PAAT
Programme
Against
African
Trypanosomosis

TSETSE AND
TRYPANOSOMOSIS
INFORMATION
Numbers 17249-17428

Edited by
James Dargie
Bisamberg
Austria

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2015
Tsetse and Trypanosomosis Information

TSETSE AND TRYPANOSOMOSIS INFORMATION

The Tsetse and Trypanosomosis Information periodical has been established to disseminate current information on all aspects of tsetse and trypanosomosis research and control to institutions and individuals involved in the problems of African trypanosomosis. This service forms an integral part of the Programme Against African Trypanosomosis (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 Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), the Institut de recherche pour le développement (IRD) and the Institute of Tropical Medicine Antwerp (ITM).

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. Subscription is free for all recipients engaged in trypanosomosis 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.

<table>
<thead>
<tr>
<th>Distribution dates and copy deadlines</th>
<th>Copy deadline for News items</th>
<th>Distribution (English and French editions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part 1</td>
<td>15 April</td>
<td>July/August</td>
</tr>
<tr>
<td>Part 2</td>
<td>15 October</td>
<td>January/February</td>
</tr>
</tbody>
</table>
**ABBREVIATIONS USED IN TTI**

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

**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 Économique 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)
- **DNDi**: Drugs for Neglected Diseases Initiative
- **DSE**: German Foundation for International Development
- **EC/EU**: European Community/European Union
## Tsetse and Trypanosomosis Information

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

SECTION A – NEWS

From the African Union
13th PATTEC Coordinators/Focal Points Meeting, Zimbabwe 1
3rd Steering Committee of AU-PATTEC Meeting 3
6th Training Course on project planning and evaluation 4
GIS training 5
Consultation Workshop on strategies, technical advances & partnerships in T&T management 5
Participation of media in monitoring & evaluation mission of PATTEC in southern Ethiopia 7

From WHO
4th International Meeting on the Control of Neglected Zoonotic Diseases 8
French version of control and surveillance of human African trypanosomiasis. Report of a WHO Expert Committee (TRS No. 984) 9

From FAO
Improving food security in sub-Saharan Africa by supporting the progressive reduction of tsetse-transmitted trypanosomosis in the framework of the NEPAD 9

From the Joint FAO/IAEA Programme
Special issue of Acta Tropica on applying population genetics and GIS for managing livestock insect pests 11
Regional Training Course on free open source software for GIS and data management applied to tsetse and trypanosomiasis control programmes 12
Research Coordination Meeting on enhancing vector refractoriness to trypanosome infection 13

From GALVmed
Seeking the next generation of livestock trypanocides 13

From FIND
Government of Uganda intensifies fight against sleeping sickness 14

From DNDi
R&D status September 2014: Human African trypanosomiasis programme 15

SECTION B – ABSTRACTS

1. General (including land use) 18
2. Tsetse biology
   (a) Rearing of tsetse flies 27
   (b) Taxonomy, anatomy, physiology, biochemistry 28
   (c) Distribution, ecology, behaviour, population studies 31
3. Tsetse control (including environmental side effects) 34
4. Epidemiology: vector-host and vector-parasite interactions 40
5. Human trypanosomiasis
   (a) Surveillance 46
   (b) Pathology and immunology 47
   (c) Treatment 52
6. Animal trypanosomosis
   (a) Survey and distribution 55
   (b) Pathology and immunology 57
### Tsetse and Trypanosomosis Information

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>(c) Trypanotolerance</td>
<td>58</td>
</tr>
<tr>
<td>(d) Treatment</td>
<td>58</td>
</tr>
<tr>
<td>7. Experimental trypanosomosis</td>
<td></td>
</tr>
<tr>
<td>(a) Diagnostics</td>
<td>58</td>
</tr>
<tr>
<td>(b) Pathology and immunology</td>
<td>64</td>
</tr>
<tr>
<td>(c) Chemotherapeutics</td>
<td>70</td>
</tr>
<tr>
<td>8. Trypanosome research</td>
<td></td>
</tr>
<tr>
<td>(a) Cultivation of trypanosomes</td>
<td>86</td>
</tr>
<tr>
<td>(b) Taxonomy, characterization of isolates</td>
<td>87</td>
</tr>
<tr>
<td>(c) Life cycle, morphology, biochemical and</td>
<td>90</td>
</tr>
<tr>
<td>molecular studies</td>
<td></td>
</tr>
</tbody>
</table>
SECTION A – NEWS

FROM THE AFRICAN UNION

13TH PATTEC COORDINATORS’/FOCAL POINTS’ MEETING
24-27 NOVEMBER 2014, HARARE, ZIMBABWE

Within the framework of its role and mandate in initiating action and providing technical support for the implementation of the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), the PATTEC Coordination Office organized this meeting in collaboration with the Government of Zimbabwe. It also arranged a field visit to PATTEC project sites to meet members of the rural communities benefiting from the project’s activities. About 90 National PATTEC Coordinators and Focal Points from 29 African countries, representatives of international organizations, research institutions and private and public partners attended the opening ceremony of the meeting which was held at the Rainbow Towers Hotel in Harare.

Opening Ceremony of the PATTEC Coordinators’/Focal Points’ Meeting

Welcome remarks were made by the Principal Director, Department of Livestock and Veterinary Services, Dr Unesu Ushewokunze-Obatulo, the Secretary for Agriculture, Mechanization and Irrigation Development, Mr R. J. Chitsiko, and a representative of the PATTEC Coordinator, Dr Gift Wanda. Opening remarks were made by the representative of the Commissioner for Rural Economy and Agriculture, Dr Hassane Mahamat Hassane, AU-PATTEC Coordinator.

In his opening remarks, Dr Hassane thanked the Government of the Republic of Zimbabwe for hosting the meeting, and resource partners for their continued support to the PATTEC Initiative. He reminded delegates that the objectives of the meeting were to review progress towards the PATTEC Initiative, and to highlight and discuss lessons learned and challenges being faced by tsetse and trypanosomosis (T&T) affected countries. Dr Hassane hailed Zimbabwe for the significant success that the country has registered in the fight against T&T, and expressed confidence that other countries would use the opportunity of being here to emulate the best practices demonstrated by Zimbabwe. Dr Hassane further noted that despite the numerous successes that have been registered to date, more needs to be done to address the T&T challenge. He urged T&T affected countries to integrate T&T interventions in their national development policies.
The meeting was officially opened by the Deputy Minister of Agriculture, Hon. Paddy Zhanda who is responsible for livestock in Zimbabwe. In his opening remarks, he extended a warm welcome on behalf of the Government and people of Zimbabwe, to the delegation from the AU-PATTEC and to all participants from outside Zimbabwe who had come to attend this meeting. The Hon. Deputy Minister recognized the role and mandate of the PATTEC in initiating action and providing technical support to African Member States and in coordinating strategies for the total eradication of tsetse and trypanosomosis from the African continent in line with the objectives of the PATTEC Initiative. He further, acknowledged the support provided to Zimbabwe by development partners. The Hon. Deputy Minister noted with concern that the T&T problem remains one of Africa’s greatest constraints to socio-economic development, severely affecting human and livestock health, limiting land use, causing food insecurity and poverty, and perpetuating underdevelopment on the continent. With specific reference to human sleeping sickness, the Hon. Deputy Minister urged the meeting to come up with a rapid diagnostic test for *Trypanosoma rhodesiense* as has been achieved in the case of *Trypanosoma gambiense* in Central and West Africa. In concluding his opening remarks, the Hon. Deputy Minister, reminded delegates that the socio-economic impacts of trypanosomiasis on livelihoods call for greater integration of tsetse and trypanosomiasis programmes into sustainable land management initiatives to maximize benefits from the various tsetse control interventions.

Following the opening ceremony, the new office bearers of the meeting were elected as follows: Mr William Shereni, National Coordinator PATTEC Zimbabwe as Chairman, and assisted by Dr Hassane H. Mahamat, AU-PATTEC Coordinator.

After three days of presentations by invited countries, international partners and several resource persons the following major recommendations were unanimously adopted:

(i) The meeting noted with concern the disparities that exist across T&T affected countries with respect to levels of development and configurations of the T&T management structures. The meeting recognized the need for an independent T&T management structure with dedicated full time staff to ensure sustainable funding with fixed government budget lines for T&T interventions. The meeting recommended that T&T affected countries which have not yet established T&T management structures should begin the process of creating such structures and report on progress during the next meeting.

(ii) The meeting commended the support that was given by AfDB to the six countries in West and East Africa in the multinational project that drew to a close in 2012. The meeting noted with concern that the pledge to support countries that were identified to be in Phase II did not materialize. The meeting further noted that lessons learned from the first phase have not been consolidated and shared with other countries that are willing to apply for similar loans. The meeting urged the AU-PATTEC Coordination Office and relevant partners to commission an independent evaluation of the six projects and share the findings with all T&T affected countries.

(iii) The meeting noted with great concern that resource availability was still being highlighted by all countries as a major hindrance to the fight against T&T. The meeting further noted that currently there is not much evidence of the existence of national resource mobilization strategies in T&T affected countries. The meeting urged the AU-PATTEC Coordination Office to provide support to national programmes to develop resource mobilization strategies that are aligned to the AU-PATTEC Coordination Office Resource Mobilisation Strategy and report on progress during the next meeting.
(iv) The meeting commended the AU-PATTEC Coordination Office and other partners for taking the lead in identifying potential regional programmes. The meeting noted with concern that for most of these proposed programmes, resources have not been identified for their implementation. The meeting urged AU-PATTEC to come up with a strategy and feasibility studies for reviving the regional programmes and mobilizing resources for their implementation in close collaboration with Regional Economic Communities, resource partners and the concerned T&T affected countries.

(v) The meeting noted with concern that despite the availability of vast information in the T&T domain, information sharing is weak. The meeting observed that most of the pertinent information resides with partners, research and academic institutions. The meeting agreed that the best approach to address this issue is to have a central repository for information which can be easily accessed by all stakeholders. To this effect, the meeting recommended that central information repositories should be established at National PATTEC and AU-PATTEC Coordination Offices. AU-PATTEC is further urged to create web links to T&T information and data sources worldwide.

(vi) The meeting welcomed developments that are designed to accelerate improvement of food and nutrition security and rural development such as the Malabo Declaration. The meeting recognized the important role of T&T programmes in realizing the goal of the Malabo Declaration. To this effect, it urged T&T affected countries and partners to design strategies and programmes in alignment to the Malabo Declaration and other Decisions adopted by the African Union with a bearing on sustainable rural development. The meeting further recommended that awareness of the Malabo Declaration should be extended to tsetse infested areas not yet covered by active PATTEC programmes to accelerate their integration in the actions that are designed to implement the Malabo Declaration.

(vii) The meeting commended all partners who have been consistently supporting the PATTEC Initiative and recognized that more can be done. It recommended that the AU-PATTEC and partners should accelerate the process of reinforcing and strengthening their partnerships through MOUs.

(viii) The meeting recognized the need for a day to raise awareness on the T&T scourge and urged the AU-PATTEC Coordination Office to seek guidance from relevant African Union organs on the most suitable day for the proposed event.

3rd STEERING COMMITTEE OF THE AU-PATTEC MEETING

The third Steering Committee of the AU-PATTEC was held on 28 November 2014 back to back with the 13th PATTEC Coordinators’ meeting at Rainbow Towers Hotel, Harare. The key PATTEC Steering Committee members were present and the agenda was fully discussed. The meeting was chaired by the Deputy Minister responsible for Livestock in Zimbabwe, Hon. Paddy Zhanda on behalf of the AUC Commissioner for Rural Economy and Agriculture, H.E. Mrs. Tumusiime Rhoda Peace.

The Chairperson welcomed all members of the SC and invited dignitaries. As the Chair had to leave the meeting to attend to other pressing issues, he was represented during the rest of the meeting by Dr Unesu Ushewokunze-Obatolu, Principal Director, Department of Livestock and Veterinary Services. The meeting was co-chaired by Prof. Josenando Theophile.
The Chair presented the draft agenda for review and adoption which was adopted without amendment.

The Chair asked one member from the Secretariat (PATTEC Coordination Office) to read the minutes. The minutes were read and adopted without amendment.

Steering Committee (SC) members asked the Secretariat to give an update on the status of implementation of the recommendations from the previous meeting. In response, the PATTEC Coordinator informed SC members that the presentations planned for this meeting were specifically designed to address the recommendations. The Committee agreed to make its observations when the presentations were made.

The Chair invited presentations to be made as per the agenda. These were made in the following order:

An update of progress on the implementation of the AU-PATTEC Coordination Office was presented by the PATTEC Coordinator.

The recommendations of the 13th PATTEC Coordinators’ meeting were presented by the Committee Secretariat. Committee members observed that the recommendations did not highlight the role of the private sector in T&T management. The Committee agreed that the private sector should be included in the column of responsibilities against the recommendations that require private sector involvement.

The Secretariat presented a Work Plan for 2015. SC members recommended as follows:

- That any activity to be undertaken in T&T affected countries should specify the countries involved so that other partners plan for their complementary activities accordingly to avoid duplication.
- That for activities which do not have secured funding, the timelines should not be indicated.
- That for the independent evaluation of the AfDB supported multinational project on creation of tsetse free areas in East and West Africa, the AU-PATTEC Coordination Office should liaise with other partners for support.
- That for the activity on developing a TCP for updating current tsetse infestation, some partners can be approached for support directly instead of developing a project proposal.
- AU-PATTEC should explore the possibility of engaging on internship arrangements for students from public health schools.
- In addition to recommendations highlighted under each section above, Dr Chris Schofield proposed to adopt the recommendations of the 13th Coordinators Meeting. He was seconded by Mr Francis Oloo and Professor Joseph Dung’u.

6th PATTEC TRAINING COURSE ON PROJECT PLANNING AND EXECUTION

This course was held at the École de Lutte Anti Tsetse (ELAT) in Bobo Diulasso, Burkina Faso. Twenty-three participants from 17 countries attended this course. The training aimed at building the technical capacity and competence required to carry out activities in the implementation of PATTEC, in developing tsetse eradication project proposals for specific areas, and in seeking financial and technical support for executing identified tsetse eradication projects in the affected countries.
GIS TRAINING

(i) The PATTEC Coordination Office conducted two national training events on GIS and data management. Thirty-four participants from Gabon PATTEC projects and stakeholder institutions benefited from the training which was conducted in Libreville from 17-26 May 2014. Similarly 14 staff from the Cameroon PATTEC project attended a training workshop on Open Source GIS application in tsetse and trypanosomosis eradication projects that was held from 11-19 December 2014, at Yaoundé. These training events started with a two-day workshop focusing on evaluating, developing and appraising the project documents and work plans for 2015.

(ii) In collaboration with FAO, PATTEC actively participated in the organization of two training workshops on spatial database management training for national project staff in Kenya (June 2014) and Ghana (August 2014). Two regional training courses on Open Source GIS applications were also organized by IAEA, FAO and PATTEC for English and French speaking T&T affected countries at the AU Commission in Addis Ababa (May 2014) and at IAEA Headquarters, Vienna (January 2015) respectively.

CONSULTATION WORKSHOP ON STRATEGIES, TECHNICAL ADVANCES AND PARTNERSHIPS IN T&T MANAGEMENT, LIVINGSTONE, ZAMBIA, 8-11 SEPTEMBER 2014

About 60 National PATTEC Coordinators and Focal Points from 17 African countries (Angola, Botswana, Burkina Faso, Burundi, Cameroon, Ethiopia, Ghana, Kenya, Malawi, Niger, Rwanda, South Sudan, Sudan, Tanzania, Uganda, Zambia and Zimbabwe), and representatives of international organizations, research institutions, private and public partners including WHO, OIE, Liverpool School of Tropical Medicine (LSTM - Vector Group), London School of Hygiene & Tropical Medicine (LSHTM), ILRI, ICIPE, COCTU, Kenya Tsetse & Trypanosomiasis Eradication Council (KENTTEC), NITR, NICETT, IRD, Orsmonds Aviation, AVIMA, Vestergaard-Frandsen, CEVA and SANOFI, FIND, GALVmed, IAEA, FAO, CIRAD, Swiss Tropical Institute of Public Health (STIPH), Arab Bank for Economic Development (BADEA), Bill & Melinda Gates Foundation (BMGF), African Development Bank (AfDB), Bio-Economy Africa and Biovision Foundation attended this consultation workshop.
The workshop was organized against the background that despite the numerous efforts over the past ten decades to address the tsetse and trypanosomosis (T&T) challenge on the African continent, the problem is still very much visible in many T&T affected countries. This is in contrast to the fact that there have been a number of recent technical advances in the field of tsetse control and trypanosomosis management, which should be translated into more effective programmes and greater commitment of policy makers and technical personnel. The aerial spraying operations in Botswana which benefited greatly from the advent of global positioning system (GPS) and its associated geographical information system (GIS) technologies, and recent developments in remote sensing, population genetics, aerial release techniques, bait technology, T&T surveillance, diagnostics and management serve as good examples of such advances.

