland puncture, fresh blood examination, stained thick blood film, mAECT, CTC and CATT titration. Sensitivity and specificity of the confirmation tests were calculated using the combination of all parasitological tests as a reference standard. Each method was costed and its feasibility was assessed with structured interviews of the

technicians. Sensitivity of classical parasitological methods was 44.8 percent (36.8-53.0), of CTC 56.5 percent (48.3-64.5) and of mAECT 75.3 percent (95 percent CI: 67.7-81.9). Cost per test was 2.82 Euro for mAECT and 0.76 Euro for CTC. Time per test was 29.78 min for mAECT and 18.25 min for CTC. These two tests were judged feasible in field conditions. It was concluded that CTC and mAECT used alone or in combination would bring a considerable improvement to HAT active case finding when used as confirmation tests in CATT-whole blood-positive persons. They proved feasible in operational conditions if a 220 V power supply can be guaranteed. As mAECT is more sensitive but also considerably more expensive, efficiency as well as feasibility considerations will have to guide the choice of the best algorithm.

13662. **Magai, T., Kaare, Picozzi, K., Mlengeya, T., Fèvre, E.M., Mellau, L.S., Mtambo, M.M., Cleaveland, S. & Welburn, S.C., 2006.** Sleeping sickness: a re-emerging disease in the Serengeti? *Travel Medicine and Infectious Disease***, In press; corrected proof.**

Centre for Tropical Medicine, University of Edinburgh, Easter Bush, Roslin, Scotland, EH25 9RG, UK.

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.

13663. **Simarro, P. P.; Franco, J. R.; Ndongo, P.; Nguema, E.; Louis, F. & Jannin, J., 2006.** The elimination of *Trypanosoma brucei gambiense* sleeping sickness in the focus of Luba, Bioko Island, Equatorial Guinea. *Tropical Medicine and International Health.* **11** (5); 636-646.

Sleeping Sickness Control Centre, Bata, Equatorial Guinea. [simarrop@who.int]

After the resurgence of sleeping sickness in Luba, Equatorial Guinea, a major campaign to control the disease was established in 1985. The campaign comprised no vector control, but intensive active and passive surveillance using serology for screening, and treatment of all parasitological and suspected serological cases. Total prevalence was used to classify villages as endemic, at risk, anecdotal and non-endemic which also allowed defining the geographic extent of the focus. Active case-finding was implemented from 1985 to 2004. The frequency of surveys was based on parasitological prevalence: twice a year during intensified control, once a year during ordinary control and once every 2 years during the control consolidation phase, when the parasitological prevalence in the whole focus fell to 0.1 percent. From 1985 to 1999, the indirect immunofluorescent antibody test (IFAT) was used as an initial screening tool, followed by parasitological confirmation of IFAT positive cases, and the Card Agglutination Trypanosomiasis Test (CATT) if necessary. In 2000, the IFAT

was replaced by the CATT. Serum-positive individuals without parasitological confirmation were subsequently tested on serial dilution. All cases underwent lumbar puncture to determine the stage of the disease. First-stage cases were treated with pentamidine and second-stage cases with melarsoprol. A few relapses and very advanced cases were treated with eflornithine. The last sleeping sickness case was identified and treated in 1995.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **29**: 13621, 13714, 13715, 13719, 13722, 13723, 13727, 13871, 13873, 13878, 13881, 13984, 13987, 13906, 13923, 13943, 13944, 13970, 13977, 13978, 13999]

13664. **Braakman, H.M., van de Molengraft, F.J., Hubert, W.W. & Boerman, D.H., 2006.** Lethal African trypanosomiasis in a traveller: MRI and neuropathology. *Neurology*, **66** (7): 1094-1096.

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The authors report a case of human African trypanosomiasis with CNS involvement caused by *Trypanosoma brucei rhodesiense* in a 52-year-old woman, which relapsed after melarsoprol treatment. After a second regimen, she developed a severe toxic polyneuropathy, progressing to coma and eventually death. MRI revealed rapidly progressive multiple white matter lesions as well as damage of the central gray matter and cortex. The autopsy results confirmed the diagnosis of human African trypanosomiasis.

13665. **Calderoni, D.R., Andrade Tdos, S. & Grotto, H.Z., 2006**. Haptoglobin phenotype appears to affect the pathogenesis of American trypanosomiasis. *Annals of Tropical Medicine and Parasitology*, **100** (3): 213-221.

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In Latin America, 16-18 million people are thought to be infected with *Trypanosoma cruzi*, the parasite that causes American trypanosomiasis. The pathophysiology of this disease, particularly that of its chronic phase, has yet to be fully elucidated. The major function of haptoglobin, an acute-phase plasma protein found in three different phenotypes (Hp1-1, Hp2-1 and Hp2-2), is to bind to free haemoglobin and so prevent the accumulation of reactive hydroxyl radicals and renal damage. The haptoglobin phenotype present can influence the severity and progression of many diseases, including infectious ones. The aim of the present study was to see if any haptoglobin phenotype could be associated with any of the various clinical forms of American trypanosomiasis, and so explore the possibility that haptoglobin and iron metabolism have a role in the pathophysiology of this disease. The Brazilian subjects investigated were either suffering from the "indeterminate" (N=16), chronic cardiac (N=34), chronic digestive (N=13) or chronic "combined" (i.e. cardiac plus digestive; N=29) forms of the disease or were apparently healthy blood donors from the same

region as the patients (N=197). Haptoglobin phenotypes were determined by polyacrylamide-gel electrophoresis. Among the iron-related parameters investigated in the patients, only total iron-binding capacity and the serum concentration of haptoglobin differed significantly with haptoglobin phenotype. Compared with its frequency in the healthy controls, the Hp2-2 phenotype was much more frequent in the patients with any form of American trypanosomiasis, in the patients with the indeterminate form of the disease, and in the patients with the chronic combined form (p <or=0.0001 for each). It therefore appears that, in terms of the pathogenesis in those exposed to *T. cruzi*, possession of the 2-2 phenotype of haptoglobin may be detrimental.

13666. **Courtin, D., Jamonneau, V., Mathieu, J.F., Koffi, M., Milet, J., Yeminanga, C.S., Kumeso, V.K., Cuny, G., Bilengue, C.M. & Garcia, A., 2006.** Comparison of cytokine plasma levels in human African trypanosomiasis. *Tropical Medicine and International Health,* **11** (5): 647-653.

Institut de Recherche pour le Développement, Santé de la mère et de l'enfant en milieu tropical, Paris, France.

Immunological studies suggest that human African trypanosomiasis (HAT) is associated with inflammatory responses. A better understanding of the complex cytokine interactions regulating HAT infections is essential to elucidate the mechanisms of generalized immunosuppression. We determined levels of interleukin (IL)-2, IL-4, IL-6, IL-10, tumour necrosis factor (TNF)-alpha and interferon (IFN)-gamma protein levels in plasma samples from three groups of individuals from the Democratic Republic of Congo: (i) HAT cases; (ii) seropositive individuals for whom parasite detection was negative and (ii) controls. Plasma levels of six cytokines were significantly higher in HAT cases than in both controls (P<0.003) and seropositive individuals (P<0.016). IL-2 and IL-10 concentrations were significantly lower (P<0.02) in the seropositive group than in the control one. It was concluded that HAT leads to the development of strong cytokine responses, indicating the potential involvement of IL-2 and IL-10 in the phenomenon of seropositivity without parasitological confirmation. This strongly suggests the involvement of immunity in this particular aspect of HAT epidemiology.

13667. **Courtin, D., Milet, J., Jamonneau, V., Yeminanga, C.S., Kumeso, V.K., Bilengue, C.M., Betard, C. & Garcia, A., 2006.** Association between human African trypanosomiasis and the IL6 gene in a Congolese population. *Infection Genetics and Evolution.* **In press; corrected proof.**

Institut de Recherche pour le Développement (IRD), Unité de recherche 010: Santé de la mère et de l'enfant en milieu tropical, Faculté de pharmacie, 4 Avenue de l'observatoire, 75270 Paris, France.

Despite the importance of behavioural and environmental risk factors, there are arguments consistent with the existence of a genetic susceptibility to human African trypanosomiasis (HAT). A candidate gene association study was conducted in the Democratic Republic of Congo using a family-based sample which included a total of 353 subjects (86 trios; one case and parents (n=258) and 23 families with more than one case and parents

(n=95)). Polymorphisms located on the IL1alpha, IL4, IL6, IL8, IL10, TNFalpha and IFNgamma genes were genotyped after re-sequencing of the genes for extensive SNP search. The T allele of the IL6 (4339) SNP was significantly associated with a decreased risk of developing the disease (p=0.0006) and a suggestive association was observed for the IL1alpha (5417 T) SNP and an increased risk of developing the disease. These results suggest that genetic variability of the IL6 and to a lesser extent the IL1alpha gene are involved in the development of HAT. For the TNFalpha and IL10 gene polymorphisms, association results obtained here were different from those we observed in another population living under different epidemiologic conditions. This underlines the complexity of the interactions existing between host genetic polymorphisms, parasite diversity and behavioural and environmental risk factors in HAT.

13668. **Courtioux, B., Boda, C., Vatunga, G., Pervieux, L., Josenando, T., M'Eyi, P.M., Bouteille, B., Jauberteau-Marchan, M.O. & Bisser, S., 2006.** A link between chemokine levels and disease severity in human African trypanosomiasis. *International Journal of Parasitology*, **36** (9): 1057-1065.

Équipe Accueil 3174 Neuroparasitologie et Neuroépidémiologie Tropicale, Institut d'Épidémiologie Neurologique et de Neurologie Tropicale, Faculté de Médecine, 2 Rue du Dr Raymond Marcland, 87025 Limoges Cedex, France. [bertrand.courtioux@unilim.fr]

Trypanosoma brucei gambiense infection is an important public health challenge in sub-Saharan Africa. This parasitic disease is difficult to diagnose due to insidious clinical signs and transient parasitaemias. The clinical course is marked by two stages of increasing disease severity. An early systemic parasitic invasion is followed by the development of a progressive meningo-encephalitis. During this latter stage, a broad spectrum of neurological signs appears, which finally lead to a demyelinating and fatal stage if untreated. Treatment is toxic and difficult to administer when the CNS is invaded. Therefore, accurate diagnostic methods for stage determination are needed. The classically used criteria are not sufficiently specific and mechanisms of parasite invasion through the blood-brain barrier remain poorly understood. As cytokines/chemokines are involved in the early recruitment of leukocytes into the CNS, this study has focused on their potential value to define the onset of CNS involvement. Levels of monocyte chemoattractant protein-1/CCL-2, macrophage inflammatory protein-1alpha/CCL-3, IL-8/CXCL-8, regulated upon activation T cell expressed and secreted (RANTES)/CCL-5 and IL-1beta were measured in paired sera, and in CSF from 57 patients and four controls. Patients were classified into three groups (stage 1, intermediate and stage 2) according to current field criteria for stage determination (CSF cell count, presence of trypanosomes in CSF and neurological signs). In sera, cytokine/chemokine levels were poorly related to disease stage. Only CXCL-8 was higher in stage 1 patients when compared with stage 2 and CCL-5 was higher in controls when compared with patients. In contrast, in CSF the expression of the selected cytokines, except CCL-5, was associated with the presence of neurological signs, demonstrating their diagnostic value. We observed a relationship between the presence of trypanosomes or trypanosome-related compounds in CSF and levels of IL-1beta, CXCL-8, CCL-2 and CCL-3. These cytokines and chemokines may be triggered by the parasite and hence are potential markers of CNS invasion.

13669. **Cross, P., Doua, F. & Jaffar, S., 2006**. The risk factors for relapse among patients with African trypanosomiasis in Daloa, Cote d'Ivoire. *Tropical Doctor*, **36** (2): 90-93.

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK.

We describe rates of follow-up and the risk factors for relapse in a cohort of adult patients treated for *Trypanosoma brucei gambiense* African trypanosomiasis. 812 patients were discharged from hospital between 6 January 1983 and 16 January 1992. The numbers who did not attend a scheduled follow-up appointment at 6, 12, 18 and 24 months were 383 (47 percent), 467 (58 percent), 536 (66 percent) and 533 (66 percent), respectively. Thirty-two patients relapsed over the 2 years follow-up: 24 (75 percent) before the 12-month follow-up appointment. The presence of antibody to trypanosomes in the cerebrospinal fluid (CSF) at discharge from hospital was associated significantly with the risk of relapse at any time. When the analysis was restricted to a follow-up of 1 year, a protein level in the CSF above the median and the presence of antibody in the CSF (both at discharge) were associated in univariate analysis with relapse. A high number of patients were lost to follow-up, which may have resulted in bias. From the data available, the majority of the relapses were recorded within 12 months and the presence of antibody in the CSF at hospital discharge was identified as an independent predictor of future relapse at any time.

13670. **Kibiki, G.S. & Murphy, D.K., 2006.** Transverse myelitis due to trypanosomiasis in a middle aged Tanzanian man. *Journal of Neurology, Neurosurgery and Psychiatry*, **77** (5): 684-685.

Department of Internal Medicine, Kilimanjaro Christian Medical Centre, Tumaini University, Moshi, Tanzania.

We report the case of a middle aged Tanzanian man who developed a spinal cord syndrome over 6 weeks, along with a mild encephalopathy. Investigations ruled out the usual major causes of such a syndrome in our setting in northern Tanzania. Examination of his cerebrospinal fluid revealed trypanosomes, and he made a slow but dramatic improvement after a full course of suramine and melarsoprol. We postulate that he had a transverse myelitis due to African trypanosomiasis, a rare and barely recognised cause.

13671. **Kumar, N., Orenstein, R., Uslan, D.Z., Berbari, E.F., Klein, C.J. & Windebank, A.J., 2006.** Melarsoprol-associated multifocal inflammatory CNS illness in African trypanosomiasis. *Neurology*, **66** (7): 1120-1121.

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Abstract not available.

(c) TREATMENT

[See also **29**: 13615, 13617, 13736, 13737, 13744, 13748, 13769, 13770, 13776, 13828, 13865, 13875, 13880, 13959, 13973, 14008]

13672. **Garcia, A., Courtin, D., Solano, P., Koffi, M. & Jamonneau, V., 2006.** Human African trypanosomiasis: connecting parasite and host genetics. *Trends in Parasitology*, **22** (9): 405-409.

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In West and Central Africa, the protozoan parasite *Trypanosoma brucei (T. b.) gambiense* causes a chronic form of Human African trypanosomiasis (HAT) that might last several years, whereas *T. b. rhodesiense* refers to an acute form in East Africa that lasts weeks to months. Without treatment, both forms can cause death. Diagnosis relies on detecting parasites in blood, lymph or cerebrospinal fluid. HAT was no longer considered a public health problem in the 1960s, but it returned to alarming levels in the 1990s. After intensifying case detection and treatment, WHO recently declared the situation is under control. However, research based on host and trypanosome interactions should be encouraged to help develop innovative tools for HAT diagnosis and treatment to prevent re-emergence.

6. ANIMAL TRYPANOSOMIASIS

(a) SURVEY AND DISTRIBUTION

[See also **29**: 13648, 13689, 13690, 13691, 13692, 13693]

13673. **Bouyer, J., Guerrini, L., Desquesnes, M., de la Rocque, S. & Cuisance, D., 2006.** Mapping African animal trypanosomosis risk from the sky. *Veterinary Research*, **37** (5): 633-645.

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In Burkina Faso, African Animal Trypanosomosis (AAT) is still a major hindrance to cattle breeding, especially in the Mouhoun river basin, which was identified as a priority area for tsetse control. The attempt of the present work was to assess the abundance of tsetse flies and AAT risk using remote sensing coupled to field environmental data, along a Mouhoun river section of 234 km long, harbouring an open riverine forest where *G. tachinoides* Westwood is the predominant tsetse species. The water course was classified into three epidemiological landscapes, corresponding to a "disturbed", "natural" and finally "border" vegetation formation at the interface of the two formers. Using the mean number of infected flies by trap and by day as a risk indicator, the border landscape was found to be 5.4 (1.3-12.0) and 15.8 (4.7-41.6) times more risky than the natural and disturbed ones respectively.

These results led to propose that a campaign against tsetse, undertaken by a development project called PAEOB (Projet d'Appui a l'Élevage dans l'Ouest du Burkina Faso), should be focussed on only 34 percent of the hydrographic network.

13674. **Sow, A., Sidibe, I., Desquesnes, M., Bengaly, Z. & Pangui, L.J., 2006.** The application of PCR-ELISA to the detection of *Trypanosoma congolense* type savannah (TCS) in bovine blood samples. *Tropical Biomedicine*, **23** (1): 123-129.

Laboratoire National d'Élevage, 03 BP. 7026 Ouagadougou 03, Burkina Faso.

PCR-ELISA was set up to detect strains of *Trypanosoma congolense* type savannah (TCS) in field samples of buffy coats. Results of PCR-ELISA and PCR were compared and the effectiveness of both techniques was also compared with Murray's method for the detection of TCS in 257 bovine buffy coats. The PCR products were labelled with digoxigenin (DIG-dUTP) during amplification cycles of the repetitive satellite DNA. A biotinylated DNA capture probe was used to detect the PCR products by ELISA in streptavidin coated microplates. Both the PCR-ELISA and PCR were more sensitive and more specific than Murray's method. Of the 257 buffy coats analysed by the three techniques, PCR-ELISA and PCR detected TCS in 98 and 97 buffy coats respectively, whereas Murray's method detected only 39 samples. PCR-ELISA and PCR had almost the same sensitivity and specificity. PCR-ELISA and PCR respectively detected TCS in 39.2 percent and 38.6 percent in all the 334 samples analysed by both techniques in this study.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **29**: 13621, 13695, 13696, 13699, 13703, 13704, 13706, 13707, 13711, 13731, 13871, 13873, 13878, 13881, 13984, 13987, 13906, 13923, 13943, 13944, 13970, 13977, 13978, 13999]

13675. **Batista, J.S., Riet-Correa, F., Teixeira, M.M., Madruga, C.R., Simoes, S.D. & Maia, T.F., 2006**. Trypanosomiasis by *Trypanosoma vivax* in cattle in the Brazilian semiarid: Description of an outbreak and lesions in the nervous system. *Veterinary Parasitology*. **In press; corrected proof.**

Escola Superior de Agricultura de Mossoro, Av. Francisco Mota S/N, Br 110, Km 47, 59 Rio Grande do Norte, Brazil.

An outbreak of trypanosomiasis by *Trypanosoma vivax* is reported in the semiarid of Paraiba, North eastern Brazil from May to August 2002. Sixty-four cows out of 130 were affected; 11 died and the other recovered after treatment with diminazene aceturate. Affected animals had fever, anaemia, weight loss, hypoglycaemia, increased serum levels of aspartate aminotransferase and, in nine cows, nervous signs. All cows with nervous signs died; six of them recovered after treatment, but the disease relapsed. Six cows aborted and one delivered a calf that died immediately after parturition. Thirty-two out of 100 calves were affected and five died. Nervous signs were not observed in the calves. Gross lesions were thickening of the meninges, enlarged lymph nodes and prominent white pulp of the spleen. The main

histological lesion was meningoencephalitis and malacia in the brain of cows with nervous signs. No antibodies against trypanosomes were found in 33 blood samples collected before the outbreak in the affected farm and in 29 samples collected at the same time in two other neighbouring farms. Until January 2003, all 89 animals tested had antibodies against *T. vivax*, suggesting the occurrence of sub clinical infections in cattle without clinical signs. Only two out of 85 serum samples collected on April 2004 were positive for *T. vivax* antibodies. Data obtained suggested that the semiarid region is non-endemic for trypanosomiasis and that disease occurred due to introduction of the parasite in a susceptible population after an apparent rise in the *Tabanus* spp. population.

13676. **Dhollander, S., Jallow, A., Mbodge, K., Kora, S., Sanneh, M., Gaye, M., Bos, J., Leak, S., Berkvens, D. & Geerts, S., 2006.** Equine trypanosomosis in the Central River Division of The Gambia: a study of veterinary gate-clinic consultation records. *Preventive Veterinary Medicine*, **75** (3-4): 152-162.

International Trypanotolerance Centre, PMB 14 Banjul, The Gambia. [sofiedhollander@yahoo.co.uk]

The objective of this study was to provide epidemiological information of equine trypanosomosis in the Central River Division (CRD) of The Gambia. Therefore, 2,285 consultations records of equines, admitted in a gate-clinic at Sololo in CRD, were studied retrospectively. The data were recorded in the period between September 1995 and July 2002 and comprised consultations of 2,113 horses and 172 donkeys. “Trypanosome infection” was the most frequently diagnosed condition and accounted for 61 percent of the cases. Horses were more frequently diagnosed with trypanosome infections than donkeys ($p<0.001$), with an occurrence of 63 percent compared to 43 percent in donkeys. In both horses and donkeys, trypanosome infections were mainly due to *Trypanosoma congolense* (64 percent) and *T. vivax* (32 percent). There was no difference observed in the occurrence of trypanosome infections in male or female donkeys ($p=0.585$), but there were more female (67.8 percent) horses observed with trypanosome infections than male horses (60.7 percent; $p=0.003$). There was no difference observed in the occurrence of trypanosome infections in donkeys older or younger than 1 year ($p=0.130$), but older horses (63.2 percent >1 year) were observed with trypanosome infections than young horses (54.5 percent <1 year; $p=0.033$). The number of donkeys and horses with trypanosome infections decreased during the rainy season (June-September). The majority of equines that were admitted with trypanosome infections were severely anaemic. The average packed cell volume (PCV) declined with increasing parasitaemia ($p=0.006$). Seventy-four percent of the farmers' predictions of trypanosome infections in their equines were confirmed by darkground-microscopy. That proved that farmers had a fairly accurate knowledge of the diseases affecting their equines. The treatments executed at the gate-clinic were generally effective. The few (0.4 percent) relapses of the *T. vivax* infections that were previously treated with diminazene aceturate in this study were not sufficient to prove drug resistance. The study showed that the analysis of consultation records at a gate-clinic can provide complementary information to conventional epidemiological studies in the same research area.

13677. **Garcia, H., Garcia, M.E., Perez, G., Bethencourt, A., Zerpa, E., Perez, H. & Mendoza-Leon, A., 2006.** Trypanosomiasis in Venezuelan water buffaloes: association of packed cell volumes with seroprevalence and current trypanosome infection. *Annals of Tropical Medicine and Parasitology*, **100** (4): 297-305.

Laboratorio de Hemoparasitos, Catedra de Parasitología, Departamento de Patología Veterinaria, Facultad de Ciencias Veterinarias, Universidad Central de Venezuela, Apartado 4563/2101A, Maracay, Venezuela. [heraklesantonio@yahoo.com]

The seroprevalence of trypanosomiasis and the prevalence of current trypanosome infection in water buffaloes from the most important livestock areas of Venezuela were evaluated by IFAT and the microhaematocrit centrifugation technique, respectively. The usefulness of a PCR-based assay for identifying the trypanosome species in the buffaloes was also evaluated. Of the 644 animals investigated, 40 (6.2 percent) were found infected with trypanosomes by blood centrifugation, and 196 (30.4 percent) were found positive for anti-trypanosome antibodies, by IFAT. The results of the PCR-based assay indicated that 92.5 percent of the animals with current infections were infected with *Trypanosoma vivax* and the rest with *T. theileri* (the first molecular confirmation of *T. theileri* in Venezuelan water buffaloes). The national programme to treat and prevent trypanosome infections in the buffaloes does not appear to be meeting with great success, even though it is focused on *T. vivax*. Although the level of parasitaemia was categorized as low for 28 (70 percent) of the infections detected (and packed-cell volumes appeared to be unassociated with the IFAT results, and uncorrelated in the infected animals with level of parasitaemia), the 40 infected buffaloes had a significantly lower mean packed-cell volume than the uninfected animals ($P<0.05$). Farmers should therefore be made aware of the probability of trypanosome-attributable losses in buffalo productivity.

13678. **Muhammad, G., Jabbar, A., Iqbal, Z., Athar, M. & Saqib, M., 2006.** A preliminary passive surveillance of clinical diseases of cart pulling camels in Faisalabad metropolis (Pakistan). *Preventive Veterinary Medicine*, **76** (3-4): 273-279.

Department of Veterinary Clinical Medicine and Surgery, University of Agriculture, Faisalabad 38040, Pakistan.

We identified clinical disorders of all 200 city-dwelling cart pulling male camels attending the Veterinary Teaching Hospital, University of Agriculture, Faisalabad, Pakistan during a 7-year period (1993-1999). Data were collected prospectively on a predesigned form and collated. Diagnoses of different diseases/disorders were based on clinical examination supplemented with relevant laboratory tests. A total of 463 entries of 34 different clinical diseases/disorders were recorded. Sarcoptic mange (35 percent of 200 camels) followed by anhidrosis (23 percent) and trypanosomosis (19 percent) were the three most frequently encountered disorders. The body system most often involved was the integument (31 percent) followed by gastrointestinal (21 percent), locomotory (12 percent), thermoregulatory (6 percent), blood (6 percent), urogenital (6 percent), lymphatic (3 percent), nervous (3 percent), respiratory (3 percent) and ocular (3 percent).

13679. **Singla, L.D.; Aulakh, G. S.; Juyal, P.D. & Singh, J., 2004.** Bovine trypanosomosis in Punjab, India. In: *Proc. 11th International Conference of the Association of Institutions for Tropical Veterinary Medicine and 16th Veterinary Association Malaysia Congress*, Petaling Jaya, Malaysia, pp. 283-285.

Department of Veterinary Parasitology, College of Veterinary Science, Punjab Agricultural University, Ludhiana-141004, India.

Trypanosomosis (Surra), caused by mechanically transmitted blood protozoan *Trypanosoma evansi*, is a widely prevalent serious haemoprotozoan disease of domestic animals of considerable economic importance. The impact of "Surra" has been underestimated in bovines because they usually suffer from sub-clinical infection; however, various stress factors result in flaring up of dormant infection. In the present study the prevalence, clinical signs, haemato-pathological, biochemical, immunomodulatory and chemotherapeutic response in natural and experimental trypanosomosis in bovines is discussed. The percentage of prevalence of "Surra" was found to be 7.92, with more cases of subclinical nature in buffaloes than in cattle. An outbreak of dexamethasone flared up trypanosomosis was also reported. Haemato-biochemical changes revealed anaemia, leucocytosis, neutrophilia, lymphopaenia, increase in blood urea nitrogen, circulating immune complexes and immunoglobulins. Marked improvement in these parameters was observed after treatment. Experimentally dexamethasone immunomodulation of *T. evansi* infected male buffalo calves increased parasitaemia, intensity of clinical signs and mortality. Severe anaemia was observed in immunomodulated calves. The biochemical parameters were adversely affected. Higher levels of pyruvate corresponded with parasitaemia. Relapse of parasitaemia may be due to sequestering of parasites into central parenchyma and cerebrospinal fluid. Prognosis of "Surra" is good if diagnosed and treated with trypanocides in early stages of infection.

13680. **Sinshaw, A., Abebe, G., Desquesnes, M. & Yoni, W., 2006**. Biting flies and *Trypanosoma vivax* infection in three highland districts bordering Lake Tana, Ethiopia. *Veterinary Parasitology*, **142** (1-2): 35-46.

Bureau of Agriculture, Amhara National Regional State, P.O. Box 437, Bahir Dar, Ethiopia.

An epidemiological study was conducted to determine the prevalence of trypanosomosis in cattle, small ruminants and *Equidae*, and to identify biting flies; potential mechanical vectors of trypanosomes in the three districts of Bahir Dar Zuria, Dembia and Fogera, bordering Lake Tana, Ethiopia. About 1509 cattle, 798 small ruminants and 749 Equidae were bled for the prevalence study using the buffy-coat method and the measurement of the hematocrit value. Sixty-six NGU and 20 monoconical traps were deployed for the fly survey. The results indicated the presence of trypanosomes in 6.1 percent (92/1509) of the cattle with a maximum during the late rainy season (9.6 percent) than during the early dry season (3.6 percent) at Fogera district. Prevalence at the district level varied from 4 percent to 9.6 percent. Only one sheep (1/122) and one goat (1/676) were found positive for *T. vivax*-like trypanosomes and none of the *Equidae* was positive. All the

trypanosomes encountered in cattle belong to the single species of *T. vivax*. The PCV was negatively associated with detection of *T. vivax* (21.6 percent in infected versus 25.4 percent in non-infected cattle). A total of 55,398 biting flies were caught of which 49,353 (89.08 percent) belong to *Stomoxys*, 4,715 (8.51 percent) to horse flies and 1,330 (2.4 percent) to *Chrysops* species. There was no tsetse fly. Species identification has indicated the presence of *Atylotus agrestis*, *Chrysops streptobalia*, *Stomoxys calcitrans, S. nigra, S. pulla, S. pallida, S. sitiens, S. taeniata, S. uruma, Haematopota lasiops* and *Hippobosca variegata*. The overall apparent density was 214.7 flies/trap/day. Seasonal comparison showed higher fly catches in the late rainy season than the early dry season. This study indicated that *T. vivax* infections culminate in cattle at the same time as mechanical vectors such as *Stomoxys* sp. and *Atylotus agrestis*. Therefore, attention towards *T. vivax* infection in cattle is essential to control the impact of the disease on productivity. A further study on biting flies is recommended.

(c) TRYPANOTOLERANCE

13681. **Jaitner, J., Njie, M., Corr, N. & Dempfle, L., 2006.** Milk production of West African Dwarf goats in the Gambia. *Tropical Animal Health and Production*, **38** (3): 261-266.

International Trypanotolerance Centre, Banjul, The Gambia. [Jutta.Jaitner@t-online.de]

Goats are important in the low-input systems of West Africa and their main importance lies in their role for income and saving. In addition, it is known that milk offtake for home consumption is also important. In order to obtain information about the real importance of milk offtake, a recording scheme was operated in 27 villages in the Central River Division of The Gambia from July 1998 until January 2000. Detailed information was obtained from about 1,500 kiddings. In the recording scheme, any sheep being milked as well as the goats of the International Trypanotolerance Centre nucleus flock were also recorded. In the villages, 36 percent of all lactations were used for milk offtake, but the fraction milked was lower for the first two lactations. The average length of lactation was 127 days and the average daily milk offtake was 0.18 litres. Goats are milked once a day and the residual milk is left for the kids. Milking starts about one week after parturition and stops when the goat becomes pregnant or the kid(s) die or the goat is drying off. The repeatability of the 90-day milk offtake was 0.24 +/- 0.09. Sixty-five percent of goat owner were women and a large fraction of goat owners also owned cattle. Goat milk was used exclusively for home consumption. It is concluded that in breeding and extension work more attention should be given to aspects of milk production.

13682. **Lemecha, H., Mulatu, W., Hussein, I., Rege, E., Tekle, T., Abdicho, S. & Ayalew, W., 2006.** Response of four indigenous cattle breeds to natural tsetse and trypanosomosis challenge in the Ghibe valley of Ethiopia. *Veterinary Parasitology*, **141** (1-2): 165-176.

National Animal Health Research Center, Sebeta, Ethiopia.

A comparative study on the response of four indigenous cattle breeds of Ethiopia, namely Abigar, Horro, Sheko and Gurage, to natural challenge of trypanosomosis in the Tolley-Gullele area of the Ghibe valley has been undertaken from August 2000 until August 2004. Fifty female yearlings each of Horro, Sheko and Abigar and 31 of the Gurage were purchased from their natural habitats and introduced in to medium to high tsetse-trypanosomosis challenge area of the Ghibe valley. While the natural habitats of first three breeds are naturally infested with tsetse flies and trypanosomosis, that of the Gurage is known to be very minimal, if any, and hence the Gurage breed was used in this study as the known susceptible breed. During the study animal health, production performance and tsetse fly situation were monitored monthly. The Sheko breed has manifested very significantly ($p<0.001$) high overall average packed cell volume (PCV) values (25 percent) compared to that of Abigar (24 percent), Horro (23 percent) and Gurage (22 percent). It also had the lowest mean trypanosome prevalence rate of 9 percent against 23 percent of Horro, 26 percent of Abigar and 27 percent of Gurage, and the least number of Berenil treatments (1.36) compared to Abigar (4.0), Horro (4.6) and Gurage (6.7). While the Abigar manifested high sensitivity and frequent death to PCV depression, the Horro showed strong resilience to PCV depression and better response to Berenil treatment assistance. At this stage the Sheko breed was also found to be equal to the other breeds in its reproductive performance. These results need to be substantiated with further in-depth investigation including immune response, animal behaviour and environmental influences.