The workshop was designed to bring together various partners/stakeholders and field implementers to share practical knowledge, information and experience regarding new technologies and strategies in the T&T domain, and to make recommendations that would help T&T affected countries achieve the main objectives of reducing the burden of T&T and creating T&T free areas which can be sustainably utilized for increased agricultural productivity.

The themes of the workshop included policies, strategies and standards, T&T management structures, new technical advances and lessons learned from field operations. The consultative workshop was also accompanied by a field visit to PATTEC Zambia field operation sites to witness the impact of aerial spraying conducted in the area.

After three days of deliberations, participants came up with the following recommendations for action by all stakeholders as per their respective responsibilities:

**Considering** the prevailing negative socio-economic impact of the tsetse and trypanosomosis challenge on the African continent;

**Recognizing** the diversity and high number of competent partners and stakeholders in the T&T domain;

**Realizing** the need for a coherent multi-stakeholder partnership framework for effective and efficient management of the T&T challenge;

**Considering** the weak link between research and policy makers in the T&T domain;

**Cognisant** of the availability of new technical advances and strategies which can be translated into more effective programmes for the management of the T&T challenge;
The workshop recommends that:

(i) The existing gaps prevailing between research and policy need to be bridged. T&T research results should be availed by partners and national offices in order to allow AU-PATTEC to create a data repository at continental level to facilitate accessibility by all partners.

(ii) The trypanocides resistance problem should be analysed in greater depth. T&T countries should build a more complete picture of drug resistance using research to encourage rational trypanocides use.

(iii) Confusion arising from inconsistent use of T&T terminology needs to be addressed urgently. The PATTEC Coordination Office should work on the definitions of terminology related to T&T and disseminate these to all stakeholders in an effort to communicate consistent messages on T&T management.

(iv) The persistence and epidemiology of non tsetse transmitted trypanosomosis (NTTAT) in tsetse free areas need to be studied further. Studies should be undertaken to better understand the role of mechanical vectors of NTTAT in disease spread and maintenance.

(v) The PATTEC Coordination Office should consider promoting greater integration of T&T programmes into Sustainable Land Management Initiatives. The PATTEC Coordination Office should facilitate the formulation of guidelines for sustainable land use management of tsetse freed areas.

(vi) T&T countries should use M&E tools to evaluate the socio-economic benefits/success before and after T&T interventions.

(vii) The PATTEC Coordination Office and countries must work together to strengthen regional level intervention mechanisms through collaboration with Regional Economic Communities (RECs).

(viii) The PATTEC's initiative of accelerating the formation of effective partnerships in the management of T&T is a welcome development. As a starting point, the African Union Commission and Member States should establish an open source database of potential partners including their profiles.

(ix) Lessons learned from the implementation of the PATTEC programme supported by the African Development Bank (AfDB) should be disseminated to other countries to promote better programme formulation and implementation.

PARTICIPATION OF MEDIA REPRESENTATIVES IN A MONITORING AND EVALUATION MISSION OF PATTEC IN SOUTHERN ETHIOPIA

14-19 OCTOBER 2014

The AU-PATTEC Coordination Office organized this mission in collaboration with the Ethiopian National Institute for Control and Eradication of Tsetse and Trypanosomosis (NICETT) to give media representatives the opportunity to evaluate and report on work related to T&T eradication.

The main results of the mission were as follows:

(i) The demonstration that tsetse suppression and eradication are possible and that the Ethiopian STEP project successfully demonstrated the different techniques available to do it.

(ii) Capacity building and participation of beneficiary communities are two important keys for success.

(iii) The SIT technique is an ultimate solution as shown during the mission.
(iv) STEP is a good pilot project on T&T, leading to the upgrading of the National Institute for Control of Tsetse and Trypanosomosis (NICETT).

In conclusion, the mission was a success and the media representatives from RFI and France 24 witnessed T&T activities being carried out from the office, insectary and through field activities. They conducted interviews with the State Minister for Livestock, the AU-PATTEC Coordinators, project staff and beneficiaries.

FROM WHO

4TH INTERNATIONAL MEETING ON THE CONTROL OF NEGLECTED ZOONOTIC DISEASES, 19-20 NOVEMBER 2014, GENEVA

This meeting on Neglected Zoonotic Disease (NZDs) was supported financially by the European Union seventh framework programme through the ADVANZ (Advocacy for Neglected Zoonotic Diseases) and ICONZ (Integrated Control of Neglected Zoonoses) projects. It was hosted by WHO at its headquarters in Geneva, Switzerland, and opened by the Assistant Director-General, Dr Nakatani.

NZDs are found in communities in low-resource settings across the world, where they impose a dual burden on people’s health and that of the livestock they depend upon. National governments are increasingly seeking to mitigate the impact of NZDs on their citizens by implementing control programmes to address these burdens. These initiatives have been strongly endorsed by the Food and Agriculture Organization of the United Nations, the World Organization for Animal Health and WHO tripartite and financially supported by members of the broader international community including the Bill & Melinda Gates Foundation, the UK Department for International Development, the European Union, the International Development Research Centre and the CGIAR. WHO’s 2012 Roadmap on accelerating work to overcome the global impact of neglected tropical diseases (NTDs) and Resolution WHA66.12 on NTDs adopted by the World Health Assembly in May 2013 have enhanced the visibility of zoonotic NTDs’ – notably rabies, cysticercosis, echinococcosis, human African trypanosomiasis, foodborne trematodiases and leishmaniasis. Although not specifically included in the WHO Roadmap, other diseases have been addressed by the NZD community such as anthrax, bovine tuberculosis, brucellosis and leptospirosis.

Much of the initial momentum for action against NZDs was catalysed by the inaugural meeting on NZD control in 2005. Whilst the priority at that time was a need for evidence, a decade later the focus is on better implementation of proven pathways for control and mobilizing central governments and donors within broader health and development agendas. The fourth international meeting on NZDs acknowledged the momentum generated by the NZD community over the past decade, urging the more than 100 participants – including representatives from national governments, international organizations, academia, foundations, the private sector and NGOs – to exert their influence and focus on operations, especially for the NZDs included in the WHO Roadmap.

Clear themes that emerged throughout this meeting were the need for political commitment, sustainable One Health collaborations and the identification of local champions to drive community participation in control. Examples of programmes making significant progress in the control of some NZDs, both at national and local levels from across three continents, were provided by many countries. For example, Charles Waiswa, from Control of Trypanosomiasis in Uganda (COCTU), described how sharing resources and working together with medical, veterinary and agriculture colleagues had brought about a reduction in cases of trypanosomiasis. Early warning systems had been developed by investing in and improving infrastructure and disease surveillance, and by bringing more people in at every
level. “Catalytic Centres” had been developed which were a focus for community empowerment. A strategic plan for 2015-2030 was now agreed, with the aim to make further progress.

Dissemination of the knowledge gained through these programmes provides significant encouragement to country partners that the control of NZDs can indeed be achieved with currently available tools. There are opportunities for innovative funding mechanisms to support NZD control outside traditional donor models, including initiatives stemming from national bodies and from the private sector. Whilst challenges undoubtedly remain regarding refinement of control tools and their application in low-income settings, these should not prevent large-scale implementation of control programmes. There is now the opportunity to capitalize on the existing knowledge, experience and political will to move “From Advocacy to Action”. WHO will continue to report progress and follow-up on actions recommended during the meeting through its Strategic and Technical Advisory Group for Neglected Tropical Diseases (STAG-NTDs).

**Note:** An article written by Suzanne Jarvis provides in depth coverage of this meeting. It is published in Veterinary Record (2015), **176**: 85-86. It can be downloaded from: [http://veterinaryrecord.bmj.com/](http://veterinaryrecord.bmj.com/).

**FRENCH VERSION OF CONTROL AND SURVEILLANCE OF HUMAN AFRICAN TRYPANOSOMIASIS. REPORT OF A WHO EXPERT COMMITTEE (TRS N°. 984)**

This report: “**Trypanosomiase humaine africaine: lutte et surveillance**” is now available and can be downloaded from [http://apps.who.int/iris/bitstream/10665/148113/1/9789240692230_fre.pdf?ua=1](http://apps.who.int/iris/bitstream/10665/148113/1/9789240692230_fre.pdf?ua=1)

**FROM FAO**

**PROJECT ON IMPROVING FOOD SECURITY IN SUB-SAHARAN AFRICA BY SUPPORTING THE PROGRESSIVE REDUCTION OF TSETSE-TRANSMITTED TRYPANOSOMOSIS IN THE FRAMEWORK OF THE NEPAD**

In the second half of 2014, this project (GTFS/RAF/474/ITA) implemented a range of activities. Strong emphasis continued to be placed on capacity development. Demand-driven courses and workshops were conducted in Angola, Burkina Faso, Ghana and Uganda. The focus of the training was on Geographic Information Systems (GIS), risk assessment and data management for decision making on tsetse and trypanosomosis (T&T) interventions and related Sustainable Agriculture and Rural Development (SARD). A regional-level training workshop on Free GIS Open Source Software applied to tsetse and trypanosomosis control programmes was carried out in Vienna (Austria) for 15 trainees from 10 countries (Angola, Burkina Faso, Chad, Côte d’Ivoire, Democratic Republic of the Congo, Gabon, Mali, Niger, Senegal and Togo). Support was also provided to the training of two staff from the Democratic Republic of the Congo: the training, jointly organized by the World Health Organization (WHO) and FAO, focused on the Atlas of HAT and it was held in Addis Ababa, Ethiopia.

All training activities were organized and conducted in close collaboration with project partners, most notably the African Union – the Pan African Tsetse and Trypanosomosis Eradication Campaign (AU-PATTEC), the International Atomic Energy Agency (IAEA), and
WHO. Inputs were also provided by trypanosomosis-affected countries, which shared the costs of the training activities.

Photos of training courses and workshops held in Angola, Burkina Faso, and Uganda.

The project also supported the organization and execution of a workshop on the integration of Gender into Livestock Production and Health. The workshop, held in Harare, Zimbabwe (2-4 December 2014), benefitted 21 participants from 10 countries in the Southern Africa region.
Photo of a workshop on the integration of Gender into Livestock Production and Health.

The project is also enabling FAO to continue assisting WHO in the upgrade, update and dissemination of the Atlas of human African trypanosomiasis (HAT). In this regard, an update to the year 2012 of the geographic distribution and risk of *gambiense*-HAT was achieved.

Field activities are being conducted in Southern Ethiopia, by piloting the deployment of integrated animal health and production packages. Focus is placed on livestock protective fences.

Livestock protective fences in Arba Minch, Southern Ethiopia.

For more information, contact Giuliano Cecchi (Giuliano.Cecchi@fao.org) and Raffaele Mattioli (Raffaele.Mattioli@fao.org).

**FROM THE JOINT FAO/IAEA PROGRAMME**

**SPECIAL ISSUE ON APPLYING POPULATION GENETICS AND GIS FOR MANAGING LIVESTOCK INSECT PESTS**

The Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) have supported from 2008 to 2013 a Coordinated Research Project (CRP) on “Applying GIS and population genetics for
managing livestock insect pests”. This six-year CRP focused on underpinning the area-wide integrated pest management (AWIPM) of tsetse and screwworm flies, and an introductory paper to the special issue in *Acta Tropica* includes the findings of the CRP participants and discusses them in a broader context. Techniques for mapping and modelling the distributions of genetically populations of tsetse and screwworm flies are increasingly used by researchers and managers for more effective decision-making in AW-IPM programmes, as illustrated by the reports in this special issue. Currently, insect pests are often characterized only by neutral genetic markers suitable for recognizing spatially isolated populations that are sometimes associated with specific environments. Two challenges for those involved in AW-IPM programmes are the standardization of best practice to permit the efficient application of GIS and genetic tools by regional teams, and the need to develop further the mapping and modelling of parasite and pest phenotypes that are epidemiologically important.

The *Acta Tropica* Special Issue Vol. 138 (October 2014) includes 13 scientific papers that can be freely downloaded until September 2015 at: http://www.sciencedirect.com/science/journal/0001706X/138/supp/S.

**REGIONAL TRAINING COURSE ON FREE OPEN SOURCE SOFTWARE FOR GIS AND DATA MANAGEMENT APPLIED TO TSETSE AND TSETTE CONTROL PROGRAMMES**

The training course, held in Addis Ababa, Ethiopia from 6 - 16 May 2014, was organized by AU-PATTEC, FAO and IAEA and was attended by 22 participants from 11 Member States (Ethiopia, Ghana, Kenya, Malawi, Mozambique, Sudan, Uganda, United Republic of Tanzania, and Zimbabwe).
SECOND RESEARCH COORDINATION MEETING OF COORDINATED RESEARCH PROJECT ON ENHANCING VECTOR REFRACTORINESS TO TRYPANOSOME INFECTION. 1–5 DECEMBER 2014, ADDIS ABABA, ETHIOPIA

The 2nd Research Coordination Meeting (RCM) of the Coordinated Research project (CRP) entitled “Enhancing vector refractoriness to trypanosome infection”, had 26 participants including one consultant and three observers. The RCM was officially opened by Dr Thomas Cherenet, Director General of NICETT (the local organizer of the meeting). The first two days of the RCM were dedicated to oral presentations by the participants, who provided updates on progress made and results achieved. Professor Serap Aksoy reported the completion of the genome sequence of six tsetse species, while Dr Martin Kaltenpoth described the impact of antibiotic treatment on the hydrocarbon signal of tsetse flies. One oral presentation was made by Dr Solomon Mekonnen, the technical director of NICETT, on the mass rearing facility and the male releases in the frame of the SIT programme in Ethiopia. The following two days were dedicated to group discussions (one on the parasite and pathogens and the second on the symbionts).

FROM GALVmed
SEEKING THE NEXT GENERATION OF LIVESTOCK TRYPANOCIDES

The loss of livestock productivity and mortality caused by the protozoan parasites Trypanosoma congolense and T. vivax, which are transmitted by tsetse and other biting flies, leads to loss of assets and income to farmers in Africa. The estimated cost is almost US$ 5 billion a year with over 150 million ruminants at risk in tsetse-infested areas.

The losses to trypanosomosis or Nagana are not limited to but are most heavily felt by the poorest livestock keepers. In 2011, the UK Department for International Development asked GALVmed to seek new trypanocides, vaccines and diagnostics to help control this disease. During Phase 1 of the programme that ended in December 2013, GALVmed developed partnerships to establish test systems to evaluate available technologies. Laboratory testing was carried out primarily at the Swiss Tropical and Public Health Institute (TPH) and then, with formulation support from the University of Greenwich, compounds were progressed and further testing occurred at sites in Mozambique (University Eduardo Modlane), South Africa (ClinVet) and Burkina Faso (CIRDES). Although none of the potential vaccines was shown to work in a rigorous model, good progress was made in identifying new diagnostics and trypanocidal compounds. This work is continuing in Phase 2. This article describes our strategy, some of the challenges faced and progress made towards identifying new trypanocides.