13683. **O'Gorman, G.M., Park, S.D., Hill, E.W., Meade, K.G., Mitchell, L.C., Agaba, M., Gibson, J.P., Hanotte, O., Naessens, J., Kemp, S.J. & Machugh, D.E., 2006**. Cytokine mRNA profiling of peripheral blood mononuclear cells (PBMC) from trypanotolerant and trypanosusceptible cattle infected with *Trypanosoma congolense*. *Physiological Genomics.* **In press; corrected proof.**

School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Dublin, Belfield, Ireland.

To examine differences in cytokine profiles that may confer tolerance/susceptibility to bovine African trypanosomiasis, N'Dama (trypanotolerant, n = 8) and Boran (trypanosusceptible, n = 8) cattle were experimentally challenged with *Trypanosoma congolense.* Blood samples were collected over a 34 day period and RNA was extracted from peripheral blood mononuclear cells (PBMC). The expression levels of a panel of 14 cytokines were profiled over the time course of infection and between breeds. Messenger RNA (mRNA) transcript levels for the IL2, IL8 and IL1RN genes were significantly downregulated across the time course of infection in both breeds. There was an early increase in transcripts for genes encoding proinflammatory mediators (IFNG, IL1A, TNF and IL12) in N'Dama by 14 days post infection (dpi) compared to pre-infection levels that was not detected in the susceptible Boran breed. By the time of peak parasitaemia, a TH2-like cytokine environment was prevalent, which was particularly evident in the Boran. Increases in transcripts for the IL6 (29 and 34 dpi) and IL10 (21, 25 and 29 dpi) genes were detected that were higher in the Boran compared to N'Dama. These findings highlight the implications for using murine models to study the bovine immune response to trypanosomiasis, where in some cases cytokine expression patterns differ. Overall, these data suggest that the trypanotolerant N'Dama are more capable of responding very early in infection with

proinflammatory and TH1 type cytokines than the trypanosusceptible Boran and may explain why N'Dama control parasitaemia more efficiently than Boran during the early stages of infection.

(d) TREATMENT

[See also **29**: 13651, 13653, 13736, 13776, 13777, 13828, 13865, 13875, 13880, 13959, 13973, 14008, 14024]

13684. **Awa, D.N. & Ndamkou, C.N., 2006**. Response of *Trypanosoma vivax* and *Trypanosoma congolense* in zebu cattle in North Cameroon to prophylactic treatment with two formulations of isometamidium. *Preventive Veterinary Medicine*, **76** (1-2): 90-96.

Institute of Agricultural Research for Development (IRAD), P.O. Box 1073, Garoua, Cameroon.[ndzingu_awa@yahoo.fr]

We tested the efficacy of two formulations of isometamidium in a tsetse-infested farm in North Cameroon from 20 August 2000 to 5 January 2001. A total of 90 adult cattle were used in three groups of 30 each corresponding to two treated and one untreated control. Drug efficacies were evaluated in terms of reduction of parasite incidence in the host's blood, maintenance of packed-cell volume (PCV) and weight gains. Both drugs reduced the incidence of parasites even though re-infections 2 weeks after treatment were common. PCV values were similar in both treated groups but higher than in the untreated control. Body weight changes followed a similar trend with the control losing weight from a mean of 427+/-119kg at the beginning to 398+/-93kg in 4 months. Weights increased from 375+/-76 and 396+/-110 to 396+/-69 and 418+/-112kg in the Veridium and Trypamidium groups, respectively. Efficacy was similar between the two formulations of isometamidium in the prophylaxis of bovine trypanosomosis. However, the presence of parasites in some animals barely 2 weeks after treatment suggested that either infections were not cleared or residual drug effects were not sufficient to prevent re-infections.

13685. **Clausen, P.H., 2005**. Studies on the diagnosis, development and distribution of drug-resistant trypanosomes in cattle herds in selected areas of East and West Africa. *Thesis, Freie Universitat Berlin.* 136 pp.

Institut für Parasitologie und Tropenveterinärmedizin, Königsweg 67, 14163 Berlin, Germany.

This study aimed to assess the development and distribution of drug-resistant trypanosomes in cattle herds in various sites in East Africa (Metekel, north west. Ethiopia; Upper Didessa Valley, west Ethiopia; Mukuno County, south east Uganda) and West Africa (province of Kenedougou, south west Burkina Faso). Longitudinal field studies were done to estimate the incidence of trypanocidal drug resistance in high risk areas. Several *in vivo* and *in vitro* tests were used to characterize the drug sensitivity of trypanosome field strains. The polymerase chain reaction was evaluated and its diagnostic potential to monitor the efficacy of prophylactic and curative treatments tested. Isometamidium resistance was widespread in *Trypanosoma congolense* in Metekel, Upper Didessa Valley and Kenedougou, but its

incidence varied between villages. Resistance to diminazene was also demonstrated in the various tests done. No resistance of trypanosomes to the drugs commonly used in cattle in Mukuno could be detected. The PCR proved to be a highly sensitive and specific tool to monitor the therapeutic and prophylactic efficacy and disease progression in bovine trypanosomiasis. Recommendations are given for measures to avoid or delay the development of resistance and to maintain the efficacy of currently available drugs. This thesis contains reprints of 14 papers published by the author, either as main or co-author, between 1992 and 2006 in the scientific literature.

13686. **Machila, N., Emongor, R., Shaw, A.P., Welburn, S.C., McDermott, J., Maudlin, I. & Eisler, M.C., 2006**. A community education intervention to improve bovine trypanosomiasis knowledge and appropriate use of trypanocidal drugs on smallholder farms in Kenya. *Agricultural Systems.* **In press; corrected proof.**

University of Edinburgh, Royal (Dick) School of Veterinary Studies, Centre for Tropical Veterinary Medicine, Kenya Agriculture Research Institute, Muguga,Kenya, and International Livestock Research Institute, Nairobi, Kenya.

This paper describes the development, design, dissemination and evaluation of a communication intervention designed to promote appropriate usage of trypanocidal drugs in trypanosomiasis endemic areas of western and coastal Kenya. Following a baseline study on current trypanosomiasis knowledge, attitudes and practices by smallholder farmers, a communication intervention strategy was developed involving dissemination through school children, village elders, animal health centres and Agrovet shops, and using layered messages in posters and leaflets. A participatory research approach was used to develop, design and assess the impact of animal health messages on the control of bovine trypanosomiasis for smallholder farmers in tsetse and trypanosomiasis endemic areas in Busia (two administrative divisions) and Kwale Districts (two administrative divisions) of Kenya. Communication intervention materials (in poster and leaflet formats) were developed and disseminated to residents in villages in one administrative division in each district (intervention area) while those from the other division in each district were not deliberately exposed to the animal health messages (control area). Several communication impact indicators were derived and these were measured 4–6 weeks after dissemination of the print media through questionnaires on trypanosomiasis knowledge administered to school children and cattle-keeping smallholders in the intervention and control study sites. School children's post-communication intervention trypanosomiasis signs knowledge was much higher than that observed during the pre-communication intervention survey. More trypanocides were named by school children during the post-intervention questionnaire survey compared to those known during the pre-intervention survey. The trypanosomiasis signs knowledge score obtained by the smallholder farmers exposed to the extension materials was higher than that obtained by those not exposed to them. Similarly, farmers' exposure to extension materials resulted in higher trypanocidal drug knowledge scores among exposed farmers than among those not exposed. These results indicate that over the period monitored, the routes (i.e. school children, village elders, animal health centres and Agrovet shops) and media (posters and leaflets) selected were effective in promoting a significant increase in knowledge of trypanosomiasis its causes and ways of dealing with it among livestock keepers.

13687. **Somda, J., Kamuanga, M., & Tollens, E., 2006**. Prospective analysis for community participation in trypanosomosis control in The Gambia. *Tropical Animal Health and Production*, **38** (2): 103-111.

International Trypanotolerance Centre, Banjul, The Gambia, and Department of Agricultural and Environmental Economics, Katholieke Universiteit Leuven, Belgium; International Livestock Research Institute, Bobo Dioulasso, Burkina Faso; Department of Agricultural and Environmental Economics, Katholieke Universiteit Leuven, Belgium.

The shift towards community participation in the eradication of trypanosomiasis calls for the investigation of the underlying incentive structure for individuals in the community to cooperate in the provision of various control methods. Survey data were used to assess patterns of the community's demand for insecticide pour-ons and trypanocidal drugs and factors affecting individual demand in The Gambia. It was shown that insecticide pour-on formulations are strongly preferred. Similarly, farmers revealed a preference for community-based provision scheme. Factors affecting an individual farmer's decision to invest in either pour-on or trypanocidal drugs were highlighted. While there are many factors associated with farmers' decisions to invest in trypanosomiasis control methods and to participate in collective actions, the results indicate that farmers are ready to anticipate complete privatization of veterinary services through community-based schemes.

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

[See also **29**: 13619]

13688. **Agranoff, D., Stich, A., Abel, P. & Krishna, S., 2005**. Proteomic fingerprinting for the diagnosis of human African trypanosomiasis. *Trends in Parasitology,* **21** (4): 154-157.

Department of Cellular and Molecular Medicine (Infectious Diseases), St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK.

Papadopoulos *et al.* recently reported the discovery of a diagnostic serum proteomic signature for human African trypanosomiasis (HAT), using a combination of surface-enhanced laser desorption-ionization time-of-flight (SELDI-TOF) mass spectrometry and data-mining algorithms. This novel approach, coupled with biochemical characterization of the proteins that contribute to the signature, provides powerful new tools for the development of improved diagnostic tests, disease staging and identification of potential novel drug targets in HAT.

13689. **Aradaib, I.E. & Majid, A.A., 2006**. A simple and rapid method for detection of *Trypanosoma evansi* in the dromedary camel using a nested polymerase chain reaction. *Kinetoplastid Biology and Disease*, **5**: 2.

Molecular Biology Research Unit, National Ribat University, Khartoum, Sudan. [aradaib@yahoo.com].

A nested polymerase chain reaction (nPCR)-based assay, was developed and evaluated for rapid detection of *Trypanosoma evansi* in experimentally infected mice and naturally infected camels (*Camelus dromedarius*). Four oligonucleotide primers (TE1, TE2, TE3 and TE4), selected from nuclear repetitive gene of *T. evansi*, were designed and used for PCR amplifications. The first amplification, using a pair of outer primers TE1 and TE2, produced a 821-bp primary PCR product from *T. evansi* DNA. The second amplification, using nested (internal) pair of primers TE3 and TE4, produced a 270-bp PCR product. *T. evansi* DNAs extracted from blood samples of experimentally infected mice and naturally infected Sudanese breed of dromedary camels were detected by this nested PCR-based assay. The nested primers TE3 and TE4 increased the sensitivity of the PCR assay and as little as 10 fg of *T. evansi* DNA (equivalent to a single copy of the putative gene of the parasite) was amplified and visualized onto ethidium bromide-stained agarose gels. Amplification products were not detected when the PCR-based assay was applied to DNA from other blood parasites including *Thieleria annulata*, *Babesia bigemina* or nucleic acid free samples. Application of this nPCR-based assay to clinical samples resulted in direct detection of *T. evansi* from a variety of tissue samples collected from experimentally infected mice and blood from naturally infected camels. The described nPCR-based assay provides a valuable tool to study the epidemiology of *T. evansi* infection in camels and other susceptible animal populations.

13690. **Gonzales, J.L., Loza, A. & Chacon, E., 2006**. Sensitivity of different *Trypanosoma vivax* specific primers for the diagnosis of livestock trypanosomosis using different DNA extraction methods. *Veterinary Parasitology* **136** (2): 119-126.

Laboratorio de Investigacíon y Diagnostico Veterinario "LIDIVET", Av. Ejercito Nacional 153, P.O. Box 29, Santa Cruz, Bolivia. [pepevet@yahoo.com].

There are several *T. vivax* specific primers developed for PCR diagnosis. Most of these primers were validated under different DNA extraction methods and study designs leading to heterogeneity of results. The objective of the present study was to validate PCR as a diagnostic test for *T. vivax* trypanosomosis by means of determining the test sensitivity of different published specific primers with different sample preparations. Four different DNA extraction methods were used to test the sensitivity of PCR with four different primer sets. DNA was extracted directly from whole blood samples, blood dried on filter papers or blood dried on FTA cards. The results showed that the sensitivity of PCR with each primer set was highly dependant of the sample preparation and DNA extraction method. The highest sensitivities for all the primers tested were determined using DNA extracted from whole blood samples, while the lowest sensitivities were obtained when DNA was extracted from filter paper preparations. To conclude, the obtained results are discussed and a protocol for diagnosis and surveillance for *T. vivax* trypanosomosis is recommended.

13691. **Lejon, V., Claes, F., Verloo, D., Maina, M., Urakawa, T., Majiwa, P.A.O. & Buscher, P., 2005**. Recombinant RoTat 1.2 variable surface glycoprotein as

antigen for diagnosis of *Trypanosoma evansi* in dromedary camels. *International Journal for Parasitology,* **35** (4): 455-460.

Department of Parasitology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium.

The transcript encoding a predominant *Trypanosoma evansi* variable surface glycoprotein RoTat 1.2 was cloned and expressed as a recombinant protein in *Spodoptera frugiperda* and *Trichoplusia ni* (insect) cells. Its potential as an antigen for specific detection of antibody in serum of dromedary camels affected by surra, was evaluated. In ELISA, the reactivity of the recombinant RoTat 1.2 VSG was similar to that of native RoTat 1.2 VSG. An indirect agglutination reagent was therefore prepared by coupling the recombinant RoTat 1.2 VSG onto latex particles. The performance of the latex agglutination test was evaluated on camel sera, and compared with the performance of CATT/*T. evansi* and LATEX/*T. evansi* tests, using the immune trypanolysis assay with *T. evansi* RoTat 1.2 as a reference test. The relative sensitivity and specificity of the latex coated with recombinant RoTat 1.2 VSG, using a 1:4 serum dilution, were respectively, 89.3 and 99.1 percent. No differences were observed between the performance of latex coated with recombinant RoTat 1.2 VSG and LATEX/*T. evansi* or CATT/*T. evansi*. Here, we describe the successful use of the recombinant RoTat 1.2 VSG for detection of specific antibodies induced by *T. evansi* infections.

13692. **Li, F.J., Gasser, R.B., Lai, D.H., Claes, F., Zhu, X.Q. & Lun, Z.R., 2006.** PCR approach for the detection of *Trypanosoma brucei* and *T. equiperdum* and their differentiation from *T. evansi* based on maxicircle kinetoplast DNA. *Molecular and Cellular Probes.* **In press; corrected proof.**

Center for Parasitic Organisms and State Key Laboratory of Biocontrol, School of Life Sciences, Zhongshan (Sun Yat-sen) University, Guangzhou 510275, PR China.

The goal of this study was to develop a PCR approach based on the sequence of maxicircle kinetoplast DNA (kDNA) of *Trypanosoma brucei* to distinguish *T. brucei/T. equiperdum* from *T. evansi* and to evaluate its diagnostic use for their detection in blood samples. Primers derived from the sequence of the maxicircle kDNA of *T. brucei*, encoding the NADH dehydrogenase subunit 5 (nad5) gene, were used to test the PCR-amplification from *T. brucei* (including *T. b. brucei* and *T. b. rhodesiense*), *T. equiperdum*, *T. evansi*, *T. vivax* and *T. congolense*. A primer pair to a nuclear DNA region incorporated into a separate PCR was employed to control for the presence of amplifiable genomic DNA (representing the subgenus *Trypanozoon*) in each sample subjected to the PCR. Products of approximately 395bp were amplified from all *T. brucei* and *T. equiperdum* samples tested using the nad5-PCR, but not from *T. evansi* DNA samples or any of the control samples representing *T. vivax, T. congolense*, or host. The current PCR approach allows the rapid differentiation of *T. brucei/T.equiperdum* from *T. evansi* and can detect the equivalent of 20-25 cells of *T. brucei* or *T. equiperdum* in purified genomic DNA or infected blood samples.

13693. **Thekisoe, O.M.M., Inoue, N., Kuboki, N., Tuntasuvan, D., Bunnoy, W., Borisutsuwan, S., Igarashi, I. & Sugimoto, C., 2005**. Evaluation of loop-

mediated isothermal amplification (LAMP), PCR and parasitological tests for detection of *Trypanosoma evansi* in experimentally infected pigs. *Veterinary Parasitology,* **130** (3-4): 327-330.

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan.

Six surra negative piglets (6-week-old) were infected with *Trypanosoma evansi* and two uninfected piglets were used as negative controls. Detection performances of various diagnostic tests (LAMP, PCR and parasitological tests) were compared by analysing blood samples collected weekly over a period of 11 weeks. With a two by two analysis without a gold standard, all methods were 100 percent specific. MI had the highest sensitivity of 65 percent, while LAMP, PCR, MHCT and TBS had sensitivities of 45, 33, 38 and 24 percent, respectively. However, when the analysis was done using MI as a gold standard, the sensitivity of MHCT was the highest at 53 percent followed by LAMP, PCR and TBS at 49, 44 and 35 percent, respectively. All methods gave high specificity above 60 percent. This study validates LAMP as an alternative method for the diagnosis of surra.

(b) PATHOLOGY AND IMMUNOLOGY

13694. **Abenga, J.N., David, K., Ezebuiro, C.O.G. & Lawani, F.A.G., 2005**. Observations on the tolerance of young dogs (puppies) to infection with *Trypanosoma congolense*. *African Journal of Clinical and Experimental Microbiology,* **6** (1): 28-33.

Nigerian Institute for Trypanosomiasis Research, PMB 2077, Kaduna, Nigeria.

Studies were undertaken to assess the susceptibility of young local dogs to infection with *Trypanosoma congolense*. Six puppies (7 weeks old) were used for the study. Although the puppies became parasitaemic 6 to 7 days post infection, they were tolerant to infection as the parasitaemia remained low throughout the first seven weeks of the eight-week observation period. The packed cell volume (PCV) also only dropped slightly during the last four weeks attaining the value of 25.6+3.8 ($p>0.05$) by the eighth week while the mean body weight continued to increase. Similarly, the mean daily body temperature did not differ significantly from that of uninfected control. The significance of trypanotolerance in Nigerian local dogs is discussed.

13695. **Adamu, S., Fatihu, M.Y., Useh, N.M., Mamman, M., Sekoni, V.O. & Esievo, K.A., 2006.** Sequential testicular and epididymal damage in Zebu bulls experimentally infected with *Trypanosoma vivax*. *Veterinary Parasitology.* **In press; corrected proof**.

Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

Six Zebu bulls aged between 31 and 34 months exhibiting good libido were used to study sequential testicular and epididymal damage in *Trypanosoma vivax* infection. Three bulls were infected with *T. vivax*, while the other three served as controls. All infected bulls became parasitaemic by day 5 post-infection and developed clinical trypanosomosis with rapidly developing anaemia. Representative bulls, one from each of the infected and control groups, were sacrificed on days 14, 28 and 56 post-infection. Testes and epididymides from these animals were studied histopathologically after processing and staining with haematoxylin and eosin (H and E). Testicular degeneration developed in all the infected bulls characterized by depletion of spermatogenic cells and destruction of interstitial tissue. The most severe testicular degeneration occurred in the bull that was sacrificed 56 days post-infection. Epididymal sperm reserves were 36 percent, 4 percent and 0 percent, respectively, in infected bulls that were sacrificed on days 14, 28 and 56 post-infection. The 0 percent epididymal sperm reserve may suggest complete cessation of spermatogenesis. It was concluded from this study that *T. vivax* infection of Zebu bulls could cause severe testicular and epididymal damage that may result in infertility or even sterility of the affected animals at early infection stages not previously thought.

13696. **Akinwale, O.P., Nock, I.H., Esievo, K.A.N., Edeghere, H.U. & Olukosi, Y.A., 2006**. Study on the susceptibility of Sahel goats to experimental *Trypanosoma vivax* infection. *Veterinary Parasitology,* **137** (3-4): 210-213.

Public Health Division, Nigerian Institute of Medical Research, PMB2013 Yaba, Lagos, Nigeria.[pheabian@yahoo.co.uk].

Sahel goats, also known as Borno whites are found in the northern semi-arid, tsetse free Sahel region of Nigeria. They are transported alongside cattle from this zone to all other zones in the country, including the tsetse-infested zones, for commercial purposes and are kept for some time in these tsetse-infested zones until they are sold. This study therefore assessed the susceptibility of this breed of goats to trypanosome infection and its response to treatment with Berenil. Six bucks were inoculated intravenously with *Trypanosoma vivax* through the jugular vein while two served as uninfected control. The mean pre-patent period was 4.5 days and increasing parasitaemia followed the establishment of infection. Onset of parasitaemia was associated with increase in rectal temperature in all the infected goats and the temperature peak coincided with the only parasitaemic peak second week post-infection. The infected goats were treated with Berenil (Hoechst, Germany) 3.5 mg/kg body weight at 4 weeks post-infection. The packed cell volume (PCV) continued to fall from a mean of 30.73 pre-infection to a mean 13.21 at 1 week post-treatment. Deaths were recorded for 4 of the infected goats 1 week post-treatment while the remaining two died 2 weeks post-treatment, not responding to treatment.

13697. **Al-Mohammed, H.I., 2006.** Parasitological and immunological studies on rats experimental infected with Saudi Arabian strain of *Trypanosoma evansi. Journal of the Egyptian Society of Parasitology*, **36** (2): 363-371.

College of Medicine, King Faisal University, P.O. Box 55017, Al-Ahsa, 31982, Saudi Arabia. [hamdan@kfu.edu.sa]

Surra, an enzootic disease caused by *Trypanosona evansi*, is one of the most important trypanosomiasis in Kingdom of Saudi Arabia. The state of parasitaemia in relation to the corresponding humoral response in experimentally infected Wister rats for 15 days were investigated. The prepatent period was found to be 5 days. The disease was characterized by intermittent fluctuation of parasitaemia and a significant difference in the level of parasitaemia ($P > 0.05$) was detected in specimens of day 7, 9, 13 & 14. There was a difference in the mean number of blood parasites in relation to sex throughout the 15 days of the study. This difference was statistically significant ($p < 0.05$). Using indirect haemagglutination serological test, almost all inoculated rats displayed specific antibodies of diagnostic value on day 7 after infection ranging between 1/80-1/160. Thereafter, antibody titres increased progressively to reach very high positive dilutions of > 2,048 in all animals at the end of study on day 15. No sex difference could be observed in both serological specimens of 7 & 15 days. Also no correlation was observed between the state of parasitaemia and the serological titres in infected rats.

13698. **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.

Department of Medicine, University of California, Los Angeles, CA 90095, USA.

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.

13699. **Biryomumaisho, S. & Katunguka-Rwakishaya, E., 2006.** The pathogenesis of anaemia in goats experimentally infected with *Trypanosoma congolense* or *Trypanosoma brucei*: use of the myeloid:erythroid ratio. *Veterinary Parasitology.* **In press; corrected proof**.

Department of Veterinary Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda.

The present study examined the development of anaemia in Small East African goats experimentally infected with *Trypanosoma congolense* or *Trypanosoma brucei*. Experimental

goats received a primary trypanosome challenge on day 0, treated with diminazene aceturate on day 49 and received a secondary trypanosome challenge on day 77 of the 136-day experiment. Both primary and secondary challenges were characterised by reduced peripheral erythrocyte counts, fall in packed cell volume (PCV), hypohaemoglobinaemia and reductions in the myeloid:erythroid ratios (M:E) compared with the uninfected goats. The progressive reduction in the M:E ratios denoted increased erythrogenesis in response to increased destruction of erythrocytes in blood by infecting trypanosomes or their products. The more rapid fall in M:E ratio in *T. congolense* infections shows that this parasite causes more severe clinical pathological effects in goats than *T. brucei.*

13700. **Budovsky, A., Prinsloo, I. & El-On, J., 2006.** Pathological developments mediated by cyclophosphamide in rats infected with *Trypanosoma lewisi*. *Parasitology International*, **55** (4): 237-242.

Department of Microbiology and Immunology, Ben-Gurion University of the Negev, Israel.

Trypanosoma lewisi is an obligatory, flagellated parasite of the rat. Despite the fact that naturally the rats overcome the disease, a lethal infection can be induced by the administration of an immunosuppressive agent, i.e. cyclophosphamide (Cy). In the Cy treated infected rats (CyI) the severity of the trypanosome infection was demonstrated in the internal organs, in the following order: lungs>liver>heart>spleen>kidney. The parasites were not detected in the brain. The accumulation of the parasites in the lungs led to the development of hemorrhagic inflammatory foci. The rupture of blood vessels was accompanied by lymphocyte infiltrations into the damaged tissues and multiple foci of oedema around the blood vessels. In most cases the lungs were dark brown in colour due to intra-alveolar haemorrhages. The spleen of the CyI rats showed general deformation of the tissue's architecture, migration of macrophages and cell depletion due to the Cy action. The liver showed inflammatory hemorrhagic foci associated with massive destruction of the parenchyma. In spite of the heavy parasite (>50 percent) developed in the CyI rats the brain remained free of parasites, which might explain the non-virulent character of this parasite compared to the African trypanosomes.

13701. **Caljon, G., Van Den Abbeele, J., Stijlemans, B., Coosemans, M., De Baetselier, P. & Magez, S., 2006.** Tsetse fly saliva accelerates the onset of *Trypanosoma brucei* infection in a mouse model associated with a reduced host inflammatory response. *Infection and Immunity*, **74** (11): 6324-6330.

Unit of Cellular and Molecular Immunology, Flanders Interuniversity Institute for Biotechnology (VIB), Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium. [gucaljon@vub.ac.be]

Tsetse flies (*Glossina* sp.) are the vectors that transmit African trypanosomes, protozoan parasites that cause human sleeping sickness and veterinary infections in the African continent. These blood-feeding dipteran insects deposit saliva at the feeding site that enables the blood-feeding process. Here we demonstrate that tsetse fly saliva also accelerates the onset of a *Trypanosoma brucei* infection. This effect was associated with a reduced

inflammatory reaction at the site of infection initiation (reflected by a decrease of interleukin-6 [IL-6] and IL-12 mRNA) as well as lower serum concentrations of the trypanocidal cytokine tumour necrosis factor. Variant-specific surface glycoprotein-specific antibody isotypes immunoglobulin M (IgM) and IgG2a, implicated in trypanosome clearance, were not suppressed. We propose that tsetse fly saliva accelerates the onset of trypanosome infection by inhibiting local and systemic inflammatory responses involved in parasite control.

13702. **Ching, S., He, L., Lai, W. & Quan, N., 2005**. IL-1 type I receptor plays a key role in mediating the recruitment of leukocytes into the central nervous system. *Brain, Behavior, and Immunity*, **19** (2): 127-137.

Department of Oral Biology, Ohio State University, Columbus, OH 43210-1094, USA.

This study investigates the role of type I IL-1 receptor (IL-1R1) in mediating the recruitment of leukocytes into the brain parenchyma in mice. Chronic infection with *Trypanosoma brucei* resulted in the recruitment of T cells, but no other cell types, into the brain. This did not occur in IL-1R1-knockout mice. Thus, IL-1R1 appears to be important for the recruitment of leukocytes across the blood-brain barrier.

13703. **Dargantes, A.P., Campbell, R.S.F., Copeman, D.B. & Reid, S.A., 2005**. Experimental *Trypanosoma evansi* infection in the goat. II. Pathology. *Journal of Comparative Pathology,* **133** (4): 267-276.

College of Veterinary Medicine, Central Mindanao University, Musuan 8710, Bukidnon, Philippines.

Infection of male goats aged 8-10 months with 5,000 or 50,000 organisms of a Mindanao strain of *Trypanosoma evansi* was observed over a period of 90 days. The infection induced clinical disease which was lethal, especially at the higher dose rate. Lesions were more acute in goats that received the higher dose. Gross and microscopical changes were not pathognomonic, except in the presence of demonstrable trypanosomes. At necropsy, a combination of lymphadenopathy, splenomegaly, hepatomegaly, testicular enlargement, anaemic signs and consolidation of the anterior lobes of the lungs was suggestive of surra. Testicular changes, especially aspermia, indicated probable infertility. The cytopathology of the lungs, liver, intestine, kidneys, testes, bone marrow, brain and other organs was immunological in nature, characterized by mononuclear infiltration of interstitial tissues, with minor cellular damage and the presence of trypanosomes. B- and T- cell responses were observed in the lymphatic system, but the findings indicated immunosuppression in the lymph nodes, spleen and bone marrow during the third month after infection. Exudative inflammatory changes were mild. It is suggested that the cytopathology of most haemophilic trypanosomal infections is predominantly an immunological process.

13704. **Dargantes, A.P., Reid, S.A. & Copeman, D.B., 2005**. Experimental *Trypanosoma evansi* infection in the goat. I. Clinical signs and clinical pathology. *Journal of Comparative Pathology,* **133** (4): 261-266.

College of Veterinary Medicine, Central Mindanao University, Musuan 8710, Bukidnon, Philippines.

A strain of *Trypanosoma evansi* isolated from an equine case of surra in Mindanao, Philippines was used to infect intravenously two groups (A and B) of five male goats aged 8-10 months. Animals of groups A and B received 5,000 and 50,000 trypanosomes, respectively, and five further animals (group C) served as uninfected controls. Four of the 10 infected goats died 8-78 days after inoculation. Group C goats gained weight (mean 22.8 g/day) while infected goats in groups A and B lost weight (means of 21.4 and 45.0 g/day, respectively). Parasitaemia fluctuated regularly between peaks and troughs, with repeated periods of about 6 days during which no trypanosomes were detected in the blood. Clinical signs and clinico-pathological changes in infected goats were not pathognomonic in the absence of parasites in the blood, and leucocytosis was not a reliable indicator of infection. It was concluded that in endemic areas fluctuating fever, progressive emaciation, anaemia, coughing, testicular enlargement and diarrhoea are suggestive of surra; confirmation, however, may necessitate examination of blood every few days for trypanosomes, and possibly other diagnostic tests.

13705. **Egbe-Nwiyi, T.N., Nwaosu, A. & Gazdama, M.A., 2005**. The effects of oral manganese chloride supplementation on the severity of *Trypanosoma brucei* and *Trypanosoma congolense* infections in rats. *Veterinarski Arhiv,* **75:** 541-547.

Department of Veterinary Physiology and Pharmacology, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.

Eighty healthy adult albino rats of both sexes were used in two experiments to study the effects of manganese chloride supplementation on the severity of *Trypanosoma brucei* and *Trypanosoma congolense* infections. In each experiment, forty rats were divided into four groups of 10 each: A. infected unsupplemented; B. infected supplemented; C. uninfected unsupplemented control; d. Uninfected supplemented control. Aqueous solution (5 percent) of MnCl2 was administered daily using stomach tube to each rat at 50 mg/kg body weight in groups b and d from 10 days before infection to the end of the experiment. Each rat in groups a and b was infected by intraperitoneal injection of $1x10^6$ trypanosomes (*T. brucei* or *T. congolense*) in diluted donor blood. The prepatent periods were shorter ($P<0.05$) in *T. brucei* than *T. congolense* infections, and shorter ($P<0.05$) in infected unsupplemented than in infected supplemented rats. The infected unsupplemented groups had higher ($P<0.05$) parasitaemia and more severe anaemia than the infected supplemented groups. Therefore, oral manganese chloride supplementation in rats appeared to reduce the severity of trypanosome infections by delaying the onset of parasitaemia, reducing the levels of parasitaemia and accompanying anaemia.

13706. **Faye, D., Fall, A., Leak, S., Losson, B. & Geerts, S., 2005**. Influence of an experimental *Trypanosoma congolense* infection and plane of nutrition on milk production and some some biochemical parameters in West African Dwarf goats**.** *Acta Tropica,* **93** (3): 247-257.

International Trypanotolerance Centre, PMB 14, Banjul, The Gambia.

The interactions of trypanosomosis and plane of nutrition on health and productivity of multiparous and primiparous West African Dwarf (WAD) does were studied in a multi-factorial experiment including diet (supplementation or basal diet).

13707. **Hilali, M., Abdel-Gawad, A., Nassar, A. & Abdel-Wahab, A., 2006**. Hematological and biochemical changes in water buffalo calves (*Bubalus bubalis*) infected with *Trypanosoma evansi*. *Veterinary Parasitology.* **In press, corrected proof**.

Parasitology Department, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.