Trypanocides are available both to treat (Diminazene), and prevent (Isometamidium, Homidium) trypanosomosis in cattle and other livestock. Indeed, trypanocides represent one of the largest segments of the animal health market in Africa with estimated sales of branded and generic products estimated at around US$ 100 million. However, these compounds are over 50 years old and not only do they have narrow therapeutic margins but field resistance to these compounds is growing steadily. Unfortunately, the potential value of this market has been reduced by widespread availability of sub-standard and fake products. The lack of understanding of the disease, poor diagnosis and poor efficacy of current products due to drug resistance or intentional and accidental under-dosing, coupled with inconvenient to use formulations, means that usage of trypanocides is much lower than required to tackle the
Tsetse and Trypanosomosis Information

disease. This market risk has resulted in a reluctance of animal health companies to invest precious research and development funding in the search for new trypanocides.

In contrast, a partnership of academics, pharma companies and not-for-profit organizations such as DNDi has made great progress in addressing human African trypanosomiasis (HAT) or sleeping sickness caused by the related species, *T. brucei rhodesiense* and *gambiense*. As well as declining cases, there is now a strong pipeline of new, highly-effective drug candidates in development, e.g. fexinidazole and SCYX7158.

GALVmed has adopted a strategy to build on the discoveries made by these groups to leverage their compounds for potential application to animal trypanosomosis. Our Phase 1 work leveraging the human drug candidate programmes almost achieved our goal of identifying a development candidate. AN7973 from Anacor was shown to be very safe and could cure cattle infected with *T. congolense* by a single 10mg/kg injection. Unfortunately, *T. vivax* proved less susceptible to this compound and could not be treated by an economic dose.

Through support from our joint funders, the Bill & Melinda Gates Foundation and the UK Government, we have been encouraged to expand our efforts in Phase 2 to leverage the human research with additional collaborations. Many of the drugs identified for HAT failed due to lack of efficacy against Stage 2 of the disease in which the parasite enters the central nervous system where it is harder to access with the drug. Other lead compounds failed to provide activity by oral dosing. However, neither of these features is required to treat animal African trypanosomosis since the parasites largely live in the peripheral tissues and bloodstream and treatment is more convenient by injection. We need a compound that is potent and long-lasting so that it can cure or provide prophylaxis by a single injection. It also needs to be very safe both to the animal and to humans who might wish to subsequently eat the animal or use its milk.

GALVmed has developed screening partnerships with more than a dozen academic and industry groups who have been active in seeking treatments for HAT. Our partnerships span the globe and include the Universities of Dundee, Strathclyde, Cape Town, Washington, North Eastern (U.S.), Monash (Australia); research institutes e.g. Broad, St. Jude Children’s Hospital; and companies such as Novartis, Anacor and Celgene. We have been testing their compounds in the laboratory against the livestock trypanosomes, *T. vivax* and *T. congolense* and have identified a number of very exciting lead structures. We have already commenced lead optimization programmes with Anacor, the University of Dundee and Celgene Global Health to seek close analogues of these lead compounds that might have the ideal combination of properties to make them candidates for development. The best of these compounds are now being progressed to cattle studies and results are very encouraging.

FROM FIND

THE GOVERNMENT OF UGANDA INTENSIFIES FIGHT AGAINST SLEEPING SICKNESS

The Government of Uganda, in a partnership with FIND, is intensifying surveillance and control of *Trypanosoma brucei gambiense* human African trypanosomiasis (HAT, or sleeping sickness) in the West Nile region and Amuru district. This follows the signing of a three year collaborative agreement between the Coordinating Office for Control of Trypanosomiasis in Uganda (COCTU) of the Uganda Trypanosomiasis Control Council (UTCC) and FIND. The project will include the use of new tests developed with the support of FIND, and a new approach that is intended to shorten the distance that a sick person has to travel to seek diagnosis. Experts from the Ministry of Health (MOH), the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) and Makerere University are leading
this initiative, which if successful could lead to elimination of *T. b. gambiense* HAT in the country, and serve as a model for replication in other endemic countries.

The new project is being implemented in 166 health facilities spread across the *T. b. gambiense* sleeping sickness belt of Uganda, in an approach that combines screening of suspected patients with a recently developed rapid test, followed by confirmation of these cases by LED fluorescence microscopy. This will be complemented with detection of parasite DNA using a highly sensitive and simple molecular method known as LAMP. All the health facilities in the project area were geo-referenced and characterized by the government of Uganda in an earlier project with support from FIND. This has made it easier to identify the facilities in which to install different diagnostics for HAT.

All participating health facilities will be stocked with the rapid tests after appropriate training is carried out, while microscopy and molecular tests are being located at strategic centres in all the districts. In this new approach, a sick person who visits the nearest health centre will be tested using the rapid test. Those who are positive with the rapid test will be referred to the nearest microscopy centre for confirmatory diagnosis, and if they are found to be negative by microscopy, a blood sample will be taken on filter paper and sent to the LAMP centre for molecular testing. The results of the LAMP test will be sent by cell phone to the health centre that first saw the patient, and that centre will take appropriate action depending on the results. All the data on individuals tested and on stocks of kits available at each health facility will be communicated to the coordinating office at the MOH, which is responsible for the day to day running of the project.

Various departments in Uganda have been collaborating with FIND to develop the tests that are being implemented in this new project. Most of the work on LED fluorescence microscopy was carried out in partnership with Makerere University, Lwala Hospital and the National Livestock Resources Research Institute (NALIRRI), while Makerere University and Lwala Hospital have been at the forefront of research on LAMP for both *T. b. rhodesiense* and *T. b. gambiense* in Uganda and the DRC. The new project is an extension of the long-term relationship between FIND and the government of Uganda and their collaboration on other diseases such as tuberculosis and malaria.

Funding for this project comes from the government of Uganda, as well as through FIND, the Bill and Melinda Gates Foundation (BMGF), Department for International Development (DFID) of the UK, the German Federal Ministry of Education and Research, the Republic and Canton of Geneva, Switzerland, and the Swiss Agency for Development and Cooperation (SDC).

**FROM DNDi**

**R&D STATUS SEPTEMBER 2014: HUMAN AFRICAN TRYpanosomiasis PROGRAMME**

Human African trypanosomiasis (HAT), also known as ‘sleeping sickness’, is transmitted by the tsetse fly. While currently its prevalence is declining, HAT is still a threat to millions of people across Sub-Saharan Africa with 83 percent (2013) of all cases in the Democratic Republic of Congo (DRC). The WHO Roadmap (2012) to overcome the global impact of neglected tropical diseases (NTDs) has set the objective to eliminate HAT disease by 2020\(^1\). In support of this strategy, DNDi is carrying out an ambitious clinical development programme in close collaboration with NGOs, research, and industrial partners, and national control programmes. The current HAT portfolio includes:

\(^1\) Less than one case per 10 000 inhabitants in at least 90 percent of endemic foci is expected.
Two projects in the research phase:

Two backup candidates have been identified and are currently on hold in pre-clinical development in case SCYX-7158 (see project in clinical development) is precluded from further clinical development:

- SCYX-1608210 from the oxaborole class was identified and selected among a range of structurally diverse oxaboroles with good activity against *T. brucei* that were profiled in a laboratory pharmacokinetic (PK) study.

- SCYX-2035811 is from the nitroimidazole class. The nitroimidazole backup programme for HAT has been searching for a compound with a lower projected human dose than fexinidazole to simplify dosing and mitigate any potential issues with tolerance.

Currently, two oral drug candidates that are both new chemical entities (NCEs) are in clinical development for HAT which, if successful, could become the first-ever oral-only treatments for the disease:

One project in the translation phase:

- A Phase I study with oxaborole SCYX-7158, will be completed by the end of this year. SCYX-7158, the first clinical candidate issued from the oxaboroles class provided by Anacor Pharmaceuticals and developed in partnership with SCYNEXIS, is also intended to treat stage 1 and 2 HAT. If successful, as a one-dose therapy (i.e. cure with one pill only), would provide significant advantages over NECT and even fexinidazole. It is expected to enter into a Phase II/III study in 2015.

One project in the development phase:

- A pivotal Phase II/III study with fexinidazole started in 2012 at nine clinical sites in the Democratic Republic of Congo and the Central African Republic. Fexinidazole, the first success of the extensive compound mining efforts pursued by DNDi aimed to explore new and old nitroimidazole drug leads, would provide significant advantages over the current treatment standard NECT, which, while very effective, still requires hospital stays and specialized healthcare staff. A simple oral treatment, fexinidazole, if successful, will be easy to administer at the primary healthcare level and will allow patients to take their treatments home. The study has so far recruited 285 stage 2 HAT adult patients out of the 390 in total. This year, following positive initial results of the trial, the study has also been extended both adult patients with stage 1 and early stage 2 of the disease, and in children between 6 and 14 years of age. Sanofi is DNDi’s industrial partner for this project.

If ultimately successful, fexinidazole and/or SCYX-7158 would be the first oral treatments to be used for both stage 1 and stage 2 sleeping sickness, thereby replacing the complicated diagnosis and treatment paradigm, which includes systematic lumbar punctures of every diagnosed patient to determine the stage of the disease before deciding which treatment to administer.
Implementation and access

DNDi continues to support the implementation and access to the Nifurtimox-Eflornithine combination treatment (NECT) that DNDi and its partners delivered in 2009. NECT has been included in the WHO Essential Medicines List since 2009 and is now on the Essential Medicines List for children (April 2013). Since June 2014, all countries endemic to T. b. gambiense are using NECT as first-line treatment for second stage HAT.
SECTION B – ABSTRACTS

1. GENERAL (INCLUDING LAND USE)


Eskitis Institute for Drug Discovery, Griffith University, Nathan, Queensland, Australia. [k.andrews@griffith.edu.au].

Parasitic diseases have an enormous health, social and economic impact and are a particular problem in tropical regions of the world. Diseases caused by protozoa and helminths such as malaria and schistosomiasis are the cause of most parasite related morbidity and mortality, with an estimated 1.1 million combined deaths annually. The global burden of these diseases is exacerbated by the lack of licensed vaccines, making safe and effective drugs vital to their prevention and treatment. Unfortunately, where drugs are available, their usefulness is being increasingly threatened by parasite drug resistance. The need for new drugs drives antiparasitic drug discovery research globally and requires a range of innovative strategies to ensure a sustainable pipeline of lead compounds. In this review we discuss one of these approaches, drug repurposing or repositioning, with a focus on major human parasitic protozoan diseases such as malaria, trypanosomiasis, toxoplasmosis, cryptosporidiosis and leishmaniasis.


Fundacion MEDINA, Parque Tecnologico de Ciencias de la Salud, Armilla (Granada), Spain; Instituto de Parasitologia y Biomedicina "Lopez-Neyra," Consejo Superior de Investigaciones Cientificas, Parque Tecnologico de Ciencias de la Salud, Armilla (Granada), Spain. [dgonzalez@ipb.csic.es].

African trypanosomiasis, leishmaniasis, and Chagas disease are three neglected tropical diseases for which current therapeutic interventions are inadequate or toxic. There is an urgent need to find new lead compounds against these diseases. Most drug discovery strategies rely on high-throughput screening (HTS) of synthetic chemical libraries using phenotypic and target-based approaches. Combinatorial chemistry libraries contain hundreds of thousands of compounds; however, they lack the structural diversity required to find entirely novel chemotypes. Natural products, in contrast, are a highly underexplored pool of unique chemical diversity that can serve as excellent templates for the synthesis of novel, biologically active molecules. We report here a validated HTS platform for the screening of microbial extracts against the three diseases. We have used this platform in a pilot project to screen a subset (5, 976) of microbial extracts from the MEDINA natural products library. Tandem liquid chromatography-mass spectrometry showed that 48 extracts contain potentially new compounds that are currently undergoing de-replication for future isolation.
and characterization. Known active components included actinomycin D, bafilomycin B1, chromomycin A3, echinomycin, hygrolidin, and nonactins, among others. The report here is, to our knowledge, the first HTS of microbial natural product extracts against the above-mentioned kinetoplastid parasites.


South African Medical Research Council Bioinformatics Unit, South African National Bioinformatics Institute, University of the Western Cape, Bellville, South Africa; Molecular Biology and Bioinformatics Unit, International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya; The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK; Vector Group, Liverpool School of Tropical Medicine, Liverpool, UK; Vector, Environment and Society Unit, Tropical Diseases Research (TDR), World Health Organization, Geneva, Switzerland; Yale School of Public Health, Department of Epidemiology and Public Health, New Haven, Connecticut, USA. [alan@sanbi.ac.za].

The genomic activities of the IGGI consortium over the past ten years have provided a skill set for African researchers to exploit the recently sequenced Glossina morsitans morsitans genome. The completion of a ten-year genome project has cemented a scientific network of trypanosomiasis researchers that will be required to provide mentoring to new students and researchers as strategies are sought to best integrate the valuable genomic resources for the tsetse and the Tritryp genomes. Moreover, the involvement of scientists from disease endemic countries from the start sets the tsetse genome project apart from other vector or parasite projects and will help to ensure a greater sense of ownership and long term commitment. Access to the human genome sequence, the trypanosomatid genomes, and trypanosome “omics” data has provided insight into host-parasite interactions and the identification of new vaccine candidates or chemotherapeutic targets. Targeting tsetse-trypanosome interactions with the goal of identifying genes that modulate trypanosome transmission has entered the realm of feasibility with the completion of the tsetse genome sequencing project.


Laboratory for Cellular and Molecular Immunology, Vrije Universiteit Brussel, Building E8.01, Pleinlaan 2, 1050 Brussels, Belgium. [jcncps@vub.ac.be].

African trypanosomes have been around for more than 100 million years, and have adapted to survival in a very wide host range. While various indigenous African mammalian host species display a tolerant phenotype towards this parasitic infection, and hence serve as perpetual reservoirs, many commercially important livestock species are highly disease susceptible. When considering humans, they too display a highly sensitive disease progression phenotype for infections with Trypanosoma brucei rhodesiense or Trypanosoma brucei gambiense, while being intrinsically resistant to infections with other trypanosome species. As extracellular trypanosomes proliferate and live freely in the bloodstream and
lymphatics, they are constantly exposed to the immune system. Due to co-evolution, this environment no longer poses a hostile threat, but has become the niche environment where trypanosomes thrive and await transmission through the bites of tsetse flies or other haematophagic vectors, ideally without causing severe side infection-associated pathology to their hosts. Hence, African trypanosomes have acquired various mechanisms to manipulate and control the host immune response, evading effective elimination. Despite the extensive research into trypanosomosis over the past 40 years, many aspects of the anti-parasite immune response remain to be solved and no vaccine is currently available. Here we review recent work on the different escape mechanisms employed by African trypanosomes to ensure infection chronicity and transmission potential.


Division of Women & Child Health, The Aga Khan University, Karachi, Pakistan; Brown University, Providence, RI, USA; Center of Excellence in Women & Child Health, The Aga Khan University, Karachi, Pakistan; Center for Global Child Health, Hospital for Sick Children, Toronto, Canada. [zulfiqar.bhutta@aku.edu].

In this paper, we aim to systematically analyse the effectiveness of community based interventions (CBI) for the prevention and control of non-helminthic diseases including dengue, trypanosomiasis, Chagas, leishmaniasis, buruli ulcer, leprosy and trachoma. We systematically reviewed literature published up to May 2013 and included 62 studies. Findings from our review suggest that CBI including insecticide spraying, insecticide treated bed nets and curtains, community education and cleanliness campaigns, chemoprophylaxis through mass drug administration, and treatment have the potential to reduce the incidence and burden of non-helminthic diseases. Lack of data limited the subgroup analysis for integrated and non-integrated delivery strategies, but qualitative synthesis suggest that integrated delivery is more effective when compared with vertical interventions. However, such integration was possible only because of the existing vertical vector control programmes. Community delivered interventions have the potential to achieve wider coverage and sustained community acceptance. Eradicating these diseases will require a multipronged approach including drug administration, health education, vector control and clean water and sanitation facilities. This would require high levels of governmental commitment along with strong partnerships among major stakeholders.


Bio-health Informatics Group, School of Computer Science, University of Manchester, Manchester, UK; School of Biological Science, University of Liverpool, Liverpool, UK; Manchester Immunology Group, Faculty of Life Science, University of Manchester, Manchester, UK. [andy.brass@manchester.ac.uk].

There is a growing concern both inside and outside the scientific community over the lack of reproducibility of experiments. The depth and detail of reported methods are critical to the reproducibility of findings, but also for making it possible to compare and integrate data from different studies. In this study, we evaluated in detail the methods reporting in a
comprehensive set of trypanosomiasis experiments that should enable valid reproduction, integration and comparison of research findings. We evaluated a subset of other parasitic (Leishmania, Toxoplasma, Plasmodium, Trichuris and Schistosoma) and non-parasitic (Mycobacterium) experimental infections in order to compare the quality of method reporting more generally. A systematic review using PubMed (2000-2012) of all publications describing gene expression in cells and animals infected with *Trypanosoma* spp. was undertaken based on PRISMA guidelines; 23 papers were identified and included. We defined a checklist of essential parameters that should be reported and have scored the number of those parameters that are reported for each publication. Bibliometric parameters (impact factor, citations and h-index) were used to look for association between journal and author status and the quality of method reporting. Trichuriasis experiments achieved the highest scores and included the only paper to score 100 percent in all criteria. The mean of scores achieved by *Trypanosoma* articles through the checklist was 65.5 percent (range 32-90 percent). Bibliometric parameters were not correlated with the quality of method reporting (Spearman's rank correlation coefficient < -0.5; p > 0.05). Our results indicate that the quality of methods reporting in experimental parasitology is a cause for concern and it has not improved over time, despite there being evidence that most of the assessed parameters do influence the results. We propose that our set of parameters be used as guidelines to improve the quality of the reporting of experimental infection models as a pre-requisite for integrating and comparing sets of data.


Department of Molecular and Translational Medicine, Medical School, University of Brescia, Italy; African Bioeconomy Capacity Development Institute, BioEconomy Africa, Addis Ababa, Ethiopia; Millennium Institute, Washington, DC., USA. [gianni.gilioli@med.unibs.it].

Recently, there has been growing interest in studying the link between human, animal and environmental health. The connection between these different dimensions is particularly important for developing countries in which people face the challenge of escaping the vicious cycle of high disease prevalence, food insecurity driven by absolute poverty and population growth, and natural capital as a poverty trap. This paper presents a framework for the development of socio-ecological systems towards enhanced sustainability. Emphasis is given to the dynamic properties of complex, adaptive social-ecological systems, their structure and to the fundamental role of agriculture. The tangible components that meet the needs of specific projects executed in Kenya and Ethiopia encompass project objectives, innovation, facilitation, continuous recording and analyses of monitoring data that allow adaptive management and system navigation. Two case studies deal with system navigation through the mitigation of key constraints; they aim to improve human health thanks to anopheline malaria vectors control in Nyabondo (Kenya), and to improve cattle health through tsetse control and antitrypanosomal drug administration to cattle in Luke (Ethiopia). The second case deals with a socio-ecological navigation system to enhance sustainability, establishing a periurban diversified enterprise in Addis Ababa (Ethiopia) and developing a rural sustainable social-ecological system in Luke (Ethiopia). The project procedures are briefly described here and their outcomes are analysed in relation to the stated objectives. The methodology for human and cattle disease vector control was easier to implement than the navigation of
social-ecological systems towards sustainability enhancement. The achievements differed considerably between key constraints removal and sustainability enhancement projects. Some recommendations are made to rationalize human and cattle health improvement efforts and to smoothe the road towards enhanced sustainability: (i) technology system implementation should be carried out through an innovation system; (ii) transparent monitoring information should be continuously acquired and evaluated for assessing the state of the system in relation to stated objectives for improving insight into the systems behaviour and rationalizing decision support; (iii) the different views of all stakeholders should be reconciled in a pragmatic approach to social-ecological system management.


University of Glasgow, Glasgow, Scotland, UK. [Peter.Holmes@glasgow.ac.uk].

Human African trypanosomiasis (HAT), also known as sleeping sickness, has been one of the most important human diseases in Africa because of widespread epidemics in the past, its very high level of mortality, and its negative influence on the development of rural populations. Two forms of the disease exist, one chronic form in West and Central Africa caused by Trypanosoma brucei gambiense (> 95 percent of current cases), and an acute form in East and Southern Africa caused by Trypanosoma brucei rhodesiense (< 5 percent of current cases). During the 1960s HAT was brought under control by the colonial health systems, but unfortunately the rarity of HAT cases led to a decline in awareness of how the disease could return and subsequently, to a lack of interest in disease surveillance and a decrease in disease control. With this decrease in control and surveillance activities, the disease re-emerged, reaching epidemic proportions by the end of the 20th century, with the majority of human infections caused by T. b. gambiense. The alarming rise in the number of cases stimulated international efforts to reverse the epidemiological trend and reduce the incidence of the disease, using enhanced surveillance and improved access to diagnosis and treatment in endemic countries. These activities have been complemented by improvements in the epidemiological knowledge of the disease and the production of highly detailed disease distribution maps of affected countries. As a result of these outstanding efforts by national sleeping sickness programmes and the support of international organizations led by the World Health Organization (WHO), and involving key pharmaceutical companies and major international donors, remarkable results have been achieved during the past 15 years, with the number of new reported cases falling to 6 228 in 2013. This has involved strong collaboration and coordination of all these stakeholders and the maintenance of a permanent and open dialogue. Based on the advances achieved in the control of the disease, in 2012 the WHO Strategic and Technical Advisory Group for Neglected Tropical Diseases made the decision to target elimination of gambiense HAT as a public health problem by 2020 and to target zero incidence of the disease by 2030. The 2020 target was included in the WHO roadmap for elimination and control of neglected tropical diseases, and it is defined as the reduction of gambiense HAT incidence to less than one new case per 10 000 population at risk, in at least 90 percent of foci with fewer than 2 000 cases reported globally. In 2013 this elimination target was endorsed by the disease endemic countries, the WHO Expert Committee on Control and Surveillance of HAT, and the London Declaration on Neglected Tropical Diseases. More recently it was adopted by the 66th World Health Assembly in the resolution WHA66.12.

In order to move forward towards gambiense HAT elimination, the first WHO meeting of stakeholders on elimination of gambiense HAT was held in Geneva (Switzerland) from 25
Tsetse and Trypanosomosis Information

to 27 March 2014. Participants came from national sleeping sickness control programmes, groups developing new tools to fight HAT, international and non-governmental organizations involved in HAT control and major international donors including private sector companies Sanofi and Bayer Healthcare, bilateral agencies like the Belgian Development Cooperation, and philanthropic organizations such as the Bill and Melinda Gates Foundation and the Wellcome Trust. The meeting reviewed the current epidemiological status of the disease and the recent achievements and challenges for moving towards the gambiense HAT elimination goal. The stakeholders also analysed the current status of important technical aspects of HAT control that are currently in development and will greatly assist in bringing about the elimination of HAT. These include the development of new drugs currently in clinical trials and new diagnostics tools that are becoming available, research on some unresolved epidemiological aspects such as the potential role of asymptomatic carriers and animal reservoirs and recently improved methods of vector control. The meeting also examined the establishment of mechanisms for monitoring and evaluation of the elimination process as well as the confirmation of outcomes. Finally, the ways and mechanisms through which collaboration and coordination among stakeholders can be strengthened and organized were considered. As the number of cases of HAT continue to decrease it will be critical that the international stakeholders maintain their support, that HAT surveillance, treatment, and control activities are integrated within health services, and that the national ownership of the control programmes is achieved in order to bring about the sustainable elimination of the disease and to avoid the repetition of the painful experience of the last century. The first WHO meeting of stakeholders on elimination of gambiense HAT held in Geneva, Switzerland in March 2014 concluded by issuing a declaration for the elimination of gambiense HAT, which appeals to the international community at large and to disease-endemic countries for their commitment, political support, and essential resources to achieve the elimination goal and establishes a network under the leadership of WHO to ensure coordinated, strengthened, and sustained efforts to eliminate the disease. The declaration of the first stakeholders meeting on gambiense HAT elimination can be viewed at: http://www.who.int/trypanosomiasis_african/meeting_declaration_2014/en/.


Department of Laboratories and Blood Safety, Ministry of Public Health, and University of Yaoundé I, Yaoundé, Cameroon; Laboratoire de Virologie, Hôpital Européen Georges Pompidou, and Faculté de Médecine Paris Descartes, Université Paris Descartes (Paris V), Paris, France; UNAIDS, Dakar, Senegal; Division of Viral Hepatitis, Centers for Disease Control and Prevention, Atlanta, Georgia, USA. [fxmkeou@hotmail.com].

Abstract not available.


Laboratorio de Biologia Celular, Instituto Oswaldo Cruz, Fundacao Oswaldo Cruz, Avenida Brasil 4365, 21040-360 Manguinhos, RJ, Brazil. [bmri@hindawi.com].

The pathogenic trypanosomatids *Trypanosoma brucei*, *Trypanosoma cruzi*, and
**Leishmania** spp. are the causative agents of African trypanosomiasis, Chagas disease, and leishmaniasis, respectively. These diseases are considered to be neglected tropical illnesses that persist under conditions of poverty and are concentrated in impoverished populations in the developing world. Novel efficient and non-toxic drugs are urgently needed as substitutes for the currently limited chemotherapy. Trypanosomatids display a single mitochondrion with several peculiar features, such as the presence of different energetic and antioxidant enzymes and a specific arrangement of mitochondrial DNA (kinetoplast DNA). Due to mitochondrial differences between mammals and trypanosomatids, this organelle is an excellent candidate for drug intervention. Additionally, during the trypanosomatids' life cycles, the shape and functional plasticity of their single mitochondrion undergo profound alterations, reflecting adaptation to different environments. In an uncoupling situation, the organelle produces high amounts of reactive oxygen species. However, the role of these in parasite biology is still controversial, involving parasite death, cell signalling, or even proliferation. Novel perspectives on trypanosomatid-targeting chemotherapy could be developed based on better comprehension of mitochondrial oxidative regulation processes.


Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, California 92121, USA.

**Abstract not available.**


Division of Pathway Medicine, Centre for Infectious Diseases, School of Biomedical Sciences, College of Medicine and Veterinary Medicine, The University of Edinburgh, Edinburgh, UK. [anna.okello@ed.ac.uk].

Rapid changes in human behaviour, resource utilization, and other extrinsic environmental factors continue to threaten the current distribution of several endemic and historically neglected zoonoses in many developing regions worldwide. There are numerous examples of zoonotic diseases which have circulated within relatively localized geographical areas for some time, before emerging into new regions as a result of changing human, environmental, or behavioural dynamics. While the world's focus is currently on the Ebola virus gaining momentum in western Africa, another pertinent example of this phenomenon is zoonotic human African trypanosomiasis (HAT), endemic to southern and eastern Africa, and spread via infected cattle. In recent years, the ongoing northwards spread of this disease in the country has posed a serious public health threat to the human population of Uganda, increasing the pressure on both individual families and government services to control the disease. Moreover, the emergence of HAT into new areas of Uganda in recent years exemplifies the important role of veterinary policy in mitigating the severe human health and economic impacts of zoonotic disease. The systemic challenges surrounding the development and enforcement of veterinary policy described here are similar across sub-Saharan Africa, highlighting the necessity to consider and support zoonotic disease control in broader human
and animal health systems strengthening and associated development programmes on the continent.


Division of Biological Chemistry and Drug Discovery, Sir James Black Centre, College of Life Sciences, University of Dundee, Dundee, UK. [s.z.patterson@dundee.ac.uk].

There is an urgent need for new, safer, and effective treatments for the diseases caused by the protozoan parasites *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania* spp. In the search for more effective drugs to treat these “neglected diseases” researchers have chosen to reassess the therapeutic value of nitroaromatic compounds. Previously avoided in drug discovery programmes owing to potential toxicity issues, a nitro drug is now being used successfully as part of a combination therapy for human African trypanosomiasis. We describe here the rehabilitation of nitro drugs for the treatment of trypanosomatid diseases and discuss the future prospects for this compound class.


King’s College London, Institute of Pharmaceutical Sciences, London, UK. [sarah.thomas@kcl.ac.uk].

Human African trypanosomiasis (HAT or sleeping sickness) is a potentially fatal disease caused by the parasite, *Trypanosoma brucei* sp. The parasites are transmitted by the bite of insect vectors belonging to the genus *Glossina* (tsetse flies) and display a life cycle strategy that is equally spread between human and insect hosts. *T. b. gambiense* is found in western and central Africa whereas, *T. b. rhodesiense* is found in eastern and southern Africa. The disease has two clinical stages: a blood stage after the bite of an infected tsetse fly, followed by a central nervous system (CNS) stage where the parasite penetrates the brain; causing death if left untreated. The blood-brain barrier (BBB) makes the CNS stage difficult to treat because it prevents 98 percent of all known compounds from entering the brain, including some anti-HAT drugs. Those that do enter the brain are toxic compounds in their own right and have serious side effects. There are only a few drugs available to treat HAT and those that do are stage specific. This review summarizes the incidence, diagnosis, and treatment of HAT and provides a close examination of the BBB transport of anti-HAT drugs and an overview of the latest drugs in development.


Faculdade de Ciências Farmacêuticas, Departamento de Análises Clínicas, Universidade Estadual Paulista “Julio de Mesquita Filho”, Araraquara, SP, Brazil. [graminha@fcfar.unesp.br].
The use of indigenous or remote popular knowledge to identify new drugs against diseases or infections is a well-known approach in medicine. The inhabitants of coastal regions are known to prepare algae extracts for the treatment of disorders and ailments such as wounds, fever and stomach aches, as for the prevention of arrhythmia. Recent trends in drug research from natural sources have indicated that marine algae are promising sources of novel biochemically active compounds, especially with antiprotozoal activity. Algae survive in a competitive environment and, therefore, developed defence strategies that have resulted in a significant level of chemical structural diversity in various metabolic pathways. The exploration of these organisms for pharmaceutical and medical purposes has provided important chemical candidates for the discovery of new agents against neglected tropical diseases, stimulating the use of sophisticated physical techniques. This review describes the main substances biosynthesized by benthic marine algae with activity against *Leishmania* spp., *Trypanosoma cruzi* and *Trypanosoma brucei*; the causative agents of leishmaniasis, Chagas disease and African trypanosomiasis, respectively. Emphasis is given to secondary metabolites and crude extracts prepared from marine algae.


The Glasgow Polyomics Facility and Wellcome Trust Centre for Molecular Parasitology, University of Glasgow, Glasgow, UK. [Isabel.vincent@glasgow.ac.uk].

Metabolomics-based studies are proving of great utility in the analysis of modes of action (MOAs) and resistance mechanisms of drugs in parasitic protozoa. They have helped to determine the MOA of eflornithine, half of the gold standard combination therapy in use against human African trypanosomiasis (HAT), as well as the mechanism of resistance to this drug. In *Leishmania*, metabolomics has also given insight into the MOA of miltefosine, an alkylphospholipid. Several studies on antimony resistance in *Leishmania* have been conducted, analysing the metabolic content of resistant lines, and offering clues as to the MOA of this class of drugs. A study of chloroquine resistance in *Plasmodium falciparum* combined metabolomics techniques with other genetic and proteomic techniques to offer new insight into the role of the PfCRT protein. The MOA and mechanism of resistance to a group of halogenated pyrimidines in *Trypanosoma brucei* have also recently been elucidated. Effective as metabolomics techniques are, care must be taken in the design and implementation of these experiments, to ensure the resulting data are meaningful. This review outlines the steps required to conduct a metabolomics experiment and provides an overview of metabolomics-based drug research in protozoa to date.