Four water buffalo calves (*Bubalus bubalis*) were each inoculated intravenously with 106 *T. evansi* (camel isolate) and the fifth calf kept as non-infected control. The blood and sera of all calves were examined every 4 days during the first month post-inoculation (pi) and then once weekly until the end of the experiment (88 days pi). They were examined for haematological and biochemical changes, liver and kidney function tests. Haemoglobin concentration (Hb percent), packed cell volume (PCV) and red blood cell count were significantly decreased. Total leucocytic count, lymphocytes and monocytes showed significant increase. Liver function tests revealed significant elevation in the activity of lactate dehydrogenase enzyme (LDH), globulin, total bilirubin and indirect bilirubin while alkaline phosphatase enzyme showed significant decrease. Kidney function tests revealed significant decrease of both creatinine and urea.

13708. **Kagira, J.M.M., N. W., Thuita, J. K., Ngotho, M. & Hau, J., 2005**. Influence of cyclophosphamide on the haematological profile of laboratory bred African soft-furred rats (*Mastomys natalensis*). *Scandinavian Journal of Laboratory Animal Science,* **32** (3): 153-158.

Kenya Agricultural Research Institute, Trypanosomiasis Research Centre (KARI-TRC), P.O. Box 362, Kikuyu, Kenya

The African soft-furred rat (*Mastomys natalensis*) has been shown to be a possible model for propagation of *Trypanosoma brucei gambiense*. This study aimed at determining the baseline biological reference values and reproductive data of a laboratory bred *Mastomys colony*, which was established at TRC. In addition, the effect of cyclophosphamide (an immunosuppressant) treatment (s) on the haematological profile was investigated. The mean gestation period was 23 days and the mean litter size was eight. At birth, the pups weighed 2.4±0.23 g and the weights increased to 78.0±10.6 g in males and 53.9±4.5 g in females by 90 days. The mean haematological values were significantly ($p<0.05$) higher in adults than juveniles. However, there was no statistical difference of haematological values between the sexes. Cyclophosphamide treatment caused a macrocytic hypochromic anaemia, which was noted 24 hours after treatment and was more severe in animals treated more than once. Thus, in studies involving a disease that causes anaemia, repeated cyclophosphamide treatment should be limited. Our study is a contribution to the clinical and biological characterization of the disease pattern in this preferred rodent model of *T. b. gambiense*.

13709. **Magez, S., Radwanska, M., Drennan, M., Fick, L., Baral, T.N., Brombacher, F. & De Baetselier, P., 2006.** Interferon-gamma and nitric oxide in combination with antibodies are key protective host immune factors during *Trypanosoma congolense* Tc13 infections. *Journal of Infectious Diseases*, **193** (11): 1575-1583.

Laboratory of Cellular and Molecular Immunology, Department of Molecular and Cellular Recognition, Flanders Interuniversity Institute for Biotechnology, Vrije Universiteit Brussel, Brussels, Belgium.

The control of chronic *Trypanosoma congolense* trypanosomiasis was analyzed using several gene-deficient mouse strains. First, interferon (IFN)-gamma receptor (IFN-gamma-R)-deficient mice were used to show that IFN- gamma -mediated immune activation is crucial for parasitaemia control. Second, infections in major histocompatibility complex (MHC) class II-deficient mice indicate that this molecule is needed for initiation of IFN-gamma and subsequent tumour necrosis factor (TNF) production. Downstream of IFN-gamma-R signalling, inducible NO synthase (iNOS)-dependent trypanosome killing occurs, as is shown by the hypersusceptible phenotype of iNOS-deficient mice. Besides proinflammatory responses, B cells and, more specifically, immunoglobulin (Ig) G antibodies are crucial for parasite killing. Hence, parasitaemia control is abolished in B cell-deficient mice, whereas IgM-deficient mice control the infection as efficiently as do wild-type mice. In addition, splenectomized mice that have a normal IgM response but an impaired IgG2a/3 response fail to control *T. congolense* infection. Collectively, these results suggest that host protective immunity against *T. congolense* is critically dependent on the combined action of the proinflammatory mediators/effectors IFN- gamma , TNF, and NO and antiparasite IgGs.

13710. **Merschjohann, K. & Steverding, D., 2006**. *In vitro* growth inhibition of bloodstream forms of *Trypanosoma brucei* and *Trypanosoma congolense* by iron chelators. *Kinetoplastid Biology and Disease*, **5**: 3.

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African trypanosomes exert significant morbidity and mortality in man and livestock. Only a few drugs are available for the treatment of trypanosome infections and therefore, the development of new anti-trypanosomal agents is required. Previously it has been shown that bloodstream-form trypanosomes are sensitive to the iron cheater deferoxamine. In this study the effect of 13 iron cheaters on the growth of *Trypanosoma brucei*, *T. congolense* and human HL-60 cells was tested *in vitro*. With the exception of 2 compounds, all cheaters exhibited anti-trypanosomal activities, with 50 percent inhibitory concentration (IC50) values ranging between 2.1 - 220 muM. However, the iron cheaters also displayed cytotoxicity towards human HL-60 cells and therefore, only less favourable selectivity indices compared to commercially available drugs. Interfering with iron metabolism may be a new strategy in the treatment of trypanosome infections. More specifically, lipophilic iron-chelating agents may serve as lead compounds for novel anti-trypanosomal drug development.

13711. **Morales, I., de Leon, M., Morales, M., Dalla, F. & Gutierrez, C., 2006.** Ocular lesions associated with *Trypanosoma evansi* in experimentally infected goats. *Veterinary Parasitology*, **141** (3-4): 325-329.

Veterinary Faculty, University of Las Palmas, 35416 Arucas, Las Palmas, Canary Islands, Spain.

Ocular lesions associated with *Trypanosoma* spp. infection have been described in man and many animal species. However, loss of vision has not been demonstrated in humans presenting Chagas disease or in animals affected by different trypanosome species. In order to assess the possible ocular disorders caused by *Trypanosoma evansi* infection, six goats were inoculated with $1x10^5$ *T. evansi* and maintained for 12 months and four goats were used as control. The inoculated animals became positive at serological and parasitological tests at 1-month post-inoculation and showed a subclinical course of the disease. Unilateral superficial corneal ulceration and retinochoroiditis were observed in two inoculated animals. Data from ocular neurologic examination and electroretinography showed no significant differences between inoculated and non-inoculated goats. It could be concluded that *Trypanosoma evansi* can produce ocular lesion but without apparent loss of vision in goats.

13712. **Morty, R.E., Bulau, P., Pelle, R., Wilk, S. & Abe, K., 2006.** Pyroglutamyl peptidase type I from *Trypanosoma brucei*: a new virulence factor from African trypanosomes that de-blocks regulatory peptides in the plasma of infected hosts. *Biochemical Journal*, **394** (3): 635-645.

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Peptidases of parasitic protozoans are emerging as novel virulence factors and therapeutic targets in parasitic infections. A trypanosome-derived aminopeptidase that exclusively hydrolysed substrates with Glp (pyroglutamic acid) in P1 was purified 9248-fold from the plasma of rats infected with *Trypanosoma brucei brucei*. The enzyme responsible was cloned from a *T. brucei brucei* genomic DNA library and identified as type I PGP (pyroglutamyl peptidase), belonging to the C15 family of cysteine peptidases. We showed that PGP is expressed in all life cycle stages of *T. brucei brucei* and is expressed in four other bloodstream-form African trypanosomes. Trypanosome PGP was optimally active and stable at bloodstream pH, and was insensitive to host plasma cysteine peptidase inhibitors. Native purified and recombinant hyper-expressed trypanosome PGP removed the N-terminal Glp blocking groups from TRH (thyrotrophin-releasing hormone) and GnRH (gonadotropin-releasing hormone) with a k(cat)/K(m) value of 0.5 and 0.1 s(-1) x uM(-1) respectively. The half-life of TRH and GnRH was dramatically reduced in the plasma of trypanosome-infected rats, both *in vitro* and *in vivo*. Employing an activity-neutralizing anti-trypanosome PGP antibody, and pyroglutamyl diazomethyl ketone, a specific inhibitor of type I PGP, we demonstrated that trypanosome PGP is entirely responsible for the reduced plasma half-life of TRH, and partially responsible for the reduced plasma half-life of GnRH in a rodent model of African trypanosomiasis. The abnormal degradation of TRH and GnRH, and perhaps other

neuropeptides N-terminally blocked with a pyroglutamyl moiety, by trypanosome PGP, may contribute to some of the endocrine lesions observed in African trypanosomiasis.

13713. **Naessens, J., Kitani, H., Nakamura, Y., Yagi, Y., Sekikawa, K. & Iraqi, F., 2005**. TNF-a mediates the development of anaemia in a murine *Trypanosoma brucei rhodesiense* infection, but not the anaemia associated with a murine *Trypanosoma congolense* infection. *Clinical and Experimental Immunology,* **139**: 405-410.

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Development of anaemia in inflammatory diseases is cytokine-mediated. Specifically, the levels of tumour necrosis factor-alpha (TNF-alpha), produced by activated macrophages, are correlated with severity of disease and anaemia in infections and chronic disease. In African trypanosomiasis, anaemia develops very early in infection around the time when parasites become detectable in the blood. Since the anaemia persists after the first waves of parasitaemia when low numbers of trypanosomes are circulating in the blood, it is generally assumed that anaemia is not directly induced by a parasite factor, but might be cytokine-mediated, as in other cases of anaemia accompanying inflammation. To clarify the role of TNF-alpha in the development of anaemia, blood parameters of wild type (TNF-alpha+/+), TNF-alpha-null (TNF-alpha-/-) and TNF-alpha-hemizygous (TNF-alpha-/+) trypanotolerant mice were compared during infections with the cattle parasite *Trypanosoma congolense*. No differences in PCV, erythrocyte numbers or haemoglobin were observed between TNF-alpha-deficient and wild type mice, suggesting that the decrease in erythrocytes was not mediated by TNF-alpha. Erythropoetin (EPO) levels increased during infection and no significant differences in EPO levels were observed between the three mouse strains. In contrast, during an infection with the human pathogen *Trypanosoma brucei rhodesiense*, the number of red blood cells in TNF-alpha-deficient mice remained significantly higher than in the wild type mice. These data suggest that more than one mechanism promotes the development of anaemia associated with trypanosomiasis.

13714. **Nikolskaia, O.V., Kim, Y.V., Kovbasnjuk, O., Kim, K.J. & Grab, D.J., 2006**. Entry of *Trypanosoma brucei gambiense* into microvascular endothelial cells of the human blood-brain barrier. *International Journal for Parasitology,* **36** (5): 513-519.

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Using an *in vitro* model of the human blood-brain barrier consisting of human brain microvascular endothelial cells, we recently demonstrated that *Trypanosoma brucei gambiense* bloodstream-forms efficiently cross these cells via a paracellular route while *Trypanosoma brucei brucei* crosses these cells poorly. Using a combination of techniques that include fluorescence activated cell sorting, confocal and electron microscopy, we now show that some *T. b. gambiense* blood stream form parasites have the capacity to enter human brain microvascular endothelial cells. The intracellular location of the trypanosomes

was demonstrated in relation to the endothelial cell plasma membrane and to the actin cytoskeleton. These parasites may be a terminal stage within a lysosomal compartment or they may be viable trypanosomes that will be able to exit the brain microvascular endothelial cells. This process may provide an additional transcellular route by which the parasites cross the blood-brain barrier.

13715. **Nikolskaia, O.V., Kim, Y.V., Lonsdale-Eccles, J.D., Fukuma, T., Scharfstein, J. & Grab, D.J., 2006.** Blood-brain barrier traversal by African trypanosomes requires calcium signaling induced by parasite cysteine protease. *Journal of Clinical Investigation*, **116** (10): 2739-2747.

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In this study we investigated why bloodstream forms of *Trypanosoma brucei gambiense* cross human brain microvascular endothelial cells (BMECs), a human blood-brain barrier (BBB) model system, at much greater efficiency than do *T. b. brucei*. After noting that *T. b. gambiense* displayed higher levels of cathepsin L-like cysteine proteases, we investigated whether these enzymes contribute to parasite crossing. First, we found that *T. b. gambiense* crossing of human BMECs was abrogated by N-methylpiperazine-urea-phe-homopheylalanine-vinylsulfone-benzene (K11777), an irreversible inhibitor of cathepsin L-like cysteine proteases. Affinity labelling and immunochemical studies characterized brucipain as the K11777-sensitive cysteine protease expressed at higher levels by *T. b. gambiense.* K11777-treated *T. b. gambiense* failed to elicit calcium fluxes in BMECs, suggesting that generation of activation signals for the BBB is critically dependant on brucipain activity. Strikingly, crossing of *T. b. brucei* across the BBB was enhanced upon incubation with brucipain-rich supernatants derived from *T. b. gambiense*. The effects of the conditioned medium, which correlated with ability to evoke calcium fluxes, were cancelled by K11777, but not by the cathepsin B inhibitor CA074. Collectively, these *in vitro* studies implicate brucipain as a critical driver of *T. b. gambiense* transendothelial migration through the human BBB.

13716. **Nishimura, K., Yanase, T., Araki, N., Ohnishi, Y., Kozaki, S., Shima, K., Asakura, M., Samosomsuk, W. & Yamasaki, S., 2006**. Effects of polyamines on two strains of *Trypanosoma brucei* in infected rats and *in vitro* culture. *Journal of Parasitology*, **92** (2): 211-217.

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We studied the effects of polyamines, which are necessary for proliferation and antioxidation in *Trypanosoma brucei gambiense* Wellcome strain (WS) and *Trypanosoma brucei brucei* ILtat 1.4 strain (IL). No difference was found in activity of ornithine decarboxylase (ODC), a key enzyme in polyamine synthesis in trypanosomes, in both strains maintained *in vitro*; higher ($P < 0.05$) ODC values were found in IL *in vivo*. However, WS *in vivo* exhibited higher proliferation rates with higher spermidine content and decreased host

survival times than IL. The *in vitro* proliferation and polyamine contents of WS increased with the addition of polyamine to the 1-difluoromethylornithine culture medium, but not IL. These results suggested that WS uses extracellular polyamine for proliferation. In the *in vitro* culture, WS was less tolerant of hydrogen peroxide (oxidative stress) than IL, and malondialdehyde levels in WS were higher than in IL. The expression of trypanothione synthetase mRNA in WS *in vitro* was higher than in IL. These results suggest that IL is dependent on the synthesis of polyamines for proliferation and reduction of oxidative stress, whereas WS is dependent on the uptake of extracellular polyamines. A thorough understanding of the differences in the metabolic capabilities of various trypanosomes is important for the design of more effective medical treatments.

13717. **Oli, M.W., Cotlin, L.F., Shiflett, A.M. & Hajduk, S.L., 2006**. Serum resistance-associated protein blocks lysosomal targeting of trypanosome lytic factor in *Trypanosoma brucei*. *Eukaryotic Cell,* **5** (1): 132-139.

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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 (apoA-I), apolipoprotein L-I (apoL-I), and haptoglobin-related protein (Hpr) provides this innate protection against *T. b. brucei* infection. This HDL subfraction, called trypanosome lytic factor (TLF), kills *T. b. brucei* following receptor binding, endocytosis, and lysosomal localization. *Trypanosoma brucei rhodesiense*, which is morphologically and physiologically indistinguishable from *T. b. brucei*, is resistant to TLF-mediated killing and causes human African sleeping sickness. Human infectivity by *T. b. rhodesiense* correlates with the evolution of a resistance-associated protein (SRA) that is able to ablate TLF killing. To examine the mechanism of TLF resistance, we transfected *T. b. brucei* with an epitope-tagged SRA gene. Transfected *T. b. brucei* expressed SRA mRNA at levels comparable to those in *T. b. rhodesiense* and was highly resistant to TLF. In the SRA-transfected cells, intracellular trafficking of TLF was altered, with TLF being mainly localized to a subset of SRA-containing cytoplasmic vesicles but not to the lysosome. These results indicate that the cellular distribution of TLF is influenced by SRA expression and may directly determine the organism's susceptibility to TLF.

13718. **Orhue, N.N., Nwanze, E.A.C., & Okafor, A., 2005**. Serum total protein, albumin and globulin levels in *Trypanosoma brucei*-infected rabbits: Effect of orally administered *Scoparia dulcis*. *African Journal of Biotechnology,* **4** (10): 1152-1155.

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The effects of orally administered *Scoparia dulcis* on *Trypanosoma brucei*-induced changes in serum total protein, albumin and globulin were investigated in rabbits over a period of twenty eight days. Results obtained show that infection resulted in

hyperproteinaemia, hyperglobulinaemia and hypoalbuminaemia. However these lesions were less severe ($p < 0.05$) in the infected and treated group relative to their untreated counterparts. We speculate that the herb may be involved in modulating the severity of these trypanosome associated lesions by some yet undefined mechanisms.

13719. **Ouwe-Missi-Oukem-Boyer, O., Mezui-Me-Ndong, J., Boda, C., Lamine, I., Labrousse, F., Bisser, S. & Bouteille, B., 2006**. The vervet monkey (*Chlorocebus aethiops*) as an experimental model for *Trypanosoma brucei gambiense* human African trypanosomiasis: a clinical, biological and pathological study. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **100** (5): 427-436.

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It has long been known that the vervet monkey, *Chlorocebus (C.) aethiops*, can be infected with *Trypanosoma rhodesiense*, but this model has not been described for *T. gambiense*. In this study, we report the development of such a model for human African trypanosomiasis. Twelve vervet monkeys infected with *T. gambiense* developed chronic disease. The duration of the disease ranged between 23 and 612 days (median 89 days) in five untreated animals. Trypanosomes were detected in the blood within the first 10 days post-infection and in the cerebrospinal fluid, with a median delay of 120 days ($n = 4$, range 28-348 days). Clinical changes included loss of weight, adenopathy, and in some cases eyelid oedema and lethargy. Haematological alterations included decreases in haemoglobin level and transitory decreases in platelet count. Biological modifications included increased gamma globulins and total proteins and decreased albumin. Pathological features of the infection were presence of Mott's cells, inflammatory infiltration of either mononuclear cells or lymphocytes and plasma cells in the brain parenchyma, and astrocytosis. These observations indicate that the development of the disease in vervet monkeys is similar to human *T. gambiense* infection. We conclude that *C. aethiops* is a promising experimental primate model for the study of *T. gambiense* trypanosomiasis.

13720**. Pan, W., Ogunremi, O., Wei, G., Shi, M. & Tabel, H., 2006**. CR3 (CD11b/CD18) is the major macrophage receptor for IgM antibody-mediated phagocytosis of African trypanosomes: Diverse effect on subsequent synthesis of tumor necrosis factor [alpha] and nitric oxide. *Microbes and Infection,* **8** (5): 1209-1218.

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Immunoglobulin M (IgM) antibodies to the variant surface glycoproteins (VSG) of African trypanosomes are the first and predominant class of anti-trypanosomal antibodies in the infected host. They are a major factor in controlling waves of parasitaemia, but not in long-term survival. The macrophage receptor(s) that enables phagocytosis of IgM anti-VSG-coated African trypanosomes is unknown. We assessed whether complement receptor CR3 (CD11b/CD18) might be involved in mediating phagocytosis of *Trypanosoma congolense*.

We show that murine complement C3 fragments are deposited onto *T. congolense* when the trypanosomes are incubated with IgM anti-VSG and fresh mouse serum. In the presence of fresh mouse serum, there is significantly and markedly less phagocytosis of IgM-opsonized *T. congolense* by CD11b-deficient macrophages compared to phagocytosis by wild-type macrophages (78 percent fewer *T. congolense* are ingested per macrophage). Significantly less tumour necrosis factor (TNF)-[alpha] (38 percent less), but significantly more nitric oxide (NO) (63 percent more) are released by CD11b-deficient macrophages that have engulfed trypanosomes than by equally treated wild-type macrophages. We conclude that CR3 is the major, but not the only, receptor involved in IgM anti-VSG-mediated phagocytosis of *T. congolense* by macrophages. We further conclude that IgM anti-VSG-mediated phagocytosis of *T. congolense* enhances synthesis of disease-producing TNF-[alpha] and inhibits synthesis of parasite-controlling NO. We suggest that signalling of inhibition of NO synthesis is mediated via CR3.

13721**. Pays, E., 2006**. The variant surface glycoprotein as a tool for adaptation in African trypanosomes. *Microbes and Infection,* **8** (3): 930-937.

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African trypanosomes (*Trypanosoma brucei*) are flagellated protozoan parasites that infect a wide variety of mammals, causing nagana in cattle and sleeping sickness in humans. These organisms can cause prolonged chronic infections due to their ability to successively expose different antigenic variants of the variant surface glycoprotein (VSG). The genomic loci where the VSG genes are expressed are telomeric and contain polycistronic transcription units with several genes that are involved in adaptation of the parasite to the host. At least three of these genes, which respectively encode the two subunits of the heterodimeric receptor for transferrin and a protein conferring resistance to the human trypanolytic factor apolipoprotein L-I, share the same origin as the VSG. The high recombination potential of the telomeric VSG expression sites, coupled to their dynamic mono-allelic expression control, provides trypanosomes with a powerful capacity for adaptation to their hosts.

13722. **Pays, E., Vanhollebeke, B., Vanhamme, L., Paturiaux-Hanocq, F., Nolan, D.P. & Perez-Morga, D., 2006.** The trypanolytic factor of human serum. *Nature Reviews Microbiology*, 4 (6): 477-486.

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African trypanosomes (the prototype of which is *Trypanosoma brucei brucei*) are protozoan parasites that infect a wide range of mammals. Human blood, unlike the blood of other mammals, has efficient trypanolytic activity, and this needs to be counteracted by these parasites. Resistance to this activity has arisen in two subspecies of Trypanosoma brucei - *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* - allowing these parasites to infect humans, and this results in sleeping sickness in East Africa and West Africa, respectively. Study of the mechanism by which *T. b. rhodesiense* escapes lysis by

human serum led to the identification of an ionic-pore-forming apolipoprotein - known as apolipoprotein L1 - that is associated with high-density-lipoprotein particles in human blood. In this article, we argue that apolipoprotein L1 is the factor that is responsible for the trypanolytic activity of human serum.

13723. **Penchenier, L., Alhadji, D., Bahebegue, S., Simo, G., Laveissiere, C. & Cuny, G., 2005**. Spontaneous cure of domestic pigs experimentally infected by *Trypanosoma brucei gambiense*. Implications for the control of sleeping sickness. *Veterinary Parasitology,* **133** (1): 7-11.

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The existence of a pig reservoir for human African trypanosomosis (HAT) due to *Trypanosoma brucei gambiense* complicates the fight against this disease. This study reports results obtained from pigs, which were inoculated with the blood of a person, suffering from HAT in Cameroon. The pigs were reared and kept in the shelter from all contact with *Glossina*, and monitored for 188 days. The seroconversion was checked by agglutination assays for trypanosomosis (CATT 1.3 and LATEX/*T.b.gambiense*). The parasitaemia was measured by quantitative buffy coat method (QBC) and by polymerase chain reaction method (PCR). In addition, growth was recorded as well as blood counting and blood formulas. The results showed that the pigs were trypanotolerant and cure themselves in less than 6 months. It is concluded that sterilisation of this reservoir could be achieved by tsetse-control measures in 1 year. It confirms the strategy to complement screening and treatment of HAT with tsetse fly control measures.

13724. **Semballa, S., Okomo-Assoumou, M.C., Holzmuller, P., Buscher, P., Magez, S., Lemesre, J.L., Daulouede, S., Courtois, P., Geffard, M. & Vincendeau, P., 2006.** Identification of a tryptophan-like epitope borne by the variable surface glycoprotein (VSG) of African trypanosomes. *Experimental Parasitology.* **In press; corrected proof).**

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Antibodies (Ab) directed against a tryptophan-like epitope (WE) were previously detected in patients with human African trypanosomiasis (HAT). We investigated whether or not these Ab resulted from immunization against trypanosome antigen(s) expressing a WE. By Western blotting, we identified an antigen having an apparent molecular weight ranging from 60 to 65kDa, recognized by purified rabbit anti-WE Ab. This antigen, present in trypomastigote forms, was absent in procyclic forms and *Trypanosoma cruzi* trypomastigotes. Using purified variable surface glycoproteins (VSG) from various trypanosomes, we showed that VSG was the parasite antigen recognized by these rabbit Ab. Anti-WE and anti-VSG Ab were purified from HAT sera by affinity chromatography. Immunoreactivity of purified antibodies eluted from affinity columns and of depleted fractions showed that WE was one of the epitopes borne by VSG. These data underline the existence of an invariant WE in the structure of VSG from several species of African trypanosomes.

13725. **Shi, M.W., G.J., Pan, W.L., Tabel, H., 2005**. Impaired Kupffer cells in highly susceptible mice infected with *Trypanosoma congolense*. *Infection and Immunity,* **73** (12): 8393-8396.

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In highly susceptible BALB/c mice infected with *Trypanosoma congolense*, the total number of Kupffer cells in the liver remains constant; however, their mean size increases fivefold towards the terminal stage. About 25 percent of Kupffer cells undergo apoptosis. We suggest that development of an impairment of the macrophage system might be a major mechanism for inefficient elimination of trypanosomes.

13726. **Shi, M., Wei, G., Pan, W. & Tabel, H., 2006**. Experimental African trypanosomiasis: a subset of pathogenic, IFN- gamma -producing, MHC class II-restricted CD4+ T cells mediates early mortality in highly susceptible mice. *Journal of Immunology*, **176** (3):1724-32.

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Infections of highly susceptible BALB/c mice with virulent strains of *Trypanosoma congolense* or *Trypanosoma brucei* result in rapid death (8 days). We have previously shown that this mortality is IFN- gamma dependent. In this study we show that IFN- gamma is produced predominantly by CD3+Thy1.2+TCR beta +CD4+ T cells shortly before the death of infected mice. Mortality may therefore be dependent on IFN- gamma -producing CD4+ T cells. Surprisingly, infected CD4+/+ and CD4-/- BALB/c mice have similar parasitaemia and survival time. In infected CD4-/- mice, the production of both IFN- gamma and IL-10 is very low, suggesting that both cytokines are predominantly produced by CD4+ T cells and that the outcome of the disease might depend on the balance of their effects. Infected BALB/c mice partially depleted of CD4+ T cells or MHC class II function have lower parasitaemia and survive significantly longer than infected normal BALB/c mice or infected BALB/c mice whose CD4+ T cells are fully depleted. Partial depletion of CD4+ T cells markedly reduces IFN- gamma secretion without a major effect on the production of IL-10 and parasite-specific IgG2a Abs. Based on our previous and current data, we conclude that a subset of a pathogenic, MHC class II-restricted CD4+ T cells (Tp cells), activated during the course of *T. congolense* infection, mediates early mortality in infected BALB/c mice via excessive synthesis of IFN- gamma. IFN- gamma , in turn, exerts its pathological effect by enhancing the cytokine release syndrome of the macrophage system activated by the phagocytosis of parasites. We speculate that IL-10-producing CD4+ T cells might counteract this effect.

13727. **Shiflett, A.M., Bishop, J.R., Pahwa, A. & Hajduk, S.L., 2005**. Human high density lipoproteins are platforms for the assembly of multi-component innate immune complexes. *Journal of Biological Chemistry*, **280** (38): 32578-32585.

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Human innate immunity to non-pathogenic species of African trypanosomes is provided by human high density lipoprotein (HDL) particles. Here we show that native human HDLs containing haptoglobin-related protein (Hpr), apolipoprotein L-I (apoL-I) and apolipoprotein A-I (apoA-I) are the principal antimicrobial molecules providing protection from trypanosome infection. Other HDL subclasses containing either apoA-I and apoL-I or apoA-I and Hpr have reduced trypanolytic activity, whereas HDL subclasses lacking apoL-I and Hpr are non-toxic to trypanosomes. Highly purified, lipid-free Hpr and apoL-I were both toxic to *Trypanosoma brucei brucei* but with specific activities at least 500-fold less than those of native HDLs, suggesting that association of these apolipoproteins within the HDL particle was necessary for optimal cytotoxicity. These studies show that HDLs can serve as platforms for the assembly of multiple synergistic proteins and that these assemblies may play a critical role in the evolution of primate-specific innate immunity to trypanosome infection.

13728. **Suzuki, T., Ueta, Y.Y., Inoue, N., Xuan, X., Saitoh, H. & Suzuki, H., 2006.** Beneficial effect of erythropoietin administration on murine infection with *Trypanosoma congolense*. *American Journal of Tropical Medicine and Hygiene*, **74** (6): 1020-1025.

Research Unit for Functional Genomics, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.

The effect of erythropoietin treatment on *Trypanosoma congolense* infection in mice was studied. Survival rates of mice were dramatically improved by treatment with recombinant human erythropoietin (r-hu-EPO; 5,000 U/kg) when infected with 1,000 cells of *T. congolense* IL3000 ($P < 0.05$). All the untreated mice infected with *T. congolense* IL3000 died by day 9 of infection; however, 100 percent, 50 percent, and 25 percent of the mice treated with r-hu-EPO for 8 days survived to day 20, day 40, and day 60 of the parasitical infection, respectively. Anti-8-hydroxy-2'-deoxyguanosine antibody, a biomarker for oxidative damage of DNA, yielded positive reactions in the cytoplasm of the parasites recovered from the mice treated with r-hu-EPO. These results, taken together, indicate that erythropoietin administration is effective for the treatment of *T. congolense* infection.

13729. **Tsujimura, Y., Watarai, S., Uemura, A., Ohnishi, Y. & Kodama, H., 2005**. Effect of anti-ganglioside antibody in experimental *Trypanosoma brucei* infection in mice. *Research in Veterinary Science,* **78** (3): 245-247.

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The effect of antibody against ganglioside antigen on *Trypanosoma brucei* parasites was examined *in vitro* and *in vivo* using anti-ganglioside GM1 (AGM-1) monoclonal

antibody. The antibody showed complement-dependent cytotoxicity against *T. brucei* with mouse complement. Furthermore, mice given AGM-1 were challenged intraperitoneally with *T. brucei*. Although all non-treated control mice died within six days after infection, all of AGM-1-injected mice had survived by six days post-infection. These data suggest that antibody against ganglioside antigen on *T. brucei* has potential in protection against *T. brucei* infection.

13730 **Utz, S., Roditi, I., Kunz Renggli, C., Almeida, I.C., Acosta-Serrano, A. & Butikofer, P., 2006.** *Trypanosoma congolense* procyclins: unmasking cryptic major surface glycoproteins in procyclic forms. *Eukaryotic Cell*, **5** (8): 1430-1440.

Institute of Biochemistry & Molecular Medicine, University of Bern, Bern, Switzerland.

In the tsetse fly, the protozoan parasite *Trypanosoma congolense* is covered by a dense layer of glycosylphosphatidylinositol (GPI)-anchored molecules. These include a protease-resistant surface molecule (PRS), which is expressed by procyclic forms early in infection, and a glutamic acid- and alanine-rich protein (GARP), which appears at later stages. Since neither of these surface antigens is expressed at intermediate stages, we investigated whether a GPI-anchored protein of 50 to 58 kDa, previously detected in procyclic culture forms, might constitute the coat of these parasites. We therefore partially purified the protein from *T. congolense* Kilifi procyclic forms, obtained an N-terminal amino acid sequence, and identified its gene. Detailed analyses showed that the mature protein consists almost exclusively of 13 heptapeptide repeats (EPGENGT). The protein is densely N glycosylated, with up to 13 high-mannose oligosaccharides ranging from Man(5)GlcNAc(2) to Man(9)GlcNAc(2) linked to the peptide repeats. The lipid moiety of the glycosylphosphatidylinositol is composed of sn-1-stearoyl-2-lyso-glycerol-3-HPO (4)-1-(2-O-acyl)-d-myo-inositol. Heavily glycosylated proteins with similar repeats were subsequently identified in *T. congolense* Savannah procyclic forms. Collectively, this group of proteins was named *T. congolense* procyclins to reflect their relationship to the EP and GPEET procyclins of *T. brucei*. Using an antiserum raised against the EPGENGT repeat, we show that *T. congolense* procyclins are expressed continuously in the fly midgut and thus form the surface coat of cells that are negative for both PRS and GARP.

13731**. Valera, Z., Parra, O., Alvarado, M., Barboza, G., Escalona, F. & Ramírez, R., 2005**. Effect of experimental *Trypanosoma vivax* infection on hematological parameters of sheep. *Revista Cientifica, Facultad de Ciencias Veterinarias, Universidad del Zulia,* **15** (5): 412-420.

Facultad de Ciencias Veterinarias, Universidad del Zulia, Maracaibo, Edo. Zulia, Venezuela.

Eight crossbred West African sheep aged 6 months to one year were randomly divided into two groups. The experimental sheep (n=4) were inoculated intravenously with 2 ml of fresh blood containing 1.3×10^6 trypanosomes/ml from a positive ewe. The remaining four animals served as controls. The effects of the parasite on body temperature, parasitaemia, haematocrit, haemoglobin, leukocytes and total serum proteins were evaluated for 90 days.

All the infected animals were positive for the parasite 2 days post-inoculation, developing undulating parasitaemia and severe anaemia which persisted until the end of the experiment. The course of experimental infection with *T. vivax* showed two phases. The first phase was observed during the first four weeks post-infection, where the infected sheep developed high levels of parasitaemia and decreased ($P<0.005$) haematocrit and haemoglobin level and total leukocyte count. The second phase was evident from the fifth week post-infection. During this period, the infected animals showed levels of parasitaemia lower than the preceding phase, remittent fever, persistence of low haematocrit and haemoglobin values, as well as, significant increase ($P<0.05$) in serum proteins. The changes in haematological parameters and body temperature were related to the appearance of trypanosomes in the circulation and to the intensity of parasitaemia.

(c) CHEMOTHERAPEUTICS

[See also **29**: 13615]

13732. **Anene, B.E., Ezeokonkwo, R.C., Mmesirionye, T.I., Tettey, J.N.A., Brock, J.M., Barrett, M.P. & De Koning, H.P., 2006**. A diminazene-resistant strain of *Trypanosoma brucei brucei* isolated from a dog is cross-resistant to pentamidine in experimentally infected albino rats. *Parasitology,* **132** (1): 127-133.

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Trypanosomosis is a major cause of mortality for dogs in Nigeria and treatment with diminazene aceturate has steadily become less effective, either as a result of low quality of the locally available diminazene preparations or of drug resistance. To investigate these alternatives, samples of locally obtained drugs were analysed for diminazene aceturate content and a strain of *Trypanosoma brucei brucei* was isolated from a diminazene-refractory dog in Nsukka, south-eastern Nigeria, and used to infect albino rats. The quality of diminazene aceturate-based preparations was variable, with two preparations containing less than 95 percent of the stated active compound. Rats infected with *T. brucei* isolated from the dog were treated 7 and 10 days after infection either with 7 mg/kg diminazene aceturate (intraperitoneally, once) or with 4 mg/kg pentamidine isethionate (intramuscularly, 7 consecutive days). Relapse rates were 100 percent for both trypanocides in the groups of rat treated 10 days post-infection, and 83 percent and 50 percent of rats treated 7 days after infection relapsed to diminazene aceturate and pentamidine isethionate, respectively. Careful consideration of physiological parameters showed that pentamidine was only marginally superior to diminazene aceturate as applied in this study. It was concluded that dogs in Nigeria are infected with genuinely diminazene aceturate-resistant trypanosomes that appear to be cross-resistant to pentamidine isethionate.

13733. **Ariyanayagam, M.R.O., S. L., Guther, M. L. S. & Fairlamb, A. H., 2005**. Phenotypic analysis of trypanothione synthetase knockdown in the African trypanosome. *Biochemical Journal*, **391** (2): 425-432.

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Trypanothione plays a pivotal role in defence against chemical and oxidant stress, thiol redox homoeostasis, ribonucleotide metabolism and drug resistance in parasitic kinetoplastids. In *Trypanosoma brucei*, trypanothione is synthesized from glutathione and spermidine by a single enzyme, TryS (trypanothione synthetase), with glutathionylspermidine as an intermediate. To examine the physiological roles of trypanothione, tetracycline-inducible RNA interference was used to reduce expression of TryS. Following induction, TryS protein was reduced >10-fold and growth rate was reduced 2-fold, with concurrent 5-10-fold decreases in glutathionylspermidine and trypanothione and an up to 14-fold increase in free glutathione content. Depleted trypanothione levels were associated with increases in sensitivity to arsenical, antimonial and nitro drugs, implicating trypanothione metabolism in their mode of action. Escape mutants arose after 2 weeks of induction, with all parameters, including growth, returning to normal. Selective inhibitors of TryS are required to fully validate this novel drug target.

13734. **Athri, P., Wenzler, T., Ruiz, P., Brun, R., Boykin, D.W., Tidwell, R. & Wilson, W.D., 2006**. 3D QSAR on a library of heterocyclic diamidine derivatives with antiparasitic activity. *Bioorganic and Medicinal Chemistry,* **14** (9): 3144-3152.

Department of Chemistry, Georgia State University, Atlanta, GA 30303, USA.

African trypanosomes, *Trypanosoma brucei rhodesiense* (TBR) and *Trypanosoma brucei gambiense* (TBG), affect hundreds of thousands of lives in tropical regions of the world. The toxicity of the diamidine pentamidine, an effective drug against TBG, necessitates the design of better drugs. An orally effective prodrug of the diamidine, furamidine (DB75), presently scheduled for phase III clinical trials, has excellent activity against TBG with toxicity lower than that of pentamidine. As part of an effort to develop additional and improved diamidines against African trypanosomes, 3D QSAR analyses have been conducted with furamidine and a set of 25 other structurally related compounds. The results have been used as a guide to design compounds that potentially have better activity against African trypanosomes.

13735**. Azema, L., Lherbet, C., Baudoin, C. & Blonski, C., 2006**. Cell permeation of a *Trypanosoma brucei* aldolase inhibitor: Evaluation of different enzyme-labile phosphate protecting groups. *Bioorganic and Medicinal Chemistry Letters,* **16** (13): 3440-3.

Laboratoire SPCMIB, Groupe de Chimie Organique Biologique, Université Paul Sabatier UMR CNRS 5068, 118 route de Narbonne, 31062 Toulouse Cedex 4, France.

13736. **Barrett, M.P. & Gilbert, I.H., 2006.** Targeting of toxic compounds to the Trypanosome's interior. *Advances in Parasitology*, **63**: 125-183.

Division of Infection & Immunity, Institute of Biomedical and Life Sciences, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow G12 8QQ, UK; University of Dundee, School of Life Sciences, MSI/WTB/CIR Complex, Dow Street, Dundee, DD1 5EH, UK.

Drugs can be targeted into African trypanosomes by exploiting carrier proteins at the surface of these parasites. This has been clearly demonstrated in the case of the melamine-based arsenical and the diamidine classes of drug that are already in use in the treatment of human African trypanosomiasis. These drugs can enter via an aminopurine transporter, termed P2, encoded by the TbAT1 gene. Other toxic compounds have also been designed to enter via this transporter. Some of these compounds enter almost exclusively through the P2 transporter, and hence loss of the P2 transporter leads to significant resistance to these particular compounds. It now appears, however, that some diamidines and melaminophenylarsenicals may also be taken up by other routes (of yet unknown function). These too may be exploited to target new drugs into trypanosomes. Additional purine nucleoside and nucleobase transporters have also been subverted to deliver toxic agents to trypanosomes. Glucose and amino acid transporters too have been investigated with a view to manipulating them to carry toxins into *Trypanosoma brucei*, and recent work has demonstrated that aquaglyceroporins may also have considerable potential for drug-targeting. Transporters, including those that carry lipids and vitamins such as folate and other pterins also deserve more attention in this regard. Some drugs, for example suramin, appear to enter via routes other than plasma-membrane-mediated transport. Receptor-mediated endocytosis has been proposed as a possible way in for suramin. Endocytosis also appears to be crucial in targeting natural trypanocides, such as trypanosome lytic factor (TLF) (apolipoprotein L1), into trypanosomes and this offers an alternative means of selectively targeting toxins to the trypanosome's interior. Other compounds may be induced to enter by increasing their capacity to diffuse over cell membranes; in this case depending exclusively on selective activity within the cell rather than selective uptake to impart selective toxicity. This review outlines studies that have aimed to exploit trypanosome nutrient uptake routes to selectively carry toxins into these parasites.

13737. **Baral, T.N., Magez, S., Stijlemans, B., Conrath, K., Vanhollebeke, B., Pays, E., Muyldermans, S. & De Baetselier, P., 2006.** Experimental therapy of African trypanosomiasis with a nanobody-conjugated human trypanolytic factor. *Nature Medicine*, **12** (5): 580-584.

Department of Cellular and Molecular Interactions, Vlaams Interuniversitair Instituut voor Biotechnologie, Laboratorium voor Cellulaire en Moleculaire Immunologie, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussel, Belgium.[tbaral@vub.ac.be]

High systemic drug toxicity and increasing prevalence of drug resistance hampers efficient treatment of human African trypanosomiasis (HAT). Hence, development of new highly specific trypanocidal drugs is necessary. Normal human serum (NHS) contains apolipoprotein L-I (apoL-I), which lyses African trypanosomes except resistant forms such as *Trypanosoma brucei rhodesiense*. *T. b. rhodesiense* expresses the apoL-I-neutralizing serum resistance-associated (SRA) protein, endowing this parasite with the ability to infect humans

and cause HAT. A truncated apoL-I (Tr-apoL-I) has been engineered by deleting its SRA-interacting domain, which makes it lytic for *T. b. rhodesiense*. Here, we conjugated Tr-apoL-I with a single-domain antibody (nanobody) that efficiently targets conserved cryptic epitopes of the variant surface glycoprotein (VSG) of trypanosomes to generate a new manmade type of immunotoxin with potential for trypanosomiasis therapy. Treatment with this engineered conjugate resulted in clear curative and alleviating effects on acute and chronic infections of mice with both NHS-resistant and NHS-sensitive trypanosomes.

13738. **Bazin, M.A., Loiseau, P.M., Bories, C., Letourneux, Y., Rault, S. & El Kihel, L., 2006.** Synthesis of oxysterols and nitrogenous sterols with antileishmanial and trypanocidal activities. *European Journal of Medical Chemistry*, **41** (10): 1109-1116.

Centre d'Études et de Recherche sur le Medicament de Normandie, UFR des Sciences Pharmaceutiques, 5, rue Vaubenard, 14032 Caen cedex, France.

13739. **Berger, H., Seebacher, W., Saf, R., Kaiser, M., Brun, R. & Weis, R., 2006**. Antiprotozoal activities of new bis-chlorophenyl derivatives of bicyclic octanes and aza-nonanes. *Bioorganic and Medicinal Chemistry Letters*, **16** (20): 5457-5461.

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens-University, Universitatsplatz 1, A-8010 Graz, Austria.

13740. **Bi, X., Lopez, C., Bacchi, C.J., Rattendi, D. & Woster, P.M., 2006.** Novel alkylpolyaminoguanidines and alkylpolyaminobiguanides with potent antitrypanosomal activity. *Bioorganic and Medicinal Chemistry Letters,* **16** (12): 3229-3232.

Department of Pharmaceutical Sciences, Wayne State University, 259 Mack Ave, Detroit, MI 48202, USA.

13741. **Bizimana, N., Tietjen, U., Zessin, K.-H., Diallo, D., Djibril, C., Melzig, M.F. & Clausen, P.-H., 2006**. Evaluation of medicinal plants from Mali for their *in vitro* and *in vivo* trypanocidal activity. *Journal of Ethnopharmacology,* **103** (3): 350-356.

Institute for Parasitology und International Animal Health, Freie Universitat Berlin, Konigsweg 67, D-l4163 Berlin, Germany.

13742. **Boda, C.E., Enanga, B., Courtioux, B., Breton, J.C. & Bouteille, B, 2006**. Trypanocidal activity of methylene blue: Evidence for *in vitro* efficacy and *in vivo* failure. *Chemotherapy*, **52** (1): 16-19.

UPRES-EA 3174 Neuroparasitologie et Neuroépidémiologie tropicale, Institut d'Épidémiologie neurologique et de Neurologie tropicale, Faculté de Médecine, Limoges, France.

13743. **Bosch, J., Robien, M.A., Mehlin, C., Boni, E., Riechers, A., Buckner, F.S., Van Voorhis, W.C., Myler, P.J., Worthey, E.A., DeTitta, G., Luft, J.R., Lauricella, A., Gulde, S., Anderson, L.A., Kalyuzhniy, O., Neely, H.M., Ross, J., Earnest, T.N., Soltis, M., Schoenfeld, L., Zucker, F., Merritt, E.A., Fan, E., Verlinde, C.L. & Hol, W.G., 2006.** Using fragment cocktail crystallography to assist inhibitor design of *Trypanosoma brucei* nucleoside 2-deoxyribosyltransferase. *Journal of Medicinal Chemistry*, **49** (20): 5939-5946.

Department of Biochemistry, Division of Infectious Disease, and Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195, USA.

13744. **Braddock, M., 2006.** Overcoming resistance with designer immunotoxins. *Expert Opinion on Pharmacotherapy*, **7** (10): 1409-1412.

Discovery Bioscience, AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, Leicestershire, LE11 5RH, UK. [martin.braddock@astrazeneca.com]

Normal human serum contains apolipoprotein L-I (apoL-I), which lyses African trypanaosomes. Resistant forms, such as *Trypanosoma brucei rhodesiense* express apoL-I-neutralising serum resistance-associated protein, which enables this parasite to infect humans and cause human African trypanosomiasis. This paper describes the construction of a mutant apoL-I conjugated to a nanobody that targets the variant surface glycoprotein of trypanosomes. Treatment with this engineered immunotoxin has resulted in both alleviating and curative effects on chronic and acute infections of mice with normal human serum-resistant and -sensitive trypanosomes.

13745. **Brigotti, M., Alfieri, R.R., Petronini, P.G. & Carnicelli, D., 2006.** Inhibition by suramin of protein synthesis *in vitro*. Ribosomes as the target of the drug. *Biochimie*, **88** (5): 497-503.

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Suramin, a drug widely used both as a therapeutic agent and in research, inhibits translation in eukaryotic cell-free systems from rabbit reticulocyte lysate (IC (50)=142-241 uM). Suramin affects both initiation (block of 43S pre-initiation complex formation) and elongation (impairment of poly (U) translation). The drug induces an increase in the pools of ribosomal subunits and the formation of high molecular weight ribosomal complexes, thus causing the disappearance of polysomes. Ribosomes isolated from suramin-treated translating mixtures are inactivated. [^{3}H]Suramin binds to ribosomes and to isolated 60S and 40S ribosomal subunits (116, 106 and 3 binding sites, respectively) showing higher affinity for the small subunit (K (d)=2 uM).

13746. **Chackal-Catoen, S., Miao, Y., Wilson, W.D., Wenzler, T., Brun, R. & Boykin, D.W., 2006.** Dicationic DNA-targeted antiprotozoal agents: naphthalene replacement of benzimidazole. *Bioorganic and Medicinal Chemistry*, **14** (22): 7434-7445.

Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30303-3083, USA.

13747. **Dardonville, C., Barrett, M.P., Brun, R., Kaiser, M., Tanious, F. & Wilson, W.D., 2006.** DNA binding affinity of bisguanidine and bis (2-aminoimidazoline) derivatives with *in vivo* antitrypanosomal activity. *Journal of Medicinal Chemistry*, **49** (12): 3748-3752.

Instituto de Química Médica, CSIC, Juan de la Cierva, 3, 28006 Madrid, Spain. [dardonville@iqm.csic.es]

A new antitrypanosomal hit compound that cures an acute (STIB 900) mouse model of *Trypanosoma brucei rhodesiense* trypanosomiasis is described. This bis (2-aminoimidazolinium) dicationic compound proved to be an excellent DNA minor groove binder, suggesting a possible mechanism for its trypanocidal activity. From these studies, the 4, 4'-diaminodiphenylamine skeleton emerged as a good scaffold for antitrypanosomal drugs.

13748**. Deterding, A., Dungey, F.A., Thompson, K.A. & Steverding, D., 2005**. Anti-trypanosomal activities of DNA topoisomerase inhibitors. *Acta Tropica,* **93** (3): 311-316.

School of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG, UK.

Only four drugs are available for chemotherapy of human African sleeping sickness with undesirable toxic side effects. The development of new anti-trypanosomal drugs is therefore urgently required. In this study, 15 DNA topoisomerase inhibitors, including approved anti-cancer drugs, were tested for *in vitro* activity against bloodstream forms of *Trypanosoma brucei* and human leukaemia HL-60 cells. All compounds exhibited anti-trypanosomal activity, with ED50 values ranging between 3 nM and 30 uM, and MIC values between 100 nM and >100 uM. The trypanocidal activities of the most effective DNA topoisomerase inhibitors, aclarubicin, doxorubicin and mitoxantrone, were comparable with those of commercial anti-trypanosomal drugs. These data support the use of DNA topoisomerase inhibitors as lead compounds for anti-trypanosomal drug development.

13749. **Egbe-Nwiyi, T.N., Igbokwe, I.O. & Onyeyili, P.A., 2005**. Diminazene aceturate resistance on the virulence of *Trypanosoma brucei* for rats. *Journal of Comparative Pathology,* **133** (4): 286-288.

Department of Veterinary Physiology and Pharmacology, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State, Nigeria.

13750. **Espinoza-Fonseca, L.M. & Trujillo-Ferrara, J.G., 2006.** Toward a rational design of selective multi-trypanosomatid inhibitors: A computational docking study. *Bioorganic and Medicinal Chemistry Letters*, **16** *(24): 6288-6292.*

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA; Escuela Superior de Medicina del Instituto Politécnico Nacional, Apartado Postal 42-161, C.P. 11340, México City, México; Departamento de Bioquímica, Escuela Superior de Medicina del Instituto Politécnico Nacional, Apartado Postal 42-161, C.P. 11340, Mexico City, México.

Compound V7, a benzothiazole which was recently found as selective inhibitor of trypanosomal TIMs, was docked into TIMs from *Trypanosoma cruzi, Trypanosoma brucei, Entamoeba histolytica, Plasmodium falciparum*, yeast, and human. Structural analyses revealed the importance of the accessibility to the two aromatic clusters located at the dimer's interface for the selective inhibition of trypanosomal TIMs. Thus, it was found that different accessibilities of the protein interface of TIMs play an important role in the inhibitory activity of benzothiazoles. These findings will contribute to the rational development and improvement of benzothiazoles to be used as multi-trypanosomatid inhibitors.

13751**. Flores-Holguin, N. & Glossman-Mitnik, D., 2005**. CHIH-DFT determination of the electrical, optical, and magnetic properties and NICS aromaticity of megazol. *Journal of Molecular Structure,* **717** (1-3): 1-3.

Grupo de Química Computacional, Simulación y Modelado Molecular and Programa Institucional de Nanotecnología, CIMAV, S.C., Miguel de Cervantes 120, Complejo Industrial Chihuahua, Chihuahua, Chih. 31109, México.

13752. **Fujii, N., Mallari, J.P., Hansell, E.J., Mackey, Z., Doyle, P., Zhou, Y.M., Gut, J., Rosenthal, P.J., McKerrow, J.H. & Guy, R.K., 2005**. Discovery of potent thiosemicarbazone inhibitors of rhodesain and cruzain. *Bioorganic and Medicinal Chemistry Letters,* **15** (1): 121-123.

Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, USA.

13753. **Gonzalez-Rey, E., Chorny, A. & Delgado, M., 2006.** VIP: an agent with license to kill infective parasites. *Annals of the New York Academy of Science*, **1070**: 303-308.

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Antimicrobial peptides are small, cationic, and amphipathic peptides of variable length, sequence, and structure. They are effector molecules of innate immunity with microbicidal and both pro- or anti-inflammatory activities. Vasoactive intestinal polypeptide (VIP) and the structurally related pituitary adenylate cyclase-activating polypeptide (PACAP) are well-

known immunomodulators. On the basis of their cationic and amphipathic structures, resembling antimicrobial peptides, we propose that their immune role could also include a direct lethal effect against pathogens. We thus investigated the potential antiparasitic activities of VIP and PACAP against the African trypanosome *Trypanosoma brucei* (*T. brucei*). Both peptides killed the bloodstream (infective) form but not the insect (noninfective) form of the parasite. VIP and PACAP caused complete destruction of the parasite integrity through a mechanism involving their entry and accumulation into the cytosol. These results provide the basis for further studies of these and other structurally related peptides as alternative treatments for parasitic diseases mainly with associated drug resistances.

13754. **Goringer, H.U., Homann, M., Zacharias, M. & Adler, A., 2006**. RNA aptamers as potential pharmaceuticals against infections with African trypanosomes. *Handbook of Experimental Pharmacology,* **173**: 375-393.

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Considerable progress has been made over the past 10 years in the development of nucleic acid-based drug molecules using a variety of different technologies. One approach is a combinatorial technology that involves an iterative Darwinian-type in vitro evolution process, which has been termed SELEX for "systematic evolution of ligands by exponential enrichment". The procedure is a highly efficient method of identifying rare ligands from combinatorial nucleic acid libraries of very high complexity. It allows the selection of nucleic acid molecules with desired functions, and it has been instrumental in the identification of a number of synthetic DNA and RNA molecules, so-called aptamers that recognize ligands of different chemical origin. Aptamers typically bind their target with high affinity and high specificity and have successfully been converted into pharmaceutically active compounds. Here we summarize the recent examples of the SELEX technique within the context of identifying high-affinity RNA ligands against the surface of the protozoan parasite *Trypanosoma brucei*, which is the causative agent of sleeping sickness.

13755. **Gros, L., Lorente, S.O., Jimenez, C.J., Yardley, V., Rattray, L., Wharton, H., Little, S., Croft, S.L., Ruiz-Perez, L.M., Gonzalez-Pacanowska, D. & Gilbert, I.H., 2006**. Evaluation of azasterols as anti-parasitics. *Journal of Medicinal Chemistry*, **49** (20): 6094-6103.

Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3XF, UK.

13756. **Gros, L., Castillo-Acosta, V.M., Jimenez Jimenez, C., Sealey-Cardona, M., Vargas, S., Manuel Estevez, A., Yardley, V., Rattray, L., Croft, S.L., Ruiz-Perez, L.M., Urbina, J.A., Gilbert, I.H. & Gonzalez-Pacanowska, D., 2006.** New azasterols against *Trypanosoma brucei*: role of 24-sterol methyltransferase in inhibitor action. *Antimicrobial Agents and Chemotherapy*, **50** (8): 2595-2601.

Welsh School of Pharmacy, Cardiff University, UK.

13757. **Hoet, S., Stevigny, C., Herent, M.F. & Quetin-Leclercq, J., 2006.** Antitrypanosomal compounds from the leaf essential oil of *Strychnos spinosa*. *Planta Medica*, **72** (5): 480-482.

Laboratoire de Pharmacognosie, Unité d'Analyse Chimique et Physico-Chimique des Médicaments, Université Catholique de Louvain, Brusseles, Belgium.

13758. **Hu, Y. & Aksoy, S., 2005**. An antimicrobial peptide with trypanocidal activity characterized from *Glossina morsitans morsitans*. *Insect Biochemistry and Molecular Biology*, **35** (2): 105-115.

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

Prior studies in trypanosome infected *Glossina morsitans morsitans* have shown induced expression and synthesis of several antimicrobial peptides in fat body tissue. Here, we have expressed one of these peptides, Attacin (GmAttA1) in *Drosophila (*S2) cells *in vitro*. We show that the purified recombinant protein (recGmAttA1) has strong antimicrobial activity against *Escherichia coli*-K12, but not against the enteric gram-negative symbiont of tsetse, *Sodalis glossinidius*. The recGmAttA1 also demonstrated inhibitory effects against both the mammalian bloodstream form and the insect stage *Trypanosoma brucei in vitro* (minimal inhibitory concentration MIC50 0.075 μM). When blood meals were supplemented with purified recGmAttA1 during the course of parasite infection, the prevalence of trypanosome infections in tsetse midgut was significantly reduced. Feeding fertile females GmAttA1 did not affect the fecundity or the longevity of mothers, nor did it affect the hatchability of their offspring. We discuss a paratransgenic strategy, which involves the expression of trypanocidal molecules such as recGmAttA1 in the midgut symbiont *Sodalis in vivo* to reduce trypanosome transmission.

13759. **Hui, X., Desrivot, J., Bories, C., Loiseau, P.M., Franck, X., Hocquemiller, R. & Figadere, B., 2006**. Synthesis and antiprotozoal activity of some new synthetic substituted quinoxalines. *Bioorganic and Medicinal Chemistry Letters,* **16 (**4): 815-20.

Laboratoire de Pharmacognosie et Groupe Chimiothérapie Antiparasitaire (associé au CNRS-BioCIS), Faculté de Pharmacie, Université de Paris-Sud, rue J.B. Clement, 92296 Chatenay-Malabry, France.

13760. **Ismail, M.A., Arafa, R.K., Brun, R., Wenzler, T., Miao, Y., Wilson, W.D., Generaux, C., Bridges, A., Hall, J.E. & Boykin, D.W., 2006.** Synthesis, DNA affinity, and antiprotozoal activity of linear dications: Terphenyl diamidines and analogues. *Journal of Medicinal Chemistry,* **49** (17): 5324-5332.

Department of Chemistry and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, Georgia 30303-3083, USA.

13761. **Ismail, M.A., Batista-Parra, A., Miao, Y., Wilson, W.D., Wenzler, T., Brun, R. & Boykin, D.W., 2005**. Dicationic near-linear biphenyl benzimidazole derivatives as DNA-targeted antiprotozoal agents. *Bioorganic and Medicinal Chemistry,* **13** (24): 6718-6726. 8b

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A series of near-linear biphenyl benzimidazole diamidines were synthesized from their respective diamidoximes, through the bis-O-acetoxyamidoxime, followed by hydrogenation in glacial acetic acid/ethanol in the presence of Pd-C. The compounds were quite active *in vitro* versus *Trypanosoma brucei rhodesiense*, giving IC 50 values ranging from 3 to 37 nM. These compounds were even more active versus *Plasmodium falciparum*, exhibiting IC 50 values ranging from 0.5 to 23 nM. The compounds showed moderate to good activity *in vivo* in the STIB900 model for acute African trypanosomiasis. The most active compounds gave 3 out of 4 cures on an i.p. dosage of 20 mg/kg.

13762. **Jagielski, A.K., Kryskiewicz, E. & Bryla, J., 2006.** Suramin-induced reciprocal changes in glucose and lactate synthesis in renal tubules contribute to its hyperglycaemic action. *European Journal of Pharmacology*, **537** (1-3): 205-209.

Department of Metabolic Regulation, Institute of Biochemistry, Warsaw University, I. Miecznikowa 1, 02-096 Warsaw, Poland.

13763. **Juyal, P.D., Singh, S., Singla, L.D. & Sood, N., 2005.** Effect of bleomycin hydrochloride on the course of *Trypanosoma evansi* infection in Swiss albino mice. *Journal of Parasitic Diseases*, **29** (2): 153-155.

Department of Veterinary Parasitology, College of veterinary Science, Punjab Agricultural University, Ludhiana-141004, India.

Bleomycin hydrochloride was used as trypanocide (5 mg per kg s/c) against experimental *T. evansi* (cattle strain) infection in Swiss albino mice. It was observed that 2 (24, 48 h) and 3 (24, 48, 72 h) consecutive injections of bleomycin had trypanocidal effect for a transient period of one and 4 days respectively, and subsequently reappearance of the trypanosomes occurred in peripheral blood. The increase in the number of treatments reduced the parasitaemia as indicated both by intensity as well the number of trypanosomes/ml of the tail blood. The survival period in treated groups was prolonged up to 12 days when compared with that of untreated control group. Histopathological changes were suggestive of mild hepatotoxicity.

13764. **Khabnadideh, S., Pez, D., Musso, A., Brun, R., Perez, L.M.R., Gonzalez-Pacanowska, D. & Gilbert, I.H., 2005**. Design, synthesis and evaluation of 2,4-

diaminoquinazolines as inhibitors of trypanosomal and leishmanial dihydrofolate reductase. *Bioorganic and Medicinal Chemistry,* **13** (7): 2637-2649.

Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3XF, UK.

13765. **Kuboki, N., Yokoyama, N., Kojima, N., Sakurai, T., Inoue, N. & Sugimoto, C., 2006.** Efficacy of dipalmitoylphosphatidylcholine liposome against African trypanosomes. *Journal of Parasitology*, **92** (2): 389-393.

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan.

We demonstrate here that dipalmitoylphosphatidylcholine (DPPC) liposome has an antitrypanosomal effect, especially against the bloodstream forms (BSFs) of African trypanosomes (*Trypanosoma congolense, T. brucei rhodesiense*, and *T. brucei brucei*). The DPPC liposome significantly decreased the *in vitro* percentage of viable and motile BSF African trypanosomes but only marginally reduced the percentage of viable and motile procyclic form (PCF) of trypanosomes. The DPPC liposome absorption was much more pronounced to BSF than to PCF trypanosomes. Administration of the DPPC liposome showed a slight but significant reduction in the early development of parasitemia in *T. congolense*-infected mice. These results suggest that parasites were killed by specific binding of the DPPC liposome to the trypanosomes. This work demonstrates for the first time that a liposome has antitrypanosomal activity.

13766. **Lamas, M.C., Villaggi, L., Nocito, I., Bassani, G., Leonardi, D., Pascutti, F., Serra, E. & Salomon, C.J., 2006**. Development of parenteral formulations and evaluation of the biological activity of the trypanocide drug benznidazole. *International Journal of Pharmaceutics,* **307** (2): 239-243.

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One of the drugs most frequently used for the treatment of Chagas disease is benznidazole (BZL). It is practically insoluble in water (0.4 mg/ml), which precludes the preparation of liquid dosage forms, in particular, parenteral formulations. Thus, the aim of this work was to investigate the solubilization of BZL at two pH values using various cosolvents such as ethyl alcohol, propylene glycol, polyethylene glycol 400, benzyl alcohol, diethylene glycol monoethyl ether (Transcutol) and surfactants such as polysorbates (Tween) 40 and 80, and sodium dioctyl sulfosuccinate (AOT). Solvent systems based on PEG 400, with the addition of ethyl alcohol and/or potassium biphthalate buffer solution, increased the BZL solubility up to 10 mg/ml. These alcoholic vehicles showed no toxicity against parasite when assayed at 1 percent. Physical and chemical stability studies showed that the formulations were stable for at least 1.5 years. In agreement with the biological activity

results, the selected formulations are suitable for further clinical studies. Moreover, increasing the aqueous solubility of BZL reduced the problems of *in vitro* testing techniques and bioassays leading to more reliable results and/or reproducibility.

13767. **Lanteri, C.A., Stewart, M.L., Brock, J.M., Alibu, V.P., Meshnick, S.R., Tidwell, R.R. & Barrett, M.P., 2006.** Roles for the *Trypanosoma brucei* P2 transporter in DB75 uptake and resistance. *Molecular Pharmacology*, **70** (5): 1585-1592.

Division of Infection and Immunity, Institute of Biomedical and Life Sciences, The Glasgow Biomedical Research Centre, University of Glasgow, Glasgow G12 8QQ, UK. [m.barrett@bio.gla.ac.uk].

13768. **Lorente, S.O., Jimenez, C.J., Gros, L., Yardley, V., de Luca-Fradley, K., Croft, S.L., J, A.U., Ruiz-Perez, L.M., Pacanowska, D.G. & Gilbert, I.H., 2005**. Preparation of transition-state analogues of sterol 24-methyl transferase as potential anti-parasitics. *Bioorganic and Medicinal Chemistry,* **13** (18): 5435-5453.

Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3XF, UK.

13769. **Mackey, Z.B., Baca, A.M., Mallari, J.P., Apsel, B., Shelat, A., Hansell, E.J., Chiang, P. K., Wolff, B., Guy, K.R., Williams, J. & McKerrow, J. H., 2006.** Discovery of trypanocidal compounds by whole cell HTS of *Trypanosoma brucei*. *Chemical Biology and Drug Design*, **67** (5): 355-363.

Department of Pathology and the Sandler Center for Basic Research in Parasitic Diseases, University of California, QB3 1700 4th St, San Francisco, CA 94158, USA.

One potentially rapid and cost-effective approach to identifying and developing new trypanocidal drugs would be high throughput-screening of existing drugs already approved for other uses, as well as clinical candidates in late development. We have developed an ATP-bioluminescence assay that could be used to rapidly and efficiently screen compound libraries against trypanosomes in a high throughput-screening format to validate this notion. We screened a collection of 2,160 FDA-approved drugs, bioactive compounds and natural products to identify hits that were cytotoxic to cultured *Trypanosoma brucei* at a concentration of 1 uM or less. This meant that any hit identified would be effective at a concentration readily achievable by standard drug dosing in humans. From the screen, 35 hits from seven different drug categories were identified. These included the two approved trypanocidal drugs, suramin and pentamidine, several other drugs suspected but never validated as trypanocidal, and 17 novel trypanocidal drugs.