School of Biological and Biomedical Sciences, Durham University, Stockton Road, Durham, UK; National Institute of Malaria Research (ICMR), New Delhi, India; Department of Environmental Sciences, Emory University, Atlanta, Georgia, USA; Fogarty International Center, National Institutes of Health, Bethesda, Maryland, USA; Department of Entomology and Nematology, University of California, Davis, California, USA; Laboratory of Entomology, Wageningen University, Wageningen, Netherlands. [s.w.lindsay@durham.ac.uk].
Tsetse and Trypanosomosis Information

Insecticide-treated nets (ITNs) are one of the main interventions used for malaria control. However, these nets may also be effective against other vector-borne diseases (VBDs). We conducted a systematic review and meta-analysis to estimate the efficacy of ITNs, insecticide-treated curtains (ITCs) and insecticide-treated house screening (ITS) against Chagas disease, cutaneous and visceral leishmaniasis, dengue, human African trypanosomiasis, Japanese encephalitis, lymphatic filariasis and onchocerciasis. MEDLINE, EMBASE, LILACS and Tropical Disease Bulletin databases were searched using intervention, vector- and disease-specific search terms. Cluster or individually randomized controlled trials, non-randomized trials with pre- and post-intervention data and rotational design studies were included. Analysis assessed the efficacy of ITNs, ITCs or ITS versus no intervention. Meta-analysis of clinical data was performed and percentage reduction in vector density calculated. Twenty-one studies were identified which met the inclusion criteria. Meta-analysis of clinical data could only be performed for four cutaneous leishmaniasis studies which together showed a protective efficacy of ITNs of 77 percent (95 percent CI: 39 percent-91 percent). Studies of ITC and ITS against cutaneous leishmaniasis also reported significant reductions in disease incidence. Single studies reported a high protective efficacy of ITS against dengue and ITNs against Japanese encephalitis. No studies of Chagas disease, human African trypanosomiasis or onchocerciasis were identified. It is concluded that there are likely to be considerable collateral benefits of ITN roll out on cutaneous leishmaniasis where this disease is co-endemic with malaria. Due to the low number of studies identified, issues with reporting of entomological outcomes, and few studies reporting clinical outcomes, it is difficult to make strong conclusions on the effect of ITNs, ITCs or ITS on other VBDs and therefore further studies should be conducted. Nonetheless, it is clear that insecticide-treated materials such as ITNs have the potential to reduce pathogen transmission and morbidity from VBDs where vectors enter houses.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES


Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, A-1400 Vienna, Austria. [gratian.mutika@gmail.com].

Procurement of sterile tsetse flies (Glossina palpalis gambiensis) from Burkina Faso for an eradication programme in Senegal that incorporates the sterile insect technique (SIT) required the development of transport and handling protocols that would allow retaining the female flies in the rearing facility and transporting of the male flies as irradiated pupae. The proposed handling scheme included the chilling of the male pupae after the female emergence and transport to Senegal under low temperatures. The effect of exposing male pupae of G. p. gambiensis to low temperature immediately prior to emergence was investigated. The parameters of interest were emergence rate, insemination potential, survival of adult males, male ability to participate in mating activities and productivity of females mated with these males. Production was assessed in laboratory rearing cages and mating behaviour in field cages. Male flies that emerged after the female emergence flush from
pupae stored at 10 °C or 12.5 °C for five or seven days were used in the investigations, with flies that emerged under standard colony conditions as control. Males that were three, six or nine days old competed for mating opportunities with three day-old females. The emergence of males after storage of pupae at low temperature (10 °C and 12.5 °C) for three, five, or seven days was similar to those kept under standard colony conditions while emergence of flies stored at 15 °C started before the storage period was over. Survival of males that emerged from pupae stored at low temperature for varying periods was more than 60 percent at 30 days post emergence (control more than 75 percent). The fecundity of females inseminated by males that emerged from pupae stored at low temperature for varying periods ranged from 0.33 +/- 0.16 to 0.73 +/- 0.04 pupae per female per 10 days (control 0.60 +/- 0.16). The older males, irrespective of treatment, out-competed the younger males and three day-old males transferred lower amounts of seminal contents to the females. Storage of male pupae at low temperature for periods up to seven days at the end of the male pupal period could not be directly associated with impairment of mating activity.


Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Wagamier Strasse 5, P.O. Box 100, A-1400 Vienna, Austria. [gratian.mutika@gmail.com].

Investigations into the possibility of using the chilled adult release system are continuing as an alternative method to the release of sterile tsetse flies, Glossina pallidipes Austen (Diptera: Glossinidae) in cardboard boxes. Exposing tsetse flies to 4 °C for six h caused negligible mortality. A combination of chilling and irradiation resulted in reduced quantities of seminal contents being transferred to females. Mortality of flies after bulk irradiation was lower when a thermos flask was used than using expanded polystyrene. Mortality after removal from cold storage increased with age. Flies that did not have a blood meal for three days prior to exposure to cold had a lower overnight survival than flies that were deprived of a blood meal for one or two days. Exposure of adult male tsetse flies to low temperature should be for as short a duration as is practical, so that the fitness of the released sterile flies is not unduly compromised. It is also necessary to ensure that losses are minimized during bulk irradiation of adult flies. It would be desirable to have minimal losses after the combined effects of irradiation, cold and transportation, such that a sufficient number of sterile male flies will still be available to successfully compete for mating opportunities with wild females.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

[See also 37: 17252]


Department of Biological Sciences, McMicken School of Arts and Sciences, University of Cincinnati, Cincinnati, OH 45221, USA; Department of Epidemiology
Tsetse flies (Glossina spp.), vectors of African trypanosomes, are distinguished by their specialized reproductive biology, defined by adenotrophic viviparity (maternal nourishment of progeny by glandular secretions and live birth). This trait has evolved infrequently among insects and requires unique reproductive mechanisms. A key event in Glossina reproduction involves the transition between periods of lactation and non-lactation (dry periods). Increased lipolysis, nutrient transfer to the milk gland, and milk-specific protein production characterize lactation, which terminates at the birth of the progeny and is followed by a period of involution. The dry stage coincides with embryogenesis of the progeny, during which lipid reserves accumulate in preparation for the next round of lactation. The obligate bacterial symbiont Wigglesworthia glossinidia is critical to tsetse reproduction and likely provides the B vitamins required for metabolic processes underlying lactation and/or progeny development. Here we describe findings that utilized transcriptomics, physiological assays, and RNA interference-based functional analysis to understand different components of adenotrophic viviparity in tsetse flies.


Laboratoire MIVEGEC, UMR 5290-224 CNRS-IRD-UM1-UM2, Centre de Recherche IRD, 34090, Montpellier, France. [olivier.duron@ird.fr].

The gamma-proteobacterium Arsenophonus and its close relatives (Arsenophonus and like organisms, ALOs) are emerging as a novel clade of endosymbionts, which are exceptionally widespread in insects. The biology of ALOs is, however, in most cases entirely unknown, and it is unclear how these endosymbionts spread across insect populations. Here, we investigate this aspect through the examination of the presence, the diversity and the evolutionary history of ALOs in 25 related species of blood-feeding flies: tsetse flies (Glossinidae), louse flies (Hippoboscidae) and bat flies (Nycteribiidae and Streblidae). While these endosymbionts were not found in tsetse flies, we identify louse flies and bat flies as harbouring the highest diversity of ALO strains reported to date, including a novel ALO clade, as well as Arsenophonus and the recently described Candidatus Aschnera chinzeii. We further show that the origin of ALO endosymbioses extends deep into the evolutionary past of louse flies and bat flies, and that it probably played a major role in the ecological specialization of their hosts. The evolutionary history of ALOs is notably complex and was shaped by both vertical transmission and horizontal transfers with frequent host turnover and apparent symbiont replacement in host lineages. In particular, ALOs have evolved repeatedly and independently close relationships with diverse groups of louse flies and bat flies, as well as phylogenetically more distant insect families, suggesting that ALO endosymbioses are exceptionally dynamic systems.


Institute of Cell Biology, University of Bern, Bern, Switzerland; Graduate School of
Tsetse and Trypanosomosis Information

The protozoan pathogen *Trypanosoma brucei* is transmitted between mammals by tsetse flies. The first compartment colonized by trypanosomes after a blood meal is the fly midgut lumen. Trypanosomes present in the lumen - designated as early procyclic forms - express the stage-specific surface glycoproteins EP and GPEET procyclin. When the trypanosomes establish a mature infection and colonize the ectoperitrophic space, GPEET is down-regulated, and EP becomes the major surface protein of late procyclic forms. A few years ago, it was discovered that procyclic form trypanosomes exhibit social motility (SoMo) when inoculated on a semi-solid surface. We demonstrate that SoMo is a feature of early procyclic forms, and that late procyclic forms are invariably SoMo-negative. In addition, we show that, apart from GPEET, other markers are differentially expressed in these two life-cycle stages, both in culture and in tsetse flies, indicating that they have different biological properties and should be considered distinct stages of the life cycle. Differentially expressed genes include two closely related adenylate cyclases, both hexokinases and calflagins. These findings link the phenomenon of SoMo *in vitro* to the parasite forms found during the first 4-7 d of a midgut infection. We postulate that ordered group movement on plates reflects the migration of parasites from the midgut lumen into the ectoperitrophic space within the tsetse fly. Moreover, the process can be uncoupled from colonization of the salivary glands. Although they are the major surface proteins of procyclic forms, EP and GPEET are not essential for SoMo, nor, as shown previously, are they required for near normal colonization of the fly midgut.


Studies on the impact of reproductive processes upon female health have yielded conflicting results, particularly in relation to the role of reproduction-associated stress. We used the viviparous tsetse fly to determine if lactation, birth and involution lead to damage from oxidative stress (OS) that impairs subsequent reproductive cycles. Tsetse females carry an intrauterine larva to full term at each pregnancy cycle, and lactate to nourish them with milk secretions produced by the accessory gland (= milk gland) organ. Unlike most K-strategists, tsetse females lack an apparent period of reproductive senescence allowing the production of 8-10 progeny over their entire life span. In a lactating female, over 47 percent of the maternal transcriptome is associated with the generation of milk proteins. The resulting single larval offspring weighs as much as the mother at birth. In studying this process we noted an increase in specific antioxidant enzyme (AOE) transcripts and enzymatic activity at critical times during lactation, birth and involution in the milk gland/fat body organ and the uterus. Suppression of superoxide dismutase (*sod*) decreased fecundity in subsequent reproductive cycles in young mothers and nearly abolished fecundity in geriatric females. Loss of fecundity was in part due to the inability of the mother to produce adequate milk to support larval growth. Longevity was also impaired after *sod* knockdown. Generation of OS
in virgin females through exogenous treatment with hydrogen peroxide at times corresponding to pregnancy intervals reduced survival, which was exacerbated by sod knockdown. AOE expression may prevent oxidative damage associated with the generation of nutrients by the milk gland, parturition and milk gland breakdown. Our results indicate that prevention of OS is essential for females to meet the growing nutritional demands of juveniles during pregnancy and to repair the damage that occurs at birth. This process is particularly important for females to remain fecund during the latter portion of their lifetime.


Vitamin B<sub>6</sub> generated by obligate symbionts is critical for maintaining proline homeostasis and fecundity in tsetse flies. Applied Environmental Microbiology, 80 (18): 5844-5853.

The viviparous tsetse fly utilizes proline as a haemolymph-borne energy source. In tsetse, biosynthesis of proline from alanine involves the enzyme alanine-glyoxylate aminotransferase (AGAT), which requires pyridoxal phosphate (vitamin B<sub>6</sub>) as a cofactor. This vitamin can be synthesized by tsetse's obligate symbiont, *Wigglesworthia glossinidia*. In this study, we examined the role of *Wigglesworthia*-produced vitamin B<sub>6</sub> for maintenance of proline homeostasis, specifically during the energetically expensive lactation period of the tsetse's reproductive cycle. We found that expression of *agat*, as well as genes involved in vitamin B<sub>6</sub> metabolism in both host and symbiont, increases in lactating flies. Removal of symbionts via antibiotic treatment of flies (aposymbiotic) led to hypoprolinaemia, reduced levels of vitamin B<sub>6</sub> in lactating females, and decreased fecundity. Proline homeostasis and fecundity recovered partially when aposymbiotic tsetse flies were fed a diet supplemented with either yeast or *Wigglesworthia* extracts. RNA interference-mediated knockdown of *agat* in wild-type flies reduced haemolymph proline levels to that of aposymbiotic females. Aposymbiotic flies treated with *agat* short interfering RNA (siRNA) remained hypoprolinaemic even upon dietary supplementation with microbial extracts or B vitamin. Flies infected with parasitic African trypanosomes display lower haemolymph proline levels, suggesting that the reduced fecundity observed in parasitized flies could result from parasite interference with proline homeostasis. This interference could be manifested by competition between tsetse and trypanosomes for vitamins, proline, or other factors involved in their synthesis. Collectively, these results indicate that the presence of *Wigglesworthia* in tsetse is critical for the maintenance of proline homeostasis through vitamin B<sub>6</sub> production.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES


Division of Pathway Medicine, School of Biomedical Sciences, College of Medicine and Veterinary Medicine, The University of Edinburgh, Chancellor's Building, 49 Little France Crescent, Edinburgh EH16 4SB, UK. [Ewan.MacLeod@ed.ac.uk].

31
Tsetse flies are the biological vectors of African trypanosomes, the causative agents of sleeping sickness in humans and nagana in animals. The tsetse endosymbiont *Sodalis glossinidius* has been suggested to play a role in tsetse susceptibility to infection. Here we investigate the prevalence of African trypanosomes within tsetse from the Luambe National Park, Zambia and if there is an association between *S. glossinidius* and presence of trypanosomes within the tsetse examined. Tsetse representing three species (*Glossina brevipalpis*, *Glossina morsitans morsitans* and *Glossina pallidipes*) were sampled from Luambe National Park, Zambia. Following DNA extraction, PCR was used to examine the tsetse for presence of trypanosomes and the secondary endosymbiont *S. glossinidius*. *S. glossinidius* infection rates varied significantly between tsetse species, with *G. brevipalpis* (93.7 percent) showing the highest levels of infection followed by *G. m. morsitans* (17.5 percent) and *G. pallidipes* (1.4 percent). ITS-PCR detected a wide variety of trypanosomes within the tsetse that were analysed. Significant differences were found in terms of trypanosome presence between the three tsetse species. A high proportion of *G. m. morsitans* was shown to carry *T. brucei* s.l. DNA (73.7 percent) and of these, around 50 percent were positive for *Trypanosoma brucei rhodesiense*. *T. vivax*, *T. godfreyi*, *T. simiae*, *T. simiae* Tsavo and *T. congolense* were also detected. No association was found between the occurrence of *S. glossinidius* and the presence of trypanosome DNA in any of the three tsetse species tested. The current work shows that *T. b. rhodesiense* was circulating in Luambe National Park, representing a risk for people living in the park or surrounding area and for tourists visiting the park. The differences in trypanosome DNA presence observed between the different tsetse species tested may indicate host feeding preferences, as the PCR will not discriminate between a fly with an active/resident infection compared with a refractory fly that has fed on an infected animal. This makes it difficult to establish if *S. glossinidius* may play a role in the susceptibility of tsetse flies to trypanosome infection.


UMR 177, Institut de Recherche pour le Développement-CIRAD Montpellier, France; Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon. [anne.geiger@ird.fr].

Tsetse flies (*Glossina* sp.) that transmit trypanosomes causing human (and animal) African trypanosomiasis (HAT and AAT, respectively) harbour symbiotic microorganisms, including the obligate primary symbiont *Wigglesworthia glossinidia*. A relationship between *Wigglesworthia* and tsetse fly infection by trypanosomes has been suggested, as removal of the symbiont results in a higher susceptibility to midgut infection in adult flies. To investigate this relationship and to decipher the role of *W. glossinidia* in the fly's susceptibility to trypanosome infection, we challenged flies with trypanosomes and subsequently analysed and compared the transcriptomes of *W. glossinidia* from susceptible and refractory tsetse flies at three time points (three, 10, and 20 d). More than 200 *W. glossinidia* genes were found to be differentially expressed between susceptible and refractory flies. The high specificity of these differentially expressed genes makes it possible to distinguish *Wigglesworthia* inhabiting these two distinct groups of flies. Furthermore, gene expression patterns were observed to evolve during the infection time course, such that very few differentially expressed genes were found in common in *Wigglesworthia* from the three-, 10- and 20-day post-feeding fly samples. The overall results clearly demonstrate that the taking up of
trypanosomes by flies, regardless of whether flies proceed with the developmental programme of *Trypanosoma brucei gambiense*, strongly alters gene expression in *Wigglesworthia*. These results therefore provide a novel framework for studies that aim to decrease or even abolish tsetse fly vector competence.


Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis, California, 95616, USA; Department of Entomology, University of Arizona, Tucson, Arizona 85721, USA. [sluckhart@ucdavis.edu].

During the process of blood feeding, insect vectors are exposed to an array of vertebrate-derived blood factors ranging from by-products of blood meal digestion to naturally occurring products in the blood including growth hormones, cytokines and factors derived from blood-borne pathogens themselves. In this review, we examine the ability of these ingested vertebrate blood factors to alter the innate pathogen defences of insect vectors. The ability of these factors to modify the immune responses of insect vectors offers new intriguing targets for blocking or reducing transmission of human disease-causing pathogens.


KARI - Trypanosomiasis Research Institute, P.O. Box 362, 00902 Kikuyu, Kenya; Department of Biochemistry & Molecular Biology, Egerton University, P.O. Box 536, 20115 Njoro, Kenya. [f.wamwiri@gmail.com].

The establishment of infection with three *Trypanosoma* spp. (Gruby) (Kinetoplastida: *Trypanosomatidae*), specifically *Trypanosoma brucei brucei* (Plimmer and Bradford), *T. b. rhodesiense* (Stephen and Fatham) and *T. congolense* (Broden) was evaluated in *Glossina pallidipes* (Austen) (Diptera: *Glossinidae*) that either harboured or were uninfected by the endosymbiont *Sodalis glossinidius* (Dale and Maudlin) (Enterobacteriales: *Enterobacteriaceae*). Temporal variation of co-infection with *T. b. rhodesiense* and *S. glossinidius* was also assessed. The results show that both *S. glossinidius* infection (chi² = 1.134, df = 2, p = 0.567) and trypanosome infection rate (chi² = 1.85, df = 2, p = 0.397) were comparable across the three infection groups. A significant association was observed between the presence of *S. glossinidius* and concurrent trypanosome infection with *T. b. rhodesiense* (p = 0.0009) and *T. congolense* (p = 0.0074) but not with *T. b. brucei* (p = 0.5491). The time-series experiment revealed a slight decrease in the incidence of *S. glossinidius* infection with increasing fly age, which may infer a fitness cost associated with *Sodalis* infection. The present findings contribute to research on the feasibility of *S. glossinidius*-based paratransgenic approaches in tsetse and trypanosomiasis control, in particular relating to *G. pallidipes* control.
3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also 37: 17255, 17265, 17276, 17290]


Unité Mixte de Recherche, Contrôle des Maladies Animales Exotiques et Émergentes, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France; Unité Mixte de Recherche, Contrôle des Maladies Animales Exotiques et Émergentes, Institut National de la Recherche Agronomique (INRA), Montpellier, France; Institut Sénégalais de Recherches Agricoles, Laboratoire National d’Elevage et de Recherches Vétérinaires, Dakar, Sénégal; Direction des Services Vétérinaires, Dakar, Sénégal; Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria. [bouyer@cirad.fr].

In 2005, the Government of Senegal embarked on a campaign to eliminate a Glossina palpalis gambiensis population from the Niayes area (approximately 1 000 km²) under the umbrella of the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC). The project was considered an ecologically sound approach to intensify cattle production. The elimination strategy includes a suppression phase using insecticide impregnated targets and cattle, and an elimination phase using the sterile insect technique, necessary to eliminate tsetse in this area. Three main cattle farming systems were identified: a traditional system using trypanotolerant cattle and two “improved” systems using more productive cattle breeds focusing on milk and meat production. In improved farming systems herd size was 45 percent lower and annual cattle sales were Euro 250 (s.d. 513) per head as compared with Euro 74 (s.d. 38) per head in traditional farming systems (p < 10^-3). Tsetse distribution significantly impacted the occurrence of these farming systems (p = 0.001), with 34 percent (s.d. 4 percent) and 6 percent (s.d. 4 percent) of improved systems in the tsetse-free and tsetse-infested areas, respectively. We calculated the potential increases in cattle sales as a result of tsetse elimination considering two scenarios, i.e. a conservative scenario with a 2 percent annual replacement rate from traditional to improved systems after elimination, and a more realistic scenario with an increased replacement rate of 10 percent five years after elimination. The final annual increase in cattle sales was estimated at approximately Euro 2 800/km² for a total cost of the elimination campaign reaching approximately Euro 6 400/km². Despite its high cost, the benefit-cost analysis indicated that the project was highly cost-effective, with internal rates of return (IRR) of 9.8 percent and 19.1 percent and payback periods of 18 and 13 years for the two scenarios, respectively. In addition to an increase in farmers' income, the benefits of tsetse elimination include a reduction of grazing pressure on the ecosystems.

Beyond insecticides, alternative methods to control insect pests for agriculture and vectors of diseases are needed. Management strategies involving the mass release of living control agents have been developed, including genetic control with sterile insects and biological control with parasitoids, for which aerial release of insects is often required. Aerial release in genetic control programmes often involves the use of chilled sterile insects, which can improve dispersal, survival and competitiveness of sterile males. Currently available means of aerially releasing chilled fruit flies are, however, insufficiently precise to ensure homogeneous distribution at low release rates and no device is available for tsetse. Here we present the smart aerial release machine, a new design based on the use of vibrating conveyors. The machine is controlled through Bluetooth by a tablet with an android operating system including a completely automatic guidance and navigation system (MaxNav software). The tablet is also connected to an online relational database facilitating the preparation of flight schedules and automatic storage of flight reports. The new machine was compared with a conveyor release machine in Mexico using two fruit flies species (Anastrepha ludens and Ceratitis capitata) and we obtained better dispersal homogeneity (percent of positive traps, p < 0.001) for both species and better recapture rates for Anastrepha ludens (p < 0.001), especially at low release densities (< 1 500/ha). We also demonstrated that the machine can replace paper boxes for aerial release of tsetse in Senegal. This technology limits damages to insects and allows a large range of release rates from 10 flies/km² for tsetse flies up to 600 000 flies/km² for fruit flies. The potential of this machine to release other species like mosquitoes is discussed. Plans and operating of the machine are provided to allow its use worldwide.


Department of Biomolecular and Biolaboratory Sciences, College of Veterinary Medicine Animal Resources and Biosecurity, Makerere University, Kampala, Uganda; Division of Pathway Medicine, Centre for Infectious Diseases, School of Biomedical Sciences, College of Medicine and Veterinary Medicine, The University of Edinburgh, Edinburgh, UK; Department of Public Health and Epidemiology, Swiss Tropical Institute, Basel, Switzerland; University of Basel, Basel, Switzerland; Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, UK. [luckydenno@covab.mak.ac.ug].

African trypanosomes constrain livestock and human health in sub-Saharan Africa, and
aggravate poverty and hunger of these otherwise largely livestock-keeping communities. To solve this, there is need to develop and use effective and cheap tsetse control methods. To this end, we aimed at determining the smallest proportion of a cattle herd that needs to be sprayed on the legs, bellies and ears (restricted application, RAP) for effective human and animal African trypanosomiasis (HAT/AAT) control. Cattle in 20 villages were ear-tagged and injected with two doses of diminazene diaceturate (DA) 40 d apart, and randomly allocated to one of five treatment regimens namely: no treatment, 25 percent, 50 percent, 75 percent monthly RAP and every three months with an Albendazole drench. Cattle trypanosome re-infection rate was determined by molecular techniques. ArcMap V10.3 was used to map apparent tsetse density (FTD) from trap catches. The effect of graded RAP on incidence risk ratios and trypanosome prevalence was determined using Poisson and logistic random effect models in R and STATA V12.1 respectively. Incidence was estimated at 9.8/100 y in RAP regimens, significantly lower compared with 25.7/100 y in the non-RAP regimens (incidence rate ratio: 0.37; 95 percent CI: 0.22-0.65; p < 0.001). Likewise, trypanosome prevalence after one year of follow up was significantly lower in RAP animals than in non-RAP animals (4 percent vs 15 percent, OR: 0.20, 95 percent CI: 0.08-0.44; p < 0.001). Contrary to our expectation, level of protection did not increase with increasing proportion of animals treated. Reduction in RAP coverage did not significantly affect efficacy of treatment. This is envisaged to improve RAP adaptability to low income livestock keepers but needs further evaluation in different tsetse challenge, HAT/AAT transmission rates and management systems before adopting it for routine tsetse control programmes.

herein with regard to endemic stability development to different TBDs. We found only a slight effect of RAP on *T. parva* infection. Since sample size determination was based on trypanosomes incidence, the study was underpowered given the low *T. parva* prevalence. While the findings need to be confirmed in future studies, the observed slight reduction in the risk of infection with *T. parva* might not compromise endemic stability.


Tsecon Consultants, Nairobi, Kenya; Department of Agriculture, Environmental and Food Sciences, University of Molise, Campobasso, Italy; College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum North, Sudan; Institute of Biology, University of Neuchatel, Neuchatel, Switzerland; Independent Scientist, Russell, Ontario, Canada. [Patrick.Guerin@unine.ch].

Riverine species of tsetse are responsible for most human African trypanosomiasis (HAT) transmission and are also important vectors of animal trypanosomiasis. This study concerns the development of visual control devices for two such species, *Glossina fuscipes fuscipes* and *Glossina tachinoides*, at the eastern limits of their continental range. The goal was to determine the most long-lasting, practical and cost-effective visually attractive device that induces the strongest landing responses in these species for use as insecticide-impregnated tools in vector population suppression. Field trials were conducted in different seasons on *G. f. fuscipes* in Kenya, Ethiopia and the Sudan and on *G. tachinoides* in Ethiopia to measure the performance of traps and 2D targets of different sizes and colours, with and without chemical baits, at different population densities and under different environmental conditions. Adhesive film was used to enumerate flies at these remote locations to compare trapping efficiencies. The findings show that targets made from black and blue fabrics (either phthalogen or turquoise) covered with adhesive film render them equal to or more efficient than traps at capturing *G. f. fuscipes* and *G. tachinoides*. Biconical trap efficiency varied between 25 percent and 33 percent for the two species. Smaller 0.25 m x 0.25 m phthalogen blue-black targets proved more efficient than the regular 1 m² target for both species, by over six times for *Glossina f. fuscipes* and two times for *G. tachinoides* based on catches per m². Overall, targets with a higher edge/surface area ratio were more efficient at capturing flies. Taking into account practical considerations and fly preferences for edges and colours, we propose a 0.5 x 0.75 m blue-black target as a simple cost-effective device for management of *G. f. fuscipes* and *G. tachinoides*, impregnated with insecticide for control and covered with adhesive film for population sampling.

17282. **Santer, R. D., 2014.** A colour opponent model that explains tsetse fly attraction to visual baits and can be used to investigate more efficacious bait materials. *PLoS Neglected Tropical Diseases*, 8 (12): e3360.

Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, Ceredigion, UK. [rds5@aber.ac.uk].

*Palpalis* group tsetse flies are the major vectors of human African trypanosomiasis, and visually-attractive targets and traps are important tools for their control. Considerable efforts are underway to optimize these visual baits, and one factor that has been investigated is
coloration. Analyses of the link between visual bait coloration and tsetse fly catches have used methods which poorly replicate sensory processing in the fly visual system, but doing so would allow the visual information driving tsetse attraction to these baits to be more fully understood, and the reflectance spectra of candidate visual baits to be more completely analysed. Following methods well established for other species, the numbers of tsetse flies caught at visual baits were reanalysed based upon the calculated photoreceptor excitations elicited by those baits. This was done for large sets of previously published data for Glossina fuscipes fuscipes (Lindh et al. (2012), G. palpalis palpalis (Green (1988), and G. pallidipes (Green and Flint (1986). Tsetse attraction to visual baits in these studies can be explained by a colour opponent mechanism to which the UV-blue photoreceptor R7y contributes positively, and both the green-yellow photoreceptor R8y, and the low-wavelength UV photoreceptor R7p, contribute negatively. A tool for calculating fly photoreceptor excitations is made available with this paper, and this will facilitate a complete and biologically authentic description of visual bait reflectance spectra that can be employed in the search for more efficacious visual baits, or the analysis of future studies of tsetse fly attraction.


Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria; Ministry of Agriculture, Natural Resources and Environment, Zanzibar, Tanzania; Vector and Vector Borne Diseases Research Institute, Tanga, Tanzania; Livestock Research, Training & Extension, Ministry of Livestock & Fisheries Development, Dar es Salaam, Tanzania; Unité Mixte de Recherche Contrôle des Maladies Animalles Exotiques et Émergentes, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France; Unité Mixte de Recherche Contrôle des Maladies Animalles Exotiques et Émergentes, Institut National de la Recherche Agronomique (INRA), Montpellier, France; Institut Sénégalais de Recherches Agricoles, Laboratoire National d'Elevage et de Recherches Vétérinaires, Dakar-Hann, Sénégal.

From mid-1994 to mid-1996, sterile male flies were dispersed by air over the entire southern part of the island, while, in the northern half of the island, the fly population continued to be suppressed using pour-on applications of deltamethrin on livestock and cattle dipping in strategic areas. The release area was expanded to include the middle and northern parts of the island in mid-1996, and 12 small offshore islands were included from January 1997 onwards. The sterile flies for the operational release phase of the project were produced at the TTRI in Tanga, Tanzania. Whereas in 1994 the G. austeni colony remained below 50 000 producing females, improvements in rearing methods and use of better quality blood for feeding the flies allowed colony expansion to 400 000 and to 900 000 by the end of 1995 and 1996, respectively. The sterile flies were released twice a week by light aircraft at an altitude of 210-270 m over fixed release paths that were separated by 1-2 km. The pilots used a global positioning system (GPS) for accurate navigation and sterile fly dispersal. The sterile flies were marked with Day Glo fluorescent powder that enabled discrimination from the wild fly population in the trap catches. The entire operational eradication phase was monitored with more than 500 sticky panel traps that were checked from once a day to once a week
depending on the importance of the area. In addition, sentinel animals were being screened every 2-5 months for AAT transmission in 38 arbitrarily assigned blocks.

During the operational sterile male release phase (August 1994-December 1997), a total of 8.5 million sterile male *G. austeni* were released over the island. Different release densities were applied over different ecosystems in relation to the suitability of that habitat to harbour *G. austeni*, i.e. from < 50 sterile males/km² over unsuitable habitats to > 300 sterile males/km² over the primary forest. During the first months of the releases, the average sterile-to-wild-male ratios remained below 10:1. As of week 13 of 1995, more than 25 000 sterile males were released per week and as a result, the average sterile-to-wild-male ratios increased gradually and reached > 100:1 by the end of 1995. The mating frequencies of the released males as evidenced by the rate of induced sterility in the trapped wild female flies were used as a direct indicator of the efficiency of the sterile male release programme. Whereas the natural abortion rate before the release of the sterile males averaged 3.5 percent, the mating frequencies of wild female flies with sterile males (i.e. the rate of abortion) increased from 19 percent in the first period of the releases to 32 percent, 48 percent, and 72 percent during the 2nd, 3rd, and 4th quarters of 1995, respectively. The adequate quality of the sterile males was epitomized by an analysis of the trap catches in the Jozani forest reserve that showed that sterile males, despite being released by air in a uniform way, aggregated in the same areas as the wild male flies, ensuring adequate sterile-to-wild male overflooding ratios. Another indicator of the success of the programme was the shift in age distribution of the female fly population, i.e. as the programme advanced, the proportion of young females in the population was gradually reduced due to the reduction in population replacement rate with each generation. As a result of the gradual increase in the rate of induced sterility in the female fly population, the fly population density as evidenced by trap catches decreased very rapidly as of mid-1995. The last indigenous fly was trapped in week 36 of 1996. The transmission rate of trypanosomes in sentinel cattle was used as an indirect indicator of the progress of the programme. The incidence of AAT in northern Unguja was already very low in 1994 (< 1 percent) due to the suppression of the fly population using the pour-on technique in previous years. Whereas *T. congolense* was still detected in 1994, it was not found any more in the period of 1995-1997. In southern Unguja, the incidence of AAT was higher, but *T. congolense* was not detected any more in 1996 and the incidence of *T. vivax* was 0.19 percent (i.e. one animal was positive out of the 520 sampled). After the releases were stopped, more than 3 000 cattle were screened for AAT in 1998-1999. None of the animals was found positive for AAT, and this has remained so ever since. AAT is occasionally reported in imported cattle from the mainland, but transmission to indigenous animals never occurs as the vector is absent.