13770. **Masocha, W., Rottenberg, M.E. & Kristensson, K., 2006.** Minocycline impedes African trypanosome invasion of the brain in a murine model. *Antimicrobial Agents and Chemotherapy*, **50** (5): 1798-1804.

Department of Neuroscience, Retzius vag 8, Karolinska Institutet, SE-171 77 Stockholm, Sweden.[willias.masocha@ki.se]

Passage of *Trypanosoma brucei* across the blood-brain barrier (BBB) is a hallmark of late-stage human African trypanosomiasis. In the present study we found that daily administration of minocycline, a tetracycline antibiotic, impedes the penetration of leukocytes and trypanosomes into the brain parenchyma of *T. brucei brucei*-infected C57BL/6 mice. The trypanosome-induced astrocytic and microglial reactions were reduced in the minocycline-treated mice, as were the levels in the brain of transcripts encoding adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and endothelial-leukocyte adhesion molecule 1 (E-selectin); the inflammatory cytokines tumor necrosis factor alpha, interleukin-1alpha (IL-1alpha), IL-1beta, IL-6, and gamma interferon; and matrix metalloprotease 3 (MMP-3), MMP-8, and MMP-12. Loss of weight occurring during infection with *T. b. brucei* was not observed after treatment of the mice with minocycline; these mice also survived longer than nontreated mice. Invasion of trypanosomes and leukocytes into the brain parenchyma most likely triggered the loss of weight and death of infected animals, since minocycline did not affect the growth of *T. b. brucei* either *in vitro* or *in vivo* or the levels of the transcripts encoding the cytokines and MMPs in the spleen. In conclusion, our data show that *T. b. brucei* invasion of the brain is related to that of leukocytes and that minocycline can ameliorate the disease in trypanosome-infected mice.

13771. **Mathis, A.M., Holman, J.L., Sturk, L.M., Ismail, M.A., Boykin, D.W., Tidwell, R.R. & Hall, J.E., 2006.** Accumulation and intracellular distribution of antitrypanosomal diamidine compounds DB75 and DB820 in African trypanosomes. *Antimicrobial Agents and Chemotherapy*, **50** (6): 2185-2191.

Division of Molecular Pharmaceutics, School of Pharmacy, University of North Carolina, Chapel Hill, Chapel 27599, USA.

13772. **Miller, D.M.S., Swan, G. E., Lobetti, R. G. & Jacobson, L. S., 2005**. The pharmacokinetics of diminazene aceturate after intramuscular administration in healthy dogs. *Journal of the South African Veterinary Association*, **76** (3): 146-150.

Department of Companion Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa.

The pharmacokinetics of diminazene aceturate following intramuscular (i.m.) administration at 4.2 mg/kg was evaluated in 8 healthy German Shepherd dogs. The results of this study indicate that diminazene is rapidly distributed and sequestered into the liver, followed by a slower terminal phase during which diminazene is both redistributed to the peripheral tissues and/or renally excreted. It is recommended that diminazene administered i.m. at 4.2 mg/kg should not be repeated within a 21-day period.

13773 **Muscia, G.C., Bollini, M., Carnevale, J.P., Bruno, A.M. & Asís, S.E. 2006.** Microwave-assisted Friedländer synthesis of quinolines derivatives as potential antiparasitic agents. *Tetrahedron Letters,* **47** (50): 8811-8815.

Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 (1113) Buenos Aires, Argentina. [elizabet@ffyb.uba.ar]

13774. **Orhan, I., Aslan, M., Sener, B., Kaiser, M. & Tasdemir, D., 2006**. *In vitro* antiprotozoal activity of the lipophilic extracts of different parts of Turkish *Pistacia vera* L. *Phytomedicine,* **13** *(9-10): 735-739.*

Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, TR-06330 Ankara, Turkey.

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K.T. Douglas: University of Manchester, School of Pharmacy and Pharmaceutical Science, Oxford Rd, Manchester M13 9PL, Lancs., England.

13776**. Pradines, B., Pages, J.M. & Barbe, J., 2005**. Chemosensitizers in drug transport mechanisms involved in protozoan resistance. *Current Drug Targets: Infectious Disorders,* **5** (4): 411-431.

Unité de Recherche en Biologie et Épidémiologie Parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Marseille, France.

The emergence and spread of antiparasitic drug resistance pose a severe and increasing public health threat. Failures in prophylaxis or those in treatment with quinolines, hydroxynaphtoquinones, sesquiterpenic lactones, antifolate drugs, arsenic and antimony containing drugs, sulfamides induce reemergence of parasite-related morbidity and mortality. Resistance is often associated with alteration of drug accumulation into parasites, which results from a reduced uptake of the drug, an increased efflux or, a combination of the two processes. Resistance to quinolines, artemisinin derivatives and arsenicals and expression of an active efflux mechanism are more or less correlated in protozoa like *Plasmodium* spp., *Leishmania* spp., and *Trypanosoma* spp. Various parasite candidate genes have been proposed to be involved in drug resistance, each concerned in membrane transport. Genes encoding membrane glycoproteins, orthologue to the P-glycoproteins identified in MDR human cancer cells, have been described in these resistant pathogens in addition to various membrane proteins involved in drug transport. Several compounds have demonstrated, in the past decade, promising capability to reverse the drug resistance in parasite isolates *in vitro*, in animal models and for human malaria. These drugs belong to different pharmacological

classes such as calcium channel blockers, tricyclic antidepressants, antipsychotic calmodulin antagonists, histamine H1-receptor antagonists, analgesic antipyretic drugs, non-steroidal anti-inflammatory drugs, and to different chemical classes such as synthetic surfactants, alkaloids from plants used in traditional medicine, pyrrolidinoaminoalkanes and derivatives, and anthracene derivatives. Here, are summarized the molecular bases of antiparasitic drug resistance emphasizing recent developments with compounds acting on trans-membrane proteins involved in drug efflux or uptake.

13777. **Puri, U.K., Sharma, S.K., Kaur, P. & Juyal, P. D., 2006**. Pharmacokinetics of suramin in uninfected and experimental *Trypanosoma evansi* infected buffalo calves. *Indian Journal of Animal Sciences*, **76** (7): 495-497.

Punjab Agricultural University,Ludhiana,Punjab 141004, India.

The pharmacokinetic pattern of suramin was studied in buffalo calves experimentally infected with *Trypanosoma evansi*. Ten male buffalo calves were divided into two groups of equal number. Group 1 was inoculated with $4.06x10^7$ trypanosome/calf and treated with suramin (0.5 g/45 kg b.w. i.v.), while Group 2 was left uninfected but treated with the same dose of suramin i.v. The level of suramin in the plasma was determined by spectrophotometry. One minute after administration, the plasma level of suramin was 103.8+or -1.63 and 160.5+or-3.60 ug/ml, which declined to 8.29+or-0.19 and 10.9+or-0.88 µg/ml 7 days after administration in infected and uninfected buffalo calves, respectively. No concentration of the drug was found in the plasma seven days after treatment. The pharmacokinetics of suramin following intravenous administration was calculated by 3-compartment open model. There were significantly lower plasma levels up to 6 h after administration and lower values of distribution, t1/2 alpha 2, AUC and CP levels in infected than uninfected buffalo calves. The significant difference between various pharmacokinetic parameters of *T. evansi* infected and uninfected buffalo calves may be due to the depletion of the drug, pathophysiological alteration and circulation changes that accompany trypanosome infection.

13778. **Rapp, M., Haubrich, T.A., Perrault, J., Mackey, Z.B., McKerrow, J.H., Chiang, P.K. & Wnuk, S.F., 2006**. Antitrypanosomal activity of 5'-deoxy-5'-(iodomethylene) adenosine and related 6-N-cyclopropyladenosine analogues. *Journal of Medical Chemistry,* **49** (6): 2096-2102.

Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33199, USA.

13779**. Reguera, R.M., Tekwani, B.L. & Balana-Fouce, R., 2005**. Polyamine transport in parasites: a potential target for new antiparasitic drug development. *Comparative Biochemistry and Physiology,* **140** (2): 151-164.

Department of Pharmacology and Toxicology (INTOXCAL), University of Leon, Campus de Vegazana (s/n), 24071 Leon, Spain.

The metabolism of the naturally occurring polyamines - putrescine, spermidine and spermine - is a highly integrated system involving biosynthesis, uptake, degradation and interconversion. Metabolic differences in polyamine metabolism have long been considered to be a potential target to arrest proliferative processes ranging from cancer to microbial and parasitic diseases. Despite the early success of polyamine inhibitors such as alpha-difluoromethylornithine (DFMO) in treating the latter stages of African sleeping sickness, in which the central nervous system is affected, they proved to be ineffective in checking other major diseases caused by parasitic protozoa, such as Chagas' disease, leishmaniasis or malaria. In the use and design of new polyamine-based inhibitors, account must be taken of the presence of up-regulated polyamine transporters in the plasma membrane of the infectious agent that are able to circumvent the effect of the drug by providing the parasite with polyamines from the host. This review contains information on the polyamine requirements and molecular, biochemical and genetic characterization of different transport mechanisms in the parasitic agents responsible for a number of the deadly diseases that afflict underdeveloped and developing countries.

13780. **Rodenko, B., Detz, R.J., Pinas, V.A., Lambertucci, C., Brun, R., Wanner, M.J. & Koomen, G.J., 2006**. Solid phase synthesis and antiprotozoal evaluation of di- and tri-substituted 5'-carboxamidoadenosine analogues. *Bioorganic and Medical Chemistry,* **14** (5): 1618-1629.

Van't Hoff Institute for Molecular Sciences, Universiteit van Amsterdam, the Netherlands.

13781. **Salem, M.M. & Werbovetz, K.A., 2006**. Isoflavonoids and other compounds from *Psorothamnus arborescens* with antiprotozoal activities. *Journal of Natural Products*, **69** (1): 43-9.

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, USA.

13782. **Seebacher, W., Schlapper, C., Brun, R., Kaiser, M., Saf, R. & Weis, R., 2005**. Antiprotozoal activities of new bicyclo[2.2.2]octan-2-imines and esters of bicyclo[2.2.2]octan-2-ols. *European Journal of Pharmaceutical Sciences,* **24** (4): 281-289.

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens University, Universitatsplatz 1, A-8010 Graz, Austria. [we.seebacher@uni-graz.at].

13783. **Seebacher, W., Schlapper, C., Brun, R., Kaiser, M., Saf, R. & Weis, R., 2006.** Synthesis of new esters and oximes with 4-aminobicyclo[2.2.2]octane structure and evaluation of their antitrypanosomal and antiplasmodial activities. *European Journal of Medicinal Chemistry*, **41** (8): 970-977.

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens-University, Universitatsplatz 1, A-8010 Graz, Austria. [we.seebacher@uni-graz.at]

13784. **Seebacher, W., Weis, R., Kaiser, M., Brun, R. & Saf, R., 2005**. Synthesis of 2-azabicyclo [3.2.2] nonanes from bicycle [2.2.2] octan-2-ones and their activities against *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum* K1. *Journal of Pharmacy and Pharmaceutical Sciences,* **8** (3): 578-585.

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens-University, A-8010 Graz, Austria. [we.seebacher@uni-graz.at].

New 2-azabicyclo [3.2.2] nonanes were prepared from antiprotozoal bicycle [2.2.2] octan-2-ones to investigate the influence of the replacement of the rigid bicyclo-octane structure by the more flexible bicyclo-nonane system on the antiplasmodial and antitrypanosomal activity. The 2-azabicyclo [3.2.2] nonanes were synthesized via a one-step procedure from bicycle [2.2.2] octan-2-ones and tested for their activities against *Trypanosoma b. rhodesiense* and *Plasmodium falciparum* K1 (resistant to chloroquine and pyrimethamine) using *in vitro* microplate assays. Due to their promising *in vitro* antiprotozoal activity and their low cytotoxicity, 2-azabicyclo [3.2.2] nonanes should serve as lead compounds for further modifications.

13785. **Staroverov, S.A., Pristensky, D.V., Yermilov, D.N., Gabalov, K.P., Zhemerichkin, D.A., Sidorkin, V.A., Shcherbakov, A.A., Shchyogolev, S.Y. & Dykman, L.A., 2006.** The effectivity analysis of accumulation of liposomal, micellar, and water-soluble forms of diminazene in cells and in organs. *Drug Delivery*, **13** (5): 351-355.

Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia.

13786. **Steverding, D., Pemberton, A.J., Royle, H., Spackman, R.W. & Rivett, A.J., 2006.** Evaluation of the anti-trypanosomal activity of tyropeptin A. *Planta Medica*, **72** (8): 761-763.

Biomedical Research Centre, School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK. [dsteverding@hotmail.com]

The natural compound tyropeptin A, a new peptidyl aldehyde proteasome inhibitor, was tested for its trypanocidal activity *in vitro* using culture-adapted bloodstream forms of *Trypanosoma brucei*. The concentrations of tyropeptin A required to reduce the growth rate by 50 percent and to kill all cells were 10 and 100 times lower for bloodstream-form trypanosomes than for human leukaemia HL-60 cells, respectively. Enzymatic analysis showed that the trypsin-like activity of the trypanosome proteasome and the chymotrypsin-like activity of the mammalian proteasome are particularly sensitive to inhibition by tyropeptin A. The results suggest that natural compounds targeting the trypsin-like activity of

the proteasome may serve as leads for rational drug development of novel anti-trypanosomal agents.

13787. **Steverding, D., Spackman, R.W., Royle, H.J. & Glenn, R.J., 2005**. Trypanocidal activities of trileucine methyl vinyl sulfone proteasome inhibitors. *Parasitology Research,* **95** (1): 73-76.

School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK.

Previous studies have shown that proteasome inhibitors are novel agents for chemotherapy of human African trypanosomiasis or sleeping sickness. In this study, five peptide trileucine methyl vinyl sulfones with different N-terminal substituents were tested for their trypanocidal activities *in vitro* using culture-adapted bloodstream forms of *Trypanosoma brucei*. Two inhibitors displayed promising anti-trypanosomal activities with ED50 values in the sub-micromolar range. Higher trypanocidal activity of the compounds generally corresponded to a higher k.obs value for inhibition of the trypsin-like activity but not for the inhibition of the chymotrypsin-like activity of the proteasome. These data suggest that inhibitors with strong activity against the trypsin-like activity of the proteasome are the rational choice for future anti-sleeping sickness drug development.

13788. **Stewart, M.B., Boussard, C., Brun, R., Gilbert, I.H. & Barrett, M.P., 2005**. Interaction of monobenzamidine-linked trypanocides with the *Trypanosoma brucei* P2 aminopurine transporter. *Antimicrobial Agents and Chemotherapy,* **49** (12): 5169-5171.

M.P. Barrett: University of Glasgow, Institute of Medical and Life Sciences, Division of Infection and Immunity, Glasgow G12 8QQ, Lanark, Scotland.

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Center for Cellular and Molecular Neurobiology, University of Liege, Liege, Belgium.

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Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Switzerland. [deniz@oci.unizh.ch].

A total of 58 extracts of different polarity were prepared from various organs of 16 species of Turkish plants and screened for their antitrypanosomal, antileishmanial and antiplasmodial activities. No significant activity was observed against *Trypanosoma cruzi,* whereas many extracts showed appreciable trypanocidal potential against *T. brucei*

rhodesiense, with the $CHCl_3$ soluble portion of *Phlomis kurdica* being the most active (IC(50) 2.7 ug/ml).

13791. **Tasdemir, D., Kaiser, M., Brun, R., Yardley, V., Schmidt, T.J., Tosun, F. & Ruedi, P., 2006.** Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: *in vitro, in vivo*, structure-activity relationship, and quantitative structure-activity relationship studies. *Antimicrobial Agents Chemotherapy*, **50** (4): 1352-1364.

Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. [deniz@oci.unizh.ch]

13792. **Tsuda, A., Witola, W.H., Konnai, S., Ohashi, K. & Onuma, M., 2006**. The effect of TAO expression on PCD-like phenomenon development and drug resistance in *Trypanosoma brucei. Parasitology International,* **55** (2): 135-142.

Laboratory of Infectious Disease, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.

13793**. Ullmann, H., Meis, S., Hongwiset, D., Marzian, C., Wiese, M., Nickel, P., Communi, D., Boeynaems, J.M., Wolf, C., Hausmann, R., Schmalzing, G. & Kassack, M.U., 2005.** Synthesis and structure-activity relationships of suramin-derived P2Y11 receptor antagonists with nanomolar potency. *Journal of Medicinal Chemistry*, **48** (22): 7040-7048.

Pharmaceutical Institute, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany.

13794. **Urbaniak, M.D., Tabudravu, J.N., Msaki, A., Matera, K.M., Brenk, R., Jaspars, M. & Ferguson, M.A., 2006.** Identification of novel inhibitors of UDP-Glc 4'-epimerase, a validated drug target for African sleeping sickness. *Bioorganic and Medicinal Chemistry Letters,* **16** (22): 5744-5747.

Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, UK.

13795. **Vega, M.C., Montero-Torres, A., Marrero-Ponce, Y., Rolon, M., Gomez-Barrio, A., Escario, J.A., Aran, V.J., Nogal, J.J., Meneses-Marcel, A. & Torrens, F., 2006.** New ligand-based approach for the discovery of antitrypanosomal compounds. *Bioorganic and Medicinal Chemistry Letters,* **16** (7): 1898-1904.

Department of Parasitology, Faculty of Pharmacy, Universidad Complutense de Madrid, 28040 Madrid, Spain.

13796. **Vicik, R., Hoerr, V., Glaser, M., Schultheis, M., Hansell, E., McKerrow, J.H., Holzgrabe, U., Caffrey, C.R., Ponte-Sucre, A. & Moll, H., 2006**. Aziridine-2,3-dicarboxylate inhibitors targeting the major cysteine protease of *Trypanosoma*

brucei as lead trypanocidal agents. *Bioorganic and Medicinal Chemistry Letters*, **16** (10): 2753-2757.

Institute of Pharmacy and Food Chemistry, University of Wuerzburg, Am Hubland, D-97074 Wuerzburg, Germany.

13797. **Weis, R., Schlapper, C., Brun, R., Kaiser, M. & Seebacher, W., 2006.** Antiplasmodial and antitrypanosomal activity of new esters and ethers of 4-dialkylaminobicyclo[2.2.2]octan-2-ols. *European Journal of Pharmacological Science*, **28** (5): 361-368.

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens-University, Universitatsplatz 1, A-8010 Graz, Austria. [robert.weis@uni-graz.at]

13798. **Weniger, B., Vonthron-Senecheau, C., Kaiser, M., Brun, R. & Anton, R., 2006.** Comparative antiplasmodial, leishmanicidal and antitrypanosomal activities of several biflavonoids. *Phytomedicine*, **13** (3): 176-180.

Pharmacognosie et Biomolécules Naturelles Actives, UMR no 7081, Faculté de Pharmacie, Université Louis Pasteur Strasbourg, Illkirch, France.

13799. **Wu, D., George, T.G., Hurh, E., Werbovetz, K.A. & Dalton, J.T., 2006**. Pre-systemic metabolism prevents *in vivo* antikinetoplastid activity of N1, N4-substituted 3,5-dinitro sulfanilamide, GB-II-150. *Life Sciences.* **In press; corrected proof.**

Division of Pharmaceutics, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA.

13800. **Wurochekke, A.U.J., James, D. B., Bello, M. I. & Ahmodu, A., 2005**. Trypanocidal activity of the leaf of *Guira senegalensis* against *Trypanosoma brucei brucei* infection in mice. *Journal of Medical Sciences (Pakistan)*, **5** (4): 333-336.

Department of Biochemistry, Federal University of Technology, Yola, Nigeria.

In vitro and *in vivo* trypanocidal activity of the leaf extract of *Guira senegalensis* against *Trypanosoma brucei brucei* has been investigated. Extract obtained from fresh leaves heated with methanol had highest *in vitro* activity against the parasite at concentration of 8.3 mg ml^{-1} of blood. Dried leaf methanolic extract also had *in vitro* activity at the same concentration after 30 min of incubation. Treatment with 100 mg/kg/day of the fresh leaf extract for five days tends to ameliorate the disease condition but did not clear the parasitaemia and pack cell volume values were not significantly affected. All other animals treated with the extract higher than the 100 mg/kg/day died before the infected controls. Addition of glycerol as an adjuvant did not show effect. The plant may be a promising trypanocide.

13801. **Yabu, Y., Suzuki, T., Nihei, C., Minagawa, N., Hosokawa, T., Nagai, K., Kita, K. & Ohta, N., 2006**. Chemotherapeutic efficacy of ascofuranone in *Trypanosoma vivax*-infected mice without glycerol. *Parasitology International*, **55** (1): 39-43.

Department of Molecular Parasitology, Nagoya City University, Graduate School of Medical Sciences, Nagoya 467-8601, Japan. [yabu@med.nagoya-cu.ac.jp].

Ascofuranone, an antibiotic isolated from *Ascochyta visiae*, showed trypanocidal activity in *Trypanosoma vivax*-infected mice. A single dose of 50 mg/kg ascofuranone effectively cured the mice without the help of glycerol. Repeated administrations of this drug further enhanced its chemotherapeutic effect. After two, three, and four consecutive days treatment, the doses needed to cure the infection decreased to 25, 12, and 6 mg/kg, so that the total doses administered were 50, 36 and 24 mg/kg, respectively. Ascofuranone (50 mg/kg) also had a prophylactic effect against *T. vivax* infection within the first two days after administration. This prophylactic activity diminished to 80 percent by day 3 and completely disappeared four days after administration. Of particular interest in this study was that ascofuranone had trypanocidal activity in *T. vivax*-infected mice in the absence of glycerol, whereas co-administration of glycerol or repeated administrations of this drug are needed for *Trypanosoma brucei brucei* infection. Our present results strongly suggest that ascofuranone is also an effective tool in chemotherapy against African trypanosomiasis in domestic animals.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

[See also **29**: 13611, 13613, 13656, 13667, 13685]

13802. **Afework, Y., Maser, P., Etschmann, B., von Samson-Himmelstjerna, G., Zessin, K.H. & Clausen, P.H., 2006**. Rapid identification of isometamidium-resistant stocks of *Trypanosoma b. brucei* by PCR-RFLP. *Parasitology Research,* **99** (3): 253-261.

Institute for Parasitology and Tropical Veterinary Medicine, Freie Universitat Berlin, Konigsweg 67, 14163, Berlin, Germany. [tropvetm@zedat.fu-berlin.de].

Analyses were made on the adenosine transporter-1 gene in *Trypanosoma brucei* (TbAT1), encoding a P2-like nucleoside transporter, from *T. brucei brucei* field stocks to investigate a possible link between the presence of mutations in this gene and isometamidium resistance. We have analysed the gene from 11 isometamidium-sensitive field stocks isolated from cattle in Uganda, two sensitive reference clones and two resistant reference clones. A sequence alignment showed that the isometamidium-sensitive *T. b. brucei* contained the wild-type sequence patterns. In contrast, the isometamidium-resistant *T. b. brucei* stocks showed

the mutant-type sequence patterns with six point mutations that had previously been reported in a laboratory-derived arsenical-resistant *T. brucei* strain. To analyse the restriction fragment length polymorphism pattern of a fragment of TbAT1 (nucleotides 430-1108), the 677-bp polymerase chain reaction products from eight of the isometamidium-sensitive and two of the isometamidium-resistant *T. b. brucei* were subjected to digestion with Sfa NI. The results revealed two different banding patterns: the digest resulted in fragment sizes of 566 and 111 bp in the case of TbAT1 from isometamidium-sensitive stocks, whereas it produced fragment sizes of 435 and 242 bp in the case of TbAT1 from isometamidium-resistant stocks. Thus, the isometamidium-sensitive and isometamidium-resistant *T. b. brucei* could be successfully distinguished by digestion with the restriction endonuclease Sfa NI.

13803. **Balmer, O. & Tostado, C., 2006**. New fluorescence markers to distinguish co-infecting *Trypanosoma brucei* strains in experimental multiple infections. *Acta Tropica,* **97** (1): 94-101.

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Multiple-genotype infections are increasingly recognized as important factors in disease evolution, parasite transmission dynamics, and the evolution of drug resistance. However, the distinction of co-infecting parasite genotypes and the tracking of their dynamics have been difficult with traditional methods based on various genotyping techniques, leaving most questions unaddressed. Here we report new fluorescence markers of various colours that are inserted into the genome of *Trypanosoma brucei* to phenotypically label live parasites of all life cycle stages. If different parasite strains are labelled with different colours they can be easily distinguished from each other in experimental studies. A total of 10 *T. brucei* strains were successfully transfected with different fluorescence markers and were monitored in culture, tsetse flies and mice, to demonstrate stability of marker expression. The use of fluorescence activated cell sorting (FACS) allowed rapid and accurate identification of parasite strains labelled with different markers. Cell counts by FACS were virtually identical to counts by traditional microscopy (n=75, Spearman's rho: 0.91, $p<0.0001$) but were considerably faster and had a significantly lower sampling error (66 percent lower, d.f.=73, t=-17.1, $p<0.0001$). Co-infecting strains transfected with fluorescence genes of different colour were easily distinguished by eye and their relative and absolute densities were reliably counted by FACS in experimental multiple infections in mice. Since the FACS can simultaneously determine the population sizes of differently labelled *T. brucei* strains or subspecies it allows detailed and efficient tracking of multiple-genotype infections within a single host or vector individual, enabling more powerful studies on parasite dynamics. In addition, it also provides a simple way to separate genotypes after experimental mixed infections, to measure responses of the single strains to an applied treatment, thus eliminating the need for laborious cloning steps. The markers presented broaden the spectrum of tools available for experimental studies on multiple-genotype infections. They are fundamentally different from isoenzyme analysis and other genotyping approaches in that they allow the distinction of parasite genotypes based on an easily recognizable phenotypic trait. They will be of specific interest to researches addressing ecological, evolutionary and epidemiological questions using trypanosomes as an experimental system.

13804. **Cortez, A.P., Ventura, R.M., Rodrigues, A.C., Batista, J.S., Paiva, F., Anez, N., Machado, R.Z., Gibson, W.C. & Teixeira, M.M., 2006**. The taxonomic and phylogenetic relationships of *Trypanosoma vivax* from South America and Africa. *Parasitology,* **133** (2): 159-169.

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The taxonomic and phylogenetic relationships of *Trypanosoma vivax* are controversial. It is generally suggested that South American, and East and West African isolates could be classified as subspecies or species allied to *T. vivax*. This is the first phylogenetic study to compare South American isolates (Brazil and Venezuela) with West/East African *T. vivax* isolates. Phylogeny using ribosomal sequences positioned all *T. vivax* isolates tightly together on the periphery of the clade containing all Salivarian trypanosomes. The same branching of isolates within *T. vivax* clade was observed in all inferred phylogenies using different data sets of sequences (SSU, SSU plus 5.8S or whole ITS rDNA). *T. vivax* from Brazil, Venezuela and West Africa (Nigeria) were closely related corroborating the West African origin of South American *T. vivax*, whereas a large genetic distance separated these isolates from the East African isolate (Kenya) analysed. Brazilian isolates from cattle asymptomatic or showing distinct pathology were highly homogeneous. This study did not disclose significant polymorphism to separate West African and South American isolates into different species/subspecies and indicate that the complexity of *T. vivax* in Africa and of the whole subgenus *Trypanosoma* (Duttonella) might be higher than previously believed.

13805. **Delespaux, V., Chitanga, S., Geysen, D., Goethals, A., Van den Bossche, P. & Geerts, S., 2006.** SSCP analysis of the P2 purine transporter TcoAT1 gene of *Trypanosoma congolense* leads to a simple PCR-RFLP test allowing the rapid identification of diminazene resistant stocks. *Acta Tropica.* **In press; corrected proof.**

Animal Health Department, Institute of Tropical Medicine (Antwerp), Nationalestraat 155, B-2000 Antwerp, Belgium.

Analyses were made on a *Trypanosoma congolense* contig coding a putative P2-like nucleoside transporter (the contig was named in this study TcoAT1). The sequence includes a start and stop codon and presents a high similarity with the gene TbAT1 of *T. brucei* (Smallest Sum Probability 2.8e-136). To investigate a possible link between point mutations and diminazene aceturate (DA) resistance in mice, the TcoAT1 putative genes of 26 T. congolense strains, characterised for DA sensitivity in the single dose mouse test, were screened by means of the Single Strand Conformation Polymorphism technique (SSCP). Results showed that the SSCP profiles of 23 out of 26 (88.5 percent) *T. congolense* strains were confirmed by the sensitivity test in mice with the commonly accepted criterion for sensitivity to diminazene being a CD80 of 20mg/kg in the mouse test. The remaining *T. congolense* strains showed a resistant SSCP profile and relapsed in mice after treatment at doses lower than 20mg/kg indicating that the SSCP is more sensitive than the single dose mouse test for the detection of resistance to diminazene. However, none of the strains used in this study showed a sensitive SSCP profile while they were resistant in the single dose mouse test. The sequencing of the TcoAT1 gene of two sensitive, two intermediate and two resistant

strains allowed the set up of a PCR-RFLP test for the discrimination between sensitive and resistant strains confirming the SSCP results for the 26 strains of this study.

13806. **El-Rayah, I.E., & El-Malik, K.H., 2006**. Characterization of quinapyramine (TrypacideReg.) drug-resistant *Trypansoma evansi*. *African Journal of Biotechnology,* **5** (10): 951-955.

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Molecular karyotyping by pulsed field gel electrophoresis was used to characterize *Trypanosoma evansi* isolates. Ten *T. evansi* isolates from camels were collected in Eastern and Western Sudan. Isolates from Eastern Sudan which were kept under continuous prophylactic treatment with quinapyramine (TrypacideReg.), were found to bear a single pattern and belonged to one karyotype group. From Western Sudan where trypanosomosis management was done by individual treatment of proven parasitaemic cases, isolates with diverse karyotype patterns were obtained. This study concluded that the occurrence of karyotype homogeneity amongst *T. evansi* isolates from field situations where anti-trypanosomal compounds have been used may infer the existence of drug resistance.

13807. **Garcia, H., Garcia, M.E., Perez, H. & Mendoza-Leon, A., 2005**. The detection and PCR-based characterization of the parasites causing trypanosomiasis in water-buffalo herds in Venezuela. *Annals of Tropical Medicine and Parasitology,* **99**: 359-370.

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The usefulness of PCR-based assays for detecting trypanosomiasis in water buffaloes and other livestock was explored, under field conditions, in Venezuela. The sensitivity and specificity of the assays, which were based on established primer pairs (21-mer/22-mer and ILO1264/ILO1265), were evaluated, partly by comparison with the results of parasitological tests (stained bloodsmears and microhaematocrit centrifugation) and immunological assays (IFAT) run in parallel. The optimised PCR-based assays showed a sensitivity of 10 pg. DNA. The use of the 21-mer/22-mer primer pair gave a test that was specific for species in the subgenus *Trypanozoon* (including *Trypanosoma evansi*), whereas use of ILO1264/ILO1265 produced a test that was specific for *T. vivax*. The results of a hybridization assay using *T. evansi*-DNA and *T. vivax*-DNA probes indicated no cross-hybridization between the *T. evansi* and *T. vivax* PCR products. The results of the bloodsmear examinations, microhaematocrit centrifugations (MHC) and IFAT indicated that 23 (6.7 percent), 39 (11.4 percent) and 135 (39.5 percent) of the 342 blood samples investigated (including 316 from water buffaloes) contained trypanosomes, respectively. The results of the PCR-based assays indicated that 68 (19.9 percent) of the same blood samples contained *T. vivax* (or at least *T. vivax* DNA), and that none contained *T. evansi* or any other member of the subgenus *Trypanozoon*. For the detection of trypanosomes, the assay therefore appeared almost twice as sensitive as the MHC. These results are the first on the molecular characterization of the

trypanosomes infecting water buffaloes in Venezuela. When the results of the MHC (which is the most practical, and frequently used, alternative detection method) were used as the gold standard, the PCR-based assay for *T. vivax* was found to have 100 percent sensitivity, 90.4 percent specificity, a positive predictive value of 0.57, a positive likelihood ratio of 10.45, and a negative likelihood ratio of 0.00. The assay therefore appears a reasonable choice for detecting *T. vivax* in the mammalian livestock of Venezuela and elsewhere.

13808. **Gibson, W., Peacock, L., Ferris, V., Williams, K. & Bailey, M., 2006**. Analysis of a cross between green and red fluorescent trypanosomes. *Biochemical Society Transactions,* **34** (4): 557-559.

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Trypanosoma brucei undergoes genetic exchange in its insect vector, but the mechanism is unknown and no one has yet seen the process. By crossing genetically engineered red and green fluorescent trypanosomes, we have been able to pinpoint the location of genetic exchange in the fly and search for intermediate stages. In experimental crosses of red and green parental trypanosomes, yellow hybrid trypanosomes first appeared in the fly salivary glands as early as 13 days after infection and were observed only in flies with a mixture of red and green trypanosomes in one or both salivary glands. Despite high numbers of flies with mixed infections, yellow trypanosomes were not detected in the fly midgut or proventriculus. The hybrid nature of yellow trypanosomes was confirmed by analysis of molecular karyotypes and microsatellite alleles. As well as yellow hybrids, hybrid trypanosomes with red, green or no fluorescence were also recovered from fly salivary glands. Analysis of microsatellite alleles in parental and progeny clones showed Mendelian inheritance. Our findings are consistent with the hypothesis that mating takes place between trypanosomes in the salivary glands of the fly before they attach to the salivary gland epithelium.

13809. **Gonzalez, L.E., Garcia, J.A., Nunez, C., Perrone, T.M., Gonzalez-Baradat, B., Gonzatti, M.I. & Reyna-Bello, A., 2005**. *Trypanosoma vivax*: A novel method for purification from experimentally infected sheep blood. *Experimental Parasitology,* **111** (2): 126-129.

Universidad Simon Bolivar, Departamento de Biología Celular, Grupo deBioquimica e Inmunologia de Hemoparasitos, Caracas, Venezuela.

Trypanosoma vivax is the principal etiological agent of bovine trypanosomosis, a widely disseminated disease in tropical and subtropical regions. Here, we present a simple and reproducible method for the purification of *T. vivax* from experimentally infected and immunosuppressed sheep, using an isopycnic Percoll gradient, followed by DEAE-cellulose chromatography, with an estimated yield of 11-15 percent. This method could be used for the purification of *T. vivax* geographical isolates from various locations and from different natural hosts.

13810. **Hamilton, P.B., Stevens, J.R., Gidley, J., Holz, P. & Gibson, W.C., 2005**. A new lineage of trypanosomes from Australian vertebrates and terrestrial bloodsucking leeches (*Haemadipsidae). International Journal for Parasitology,* **35** (4): 431-443.

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Little is known about the trypanosomes of indigenous Australian vertebrates and their vectors. We surveyed a range of vertebrates and blood-feeding invertebrates for trypanosomes by parasitological and PCR-based methods using primers specific to the small subunit ribosomal RNA (SSU rRNA) gene of genus *Trypanosoma*. Trypanosome isolates were obtained in culture from two common wombats, one swamp wallaby and an Australian bird (*Strepera* sp.). By PCR, blood samples from three wombats, one brush-tailed wallaby, three platypuses and a frog were positive for trypanosome DNA. All the blood-sucking invertebrates screened were negative for trypanosomes both by microscopy and PCR, except for specimens of terrestrial leeches (*Haemadipsidae*). Of the latter, two *Micobdella* sp. specimens from Victoria and 18 *Philaemon* sp. specimens from Queensland were positive by PCR. Four *Haemadipsa zeylanica* specimens from Sri Lanka and three *Leiobdella jawarerensis* specimens from Papua New Guinea were also PCR positive for trypanosome DNA. We sequenced the SSU rRNA and glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH) genes in order to determine the phylogenetic positions of the new vertebrate and terrestrial leech trypanosomes. In trees based on these genes, Australian vertebrate trypanosomes fell in several distinct clades, for the most part being more closely related to trypanosomes outside Australia than to each other. Two previously undescribed wallaby trypanosomes fell in a clade with *Trypanosoma theileri*, the cosmopolitan bovid trypanosome, and *Trypanosoma cyclops* from a Malaysian primate. The terrestrial leech trypanosomes were closely related to the wallaby trypanosomes, *T. cyclops* and a trypanosome from an Australian frog. We suggest that haemadipsid leeches may be significant and widespread vectors of trypanosomes in Australia and Asia.

13811. **Jamal, S., Sigauque, I., Macuamule, C., Neves, L., Penzhorn, B.L., Marcotty, T. & Bossche, P.v.d., 2005**. The susceptibility of *Trypanosoma congolense* isolated in Zambezia Province, Mozambique, to isometamidium chloride, diminazene aceturate and homidium chloride. *Onderstepoort Journal of Veterinary Research,* **72** (4): 333-8.

National Directorate of Livestock, Maputo, Mozambique.

Resistance to trypanocidal drugs has been detected in various African countries and is a serious impediment to the control of livestock trypanosomosis. To determine whether drug resistant trypanosome strains are present in the Zambezia Province of Mozambique a study was initiated. To assess the effect of the farming system and the drug-use regimen on the development of drug resistance, trypanosome isolates were collected from cattle from subsistence and commercial livestock production systems. The susceptibility of seven isolates against isometamidium chloride, diminazene aceturate and homidium chloride was tested in mice using a multiple-dose test. In four of the seven isolates high levels of drug resistance to diminazene aceturate and isometamidium chloride were detected. In most cases the observed

levels of drug resistance correlated with the drug-use practices in the particular livestock production system.

13812. **Jamnadass, R.H., Pelle, R., Pandit, P., Ricard, B. & Murphy, N.B., 2006**. *Trypanosoma brucei*: composition, organisation, plasticity, and differential transcription of NlaIII repeat elements in drug-resistant and sensitive isolates. *Experimental Parasitology,* **113** (4): 244-255.

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A *Trypanosoma brucei brucei* DNA repeat sequence termed NlaIII repeat (NR) was originally isolated from a multidrug-resistant field isolate CP547. Identification and characterisation of an extrachromosomal element from a multidrug-resistant isolate of *T. brucei brucei* was then carried out and subsequently studied in a laboratory strain. Circular extrachromosomal DNA was identified in the nuclear genome of *T. brucei* and NRs shown to be exclusively episomal. Here we show that NR sequences in CP547 are present on linear chromosomes as well as on episomal circular elements. Sequence analysis shows that NRs are composed of three classes of sub-repeat arranged in a specific order. Heterogeneity in size and sequence of an episomal 6.6kbp element was shown in successive passages of the original CP547 isolate and derived clones in mice. Its copy number was unstable and was affected by selective pressure with the trypanocide diminazene aceturate. Some of the extrachromosomal elements appear to be composed of RNA-DNA hybrids. NR sequences were transcribed in a developmentally regulated manner but transcripts did not contain the spliced-leader sequence found on all trypanosome mRNAs.

13813. **Li, F.G., Gasser, R.B., Zheng, J.Y., Claes, F., Zhu, X.Q. & Lun, Z.R., 2005.** Application of multiple DNA fingerprinting techniques to study the genetic relationships among three members of the subgenus *Trypanozoon* (Protozoa: Trypanosomatidae). *Molecular and Cellular Probes*, **19** (6): 400-407.

State Key Laboratory of Biocontrol, School of Life Sciences, Center for Parasitic Organisms, Sun Yat-sen (Zhongshan) University, Guangzhou 510275, People's Republic of China.

Three different DNA fingerprinting techniques, the mobile genetic element (MGE)-PCR, simple sequence repeat (SSR)-PCR and random amplified polymorphic DNA (RAPD)-PCR, were used to define a large set of genetic markers to study genetic similarity within and among *Trypanosoma brucei*, *Trypanosoma equiperdum* and *Trypanosoma evansi* strains (n = 18) from China, Africa and South America and to investigate their genetic relationships. Using the three fingerprinting techniques, >890 bands (ranging in size from 0.2 to 2 kb) were defined for all 18 strains of *Trypanosoma.* Within each of the strains, 39-59 bands were defined. The similarity coefficients between strains ranged from 41 to 94 percent, with a mean of 65 percent. There was more genetic similarity among strains within *T. evansi* (mean 79 percent) compared with *T. equiperdum* (65 percent) and *T. brucei* (59 percent). The similarity coefficient data were used to construct the dendrogram, which revealed that (irrespective of species) the majority of strains from China and South America grouped together to the exclusion of those from Africa. The exceptions were a *T. brucei* strain from

Africa and a *T. equiperdum* strain of unknown origin. Hence, employing data sets generated using the three different fingerprinting methods, it was not possible to unequivocally distinguish among *T. brucei*, *T. evansi* and *T. equiperdum*, although there was a tendency for *T. evansi* strains to group together to the exclusion of *T. brucei*. The findings provide support for the hypothesis that *T. evansi* originated from a mutated form of *T. equiperdum* and stimulate further investigations of the genetic make-up and evolution of members of the subgenus *Trypanozoon*.

13814. **Li, F., Zheng, J., Jia, W. & Lun, Z., 2005**. Analysis of molecular profiles among *Trypanozoon* species and subspecies by MGE-PCR method. *Chinese Journal of Parasitology and Parasitic Diseases,* **23** (5):277-82.

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To analyse the relationship between genetic variability and evolution among *Trypanosoma brucei* (including *T. b. brucei, T. b. rhodesiense* and *T. b. gambiense*, *T. evansi* and *T. equiperdum* isolates), genomic DNAs of 26 trypanosome isolates were amplified by a mobile genetic elements (MGE) -PCR technique and cluster analysis was performed based on the molecular profiles with Neighbor-Joining method. The genetic variability among trypanosome isolates examined was obvious with an average genetic distance of 41.2 percent (ranged from 0 to 100 percent). Similarity coefficient among *T. brucei* isolates was 41.15 percent which was lower than that between *T. evansi* and *T. equiperdum* isolates. The closest relationship was found between *T. evansi* and *T. brucei* isolates with a similarity coefficient of 62.94 percent. The genetic variability between *T. b. rhodesiense* and *T. b. brucei* isolates was higher than that among *T. b. gambiense* isolates. In conclusion, species and subspecies displayed a higher genetic variability; *T. equiperdum* isolates collected from China and from South America, and *T. evansi* isolates from China and from South America, should have a similar origin.

13815. **Likeufack, A.C., Brun, R., Fomena, A. & Truc, P., 2006.** Comparison of the *in vitro* drug sensitivity of *Trypanosoma brucei gambiense* strains from West and Central Africa isolated in the periods 1960–1995 and 1999–2004. *Acta Tropica*. **In press; corrected proof**.

Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale, P.O. Box 288, Yaoundé, Cameroon; Institut de Recherche pour le Développement, UR177, LRCT, TA 207/G, Campus International de Baillarguet, 34 398 Montpellier Cedex 5, France; Swiss Tropical Institute, Parasite Chemotherapy, P.O. Box, CH-4002 Basel, Switzerland; Université des Sciences de Yaoundé, Yaoundé, Cameroon.

The situation of human African trypanosomiasis remains serious with one of the main threats being the increasing number of relapses or treatment failures after melarsoprol treatment. In order to investigate and to compare drug sensitivities of trypanosomes isolated at different time periods and in different locations, two sets of *Trypanosoma brucei gambiense* strains were used. One set was isolated in the time period 1960–1981 and the

other one in 1995–2004 from different locations of West and Central Africa. These isolates were not selected based on the treatment outcome but on availability. The drug sensitivity profile for all available drugs in use and the diamidine compound DB75 was established. IC_{50} values were not significantly different between the "old" and "new" stocks. No indications for emerging drug resistance to any drug could be observed. The results indicate a relative stability of *in vitro* sensitivity of *T. b. gambiense* to trypanocidal drugs in space (West and Central Africa) and time (1960–2004).

13816**. MacLeod, A., 2004.** Minisatellites and MVR-PCR for the individual identification of parasite isolates. In: *Parasite Genomics Protocols*, Humana Press, Totowa, USA., pp.187-202.

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In recent years, a wide variety of biochemical and molecular typing systems have been employed in the study of parasite diversity aimed at investigating the level of genetic diversity and delineating the relationships among different species and subspecies. Parasite sequence-specific polymerase chain reaction (PCR)-based genotyping systems are among the most useful tools employed to date, because they can be applied to very small quantities of host-contaminated parasite material and, using repeated loci such as mini- and microsatellites, allow the identification and tracking of individual strains as well as the determination of allele and genotype frequencies in populations. Although minisatellites have been used very successfully to study parasite populations, in particular *Trypanosoma brucei* populations, there are some technical problems involved in the use of these markers. For example, minisatellite alleles tend to vary in a quasi-continuous fashion, making unambiguous allele identification difficult. The development of minisatellite variant repeat (MVR) mapping by the polymerase chain reaction (MVR-PCR) as a digital approach to DNA typing has overcome many of the drawbacks of minisatellite length analysis. The system assays the dispersion patterns of MVRs within minisatellite alleles, producing an easily interpretable code for each allele. This technique not only allows unequivocal allele identification but also reveals cladistic information that can be used to determine the possible genetic relationships among the different strains and subspecies. The MVR mapping technique has been applied successfully to minisatellites in the parasite *Plasmodium falciparum* to uniquely identify strains, and more extensively in *Trypanosoma brucei*, where it was used to determine population structure and to examine the relationships among *T. brucei* subspecies, providing evidence for multiple origins of human infectivity. In this chapter, the methods for genotyping of *T. brucei* parasites using both minisatellite allele length and MVR mapping are described in full and can be easily adapted to apply to mini-satellites in other parasites.

13817. **Masiga, D.K., Ndung'u, K., Tweedie, A., Tait, A. & Turner, C.M., 2006.** *Trypanosoma evansi*: Genetic variability detected using amplified restriction fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) analysis of Kenyan isolates. *Experimental Parasitology*, **114** (3): 147-153.

Division of Infection and Immunity, IBLS, Biomedical Research Centre, University of Glasgow, 120 University Place, Glasgow G12 8TA, UK; Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 43844, 00100 Nairobi, Kenya.

We compared two methods to generate polymorphic markers to investigate the population genetics of *Trypanosoma evansi*; random amplified polymorphic DNA (RAPD) and amplified restriction fragment length polymorphism (AFLP) analyses. AFLP accessed many more polymorphisms than RAPD. Cluster analysis of the AFLP data showed that 12 *T. evansi* isolates were very similar ('type A') whereas 2 isolates differed substantially ("type B"). Type A isolates have been generally regarded as genetically identical but AFLP analysis was able to identify multiple differences between them and split the type A *T. evansi* isolates into two distinct clades.

13818. **Masumu, J., Geysen, D., Vansnick, E., Geerts, S. & Van den Bossche, P., 2006**. A modified AFLP for *Trypanosoma congolense* isolate characterisation. *Journal of Biotechnology,* **125** (1): 22-26.

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The amplified fragment length polymorphism (AFLP) technique is a reliable and powerful DNA fingerprint tool for genetic characterisation and analysis. In this paper, we described a modified AFLP with high resolution for *Trypanosoma congolense* using one enzyme and agarose or Elchrom gel electrophoresis. Eleven allopatric and fourteen sympatric isolates of *T. congolense* savannah were used to assess the resolution of the method and its ability to characterise *T. congolense* isolates. Two enzymes (Eco RI or Bgl II) and corresponding non-selective and selective primers were used to identify the most appropriate combination. Patterns generated by Bgl II enzyme and a single selective primer A, C, G or T produced clear profiles. Each of the four selective primers produced different profiles for all the 25 *T. congolense* isolates. Due to the reduction in the number of bands, profiles could be analysed using agarose or Elchrom gels. Although comparison of a great number of samples could benefit from software help, this technique did not require flurochrome detection methods. The results of the present study demonstrated that this modified AFLP makes the characterisation of *T. congolense* easier while maintaining high resolution.

13819. **Masumu, J., Marcotty, T., Geysen, D., Geerts, S., Vercruysse, J., Dorny, P. & den Bossche, P.V., 2006**. Comparison of the virulence of *Trypanosoma congolense* strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. *International Journal of Parasitology,* **36** (4): 497-501.

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The virulence of 31 genetically different *Trypanosoma congolense* strains belonging to the Savannah subgroup and isolated from cattle at 11 sites in a trypanosomiasis endemic area of eastern Zambia was compared. Virulence testing, done in OF1 mice, revealed three

virulence categories. Strains were considered extremely virulent when the median survival time ranged between 5 and 9 days. Moderately virulent strains had a median survival time between 10 and 30 days and low virulence, more than 30 days. For each strain, the prepatent period was determined and the PCV of the infected animals was measured at regular intervals. A total of six (19.4 percent) strains belonged to the extremely virulent category with a short prepatent period (mean 2.3+/-0.3 days), high parasitaemia, decline in PCV of 15.6+/-1.1 percent during the first 7 days p.i. and a short median survival time (mean 6 days). The remainder of the strains belonged to the moderate (13 strains) or low (12 strains) virulence categories with median survival times of 13 and 60 days, respectively. They had longer prepatent periods (means 3.2+/-1.6 days and 3.5+/-1.6 days for moderately virulent and strains with low virulence, respectively) and the decline in PCV was less steep (decline of 14.2+/-0.6 and 9.7+/-0.6 percent during the first 7 days of infection with moderately virulent strains and strains with low virulence, respectively). Extremely virulent strains were isolated from cattle at four sampling sites with 60 percent of the cattle from one sampling site harbouring such extremely virulent strains. Results from this study demonstrated substantial differences in the virulence of *T. congolense* strains of the Savannah subgroup, isolated in one geographic area from a single host species. On the assumption that information on virulence obtained from tests in mice can be extrapolated to cattle, the high proportion of strains with low to moderate virulence is thought to be attributed to the important role of susceptible cattle as reservoirs of trypanosomes in the study area and the ensuing selection against extremely virulent strains.

13820. **Masumu, J., Marcotty, T., Ndeledje, N., Kubi, C., Geerts, S., Vercruysse, J., Dorny, P. & van den Bossche, P., 2006**. Comparison of the transmissibility of *Trypanosoma congolense* strains isolated in a trypanosomiasis endemic area of eastern Zambia by *Glossina morsitans morsitans*. *Parasitology,* **133** (3): 331-334.

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Transmission experiments were conducted to compare the transmissibility of genetically different *Trypanosoma congolense* (Savannah subgroup) strains isolated from cattle in a trypanosomiasis endemic area of eastern Zambia. A total of 17 strains were compared. Three strains were extremely virulent with a short pre-patent period, high parasitaemia and a short median survival time (between 5 and 9 days) in mice. The remainder of the strains belonged to the moderate (6 strains) or low (8 strains) virulence categories with median survival times between 10 and 30 days and >30 days, respectively. Batches of 40 teneral *Glossina morsitans morsitans* (Diptera: Glossinidae) were offered a single bloodmeal on mice infected with one of those strains. Flies were dissected to determine their infection status 21 days later. The proportion of flies with procyclic and metacyclic infections differed significantly between trypanosome strains and was significantly higher in flies infected with extremely virulent strains (P=0.033 and P=0.016 for the differences in the procyclic infection rate of strains with moderate and low virulence, respectively and P=0.005 and P=0.019 for the differences in the metacyclic infection rate of strains with moderate and low virulence, respectively). On the other hand, moderately virulent strains had, in general, higher procyclic and metacyclic infection rates compared to low virulent strains. But the differences were not significant (P>0.05). The outcome of those experiments shows clear differences in

transmissibility of trypanosome strains associated with their virulence. This observation confirms the theory for the evolution and maintenance of virulence in a parasite population and may explain the persistence of virulent trypanosome strains in a susceptible host population.

13821. **Ngaira, J.M., Olembo, N.K., Njagi, E.N.M. & Ngeranwa, J.J.N., 2005**. The detection of non-RoTat 1.2 *Trypanosoma evansi*. *Experimental Parasitology,* **110** (1): 30-38.

Department of Biochemistry and Biotechnology, Kenyatta University, P.O. Box 25530, Nairobi, Kenya. [Janemngaira@yahoo.com].

The majority of *Trypanosoma evansi* can be detected using diagnostic tests based on the variant surface glycoprotein (VSG) of *Trypanosoma evansi* Rode *Trypanozoon* antigen type (RoTat) 1.2. Exceptions are a number of *T. evansi* isolated in Kenya. To characterize *T. evansi* that are undetected by RoTat 1.2, we cloned and sequenced the VSG cDNA from *T. evansi* JN 2118Hu, an isolate devoid of the RoTat 1.2 VSG gene. A 273 bp DNA segment of the VSG gene was targeted in PCR amplification for the detection of non-RoTat 1.2 *T. evansi*. Genomic DNA samples from different trypanosomes were tested including 32 *T. evansi*, 10 *Trypanosoma brucei*, three *Trypanosoma congolense*, and one *Trypanosoma vivax*. Comparison was by PCR amplification of a 488 bp fragment of RoTat1.2 VSG gene. Results showed that the expected 273 bp amplification product was present in all five non-RoTat 1.2 *T. evansi* tested and was absent in all 27 RoTat 1.2-positive *T. evansi* tested. It was also absent in all other trypanosomes tested. The PCR test developed in this study is specific for non-RoTat 1.2 *T. evansi*.

13822. **Njiru, Z.K., Constantine, C.C., Masiga, D.K., Reid, S.A., Thompson, R.C.A. & Gibson, W.C., 2006**. Characterization of *Trypanosoma evansi* type B. *Infection, Genetics and Evolution,* **6** (4): 292-300.

Division of Health, School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, South Street, WA 6150, Australia; Trypanosomiasis Research Centre, Kenya Agricultural Research Institute, P.O. Box 362, Kikuyu, Kenya.

A distinctive feature of *Trypanosoma evansi* is the possession of a kinetoplast that contains homogeneous DNA minicircles, but lacks DNA maxicircles. Two major sequence variants of the minicircle have been described and here we have sequenced the type B variant and designed a specific PCR test to distinguish it from type A. Further a test based on maxicircles to distinguish *T. brucei brucei* from *T. evansi* was designed and evaluated. Using the designed PCR tests, we detected three type B isolates from camel blood samples collected in northern Kenya, more than 20 years after the first isolation of type B. Comparison of minicircle sequences from all four type B isolates shows >96 percent identity within the group, and 50-60 percent identity to type A minicircles. Phylogenetic analysis based on minicircle sequences reveals two clusters, one comprising isolates of type A and one of type B, while random amplification of polymorphic DNA show slight polymorphic bands within type B. Most *T. evansi* isolates analysed were heterozygous at a repetitive coding locus

(MORF2). All type B isolates had one genotype designated 3/5 based on the alleles present. Three camel isolates, which had homogenous type A minicircles, lacked the RoTat 1.2 gene, while another five isolates were *T. b. brucei*, based on the heterogeneity of their minicircles and presence of maxicircles as demonstrated by PCR amplification of the gene for cytochrome oxidase subunit 1. Our results confirm the existence of *T. evansi* type B isolates, *T. b. brucei* and existence of *T. evansi* type A without RoTat 1.2 gene in Kenyan isolates.

13823. **Piontkivska, H.H. & Hughes, A. L., 2005**. Environmental kinetoplastid-like 18S rRNA sequences and phylogenetic relationships among Trypanosomatidae: paraphyly of the genus *Trypanosoma*. *Molecular and Biochemical Parasitology,* **144** (1): 94-99.

Department of Biological Sciences, 242 Cunningham Hall, Kent State University, Kent, OH 44242, USA.

Using kinetoplastid-like sequences from deep-sea environmental samples as an out-group, we applied phylogenetic analysis to 18S rRNA sequences of the families Trypanosomatidae and Bodonidae (Eugelenozoa: Kinetoplastida). The monophyly of the genus *Trypanosoma* was not supported by a number of different methods. Rather, the results indicate that the American and African trypanosomes constitute distinct clades, therefore, implying that the major human disease agents *T. cruzi* (cause of Chagas' disease) and *T. brucei* (cause of African sleeping sickness) are not as closely related to each other as they were previously thought to be. Likewise, the results did not support monophyly of the genera *Leishmania, Leptomonas, Bodo* and *Cryptobia.*

13824. **Ravel, S., Patrel, D., Koffi, M., Jamonneau, V. & Cuny, G., 2006**. Cyclical transmission of *Trypanosoma brucei gambiense* in *Glossina palpalis gambiensis* displays great differences among field isolates. *Acta Tropica*. **In press; corrected proof.**

IRD, UR177, Laboratoire de Recherche et de Coordination sur les Trypanosomoses, IRD-CIRAD, TA 207/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France.

Six sets of teneral *Glossina palpalis gambiensis* (Diptera: Glossinidae) were fed on mice infected with six different isolates of *Trypanosoma brucei gambiense* (each mouse was infected with one of the isolates), previously isolated from patients in the sleeping sickness focus of Bonon, Côte d'Ivoire and in Makoua, Congo. All the tsetse flies were dissected 42 days post-infection and midgut and salivary glands were examined for trypanosomes by microscopical examination. No infection was observed with the reference stock whereas each of the five recently isolated trypanosome isolates was able to infect tsetse flies, with rates of infection varying between 9.7 and 18.2 percent depending on the isolate. Three isolates displayed only immature infections with 9.7, 17.3 and 18 percent of the flies showing trypanosomes in their midgut. One isolate gave both immature (12.1 percent) and mature infections (6.1 percent). Finally, the last isolate involved only mature infections in 9.7 percent of the *Glossina* species examined. These substantial differences in the cyclical transmission

of *T. b. gambiense* in the same fly species could have important implications for the epidemiology of the transmission of Human African Trypanosomiasis.

13825. **Truc, P., Gibson, W. & Herder, S., 2006**. Genetic characterization of *Trypanosoma evansi* isolated from a patient in India. *Infection, Genetics and Evolution.* **In press; corrected proof**..

Institut de Recherche pour le Développement, UR177, Instituto de Combate et Controlo das Tripanossomiases (ICCT), CP 2657, Luanda, Angola.

The first human case of trypanosomiasis caused by *Trypanosoma evansi* was recently discovered in India. We have focused on the parasite to investigate whether this atypical infection was due to a particular genotype of *T. evansi*. The SRA gene was not detected by PCR in the Indian human *T. evansi* (TEVH) DNA sample. TEVH appears to be closely related to Vietnam WH, with identical alleles for TRBPA and MT30-33 AC/TC microsatellites. Furthermore, *T. evansi* has homogeneous kDNA minicircles and the minicircles of isolate TEVH were shown to be of Type A. Thus, the *T. evansi* isolated from an Indian patient appears to be a typical *T. evansi* as far as we can judge, suggesting that the explanation for this unusual infection may lie with the patient.

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[See also **29**: nos. 13466, 13478, 13494, 13502, 13505, 13510, 13541, 13544, 13545, 13558, 13584, 13588, 13597]

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Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK.

13827. A**libu, V.P., Richter, C., Voncken, F., Marti, G., Sanjay, S., Renggli, C.K., Seebeck, T., Brun, R. & Clayton, C., 2006**. The role of *Trypanosoma brucei* MRPA in melarsoprol susceptibility. *Molecular and Biochemical Parasitology,* **146** (1): 38-44.

Universitat Heidelberg, Zentrum fur Molekulare Biologie (ZMBH), Im Neuenheimer Feld 282, D69120 Heidelberg, Germany.

We previously showed that over-expression of *Trypanosoma brucei* MRPA, a member of the multidrug resistance protein family in *T. brucei*, reproducibly resulted in resistance to the anti-trypanosomal drug melarsoprol *in vitro*. MRPA is predicted to mediate efflux of melarsoprol as a conjugate with trypanothione, a glutathione-spermidine conjugate which is the major small thiol in trypanosomes. Here, we show that depletion of MRPA by RNA interference resulted in moderate hypersensitivity to both melarsoprol and melarsen oxide.

Over-expression of MRPA alone is not sufficient to cause melarsoprol resistance *in vivo*, although it is sufficient *in vitro*. This discrepancy is not an effect of drug metabolism since over-expression of MRPA alone conferred resistance to melarsoprol and its principal metabolite, melarsen oxide, *in vitro*. Over-expression of MRPA was not detected in four melarsoprol-resistant trypanosome isolates from sleeping sickness patients.

13828. **Alphey, M.S., Burton, A., Urbaniak, M.D., Boons, G.J., Ferguson, M.A. & Hunter, W.N., 2006.** *Trypanosoma brucei* UDP-galactose-4'-epimerase in ternary complex with NAD+ and the substrate analogue UDP-4-deoxy-4-fluoro-alpha-D-galactose. *Acta Crystallographica Section F Structural Biology and Crystallography Communications*, **62** (9): 829-834.

Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

13829. **Alphey, M.S. & Hunter, W.N., 2006**. High-resolution complex of papain with remnants of a cysteine protease inhibitor derived from *Trypanosoma brucei*. *Acta Crystallographica Section F Structural Biology and Crystallography Communications,* **62** (6): 504-508.

Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

13830. **Alsford, S., Kawahara, T., Glover, L. & Horn, D., 2005**. Tagging a *T. brucei* rRNA locus improves stable transfection efficiency and circumvents inducible expression position effects. *Molecular and Biochemical Parasitology,* **144** (2): 142-148.

Department of Infections and Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK.

13831. **Aranda, A., Maugeri, D., Uttaro, A.D., Opperdoes, F., Cazzulo, J.J. & Nowicki, C., 2006**. The malate dehydrogenase isoforms from *Trypanosoma brucei*: Subcellular localization and differential expression in bloodstream and procyclic forms. *International Journal for Parasitology,* **36** (3): 295-307.

Instituto de Quimica y Fisicoquimica Biologica IQUIFIB-CONICET, Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires, Junin 956, CP1113, Argentina.

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C Tschudi: Yale University, School of Medicine, Department of Epidemiology and Public Health, 295 Congress Ave., New Haven, CT 06536 USA.

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Department of Internal Medicine, Yale University Medical School, BCMM 136D, 295 Congress Avenue, Box 9812, New Haven, CT 06536-8012, USA.

13834. **Arhin, G.K.S., Shen, S. Y., Ullu, E. & Tschudi, C., 2004**. A PCR-based method for gene deletion and protein tagging in *Trypanosoma brucei*. *Methods in Molecular Biology*, **270**: 277-86.

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut, USA.

Sequence information on the *Trypanosoma brucei* genome is rapidly accumulating. As a consequence, there is a need for techniques to analyse gene function systematically. Here, we describe a polymerase chain reaction (PCR)-based method for direct gene deletion and the generation of epitope-tagged fusion proteins. The approach is based on methodologies developed for *Saccharomyces cerevisiae* and involves PCR amplification of a reporter cassette using primers containing flanking sequences specific to the target gene. The PCR product is then transfected directly into procyclic *T. brucei* cells, and homologous recombinants that carry the deleted or tagged target gene are identified.

13835. **Balmer, O., Palma, C., Macleod, A. & Caccone, A., 2006.** Characterization of di-, tri- and tetranucleotide microsatellite markers with perfect repeats for *Trypanosoma brucei* and related species. *Molecular Ecology Notes* **6** (2): 508-510.

Yale University, Department of Ecology and Evolutionary Biology, 165 Prospect St., New Haven, USA.[oliver.balmer@yale.edu, oliver.balmer@pronet.ch].

Trypanosoma brucei, a unicellular parasite causing human sleeping sickness and animal nagana, has a great impact on the socioeconomic environment of sub-Saharan Africa. The dynamics of the parasite are still poorly understood. We have characterized 14 polymorphic di-, tri- and tetranucleotide microsatellite loci with perfect repeats (only one motif) exhibiting between five and 16 alleles in *T. brucei* isolates from all over Africa and from all described subspecies. The microsatellites will be useful in addressing population genetic questions in *T. brucei* to better understand the population structure and spread of this important parasite.

13836. **Bell, A., Monaghan, P. & Page, A.P., 2006**. Peptidyl-prolyl cis-trans isomerases (immunophilins) and their roles in parasite biochemistry, host-parasite interaction and antiparasitic drug action. *International Journal for Parasitology,* **36** (3): 261-276.

Department of Microbiology, Moyne Institute of Preventive Medicine, University of Dublin, Trinity College, Dublin 2, Ireland. [abell@tcd.ie].

13837. **Besteiro, S., Barrett, M.P., Riviere, L. & Bringaud, F., 2005**. Energy generation in insect stages of *Trypanosoma brucei*: metabolism in flux. *Trends in Parasitology,* **21** (4): 185-191.

Wellcome Centre for Molecular Parasitology, The Anderson College, University of Glasgow, Glasgow G11 6NU, Scotland, UK.