Two economic surveys implemented two and five years after the completion of the eradication operations concluded that the proportion of small farmers (i) holding indigenous cattle increased from 31 percent to 94 percent between 1985 and 2002, (ii) selling milk from indigenous cattle increased from 11 percent to 62 percent between 1985 and 1999, and (iii) using oxen for ploughing increased to 5 percent in 2002 (but was expected to increase thereafter). There was high demand for improved livestock breeds, and the percentage of farmers holding improved cattle breeds increased from 2 percent to 24 percent in the period of 1985-2002. In addition, from 1985 to 1999, milk production nearly tripled. In general, the average monthly income of farming households increased by 30 percent in the period of 1999-2002, and the proportion of households with a monthly income of over US$ 25 and over US$ 50 increased from 69 percent to 86 percent and from 22 percent to 36 percent, respectively. This could be associated with tsetse and trypanosomosis eradication since a strong correlation was observed between household income and milk yields, milk sales, and the use of manure and animal power for cultivation and transport. There was a clear
acceleration in the innovation trajectories of farmers. The eradication of the tsetse fly from Unguja in 1997 followed by the disappearance of AAT thereafter enabled farmers to integrate livestock keeping with cropping in areas where this had been impossible before. The increased livestock and crop productivity and the use of animals for transport and traction significantly contributed to an increase in the quality of people's lives.

The successful and sustained eradication of the G. austeni population from Unguja Island, the subsequent disappearance of the AAT disease, and the positive impact of the programme on raising the socioeconomic standards of the human population on the island incited a renewed interest from African governments. This culminated in the establishment of the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) at the African Summit in 2000 under the auspices of the African Union Commission. This political initiative provided encouragement to tsetse-affected African states for increased and renewed efforts in their struggle against tsetse and trypanosomosis and to develop and implement similar campaigns in their infested territories. The success of the eradication programme on Unguja Island was possible due to the many technical and managerial prerequisites that were in place during the planning and implementation of the programme. Beyond the complete isolation of the target population, the programme on Unguja Island benefited from: an extensive planning phase based on a strong set of baseline data that allowed a good strategic choice of control tactics that are very effective at high and low population densities, such as the SIT; sterile flies of adequate quality available in adequate numbers and released throughout the campaign without interruption; routine quality control procedures in place; an extensive monitoring component with permanent feedback between field teams and managers, allowing the making of adequate strategic choices based on sound scientific principles rather than on process-oriented bureaucracies or political wishes; adequate national and international expertise; sufficient financial resources and adequate logistics; and regular independent programme reviews. Failing to meet these prerequisites will increase the probability of failure for area-wide integrated pest management (AW-IPM) programmes, and in those cases, it is wise to encourage local IPM on a field-by-field basis that combines vector control and trypanocidal treatments aiming at suppression rather than eradication.

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


Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia. [nsheena@um.edu.my].

A total of 719 wild rats were captured from four localities representing the west (Kuala Lumpur), east (Kuantan), north (Georgetown) and south (Malacca) to determine the diversity of blood protozoan from the urban wild rat population in peninsular Malaysia. Five rat species were recovered with *Rattus rattus diardi* being the most dominant species, followed by *Rattus norvegicus*, *Rattus exulans*, *Rattus annandalei* and *Rattus argentiventer*. Two blood protozoan species were found infecting the rodent population namely, *Plasmodium* sp. (42.1 percent) and *Trypanosoma lewisi* (25.0 percent). This study reports the presence of *Plasmodium* sp. for the first time in the rodent population in Malaysia. Two main intrinsic factors were identified affecting the parasitic infections. *Trypanosoma lewisi* infections were influenced by host age and sex with higher infection rates observed in male and juvenile rats.
while *Plasmodium* sp. infections were found to be almost similar in both sexes. However, infection rates were higher in younger rats.


Myeloid Cell Immunology Laboratory, VIB Brussels, Brussels, Belgium; Cellular and Molecular Immunology Unit, Vrije Universiteit Brussel (VUB), B-1050 Brussels, Belgium; Department of Biomedical Sciences, Veterinary Protozoology Unit, Institute of Tropical Medicine Antwerp, B-2000 Antwerp, Belgium; Department of Physiology, Laboratory of Zoophysiology, University of Ghent, B-9000 Ghent, Belgium; Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles (ULB), B-6041 Gosselies, Belgium; Walloon Excellence in Life Sciences and Biotechnology (WELBIO), Wallonia, Belgium. [jvdabbeele@itg.be].

The life cycle of African trypanosomes involves adaptations to the defence mechanisms of two completely different hosts, the insect vector *Glossina* and the mammalian host. This interplay ultimately determines host resistance and/or tolerance to parasite infection. In the tsetse fly, the immune deficiency (IMD)-regulated pathway, the scavenger receptor peptidoglycan-recognition protein LB (PGRP-LB), and the reactive oxygen species (ROS)-mediated response modulate the insect's capacity to transmit the parasite. In experimental mice, control of parasite burden and tissue pathogenicity relies on timely regulated interactions between myeloid cells exhibiting distinct activation states (M1 versus M2 type). Tsetse fly saliva and various trypanosome components including adenylate cyclases, DNA, a kinesin heavy chain, and variant surface glycoprotein (VSG) interfere with resistance and tolerance to infection.


Department of Biomedical Sciences, Unit of Veterinary Protozoology, Institute of Tropical Medicine Antwerp (ITM), Antwerp, Belgium; Unit of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium; Laboratory of Myeloid Cell Immunology, VIB, Brussels, Belgium; Department of Physiology, Laboratory of Zoophysiology, University of Ghent, B-9000 Ghent, Belgium. [ldevooght@itg.be].

*Sodalis glossinidius*, a vertically transmitted microbial symbiont of the tsetse fly, is currently considered as a potential delivery system for anti-trypanosomal components that reduce or eliminate the capability of the tsetse fly host to transmit parasitic trypanosomes, an approach also known as paratransgenesis. An essential step in developing paratransgenic tsetse is the stable colonization of adult flies and their progeny with recombinant *Sodalis* bacteria expressing trypanocidal effector molecules in tissues where the parasite resides. In this study, *Sodalis* was tested for its ability to deliver functional anti-trypanosome nanobodies (Nbs) in *Glossina morsitans morsitans*. We characterized the *in vitro* and *in vivo* stability of recombinant *Sodalis* (recSodalis) expressing a potent trypanolytic nanobody, i.e. Nb_An46.
We show that recSodalis is competitive with WT Sodalis under in vivo conditions and that tsetse flies transiently cleared of their endogenous WT Sodalis population can be successfully repopulated with recSodalis at high densities. In addition, vertical transmission to the offspring was observed. Finally, we demonstrated that recSodalis expressed significant levels (ng range) of functional Nb_An46 in different tsetse fly tissues, including the midgut where an important developmental stage of the trypanosome parasite occurs. In conclusion, we demonstrated the proof-of-concept that the Sodalis symbiont can be genetically engineered to express and release significant amounts of functional anti-trypanosome Nbs in different tissues of the tsetse fly. The application of this innovative concept of using pathogen-targeting nanobodies delivered by insect symbiotic bacteria could be extended to other vector-pathogen systems.


World Health Organization, Control of Neglected Tropical Diseases, Innovative and Intensified Disease Management, Geneva, Switzerland; World Health Organization, Inter Country Support Team for Central Africa, Regional Office for Africa, Libreville, Gabon. [francoj@who.int].

Human African trypanosomiasis (HAT), or sleeping sickness, is caused by Trypanosoma brucei gambiense, which is a chronic form of the disease present in western and central Africa, and by Trypanosoma brucei rhodesiense, which is an acute disease located in eastern and southern Africa. The rhodesiense form is a zoonosis, with the occasional infection of humans, but in the gambiense form, the human being is regarded as the main reservoir that plays a key role in the transmission cycle of the disease. The gambiense form currently assumes that 98 percent of the cases are declared; the Democratic Republic of the Congo is the most affected country, with more than 75 percent of the gambiense cases declared. The epidemiology of the disease is mediated by the interaction of the parasite (trypanosome) with the vectors (tsetse flies), as well as with the human and animal hosts within a particular environment. Resulting from these interactions, the disease is confined in spatially limited areas called "foci", which are located in sub-Saharan Africa, mainly in remote rural areas. The risk of contracting HAT is, therefore, determined by the possibility of contact of a human being with an infected tsetse fly. Epidemics of HAT were described at the beginning of the 20th century; intensive activities have been set up to confront the disease, and it was under control in the 1960s, with fewer than 5 000 cases reported in the whole continent. The disease resurfaced at the end of the 1990s, but renewed efforts from endemic countries, cooperation agencies, and nongovernmental organizations led by the World Health Organization succeeded in raising awareness and resources, while reinforcing national programmes, reversing the trend of the cases reported, and bringing the disease under control again. In this context, sustainable elimination of the gambiense HAT, defined as the interruption of the transmission of the disease, was considered as a feasible target for 2030. Since rhodesiense HAT is a zoonosis, where the animal reservoir plays a key role, the interruption of the disease's transmission is not deemed feasible.


UMR 177, IRD-CIRAD, CIRAD TA A-17/G, Campus International de Baillarguet,
Montpellier Cedex 5, France; School of Biological Sciences, The University of Sydney, Sydney, New South Wales, Australia; The Charles Perkins Centre, The University of Sydney, Sydney, New South Wales, Australia; Molecular Parasitology and Entomology Unit, Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon. [anne.geiger@ird.fr].

The tsetse fly vector transmits the protozoan Trypanosoma brucei, responsible for human African trypanosomiasis, one of the most neglected tropical diseases. Despite a recent decline in new cases, it is still crucial to develop alternative strategies to combat this disease. Here, we review the literature on the factors that influence trypanosome transmission from the fly vector to its vertebrate host (particularly humans). These factors include climate change effects to pathogen and vector development (in particular climate warming), as well as the distribution of host reservoirs. Finally, we present reports on the relationships between insect vector nutrition, immune function, microbiota and infection, to demonstrate how continuing research on the evolving ecology of these complex systems will help improve control strategies. In the future, such studies will be of increasing importance to understand how vector-borne diseases are spread in a changing world.


IRD-CIRAD, UMR 177 Montpellier, France; Inserm, U844, Hôpital Saint-Eloi, Montpellier, France. [anne.geiger@ird.fr].

Tsetse flies from the subspecies Glossina morsitans morsitans and Glossina palpalis gambiensis, respectively, transmit Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense. The former causes the acute form of sleeping sickness, and the latter provokes the chronic form. Although several articles have reported G. m. morsitans gene expression following trypanosome infection, no comparable investigation has been performed for G. p. gambiensis. This report presents results on the differential expression of immune-related genes in G. p. gambiensis challenged with T. b. gambiense. The aim was to characterize transcriptomic events occurring in the tsetse gut during the parasite establishment step, which is the crucial first step in the parasite development cycle within its vector. The selected genes were chosen from those previously shown to be highly expressed in G. m. morsitans, to allow further comparison of gene expression in both Glossina species. Using quantitative PCR, genes were amplified from the dissected midguts of trypanosome-stimulated, infected, non-infected, and self-cleared flies at three sampling time points (3, 10, and 20 d) after a bloodmeal. At the 3-d sampling point, transferrin transcripts were significantly up-regulated in trypanosome-challenged flies versus flies fed on non-infected mice. In self-cleared flies, serpin-2 and thioredoxin peroxidase-3 transcripts were significantly up-regulated 10 d after trypanosome challenge, whereas nitric oxide synthase and chitin-binding protein transcripts were up-regulated after 20 d. Although the expression levels of the other genes were highly variable, the expression of immune-related genes in G. p. gambiensis appears to be a time-dependent process. The possible biological significance of these findings is discussed, and the results are compared with previous reports for G. m. morsitans.

Mathematical Biology, 76 (3): 673-696.

DST/NRF Centre of Excellence in Epidemiological Modelling and Analysis (SACEMA), University of Stellenbosch, Stellenbosch, South Africa; Department of Mathematics, Makerere University, P.O. Box 7062, Kampala, Uganda; Department of Disease Control, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel St., London, WC1E 7HT, UK; Centre for Infectious Diseases and Division of Pathway Medicine, School of Biomedical Sciences, College of Medicine and Veterinary Medicine, The University of Edinburgh, Chancellor’s Building, 49 Little France Crescent, Edinburgh, Scotland, EH16 4SB, UK. [dkajung@gmail.com].

We present a mathematical model for the transmission of Trypanosoma brucei rhodesiense by tsetse vectors to a multi-host population. To control tsetse and T. b. rhodesiense, a proportion, psi, of cattle (one of the hosts considered in the model) is taken to be kept on treatment with insecticides. Analytical expressions are obtained for the basic reproduction number, $R_0^n$ in the absence, and $R_0^T^n$ in the presence of insecticide-treated cattle (ITC). Stability analysis of the disease-free equilibrium was carried out for the case when there is one vertebrate host untreated with insecticide. By considering three vertebrate hosts (cattle, humans and wildlife) the sensitivity analysis was carried out on the basic reproduction number ($R_{03}^T$) in the absence and presence of ITC. The results show that $R_{03}^T$ is more sensitive to changes in the tsetse mortality. The model is then used to study the control of tsetse and T. b. rhodesiense in humans through application of insecticides to cattle either over the whole-body or to restricted areas of the body known to be favoured tsetse feeding sites. Numerical results show that while both ITC strategies result in decreases in tsetse density and in the incidence of T. b. rhodesiense in humans, the restricted application technique results in improved cost-effectiveness, providing a cheap, safe, environmentally friendly and farmer based strategy for the control of vectors and T. b. rhodesiense in humans.


Department of Biomolecular and Biolaboratory Sciences, College of Veterinary Medicine Animal Resources and Biosecurity, Makerere University, Kampala, Uganda; Division of Pathway Medicine, Centre for Infectious Diseases, School of Biomedical Sciences, College of Medicine and Veterinary Medicine, The University of Edinburgh, Edinburgh, UK; Department of Public Health and Epidemiology, Swiss Tropical Institute, Basel, Switzerland; University of Basel, Basel, Switzerland; Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Edinburgh, UK. [luckydenno@covab.mak.ac.ug].

African animal trypanosomiasis (AAT) is considered to be one of the greatest constraints to livestock production and livestock-crop integration in most African countries. South-eastern Uganda has suffered for more than two decades from outbreaks of zoonotic human African trypanosomiasis (HAT), adding to the burden faced by communities from AAT. There is insufficient AAT and HAT data available from contemporary, sensitive and specific molecular techniques (in the animal reservoir) to guide and prioritize AAT control programmes. This study was undertaken to evaluate the burden that AAT presents to the
small-scale cattle production systems in south-eastern Uganda. Randomized cluster sampling was used to select 14 percent (57/401) of all cattle-containing villages across Tororo District. Blood samples were taken from all cattle in the selected villages between September-December 2011; these were preserved on FTA cards and analysed for different trypanosomes using a suite of molecular techniques. Generalized estimating equation and Rogen-Gladen estimator models were used to calculate apparent and true prevalences of different trypanosomes, while intra cluster correlations were estimated using a one-way mixed effect analysis of variance (ANOVA) in R statistical software version 3.0.2. The prevalence of all trypanosome species in cattle was 15.3 percent (95 percent CI; 12.2-19.1) while herd level trypanosome species prevalence varied greatly between 0 and 43 percent. Trypanosoma vivax (17.4 percent, 95 percent CI; 10.6-16.8) and Trypanosoma brucei rhodesiense (0.03 percent) were respectively, the most and least prevalent trypanosome species identified. The prevalence of bovine trypanosomes in this study indicates that AAT remains a significant constraint to livestock health and production. There is need to implement tsetse and trypanosomiasis control efforts across Tororo District by employing effective, cheap and sustainable tsetse and trypanosomiasis control methods that could be integrated in the control of other endemic vector borne diseases like tick-borne diseases.