The generation of energy in African trypanosomes is a subject of undoubted importance. In bloodstream-form organisms, substrate-level phosphorylation of glucose is sufficient to provide the energy needs of the parasite. The situation in procyclic-form trypanosomes is more complex. For many years, it was accepted that glucose metabolism followed a conventional scheme involving glycolysis, the tricarboxylic acid cycle and ATP-producing oxidative phosphorylation linked to the electron-transport chain. However, progress in sequencing the *Trypanosoma brucei* genome and the development of gene-knockout and RNA interference technology has provided novel insight. Coupling these new technologies with classical approaches, including NMR and mass spectrometry to analyse glycolytic intermediates and end products, have yielded several surprises. In this article, we summarize how these recent data have helped to change the view of metabolism in procyclic-form *T. brucei*.

13838. **Biton, M., Mandelboim, M., Arvatz, G. & Michaeli, S., 2006.** RNAi interference of XPO1 and Sm genes and their effect on the spliced leader RNA in *Trypanosoma brucei. Molecular Biochemistry and Parasitology*, **150** (2): 132-143.

The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel.

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INSERM U565 and CNRS UMR5153 and MNHN USM0503, Museum National d'Histoire Naturelle, 43 rue Cuvier, 75231 Paris Cedex 05, France.

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Laboratoire de Génomique Fonctionnelle des Trypanosomatides, Université Victor Segalen Bordeaux 2, UMR-5162 CNRS, 146 rue Leo Saignat, 33076 Bordeaux Cedex, France.

13841. **Broadhead, R.D., Daw, H.R., Farr, H., Griffiths, S., Hart, S.R., Portman, N., Shaw, M.K., Ginger, M.L., Gaskell, S.J., McKean, P.G. & Gull, K., 2006.** Flagellar motility is required for the viability of the bloodstream trypanosome. *Nature,* **440** (7081): 224-227.

K. Gull: University of Oxford, Sir William Dunn School of Pathology, S. Parks Rd, Oxford OX1 3RE, UK.

Here we present a detailed proteomic analysis of the trypanosome flagellum. RNA interference (RNAi)-based interrogation of this proteome provides functional insights into human ciliary diseases and establishes that flagellar function is essential to the bloodstream-form trypanosome. We show that RNAi-mediated ablation of various proteins identified in the trypanosome flagellar proteome leads to a rapid and marked failure of cytokinesis in bloodstream-form (but not procyclic insect-form) trypanosomes, suggesting that impairment of flagellar function may provide a method of disease control. A postgenomic meta- analysis, comparing the evolutionarily ancient trypanosome with other eukaryotes including humans, identifies numerous trypanosome-specific flagellar proteins, suggesting new avenues for selective intervention.

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Department of Microbiology and Immunology, 253 Biomedical Research Building, University at Buffalo, Buffalo, New York 14214, USA.

13843. **Callejas, S., Leech, V., Reitter, C. & Melville, S., 2006.** Hemizygous subtelomeres of an African trypanosome chromosome may account for over 75 percent of chromosome length. *Genome Research*, **16** (9): 1109-1118.

Department of Pathology, University of Cambridge, CB2 1QP, Cambridge, UK.

We have developed a Tb927 high-resolution DNA microarray to study DNA content variation along chromosome I, one of the most size-variable chromosomes, in different strains and subspecies of *T. brucei*. Results show considerable copy number polymorphism, especially at subtelomeres, but are insufficient to explain the observed size difference. Additional sequencing reveals that >50 percent of a larger chromosome I consists of arrays of variant surface glycoprotein genes (VSGs), involved in avoidance of acquired immunity. In total, the subtelomeres appear to be three times larger than the diploid core. These results reveal that trypanosomes can utilize subtelomeres for amplification and divergence of gene families to such a remarkable extent that they may constitute most of a chromosome, and that the VSG repertoire may be even larger than reported to date. Further experimentation is required to determine if these results are applicable to all size-variable chromosomes.

13844. **Carnes, J.T., J.R., Ernst, N.L., Steinberg, A. & Stuart, K., 2005**. An essential RNase III insertion editing endonuclease in *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences,* USA, **102** (46): 16614-16619.

K. Stuart: Seattle Biomedical Research Institute, 307 Westlake Ave N.,Suite 500, Seattle, WA 98109 USA.

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Laboratorio de Biologia Molecular de la Enfermedad de Chagas-INGEBI-CONICET, Departamento de Fisiologia y Biologia Molecular y Celular, Facultad de Ciencias Exactas y Naturales, University of Buenos Aires, Buenos Aires, Argentina.

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Laboratory of Glycobiology, Fundacion Instituto Leloir, Avenida Patricias Argentinas, 435 C1405 BWE Buenos Aires, Argentina.

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Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz (IOC/FIOCRUZ), Av. Brasil, 4365, 21045-900, Rio de Janeiro, Brazil. [marcelr@fiocruz.br].

Whole sequencing of protozoan trypanosomatid genomes revealed the presence of several predicted unknown genes coding for hypothetical proteins. Pairwise, alignment-based, computational methods available online are unable to identify the function of these sequences. To detect clues to identify the function of hypothetical proteins, a user-friendly, bioinformatic tool named PROTOzoan Gene Identification Motifs (PROTOGIM, available on http://www.biowebdb.org/protogim) was developed, which allows the user to search functional patterns of hypothetical proteins through the screening of regular expression in the sequences. The analysis of 1,194 trypanosomatid hypothetical proteins through PROTOGIM resulted in an identification of motifs and domains in 98 percent of the cases, demonstrating the reliability and accuracy of the employed method. The added value of this tool is the possibility to modify or insert new regular expressions to perform an analysis against either one or several sequences at the same time. An *in silico* strategy along with biochemical and molecular characterizations creates new possibilities to find the functions of hypothetical proteins at the postgenome era.

13848. **Chanez, A.L., Hehl, A.B., Engstler, M. & Schneider, A., 2006.** Ablation of the single dynamin of *T. brucei* blocks mitochondrial fission and endocytosis and leads to a precise cytokinesis arrest. *Journal of Cell Science*, **119** (14): 2968-2974.

Department of Biology/Cell and Developmental Biology, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland.

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Department of Biology/Cell and Developmental Biology, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland.

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Department of Biology/Zoology, University of Fribourg, Chemin du Musée 10, CH-1700 Fribourg, Switzerland.

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Division of Microbial Pathogenesis and Immune Response, Department of Biomedical Sciences, Meharry Medical College, Nashville, TN 37208, USA. [mchaudhuri@mmc.edu]

Trypanosome alternative oxidase (TAO) is the cytochrome-independent terminal oxidase of the mitochondrial electron transport chain. TAO is a diiron protein that transfers electrons from ubiquinol to oxygen, reducing the oxygen to water. The mammalian bloodstream forms of *Trypanosoma brucei* depend solely on TAO for respiration. The inhibition of TAO by salicylhydroxamic acid (SHAM) or ascofuranone is trypanocidal. TAO is present at a reduced level in the procyclic form of *T. brucei*, where it is engaged in respiration and is also needed for developmental processes. Alternative oxidases similar to TAO have been found in a wide variety of organisms but not in mammals, thus rendering TAO an important chemotherapeutic target for African trypanosomiasis

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Division of Infectious Diseases, Department of Medicine, University of British Columbia, Faculty of Medicine, Vancouver Coastal Health Research Institute, Vancouver, British Columbia, Canada. [artc@interchange.ubc.ca].

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Department of Microbiology, University of Illinois, Urbana, 61801, USA.[cronan@life.uiuc.edu]

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Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, International Center for Public Health, 225 Warren Street, Newark, NJ 07103, USA.

In *Trypanosoma brucei*, transcription by RNA polymerase II accounts for the expression of the spliced leader (SL) RNA and most protein coding mRNAs. To understand the regulation of RNA polymerase II transcription in these parasites, we have purified a transcriptionally active enzyme through affinity chromatography of its essential subunit, RPB4. The enzyme preparation is active in both promoter-independent and promoter-dependent *in vitro* transcription assays. Importantly, the enzyme is sensitive to alpha-amanitin inhibition, a hallmark of eukaryotic RNA polymerase II enzymes. Using mass spectrometric analysis we have identified the previously unobserved RPB12 subunit of *T. brucei* RNA polymerase II. TbRPB12 contains a conserved CX(2)CX(10-15)CX(2)C zinc binding motif that is characteristic of other eukaryotic RPB12 polypeptides. We also identified seven proteins that associate with *T. brucei* RNA polymerase II. While both bioinformatics and biochemical analysis have focused on the subunit structure of trypanosome RNA polymerases, this is the first study that reveals a functional RNA polymerase II enzyme.

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Trypanosoma vivax is a haemoparasite affecting the livestock industry in South America and Africa. Despite the high economic relevance of the disease caused by *T. vivax*, little work has been done on its molecular characterization, in contrast with human trypanosomes, such as *T. brucei* and *T. cruzi*. The present study reports the construction of a semi-normalized genomic library and the sequencing of 160 Genome Sequence Survey (GSS) ends of *T. vivax*. The analyses of this preliminary data show that this simple and rapid approach worked well to generate some potential new markers for this species.

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Departamento de Microbiologia Geral, Instituto de Microbiologia Prof. Paulo de Goes, Centro de Ciencias da Saude, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

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LSPCMIB, UMR-CNRS 5068, Groupe de Chimie Organique Biologique, Université Paul Sabatier, Bat. IIR1, 118 Route de Narbonne 31062, Toulouse Cedex 9, France.

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Trypanosomatids are pathogenic protozoa that undergo a unique form of post-transcriptional RNA editing that inserts or deletes uridine nucleotides in many mitochondrial pre-mRNAs. Editing is catalyzed by a large multiprotein complex, the editosome. A key editosome enzyme, RNA editing terminal uridylyl transferase 2 (TUTase 2; RET2) catalyzes the uridylate addition reaction. Here, we report the 1.8 angstrom crystal structure of the *Trypanosoma brucei* RET2 apoenzyme and its complexes with uridine nucleotides. This structure reveals that the specificity of the TUTase for UTP is determined by a crucial water molecule that is exquisitely positioned by the conserved carboxylates D421 and E424 to sense a hydrogen atom on the N3 position of the uridine base. The three- domain structure also unveils a unique domain arrangement not seen before in the nucleotidyltansferase superfamily, with a large domain insertion between the catalytic aspartates. This insertion is present in all trypanosomatid TUTases. We also show that TbRET2 is essential for survival of the bloodstream form of the parasite and therefore is a potential target for drug therapy

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Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401, USA. [eyd1@cornell.edu].

Macrophages are crucial in immunity to infection. They possess potent antimicrobial function, and efficiently process and present peptide antigens for T-cell activation. Despite this, the intracellular protozoan parasites *Toxoplasma gondii*, *Trypanosoma cruzi* and *Leishmania* spp. target macrophages for infection. Each has adopted unique strategies to subvert macrophage antimicrobial functions. The parasites sabotage killing activities through

sophisticated manipulation of intracellular macrophage signaling pathways. These subversive activities are probably dictated by the need to evade microbicidal effector function, as well as to avoid proinflammatory pathology that can destabilize the host-parasite interaction. The molecular details of how intracellular protozoans manipulate macrophage signal transduction pathways for their own ends are beginning to emerge.

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Laboratory of Molecular Parasitology, Institute for Molecular Biology and Medicine (IBMM), UniversitéLibre de Bruxelles, Gosselies, Belgium.

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Department of Microbiology and Immunology, University of Maryland School of Medicine, 20 Penn Street, Baltimore, MD 21201, USA.

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Glycolysis and gluconeogenesis are, in part, driven by the interconversion of 3- and 2-phosphoglycerate (3-PG and 2-PG) which is performed by phosphoglycerate mutases (PGAMs) which can be cofactor dependant (dPGAM) or cofactor independent (iPGAM). The African trypanosome, *Trypanosoma brucei*, possesses the iPGAM form which is thought to play an important role in glycolysis. Here, we report on the use of RNA interference to down-regulate the *T. brucei* iPGAM in procyclic form *T. brucei* and evaluation of the resulting phenotype. We first demonstrated biochemically that depletion of the steady state levels of iPGAM mRNA correlates with a marked reduction of enzyme activity. We further show that iPGAM is required for cell growth in procyclic *T. brucei*.

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Trypanosoma brucei has become one of the model systems for unicellular pathogens to study fundamentally important biological phenomena. The method of choice today to examine gene function in these organisms is RNA interference (RNAi). Messenger RNA (mRNA) degradation is triggered by double-stranded RNA (dsRNA) produced *in vivo* from transgenes transcribed from opposing tetracycline (tet)-inducible T7 RNA polymerase promoters, or hairpin RNA transcribed from the tet-inducible procyclic acidic repetitive protein promoter. This chapter describes some of the methods we employ for ablation of gene expression by RNAi in *T. brucei* with particular emphasis on transfection and cloning of procyclic cells, induction of dsRNA expression, isolation of RNA, and analysis of dsRNA and target mRNA.

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African trypanosomes are protozoan parasites, most species of which are transmitted by tsetse flies. They reside in the mammalian bloodstream and evade the immune system by periodically switching the major protein on their surface-a phenomenon called antigenic variation, mediated by gene rearrangements in the trypanosome genome. The trypanosomes eventually enter the central nervous system and cause a fatal disease, commonly called nagana in domestic cattle and sleeping sickness in humans. Two sub-species of *Trypanosoma brucei* infect humans (*T. b. rhodesiense* and *T. b. gambiense*) and one sub-species does not survive in humans (*T. b. brucei*) because it is lysed by the human- specific serum protein, apolipoprotein L-I. Wild animals in Africa have other (less well understood) molecular mechanisms of suppressing the number of African trypanosomes in the blood, and some indigenous breeds of African cattle also display a partial "trypanotolerance" whose genetic loci have recently been mapped.

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Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.

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Laboratory of Molecular Parasitology, The Rockefeller University, New York, USA.

Trypanosoma brucei evades the host immune response by sequential expression of a large family of variant surface glycoproteins (VSG) from one of approximately 20

subtelomeric expression sites (ES). VSG transcription is monoallelic, and little is known about the regulation of antigenic switching. To explore whether telomere length could affect antigenic switching, we created a telomerase-deficient cell line, in which telomeres shortened at a rate of 3-6 bp at each cell division. Upon reaching a critical length, short silent ES telomeres were stabilized by a telomerase-independent mechanism. The active ES telomere progressively shortened and frequently broke. Upon reaching a critical length, the short active ES telomere stabilized, but the transcribed VSG was gradually lost from the population and replaced by a new VSG through duplicative gene conversion. We propose a model in which subtelomeric break induced replication mediated repair at a short ES telomere leads to duplicative gene conversion and expression of a new VSG.

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Protein farnesylation is a form of posttranslational modification that occurs in most, if not all, eukaryotic cells. Inhibitors of protein farnesyltransferase (PFTIs) have been developed as anticancer chemotherapeutic agents. Using the knowledge gained from the development of PFTIs for the treatment of cancer, researchers are currently investigating the use of PFTIs for the treatment of eukaryotic pathogens. This "piggy-back" approach not only accelerates the development of a chemotherapeutic agent for protozoan pathogens, but it is also a means of mitigating the costs associated with *de novo* drug design. PFTIs have already been shown to be efficacious in the treatment of eukaryotic pathogens in animal models, including both *Trypanosoma brucei*, the causative agent of African sleeping sickness, and *Plasmodium falciparum*, one of the causative agents of malaria. In this paper, current evidence and progress that support the targeting of protein farnesyltransferase [farnesyl-diphosphate farnesyltransferase] for the treatment of protozoal infections are summarized.

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Department of Biological Sciences, University of Hull, Hull, HU6 7RX, UK. [k.ersfeld@hull.ac.uk].

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The host range of African trypanosomes is influenced by innate protective molecules in the blood of primates. A subfraction of human high-density lipoprotein (HDL) containing apolipoprotein A-I, apolipoprotein L-I, and haptoglobin-related protein is toxic to *Trypanosoma brucei brucei* but not the human sleeping sickness parasite *Trypanosoma brucei rhodesiense*. It is thought that *T. b. rhodesiense* evolved from a *T. b. brucei*-like ancestor and expresses a defense protein that ablates the antitrypanosomal activity of human HDL. To directly investigate this possibility, we developed an *in vitro* selection to generate human HDL-resistant *T. b. brucei*. Here we show that conversion of *T. b. brucei* from human HDL sensitive to resistant correlates with changes in the expression of the variant surface glycoprotein (VSG) and abolished uptake of the cytotoxic human HDLs. Complete transcriptome analysis of the HDL-susceptible and -resistant trypanosomes confirmed that VSG switching had occurred but failed to reveal the expression of other genes specifically associated with human HDL resistance, including the serum resistance-associated gene (SRA) of *T. b. rhodesiense*. In addition, we found that while the original active expression site was still utilized, expression of three expression site-associated genes (ESAG) was altered in the HDL-resistant trypanosomes. These findings demonstrate that resistance to human HDLs can be acquired by *T. b. brucei*.

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Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK.

We have undertaken 2-DE and MS to identify proteins associated with arsenical drug resistance in *Trypanosoma brucei*. This parasite causes sleeping sickness in humans, and arsenical drug resistance is a significant potential problem. Comparative analysis of approximately 2,000 spots resolved by 2-DE in the soluble proteomes of drug-sensitive and drug-resistant isogenic lines of *T. brucei* identified a protein spot whose absence associated with resistance to the arsenical drug, Cymelarsan. MS matched this protein to an identical pair of tandem genes Tb09.211.0120 and 0130 that encode a putative nascent polypeptide associated complex subunit. This protein also occurs as an isoform located in both resistant and sensitive lines at a similar molecular weight, but different pI. The difference between isogenic lines was confirmed by Western blot using an antibody against recombinant protein. Both genes were identical in sequence between drug-sensitive and drug-resistant lines and both were transcribed as determined by RT-PCR. We postulate that the missing protein isoform arose due to the lack of a PTM.

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Genomes of some parasites contain dozens of alternative and highly diverged surface antigens, of which only a single one is expressed in any cell. Individual cells occasionally change expression of their surface antigen, allowing them to escape immune surveillance. These switches appear to occur in a partly random way, creating a diverse set of antigenic variants. In spite of this diversity, the parasitaemia develops as a series of outbreaks, in which each outbreak is dominated by relatively few antigenic types. Host-specific immunity eventually clears the dominant antigenic types, and a new outbreak follows from antigenic types that have apparently been present all along at low frequency. This pattern of sequential dominance by different antigenic types remains unexplained. We review the five most prominent theories, which have developed mainly from studies of the protozoans *Trypanosoma* and *Plasmodium*, and the bacterial spirochete *Borrelia*. The most promising theories depend on some combination of mechanisms to create favoured connectivity pathways through the matrix of transitions between variants. Favoured pathways may arise from biased switches at the molecular level of gene expression or from biases imposed by immune selection. We illustrate the concept of connectivity pathways by reanalysis of data on transitions between variants from *Borrelia hermsii*.

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Complete or partial genome sequences have recently become available for several medically and evolutionarily important parasitic protozoa, viz. *Trypanosoma cruzi*, *T. brucei*, *Leishmania* spp., *Plasmodium falciparum*, *Toxoplasma gondii*, *Giardia* sp. and *Entamoeba histolytica*. Through the application of bioinformatics complete metabolic repertoires for these parasites can be predicted. For experimentally intractable parasites insight provided by metabolic maps generated *in silico* has been startling. At its more extreme end, such bioinformatics reckoning facilitated the discovery in some parasites of mitochondria remodelled beyond previous recognition, and the identification of a non-photosynthetic chloroplast relic in malarial parasites. However, for experimentally tractable parasites, mapping of the general metabolic terrain is only a first step in understanding how the parasite modulates its streamlined, yet still often puzzlingly complex, metabolism in order to complete life cycles within host, vector, or environment. This review provides a comparative overview and discussion of metabolic strategies used by several different parasitic protozoa in order to subvert and survive host defences, and illustrates how genomic data contribute to the elucidation of parasite metabolism.

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During human African trypanosomiasis, trypanosomes (*Trypanosoma brucei gambiense* or *T. b. rhodesiense*) invade the central nervous system (CNS). Mechanisms of blood-brain barrier and blood-cerebrospinal fluid barrier leakage remain unknown. To better understand the relationships between trypanosomes and endothelial cells, the principal cell population of those barriers, we cultured a human bone marrow endothelial cell (HBMEC) line in the presence or absence of *T. b. gambiense*, to study cell activation. As indicated by NF-kappaB translocation to the nucleus, cells were activated in the presence of trypanosomes. The expression of the adhesion molecules ICAM-1, E-selectin and VCAM-1 increased in co-culture. The parasites induced the synthesis of the pro-inflammatory cytokines TNF-alpha, IL-6 and IL-8, and of nitric oxide (NO) by HBMEC. Cells were also cultured in the presence of parasite variant surface glycoproteins (VSGs), and an increase in TNF-alpha, IL-6, IL-8, and NO synthesis was also observed. Soluble VSGs induced NF-

kappaB translocation, and the expression of adhesion molecules, indicating that they could possibly be the molecular soluble factor responsible for endothelial cell activation. The permeability coefficient of HBMEC layer increased when cells were cultured in the presence of trypanosomes, parasite culture supernatant, or VSGs. Thus, *T. b. gambiense* can activate endothelial cells *in vitro*, through the release of soluble activating factors. Consequences of endothelial cell activation by parasite products may include a potentiation of the inflammatory reaction, leukocyte recruitment, passage of trypanosomes into the CNS, and barrier dysfunction observed during CNS involvement of HAT.

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African trypanosomes (*Trypanosoma brucei*) have a digenetic lifecycle that alternates between the mammalian bloodstream and the tsetse fly vector. In the bloodstream, replicating long slender parasites transform into non-dividing short stumpy forms. Upon transmission into the fly midgut, short stumpy cells differentiate into actively dividing procyclics. A hallmark of this process is the replacement of the bloodstream-stage surface coat composed of variant surface glycoprotein (VSG) with a new coat composed of procyclin. Pre-existing VSG is shed by a zinc metalloprotease activity (MSP-B) and glycosylphosphatidylinositol-specific phospholipase C (GPI-PLC). We now provide a detailed analysis of the coordinate and inverse regulation of these activities during synchronous differentiation. MSP-B mRNA and protein levels are upregulated during differentiation at the same time as proteolysis whereas GPI-PLC levels decrease. When transcription or translation is inhibited, VSG release is incomplete and a substantial amount of protein stays cell-associated. Both modes of release are still evident under these conditions, but GPI hydrolysis plays a quantitatively minor role during normal differentiation. Nevertheless, GPI biosynthesis shifts early in differentiation from a GPI-PLC sensitive structure to a resistant procyclic-type anchor. Translation inhibition also results in a marked increase in the mRNA levels of both MSP-B and GPI-PLC, consistent with negative regulation by labile protein factors. The relegation of short stumpy surface GPI-PLC to a secondary role in differentiation suggests that it may play a more important role as a virulence factor within the mammalian host.

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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 uM. 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 uM. A higher-affinity U2 uridine transporter (Km = 4.1 uM) 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.*

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Department of Medical Microbiology and Immunology, University of Wisconsin Medical School of Medicine and Public Health, 1300 University Avenue, Madison, Wisconsin 53706-1532, USA.

Activation of a type I cytokine response is important for early resistance to infection with *Trypanosoma brucei rhodesiense*, the extracellular protozoan parasite that causes African sleeping sickness. The work presented here demonstrates that trypanosome DNA activates macrophages to produce factors that may contribute to this response. Initial results demonstrated that *T. brucei rhodesiense* DNA was present in the plasma of C57BL/6 and C57BL/6-scid mice following infection. Subsequently, the effect of trypanosome DNA on macrophages was investigated; parasite DNA was found to be less stimulatory than *Escherichia coli* DNA but more stimulatory than murine DNA, as predicted by the CG dinucleotide content. Trypanosome DNA stimulated the induction of a signal transduction cascade associated with Toll-like receptor signalling in RAW 264.7 macrophage cells. The signalling cascade led to expression of mRNAs, including interleukin-12 (IL-12) p40, IL-6, IL-10, cyclooxygenase-2, and beta interferon. The treatment of RAW 264.7 cells and bone

marrow-derived macrophages with trypanosome DNA induced the production of NO, prostaglandin E2, and the cytokines IL-6, IL-10, IL-12, and tumour necrosis factor alpha. In all cases, DNase I treatment of *T. brucei rhodesiense* DNA abolished the activation. These results suggest that *T. brucei rhodesiense* DNA serves as a ligand for innate immune cells and may play an important contributory role in early stimulation of the host immune response during trypanosomiasis.

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African trypanosomes are parasitic protozoa that cause sleeping sickness and nagana. Trypanosomes are not only of scientific interest because of their clinical importance, but also because these protozoa contain several very unusual biological features, such as their specially adapted mitochondrion and the compartmentalization of glycolytic enzymes in glycosomes. The energy metabolism of *Trypanosoma brucei* differs significantly from that of their hosts and changes drastically during the life cycle. Despite the presence of all citric acid cycle enzymes in procyclic insect-stage *T. brucei,* citric acid cycle activity is not used for energy generation. Recent investigations on the influence of substrate availability on the type of energy metabolism showed that absence of glycolytic substrates did not induce a shift from a fermentative metabolism to complete oxidation of substrates. Apparently, insect-stage *T. brucei* use parts of the citric acid cycle for other purposes than for complete degradation of mitochondrial substrates. Parts of the cycle are suggested to be used for (i) transport of acetyl-CoA units from the mitochondrion to the cytosol for the biosynthesis of fatty acids, (ii) degradation of proline and glutamate to succinate, (iii) generation of malate, which can then be used for gluconeogenesis. Therefore the citric acid cycle in trypanosomes does not function as a cycle.

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The regulation of differentiation is particularly important in microbial eukaryotes that inhabit multiple environments. The parasite *Trypanosoma brucei* is an extreme example of this, requiring exquisite gene regulation during transmission from mammals to the tsetse fly vector. Unusually, trypanosomes rely almost exclusively on post-transcriptional mechanisms for regulated gene expression. Hence, RNA binding proteins are potentially of great significance in controlling stage-regulated processes. We have previously identified TbZFP1 as a trypanosome molecule transiently enriched during differentiation to tsetse midgut

procyclic forms. This small protein (101 amino acids) contains the unusual CCCH zinc finger, an RNA binding motif. Here, we show that genetic ablation of TbZFP1 compromises repositioning of the mitochondrial genome, a specific event in the strictly regulated differentiation programme. Despite this, other events that occur both before and after this remain intact. Significantly, this phenotype correlates with the TbZFP1 expression profile during differentiation. This is the first genetic disruption of a developmental regulator in *T. brucei.* It demonstrates that programmed events in parasite development can be uncoupled at the molecular level. It also further supports the importance of CCCH proteins in key aspects of trypanosome cell function.

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African trypanosomes are extracellular blood parasites that cause sleeping sickness in humans and nagana in cattle. The therapeutics used to control and treat these diseases are very ineffective and thus, the development of new drugs is urgently needed. We have previously suggested to use trypanosome-specific RNA aptamers as tools for the development of novel trypanocidal compounds. Here, we report the selection of a 2'-NH(2)-modified RNA aptamer that binds to live trypanosomes with an affinity of 70 +/- 15 nM. The aptamer adopts a stable G-quartet structure and has a half-life in human serum of > 30 h. RNA binding is restricted to the flagellar attachment zone, located between the cell body and the flagellum of the parasite. We demonstrate that antigen-tagged preparations of the aptamer can bind to live trypanosomes and that they can be used to re-direct immunoglobulins to the parasite surface.

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We have undertaken a large scale study of the proteins expressed in the procyclic form of the parasite *Trypanosoma brucei*, which causes African sleeping sickness, using 2-DE and MS. The complete data set encompasses over 2,000 identifications, of which 770 are distinct proteins. We have discovered that multiple protein isoforms appear to be common in *T. brucei*, as most proteins have been matched to more than one gel spot. We have developed visualisation software to investigate the differences between isoforms, based on the information from the results of database searches with MS data. We are able to highlight instances where PTMs are the most likely cause of variant forms. In other cases, spots that appear reproducibly across replicates contain fragments of proteins, arising either as experimental artefacts or as part of protein degradation. We are also able to classify clusters of gel spots into different groups based on the pattern of peptides that have been matched from MS data. The entire data set is stored within a relational database system that allows complex queries (http://www.gla.ac.uk/functionalgenomics). Using specific proteins as examples, we demonstrate how the visualisation software and the database query facilities can be used.

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This study aimed to provide the foundation for an integrative approach to the identification of the mechanisms underlying the response to infection with *Trypanosoma congolense*, and to identify pathways that have previously been overlooked. We undertook a large-scale gene expression analysis study comparing susceptible A/J and more tolerant C57BL/6 mice. In an initial time course experiment, we monitored the development of parasitaemia and anaemia in every individual. Based on the kinetics of disease progression, we extracted total RNA from liver at days 0, 4, 7, 10 and 17 post infection and performed a microarray analysis. We identified 64 genes that were differentially expressed in the two strains in non-infected animals, of which nine genes remained largely unaffected by the disease. Gene expression profiling at stages of low, peak, clearance and recurrence of parasitaemia suggest that susceptibility is associated with high expression of genes coding for chemokines (e.g. Ccl24, Ccl27 and Cxcl13), complement components (C1q and C3) and interferon receptor alpha (Ifnar1). Additionally, susceptible A/J mice expressed higher levels of some potassium channel genes. In contrast, messenger RNA levels of a few immune response, metabolism and protease genes (e.g. Prss7 and Mmp13) were higher in the tolerant C57BL/6 strain as compared to A/J.

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INSERM U565, CNRS UMR5153, and MNHN USM 0503, Museum National d'Histoire Naturelle, 75231 Paris, France.

Eukaryotic cilia and flagella are cytoskeletal organelles that are remarkably conserved from protists to mammals. Their basic unit is the axoneme, a well-defined cylindrical structure composed of microtubules and up to 250 associated proteins. These complex organelles are assembled by a dynamic process called intraflagellar transport. Flagella and cilia perform diverse motility and sensitivity functions in many different organisms. Trypanosomes are flagellated protozoa, responsible for various tropical diseases such as sleeping sickness and Chagas disease. In this review, we first describe general knowledge on the flagellum: its occurrence in the living world, its molecular composition, and its mode of assembly, with special emphasis on the exciting developments that followed the discovery of intraflagellar transport. We then present recent progress regarding the characteristics of the trypanosome flagellum, highlighting the original contributions brought by this organism. The

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Trypanosomes have an unusual mitochondrial genome, called kinetoplast DNA, that is a giant network containing thousands of interlocked minicircles. During kinetoplast DNA synthesis, minicircles are released from the network for replication as theta-structures, and then the free minicircle progeny reattach to the network. We report that a mitochondrial protein, which we term p38, functions in kinetoplast DNA replication. RNA interference (RNAi) of p38 resulted in loss of kinetoplast DNA and accumulation of a novel free minicircle species named fraction S. Fraction S minicircles are so underwound that on isolation they become highly negatively super twisted and develop a region of Z-DNA. p38 binds to minicircle sequences within the replication origin. We conclude that cells with RNAi-induced loss of p38 cannot initiate minicircle replication, although they can extensively unwind free minicircles.

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Department of Biochemistry and Immunology, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Trypanosomes are protozoan parasites that cause major diseases in humans and other animals. *Trypanosoma brucei* and *Trypanosoma cruzi* are the etiologic agents of African and American Trypanosomiasis, respectively. In spite of large amounts of information regarding various aspects of their biology, including the essentially complete sequences of their genomes, studies directed towards an understanding of mechanisms related to DNA metabolism have been very limited. Recent reports, however, describing genes involved with DNA recombination and repair in *T. brucei* and *T. cruzi*, indicated the importance of these processes in the generation of genetic variability, which is crucial to the success of these parasites. Here, we review these data and discuss how the DNA repair and recombination machineries may contribute to strikingly different strategies evolved by the two trypanosomes to create genetic variability that is needed for survival in their hosts. In *T. brucei*, two genetic components are critical to the success of antigenic variation, a strategy that allows the parasite to evade the host immune system by periodically changing the expression of a group of variant surface glycoproteins (VSGs). One component is a mechanism that provides for the exclusive expression of a single VSG at any one time, and the second is a large repository of antigenically distinct VSGs. Work from various groups showing the importance of recombination reactions in *T. brucei*, primarily to move a silent VSG into an active VSG expression site, is discussed. *T. cruzi* does not use the strategy of antigenic variation for host immune evasion but counts on the extreme heterogeneity of their population for parasite adaptation to different hosts. We discuss recent evidence indicating the existence of major differences in the levels of genomic heterogeneity among *T. cruzi* strains, and suggest that metabolic changes in the mismatch repair pathway could be an important source of antigenic diversity found within the *T. cruzi* population.

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Wellcome Centre for Molecular Parasitology, Anderson College Complex, University of Glasgow, 56 Dumbarton Road, Glasgow G11 6NU, UK.

Trypanosoma brucei is the causative agent of African sleeping sickness in humans and contributes to the debilitating disease “nagana” in cattle. To date we know little about the genes that determine drug resistance, host specificity, pathogenesis and virulence in these parasites. The availability of the complete genome sequence and the ability of the parasite to undergo genetic exchange have allowed genetic investigations into this parasite and here we report the first genetic map of *T. brucei* for the genome reference stock TREU 927, comprising of 182 markers and 11 major linkage groups, that correspond to the 11 previously identified chromosomes. The genetic map provides 90 percent probability of a marker being

11 cM from any given locus. Its comparison to the available physical map has revealed the average physical size of a recombination unit to be 15.6 Kb/cM. The genetic map coupled with the genome sequence and the ability to undertake crosses presents a new approach to identifying genes relevant to the disease and its prevention in this important pathogen through forward genetic analysis and positional cloning.

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Trypanosoma brucei provides an excellent system for studies of many aspects of cell biology, including cell structure and morphology, organelle positioning, cell division and protein trafficking. However, the trypanosome has a complex life cycle in which it must adapt either to the mammalian bloodstream or to different compartments within the tsetse fly. These differentiation events require stage-specific changes to basic cell biological processes and reflect responses to environmental stimuli and programmed differentiation events that must occur within a single cell. The organization of cell structure is fundamental to the trypanosome throughout its life cycle. Modulations of the overall cell morphology and positioning of the specialized mitochondrial genome, flagellum and associated basal body provide the classical descriptions of the different life cycle stages of the parasite. The dependency relationships that govern these morphological changes are now beginning to be understood and their molecular basis identified. The overall picture emerging is of a highly organized cell in which the rules established for cell division and morphogenesis in organisms such as yeast and mammalian cells do not necessarily apply. Therefore, understanding the developmental cell biology of the African trypanosome is providing insight into both fundamentally conserved and fundamentally different aspects of the organization of the eukaryotic cell.

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Peptidases of parasitic protozoans are emerging as novel virulence factors and therapeutic targets in parasitic infections. A trypanosome-derived aminopeptidase that exclusively hydrolysed substrates with Glp (pyroglutamic acid) in P1 was purified 9,248-fold from the plasma of rats infected with *Trypanosoma brucei brucei*. The enzyme responsible was cloned from a *T. brucei brucei* genomic DNA library and identified as type I PGP (pyroglutamyl peptidase), belonging to the C15 family of cysteine peptidases. We showed that PGP is expressed in all life cycle stages of *T. brucei brucei* and is expressed in four other blood-stream-form African trypanosomes. Trypanosome PGP was optimally active and stable at bloodstream pH, and was insensitive to host plasma cysteine peptidase inhibitors. Native purified and recombinant hyper-expressed trypanosome PGP removed the N-terminal Glp blocking groups from TRH (thyrotrophin-releasing hormone) and GnRH (gonadotropin-releasing hormone) with a kcat/Km value of 0.5 and 0.1 s-1. uM^{-1} respectively. The half-life of TRH and GnRH was dramatically reduced in the plasma of trypanosome-infected rats, both *in vitro* and *in vivo*. Employing an activity-neutralizing anti-trypanosome PGP antibody, and pyroglutamyl diazomethyl ketone, a specific inhibitor of type I PGP, we demonstrated that trypanosome PGP is entirely responsible for the reduced plasma half-life of TRH, and partially responsible for the reduced plasma half-life of GnRH in a rodent model of African trypanosomiasis. The abnormal degradation of TRH and GnRH, and perhaps other neuropeptides N-terminally blocked with a pyroglutamyl moiety by trypanosome PGP may contribute to some of the endocrine lesions observed in African trypanosomiasis.

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Oligopeptidases are emerging as important pathogenic factors and therapeutic targets in trypanosome infections. We describe here the purification, cloning, and biochemical analysis of a new oligopeptidase from two pathogenic African trypanosomes. This oligopeptidase, which we have called tropolysin (encoded by the trn gene), represents an evolutionarily distant member of the M3A subfamily of metallopeptidases, ancestral to thimet oligopeptidase, neurolysin, and saccharolysin. The trn gene was present as a single copy per haploid genome, was expressed in both the mammalian and insect stages of the parasite life cycle, and encoded an 84 kDa protein. Both purified and hyperexpressed tropolysin hydrolyzed bradykinin-derived fluorogenic peptide substrates at restricted sites, with an alkaline pH optimum, and were activated by dithiothreitol and reduced glutathione and by divalent metal cations, in the order $Zn^{2+} > Co^{2+} > Mn^{2+}$. Under oxidizing conditions, tropolysin reversibly formed inactive multimers. Tropolysin exhibited a preference for acidic amino acid side chains in P(4), hydrophobic side chains in P(3), and hydrophobic or large uncharged side chains in P(1), P(1)', and P(3)', while the S(2)' site was unselective. Highly charged residues were not tolerated in P(1)'. Tropolysin was responsible for the bulk of the kinin-degrading activity in trypanosome lysates, potently (k(cat) approximately 119 s(-)(1)) inactivated the vasoactive kinins bradykinin and kallidin, and generated angiotensin(1-7) from angiotensin I. This hydrolysis both abolished the capacity of bradykinin to stimulate the bradykinin B(2) receptor and abrogated bradykinin prohypotensive properties *in vivo*, raising the possibility that tropolysin may play a role in the dysregulated kinin metabolism observed in the plasma of trypanosome-infected hosts.

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Protein kinases represent promising drug targets for a number of human and animal diseases. The recent completion of the sequenced genomes of three human-infective trypanosomatid protozoa, *Leishmania major*, *Trypanosoma brucei* and *Trypanosoma cruzi,* has allowed the kinome for each parasite to be defined as 179, 156 and 171 eukaryotic

protein kinases respectively, that is about one third of the human complement. The analysis revealed that the trypanosomatids lack members of the receptor-linked or cytosolic tyrosine kinase families, but have an abundance of STE and CMGC family protein kinases likely to be involved in regulating cell cycle control, differentiation and response to stress during their complex life cycles. In this review, we examine the prospects for exploiting differences between parasite and mammalian protein kinases to develop novel anti-parasitic chemotherapeutic agents.

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Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, 12, rue des Profs. Jeener et Brachet, B6041 Gosselies, Belgium.

The trypanosome genome is organized into long polycistronic units that seem to be permanently transcribed in proliferative stages of the parasite. Cellular differentiation is controlled primarily at the level of individual mRNA maturation and stability. The transcription units of the two major stage-specific antigens, the variant surface glycoprotein (VSG) of the bloodstream form and procyclin of the procyclic form, are subject to an

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We are studying early events in the establishment of *Trypanosoma brucei* in the tsetse midgut using fluorescent trypanosomes to increase visibility. Feeding flies with the lectin-inhibitory sugars D-glucosamine (GlcN) or N-acetyl-glucosamine (GlcNAc) has previously been shown to enhance fly susceptibility to infection with trypanosomes and, as expected, we found that both sugars increased midgut infection rates of *Glossina morsitans morsitans* with *T. brucei*. However, GlcNAc did not show the inhibitory effect on salivary gland infection rate reported previously for GlcN. Both sugars significantly slowed the movement of the bloodmeal along the midgut. GlcN also significantly increased the size of the bloodmeal taken and fly mortality. The most surprising finding was that GlcNAc stimulated trypanosome growth not only in the midgut, but also *in vitro* in the absence of any factor derived from the fly. Thus our direct comparison of the effects of GlcN and GlcNAc on the trypanosome-tsetse interaction has shown that these sugars impact on trypanosome growth and tsetse physiology in different ways and are not interchangeable as suggested in the literature. The sugars cause multiple effects, not restricted solely to the inhibition of midgut lectins. These findings have implications for current models of tsetse susceptibility to trypanosome infection.

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

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African trypanosomes undergo differentiation in order to adapt to the mammalian host and the tsetse fly vector. To characterize the role of a mitogen-activated protein (MAP) kinase homologue, TbMAPK5, in the differentiation of *Trypanosoma brucei*, we constructed a knockout in procyclic (insect) forms from a differentiation-competent (pleomorphic) stock.

Two independent knockout clones proliferated normally in culture and were not essential for other life cycle stages in the fly. They were also able to infect immunosuppressed mice, but the peak parasitemia was 16-fold lower than that of the wild type. Differentiation of the proliferating long slender to the nonproliferating short stumpy bloodstream form is triggered by an autocrine factor, stumpy induction factor (SIF). The knockout differentiated prematurely in mice and in culture, suggestive of increased sensitivity to SIF. In contrast, a null mutant of a cell line refractory to SIF was able to proliferate normally. The differentiation phenotype was partially rescued by complementation with wild-type TbMAPK5 but exacerbated by introduction of a nonactivatable mutant form. Our results indicate a regulatory function for TbMAPK5 in the differentiation of bloodstream forms of *T. brucei* that might be exploitable as a target for chemotherapy against human sleeping sickness.

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Apoptosis and programmed cell death are amongst the most fascinating new concepts for understanding the host-parasite relationship. A growing body data is raising questions about the impact of the death of an individual parasite on the survival of the parasite population as a whole. Does the parasite induce the death of the host cell as an expression of virulence or does it inhibit that death as a factor for transmissibility? Current evidence that these effects are mediated through specific highly regulated mechanisms suggests that deciphering programmed cell death could provide new tools for control of parasitic diseases.

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In *Trypanosoma brucei*, DNA recombination is crucial in antigenic variation, a strategy for evading the mammalian host immune system found in a wide variety of pathogens. *T. brucei* has the capacity to encode > 1,000 antigenically distinct variant surface glycoproteins (VSGs). By ensuring that only one VSG is expressed on the cell surface at one time, and by periodically switching the VSG gene that is expressed, *T. brucei* can evade immune killing for prolonged periods. Much of VSG switching appears to rely on a widely conserved DNA repair pathway called homologous recombination, driven by RAD51. Here, we demonstrate that *T. brucei* encodes a further five RAD51-related proteins, more than has been identified in other single-celled eukaryotes to date. We have investigated the roles of two of the RAD51-related proteins in *T. brucei*, and show that they contribute to DNA repair, homologous recombination and RAD51 function in the cell. Surprisingly, however, only one of the two proteins contributes to VSG switching, suggesting that the family of diverged RAD51 proteins present in *T. brucei* have assumed specialized functions in homologous recombination, analogous to related proteins in metazoan eukaryotes.

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The *Trypanosoma brucei* flagellum is a multifunctional organelle with critical roles in motility, cellular morphogenesis, and cell division. Although motility is thought to be important throughout the trypanosome lifecycle, most studies of flagellum structure and function have been restricted to the procyclic lifecycle stage, and our knowledge of the bloodstream form flagellum is limited. We have previously shown that trypanin functions as part of a flagellar dynein regulatory system that transmits regulatory signals from the central

pair apparatus and radial spokes to axonemal dyneins. Here we investigate the requirement for this dynein regulatory system in bloodstream form trypanosomes. We demonstrate that trypanin is localized to the flagellum of bloodstream form trypanosomes, in a pattern identical to that seen in procyclic cells. Surprisingly, trypanin RNA interference is lethal in the bloodstream form. These knockdown mutants fail to initiate cytokinesis, but undergo multiple rounds of organelle replication, accumulating multiple flagella, nuclei, kinetoplasts, mitochondria, and flagellum attachment zone structures. These findings suggest that normal flagellar beat is essential in bloodstream form trypanosomes and underscore the emerging concept that there is a dichotomy between trypanosome lifecycle stages with respect to factors that contribute to cell division and cell morphogenesis. This is the first time that a defined dynein regulatory complex has been shown to be essential in any organism and implicates the dynein regulatory complex and other enzymatic regulators of flagellar motility as candidate drug targets for the treatment of African sleeping sickness.

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The flagellum of *Trypanosoma brucei* is a multifunctional organelle with critical roles in motility and other aspects of the trypanosome life cycle. Trypanin is a flagellar protein required for directional cell motility, but its molecular function is unknown. Recently, a trypanin homologue in *Chlamydomonas reinhardtii* was reported to be part of a dynein regulatory complex (DRC) that transmits regulatory signals from central pair microtubules and radial spokes to axonemal dynein. DRC genes were identified as extragenic suppressors of central pair and/or radial spoke mutations. We used RNA interference to ablate expression of radial spoke (RSP3) and central pair (PF16) components individually or in combination with trypanin. Both rsp3 and pf16 single knockdown mutants are immotile, with severely defective flagellar beat. In the case of rsp3, this loss of motility is correlated with the loss of radial spokes, while in the case of pf16 the loss of motility correlates with an aberrant orientation of the central pair microtubules within the axoneme. Genetic interaction between trypanin and PF16 is demonstrated by the finding that loss of trypanin suppresses the pf16 beat defect, indicating that the DRC represents an evolutionarily conserved strategy for dynein regulation. Surprisingly, we discovered that four independent mutants with an impaired flagellar beat all fail in the final stage of cytokinesis, indicating that flagellar motility is necessary for normal cell division in *T. brucei*. These findings present the first evidence that flagellar beating is important for cell division and open the opportunity to exploit enzymatic activities that drive flagellar beat as drug targets for the treatment of African sleeping sickness.

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A non-toxic and versatile protein salting-out DNA extraction method is here described for convenient and rapid extraction of nuclear DNA molecules from trypanosomatids. The procedure just involves four manipulations, does not require any organic solvent, and is performed in less than 1 h in a single tube. DNA yields obtained were similar to those from commercial kits and phenol-chloroform procedures. Samples extracted by this method were suitable for PCR and subsequent analyses. The reduced manual labour involved was perceived as an important benefit in medical diagnosis routine use as well as for large-scale taxonomic and eco-epidemiological studies of trypanosomatids.

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Trypanosome U insertion and U deletion RNA editing of mitochondrial pre-mRNAs is catalyzed by multisubunit editing complexes as directed by partially complementary guide RNAs. The basic enzymatic activities and protein composition of these high-molecular mass complexes have been under intense study, but their specific protein interactions with functional pre-mRNA/gRNA substrates remains unknown. We show that editing complexes purified through extensive ion-exchange chromatography and immunoprecipitation make specific cross-linking interactions with A6 pre-mRNA containing a single 32P and photoreactive 4-thioU at the scissile bond of a functional site for full-round U deletion. At least four direct protein-RNA contacts are detected at this site by cross-linking. All four interactions are stimulated by unpaired residues just 5' of the pre-mRNA/gRNA anchor duplex, but strongly inhibited by pairing of the editing site region. Furthermore, competition analysis with homologous and heterologous transcripts suggests preferential contacts of the editing complex with the mRNA/gRNA duplex substrate. This apparent structural selectivity suggests that the RNA-protein interactions we observe may be involved in recognition of editing sites and/or catalysis in assembled complexes.

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Department of Internal Medicine, Yale University Medical School, New Haven, Connecticut 06536, USA.

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Canadian Institute for Advanced Research and Department of Biology, Dalhousie University, Halifax, Canada, B3H 4J1.

Five years ago, little was known about kinetoplastid evolution. Recent improvements in the taxon sampling for nuclear rRNA genes and several protein markers have transformed this understanding. Parasitism evolved at least four times in kinetoplastids. Obligate parasitic trypanosomatids are a relatively "derived" group within kinetoplastids; their closest relative is likely to be the free-living *Bodo saltans*, and the ancestral trypanosomatids were probably parasites of insects. Although subject to recent controversy, trypanosomes (genus *Trypanosoma*) probably constitute a monophyletic group. Several unusual features of trypanosomatid genomes (e.g. trans-splicing, mitochondrial RNA editing and intron poverty) are common in kinetoplastids and pre-date the adoption of parasitism. The framework of relationships is becoming robust enough for real comparative approaches to be used to understand kinetoplastid biology.

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Department of Parasitology, Faculty of Science, Charles University, 12844 Prague, Czech Republic.

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Department of Biology, Slippery Rock University, Slippery Rock, PA 16057, USA. [carolyn.steglich@sru.edu].

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Abteilung Parasitologie, Hygiene-Institut der Ruprecht-Karls-Universtat, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany. [dsteverding@hotmail.com].

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School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, UK; Department of Parasitology, Ruprecht-Karls-University, Heidelberg, Germany.

The transferrin (Tf) receptor of *Trypanosoma brucei* (TbTfR) is encoded by two expression-site-associated genes, ESAG6 and ESAG7. There are around 20 different expression sites containing different copies of these genes that encode TbTfRs with quite distinct affinities for Tf of various hosts. It was proposed that *T. brucei* has developed multiple expression sites encoding different TbTfRs to ensure sufficient iron uptake in the presence of antibodies competing for binding to Tf. Here it is shown that anti-TbTfR antibody titres produced during chronic murine trypanosomiasis are only one-tenth of those achieved by immunisation of mice using recombinant TbTfR. Calculations indicate that the concentrations of competing anti-TbTfR antibodies present during chronic *T. brucei* infection are too low to deprive the parasite of iron. In addition, during human African trypanosomiasis the antibody response to the TbTfR seems to be poor and transient. Altogether, the results suggest that the host antibody response to the TbTfR during chronic infection with *T. brucei* is too low, if present at all, to prevent sufficient iron uptake by bloodstream forms to promote their growth.

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H.P. de Koning, University of Bern, Institute of Cell Biology, Baltzerstr 4, CH-3012 Bern, Switzerland.

Bloodstream forms of *Trypanosoma brucei brucei* were cultivated in the presence and absence of thiamine (vitamin B-1) and pyridoxine (vitamin B-6). The vitamins do not change growth behaviour, indicating that *Trypanosoma brucei* is prototrophic for the two vitamins even though *in silico* no bona-fide thiamine-biosynthetic genes could be identified in the *T. brucei* genome. Intracellularly, thiamine is mainly present in its diphosphate form. We were unable to detect significant uptake of [^{3}H] thiamine and structural thiamine analogues such as pyrithiamine, oxithiamine and amprolium were not toxic for the bloodstream forms of *T. brucei*, indicating that the organism does not have an efficient uptake system for thiamine and its analogues. We have previously shown that, in the fission yeast *Saccharomyces pombe*, the toxicity of melarsen oxide, the pharmacologically active derivative of the frontline sleeping sickness drug melarsoprol, is abolished by thiamine and the drug is taken up by a thiamine-regulated membrane protein which is responsible for the utilization of thiamine. We show here that thiamine also has weak effects on melarsen oxide-induced growth inhibition and lysis in *T. brucei*. These effects were consistent with a low affinity of thiamine for the P2 adenosine transporter that is responsible for uptake of melaminophenyl arsenicals in African trypanosomes.

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Trypanosomatids of the order Kinetoplastida are major contributors to global disease and morbidity, and understanding their basic biology coupled with the development of new drug targets represents a critical need. Additionally, trypanosomes are among the more accessible divergent eukaryote experimental systems. The genome of *Trypanosoma brucei* contains 8,131 predicted open reading frames (ORFs), of which over half have no known homologues beyond the Kinetoplastida and a substantial number of others are poorly defined by *in silico* analysis. Thus, a major challenge following completion of the *T. brucei* genome sequence is to obtain functional data for all trypanosome ORFs. As *T. brucei* is more experimentally tractable than the related *Trypanosoma cruzi* and *Leishmania* spp. and shares >75 percent of their genes, functional analysis of *T. brucei* has the potential to inform a range of parasite biology. Here, we report methods for systematic mRNA ablation by RNA interference (RNAi) and for phenotypic analysis, together with online data dissemination. This represents the first systematic analysis of gene function in a parasitic organism. In total,

210 genes have been targeted in the bloodstream form parasite, representing an essentially complete phenotypic catalogue of chromosome I together with a validation set. Over 30 percent of the chromosome I genes generated a phenotype when targeted by RNAi; most commonly, this affected cell growth, viability, and/or cell cycle progression. RNAi against approximately 12 percent of ORFs was lethal, and an additional 11 percent had growth defects but retained short-term viability in culture. Although we found no evidence for clustering or a bias towards widely evolutionarily conserved genes within the essential ORF cohort, the putative chromosome I centromere is adjacent to a domain containing genes with no associated phenotype. Involvement of such a large proportion of genes in robust growth *in vitro* indicates that a high proportion of the expressed trypanosome genome is required for efficient propagation; many of these gene products represent potential drug targets.

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Department of Cellular Biology, University of Georgia, Athens, GA, USA.

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Differentiation in African trypanosomes (*Trypanosoma brucei*) entails passage between a mammalian host, where parasites exist as a proliferative slender form or a G0-arrested stumpy form, and the tsetse fly. Stumpy forms arise at the peak of each parasitaemia and are committed to differentiation to procyclic forms that inhabit the tsetse midgut. We have identified a protein tyrosine phosphatase (TbPTP1) that inhibits trypanosome differentiation. Consistent with a tyrosine phosphatase, recombinant TbPTP1 exhibits the anticipated substrate and inhibitor profile, and its activity is impaired by reversible oxidation. TbPTP1 inactivation in monomorphic bloodstream trypanosomes by RNA interference or pharmacological inhibition triggers spontaneous differentiation to procyclic forms in a subset of committed cells. Consistent with this observation, homogeneous populations of stumpy forms synchronously differentiate to procyclic forms when tyrosine phosphatase activity is inhibited. Our data invoke a new model for trypanosome development in which differentiation to procyclic forms is prevented in the bloodstream by tyrosine dephosphorylation. It may be possible to use PTP1B inhibitors to block trypanosomatid transmission.

13993. **Toh, H., Weiss, B.L., Perkin, S.A., Yamashita, A., Oshima, K., Hattori, M. & Aksoy, S., 2006.** Massive genome erosion and functional adaptations provide insights into the symbiotic lifestyle of *Sodalis glossinidius* in the tsetse host. *Genome Research*, **16** (2): 149-156.

Kitasato Institute for Life Sciences, Kitasato University, Sagamihara, Kanagawa 228-0829, Japan.

Sodalis glossinidius is a maternally transmitted endosymbiont of tsetse flies (*Glossina* spp.), an insect of medical and veterinary significance. Analysis of the complete sequence of *Sodalis'* chromosome (4,171,146 bp, encoding 2,432 protein coding sequences) indicates a reduced coding capacity of 51 percent. Furthermore, the chromosome contains 972 pseudogenes, an inordinately high number compared with that of other bacterial species. A high proportion of these pseudogenes are homologues of known proteins that function either in defence or in the transport and metabolism of carbohydrates and inorganic ions, suggesting *Sodalis'* degenerative adaptations to the immunity and restricted nutritional status of the host. *Sodalis* possesses three chromosomal symbiosis regions (SSR): SSR-1, SSR-2, and SSR-3, with gene inventories similar to the Type-III secretion system (TTSS) ysa from *Yersinia enterolitica* and SPI-1 and SPI-2 from *Salmonella*, respectively. While core components of the needle structure have been conserved, some of the effectors and regulators typically associated with these systems in pathogenic microbes are modified or eliminated in *Sodalis*. Analysis of SSR-specific invA transcript abundance in *Sodalis* during host development indicates that the individual symbiosis regions may exhibit different temporal expression profiles. In addition, the *Sodalis* chromosome encodes a complete flagella structure, key components of which are expressed in immature host developmental stages. These features may be important for the transmission and establishment of symbiont infections in the intra-uterine progeny. The data suggest that *Sodalis* represents an evolutionary intermediate transitioning from a free-living to a mutualistic lifestyle.

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In *Trypanosoma brucei brucei*, an invariant surface glycoprotein of molecular weight 75 kDa (ISG75) is uniformly distributed over the surface of a trypanosome and is specific for bloodstream-form parasites. For the other taxa of the *Trypanozoon* subgenus no data about this surface molecule are available. Therefore, we investigated the ISG75 in the genomes of several pathogenic *Trypanozoon* by Southern blot, PCR and RT-PCR and sequence analysis. Nucleotide sequence data reported in this paper are available in the GeneBank™, EMBL and DDBJ databases under the Accession numbers DQ200175-DQ200256. This study reveals that (i) all members of the *Trypanozoon* subgenus, i.e. *T. b. brucei, T. b. gambiense, T. b. rhodesiense, T. evansi* and *T. equiperdum*, harbour ISG75 as multiple gene copies with at least 4-16 copies per genome; (ii) ISG75 gDNA and cDNA sequences are distributed in 2 groups that share at least 75 percent and 77 percent identity respectively; (iii) sequences from both groups are transcribed in all species and subspecies of the *Trypanozoon* subgenus; (iv) the main differences between group I and group II are located in the variable region at the amino-terminus of the putative proteins; (v) however, all the sequences in both groups have some well-conserved features, such as the cysteine residues, an amino-terminal cleavable

signal peptide, a single alpha-helix transmembrane domain and a cytoplasmic domain at the carboxy-terminus.

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

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Laboratory of Infectious Disease, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.

Drug resistance in *Trypanosoma brucei* causes severe problems for people and domestic animals, but molecular mechanisms of the resistance are not well known. Programmed cell death (PCD) is a fundamental process in both multicellular and unicellular organisms, and it is speculated to be one of the important factors contributing to the emergence of drug resistance. We have previously reported that the expression of TAO appears to play a role in the inhibition of the PCD-like phenomenon development in *T. brucei*. In this study, to ascertain the correlation between the development of the PCD-like phenomenon and the expression of TAO in *T. brucei*, we genetically engineered *T. brucei* for conditional over-expression of the TAO gene. TAO over-expressing transgenic *T. brucei* was refractory to the development of the PCD-like phenomenon compared to the wild-type, indicating that expression of TAO might have a regulatory role on PCD development. Furthermore, the transgenic cells showed resistance to suramin and antrycide. We postulated that intracellular reactive oxygen species (ROS) may be involved in the mechanism of resistance to antrycide because augmentation of ROS in transgenic cells was lower than that in the wild-type cells following treatment with antrycide. These results suggest a possible correlation of PCD to drug resistance in *T. brucei.*

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College of Allied Health Professions, University of South Alabama, Mobile, AL 36688, USA.

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Laboratory of Veterinary Immunology, Division of Veterinary Science, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan.

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Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, Scotland, United Kingdom. [m.a.j.ferguson@dundee.ac.uk].

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Lysophospholipid analogues (LPAs) comprise a class of metabolically stable compounds that have been developed as anticancer agents for over two decades, but which have also potent and selective antiparasitic activity, particularly against trypanosomatid parasites such as *Leishmania* and *Trypanosoma cruzi*, both *in vitro* and *in vivo*. The *in vivo* activities of LPAs result from direct effects on their target cells and are not dependent on a functional immune system. Because of their chemical nature, LPAs have a potential for interaction with a variety of subcellular structures and biochemical pathways. However, in mammalian cells LPA-induced growth inhibition and programmed cell death are usually associated with a blockade of phosphatidylcholine (PC) biosynthesis at the level of CTP: phosphocholine citidyltransferase, probably through an increase of cellular ceramide levels due to depressed sphingomyelin synthesis. Although in trypanosomatid parasites much less information is available, inhibition of PC biosynthesis by LPA has also been documented but at the level of phosphatidylethanolamine N-methyl-transferase, as well as LPA-induced classical apoptotic phenomena. The higher activity of LPAs as inhibitors of PC biosynthesis in parasites than in mammalian cells, probably due to different biochemical pathways involved in the two types of cells, could explain their selective antiparasitic action *in vivo*.

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African trypanosomes are parasitic protozoa of the order of Kinetoplastida, which cause sleeping sickness and nagana. Trypanosomes are not only of scientific interest because of their clinical importance, but also because these protozoa contain several very unusual biological features, such as their special energy metabolism. The energy metabolism of *Trypanosoma brucei* differs significantly from that of its host, not only because it comprises distinct enzymes and metabolic pathways, but also because some of the glycolytic enzymes are localized in organelles called glycosomes. Furthermore, the energy metabolism changes drastically during the complex life cycle of this parasite. This review focuses on the recent advances made in understanding the process of ATP production in *T brucei* during its life cycle and the consequences of the special subcellular compartmentation

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Trypanosomes are unicellular parasites and like all decent parasites, they try to obtain from the host as much material as possible, including lipids. However, the needs of a parasite are not always the same as those of the host, and therefore, mostly, some biosynthetic work still has to be done by the parasite itself. Very often at least modifications of the lipid components that are acquired from the host have to be made. Furthermore, next to the lipids *Trypanosoma brucei* indeed obtains from the host, some other lipid components have to be synthesized *de novo*. Especially the processes where the metabolism of *T. brucei* differs from that of the host will be discussed as at least some of them are excellent targets for the development of urgently needed new chemotherapeutics.

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Laboratorium voor Ultrastructuur, Vrije Universiteit Brussel and Department of Molecular and Cellular Interactions, Vlaams Instituut voor Biotechnologie, Pleinlaan 2, B-1050 Brussel, Belgium.

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Superoxide dismutases (SOD) are a family of antioxidant enzymes that function by removing superoxide anions from the cellular environment. Here, we show that the African trypanosome, *Trypanosoma brucei*, expresses four SOD isoforms, three of which we have validated biochemically as iron dependent, a feature normally associated with prokaryotic SODs. Localisation studies reveal that two of the enzymes are found predominantly in a

parasite-specific organelle, the glycosome (TbSODB1 and TbSODB2), while the other two are targeted to the mitochondrion (TbSODA and TbSODC). Functional analysis of the SOD repertoire in bloodstream form parasites was performed using an inducible RNA interference (RNAi) approach. Down-regulation of the glycosomal SOD transcripts corresponded with a significant reduction in the corresponding proteins and a dramatic level of cell death within the population. The importance of one of the mitochondrial enzymes (TbSODA) only became apparent when parasites were exposed to the superoxide- generating agent paraquat following induction of RNAi. These experiments therefore identify essential components of the superoxide metabolising arm of the *T brucei* oxidative defence system and validate these enzymes as parasite-specific targets for drug design.

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Bloodstream trypanosomes take up iron needed for their propagation through the transferrin receptor that, in *Trypanosoma brucei*, is encoded by expression-site-associated genes (ESAGs), ESAG6 and 7 genes located in variant surface glycoprotein expression sites. ESAG6 and 7 genes in different expression sites have been shown to encode transferrin receptors with varying affinities for polymorphic transferrins. *T. brucei* could cope with the different host transferrins by switching between expression sites. ESAG6- and 7-encoded transferrin receptor appears to be present in *Trypanosoma evansi* but the genes have not yet been characterized. In this study, we cloned and sequenced different members of ESAG6 genes in seven isolates of *T. evansi* from geographically distinct localities in Thailand. We assessed the intra- and inter-species genetic variability in the transferrin receptor gene regions involved in transferrin binding and established that *T. evansi*, like *T. brucei*, has widely diverse ESAG6 genes. In addition, *T. evansi* possess a clade of ESAG6 variants not observed in *T. brucei* and different *T. evansi* strains share at least two conserved variants. We further noted that *T. evansi* possesses all the reported *T. equiperdum* ESAG6 variants as a subset. Our findings depict a correlation between the genetic diversity in the transferrin-binding regions of ESAG6 genes with the broad host range of *T. evansi* and *T. brucei* compared to the narrow host range of *Trypanosoma equiperdum*.

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M Onuma: Hokkaido University, Graduate School of Veterinary Medicine, Infectious Disease Laboratory, Sapporo, Hokkaido 0600818, Japan.

Drug resistance is now a severe and increasing problem in trypanosomes, but molecular details of mechanisms of resistance are only beginning to unveil. There is urgent need to clearly elucidate the different mechanisms of drug resistance in trypanosomes in order to circumvent existing resistance problems and avoid emergence of resistance to the next

generation drugs. In this study, we cloned and characterized a novel gene, TeDR40, whose expression is associated with resistance to berenil in *Trypanosoma evansi*. Expression analysis showed that the gene was at least 1,000-fold upregulated in resistant parasites and the encoded protein appeared to have a ubiquitous cellular localization. To investigate the association of TeDR40 with berenil-resistance, we genetically modified wild-type berenil-sensitive *T. evansi* for inducible overexpression of the TeDR40 gene. Induction of over-expression of TeDR40 in *T. evansi* led to decreased ($P<0.01$) sensitivity to berenil. Our findings indicate a possible correlation between over-expression of a novel gene, TeDR40, and reduced sensitivity to berenil in an *in vitro*-cultured clonal line of *T. evansi*.

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Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA 90095-1489, USA.

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