Molecular Parasitology and Entomology Unit (MPEU), Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon; National Sleeping Sickness Control Programme of Cameroon, Yaoundé, Cameroon; Faculty of Science, University of Douala, P.O. Box 24157, Douala, Cameroon; Institut de Recherche pour le Développement, Unité Mixte de Recherche 177 IRD-CIRAD, Campus International de Baillarguet, TA A17/G, 34398 Montpellier Cedex 5, France. [gsimoca@yahoo.fr].

The sleeping sickness focus of Campo lies along the Atlantic coast and extends along the Ntem River, which constitutes the Cameroonian and Equatorial Guinean border. It is a hypo-endemic focus with the disease prevalence varying from 0.3 to 0.86 percent during the last few decades. Investigations on animal reservoirs revealed a prevalence of *Trypanosoma brucei gambiense* of 0.6 percent in wild animals and 4.83 percent in domestic animals within this focus. From 2001 to 2012, about 19 931 tsetse were collected in this focus and five tsetse species including *Glossina palpalis palpalis*, *G. pallicera*, *G. nigrofusca*, *G. tabaniformis* and *G. caliginea* were identified. The analysis of blood meals of these flies showed that they feed on human, pig, goat, sheep, and wild animals such as antelope, duiker, wild pig, turtle and snake. The percentage of blood meals taken from these hosts varied according to the sampling periods. For instance, 6.8 percent of blood meals from pig were reported in 2004 and 22 percent in 2008. This variation is subject to considerable evolution because the Campo HAT focus has been subjected to socio-economic changes including the reopening of a new wood company, the construction of an autonomous port at "Kribi" as well as the dam at "Memve ele". These activities will bring more that 3 000 inhabitants around Campo and induce deforestation for the implementation of farming as well as breeding of domestic animals. Such changes will impact the transmission and epidemiology of sleeping sickness due to modifications in fauna composition, the nutritional behaviour of tsetse and the zoophilic/anthropophilic index. To achieve elimination in the sleeping sickness focus of
Tsetse and Trypanosomosis Information

Campo, we report in this paper the current epidemiological situation of the disease, research findings from the last decades notably on the population genetics of trypanosomes, the modifications in nutritional behaviour of tsetse, and the prevalence of *T. b. gambiense* in humans, domestic and wild animals. An overview of the types of changes occurring in the region is provided as well as a discussion on the strategies that can be implemented to achieve the elimination of the disease.

5. HUMAN TRYPANOSOMIASIS

(a) SURVEILLANCE

[See also 37: 17256, 17260, 17287]


Department of Clinical Tropical Medicine, Pellegrin Hospital, University Hospital Center, Bordeaux, F-33075, France; Centre of Tropical Health and Medicine, University Bordeaux Segalen, Bordeaux, F-33076, France; Department of Medicine, Hôpital du Pays d'Autan, Castres, F-81108, France; Department of Biology, Hôpital du Pays d'Autan, Castres, F-81108, France; Department of Dermatology, St-André Hospital, University Hospital Center, Bordeaux, F-33075, France. [khaled.ezzedine@chu-bordeaux.fr].

Abstract not available.


Department of Parasitology, Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo; Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; Programme National de Lutte contre la Trypanosomiase Humaine Africaine, Kinshasa, Democratic Republic of the Congo; Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; 6 UMR 177 IRD-CIRAD INTERTRYP, Institut de Recherche pour le Développement, Montpellier, France. [pbuscher@itg.be].

Human African trypanosomiasis or sleeping sickness still causes considerable suffering in sub-Saharan Africa. Diagnostics for this infectious disease constantly improve but their performance in terms of accuracy and reproducibility should be evaluated prior to implementation in control activities. We evaluated the diagnostic performance of several microscopic, serological and molecular diagnostic tests on a cohort of 237 sleeping sickness suspects in the Democratic Republic of the Congo. Since molecular diagnostics are rather sophisticated, we also assessed their repeatability and reproducibility. In the absence of a golden standard test, latent class analysis revealed that the suboptimal specificity of the
serological and molecular tests is an issue. Our study shows the superior diagnostic sensitivity of the combination of lymph node aspirate examination and separation of trypanosomes from blood by mini anion exchange centrifugation techniques.

(b) PATHOLOGY AND IMMUNOLOGY


Department of Infectious Diseases, Nottingham University Hospitals City Campus, Nottingham, UK; Hospital for Tropical Diseases, London, UK; Department of Radiology, Nottingham University Hospitals, Nottingham, UK. [pradhib.venkatesan@nuh.nhs.uk].

West African trypanosomiasis caused by Trypanosoma brucei gambiense is a rare imported infection presenting with somnolence, lymphadenopathy and wide-ranging neurological symptoms. A 67-year-old Caucasian man presented with a 10-month history of cognitive deterioration, ataxic gait, somnolence and urinary incontinence. His symptoms had progressed more rapidly over the course of a month prior to admission. Serological testing confirmed a diagnosis of West African trypanosomiasis. The patient was successfully treated with eflornithine and made a good recovery. West African trypanosomiasis should be considered in the differential diagnosis of unexplained cognitive decline in those with a relevant travel history. If left untreated, the condition is universally fatal.


Centre International de Recherche-Développement sur l'Elevage en Zones Subhumides (CIRDES), Unité de Recherches sur les Bases Biologiques de la Lutte Intégrée, Bobo-Dioulasso, Burkina Faso; Institut de Recherche pour le Développement (IRD), UMR IRD-CIRAD 177 INTERTRYP, Campus International de Baillarguet, Montpellier, France; Ministère de la Santé et de l'Hygiène Publique, Programme National de Lutte contre la Trypanosomose Humaine Africaine, Conakry, Guinea; Institut National de la Recherche Agronomique (INRA), UMR 1313 GABI, F78350 Jouy-en-Josas, France. [bruno.bucheton@ird.fr].

In West Africa, Trypanosoma brucei gambiense, causing human African trypanosomiasis (HAT), is associated with a great diversity of infection outcomes. In addition to patients who can be diagnosed in the early haemolymphatic phase (stage 1) or meningoencephalitic phase (stage 2), a number of individuals can mount long-lasting specific serological responses while the results of microscopic investigations are negative (serum trypanolysis test, SERO TL+). Evidence is now increasing to indicate that these are asymptomatic subjects with low-grade parasitaemia. The goal of our study was to investigate
the type of immune response occurring in these "trypanotolerant" subjects. Cytokine levels were measured in healthy endemic controls (n = 40), stage 1 (n = 10), early stage 2 (n = 19), and late stage 2 patients (n = 23) and in a cohort of SERO TL+ individuals (n = 60) who were followed up for two years to assess the evolution of their parasitological and serological status. In contrast to HAT patients in whom T-cell responses appeared to be activated with increased levels of IL2, IL4, and IL10, SERO TL+ exhibited high levels of proinflammatory cytokines (IL6, IL8 and TNFalpha) and an almost absence of IL12p70. In SERO TL+, high levels of IL10 and low levels of TNFalpha were associated with an increased risk of developing HAT whereas high levels of IL8 predicted that serology would become negative. Further studies using high throughput technologies will hopefully provide a more detailed view of the critical molecules or pathways underlying the trypanotolerant phenotype.


Interfaculty Institute of Biochemistry, University of Tubingen, Hoppe-Seyler-Str. 4, 72076 Tubingen, Germany; Institute of Pathology and Neuropathology, University of Tubingen, Liebermeisterstr. 8, 72076 Tubingen, Germany. [michael.duszenko@uni-tuebingen.de].

African trypanosomes induce sleeping sickness. Although it is clear that this parasite moves from the blood to the central nervous system (CNS) to induce the second stage of the disease, little is known about the molecular details of this process. Considering new findings of trypanosome localization, this opinion paper summarizes the current knowledge about CNS infection, proposes a different perception of the invasion process, and discusses possible consequences for drug development.


Department of Biochemistry, Centers for Genomics and Personalized Medicine Research and Public Health Genomics, and Section on Nephrology, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA; Division of Renal Medicine, Department of Medicine, Emory School of Medicine, Atlanta, Georgia, USA; Division of Nephrology, Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia, USA; Division of Clinical Immunology and Rheumatology, University of Alabama, Birmingham, Alabama, USA. [bfreedma@wakehealth.edu].

Apolipoprotein L1 gene (APOL1) G1 and G2 variants are strongly associated with progressive non-diabetic nephropathy in populations with recent African ancestry. Selection for these variants occurred as a result of protection from human African trypanosomiasis (HAT). Resequencing of this region in 10 genetically and geographically distinct African populations residing in HAT endemic regions identified eight single nucleotide polymorphisms (SNPs) in strong linkage disequilibrium and comprising a novel G3 haplotype. To determine whether the APOL1 G3 haplotype was associated with nephropathy, G1, G2, and G3 SNPs and 70 ancestry informative markers spanning the genome were
genotyped in 937 African Americans with non-diabetic end stage renal disease (ESRD), 965 African Americans with type 2 diabetes-associated ESRD, and 1 029 non-nephropathy controls. In analyses adjusting for age, sex, APOL1 G1/G2 risk (recessive), and global African ancestry, the G3 haplotype was not significantly associated with ESRD (p = 0.05 for non-diabetic ESRD, p = 0.57 for diabetes-associated ESRD, and p = 0.27 for all-cause ESRD). We conclude that variation in APOL1 G3 makes a nominal, if any, contribution to ESRD in African Americans; G1 and G2 variants explain the vast majority of non-diabetic nephropathy susceptibility.


To date, this case represents the longest duration of HAT infection ever reported. As our patient last visited an endemic country in 1983 and became unwell in 2012, the period from infection to stage 2 symptoms must have been at least 29 years. This case provides concrete evidence behind the emerging concept of “human trypanotolerance,” which challenges the traditionally held view that, left untreated, HAT always progresses to the fatal stage 2 phase. Checchi, et al., reviewed the prior trypanotolerance literature, which consists of cases showing an extended duration of infection, like the one discussed above, as well as cohorts demonstrating spontaneous resolution of infection. We have summarised and updated the key evidence in this review. We were able to demonstrate the presence of long standing HAT infection, using several molecular techniques that were not available in the past. First, historically, it had not been possible to confirm whether chronic infections were due to pathogenic trypanosome species, as diagnosis was based on microscopy, which does not allow differentiation of sub-species. However, PCR amplification of our patient's parasite DNA clearly indicated *T. b. gambiense* group 1. Second, retrospective testing of a serum sample in 2004 demonstrated clear evidence of serological infection prior to disease. Third, additional microsatellite typing showed clustering with field isolates taken from West Africa in the 1980s, which is the estimated time our patient would have been first infected. Our findings are consistent with a recent 15-year follow-up study of untreated stage 1 HAT patients in the Ivory Coast, which showed that whilst some patients progress to stage 2, a proportion of patients become asymptomatic carriers, and another subset appears to self cure. Elucidating the genetic basis underlying trypanotolerance is of great interest. Although few parasite factors have been discovered, associations between human genes involved in the immune response and susceptibility to HAT have been reported.

Current disease control strategies rely on case finding and treatment of patients with detectable parasites in lymph nodes or blood or laboratory evidence of stage 2 disease. Such strategies could fail to stop transmission if hidden populations of individuals exist that harbour undetectably low parasite numbers and act as reservoirs of infection. Evidence that
seropositive, microscopy-negative patients have low-level infection is supported by molecular diagnostic work demonstrating detectable circulating T. b. gambiense DNA in a proportion of these asymptomatic carriers. Their potential role in disease transmission is supported by a parasite population genetic study as well as by research in trypanotolerant cattle. Detecting asymptomatic carriers is particularly challenging due to the lack of a specific diagnostic test that can be used in remote field settings. However, a recent study using blood stored on filter paper with the immune trypanolysis test has shown promising results. As the focus of HAT programmes shifts towards elimination of the disease, it is essential that trypanotolerance is taken into account. Specifically, the following questions will need to be answered: How common are these asymptomatic carriers? Does their prevalence vary in different HAT foci? How can they best be detected and monitored? What fraction of transmission is associated with failure to detect and treat such cases?

Several features of this case generate hypotheses about the immunopathology of HAT. The factors that led to our patient's development of stage 2 disease after such a long duration of chronic infection are unknown, but it is feasible that immunosuppressive therapy (azathioprine and prednisolone) may have played a role. While immunosuppressive therapy is well described when used for other protozoal infections, this is the first such case reported for HAT. This may be due to the low prevalence of HAT in populations at risk of iatrogenic immunosuppression. HIV and HAT have considerable geographic overlap; limited evidence does not suggest an increased risk of HAT among HIV+ persons, but there is no data on the role of HIV in activation of latent HAT infections, a mechanism well established for Chagas trypanosomiasis. One can speculate that the immunosuppressive therapy somehow altered the tight balance between immune control and disease. The complex clinical course of our patient before diagnosis of HAT warrants attention. For more than a decade, our patient was diagnosed with a series of conditions (Sjögren's, Lyme, antiphospholipid disease) based on abnormal serological tests. We consider these to be false-positive results, due to nonspecific immune activation characteristic of long-standing HAT infection. Through continuous antigenic variation of their variant surface glycoprotein, trypanosomes evade the immune system, inducing polyclonal B cell activation, which produces hypergammaglobulinaemia and autoantibodies. We hypothesize that the clinical course of our patient after definitive treatment may be similarly related to an autoimmune component. Most patients presenting with stage 2 HAT in field settings respond to treatment with rapid improvement within days. Whereas it is not uncommon for patients given melarsoprol to develop a post-treatment reactive encephalopathy, such a syndrome has been described as less frequent with NECT. Our patient had an uncharacteristically slow improvement following NECT with a fluctuating sleep disorder lasting several weeks. We believe that this could be due to immune-mediated encephalopathy. Indeed, our patient was found to be positive for VGKC-complex antibodies, which are known to cause autoimmune encephalitis. This led us to administer plasma exchange, with clinical improvement mirroring the fall in antibody titres. One might postulate that VGKC-complex antibodies may therefore contribute to stage 2 immunopathogenesis. However, a stored serum sample from five years before onset of stage 2 symptoms was also strongly positive (1 628 pM; normal range < 100 pM), so other insults must be required to generate pathology, such as the blood-brain barrier breakdown that must have occurred during the trypanosomal invasion. Improved understanding of CNS immunopathology is important, as it may help to design better and safer treatments. In conclusion, although we report only on a single patient, this case adds to the accumulating evidence of human trypanotolerance, thus further stimulating debate about the impact of this phenomenon in the field and illuminating new avenues of research that must be explored if we are to provide the answers that are vital for the elimination of this neglected tropical disease.
Tsetse and Trypanosomosis Information


Translational Biomarker Group, Department of Human Protein Sciences, University of Geneva, Rue Michel Servet 1, Geneva 1211, Switzerland; Institut de Recherche pour le Développement, Unité Mixte de Recherche UMR 177 IRD-CIRAD, Montpellier, France; Department of Parasitology, Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo; Department of Biotechnical and Diagnostics Sciences, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda; Department of Biochemistry, College of Natural Sciences, Makerere University, Kampala, Uganda; Department of Tropical Medicine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo; Department of Neuroscience, Karolinska Institute, Stockholm, Sweden; INSERM UMR1094, Tropical Neuroepidemiology, Limoges, France; Institute of Neuroepidemiology and Tropical Neurology, School of Medicine, CNRS FR 3503 GEIST, University of Limoges, Limoges, France; Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium. [Jean-Charles.Sanchez@unige.ch].

The host central nervous system (CNS) response to infection with Trypanosoma brucei (T. b.) gambiense or T. b. rhodesiense parasites, the causing agents of human African trypanosomiasis (HAT), is a poorly explored area. The two parasites are responsible for respectively a chronic and an acute form of HAT. In both cases, however, the disease progresses from a haemolymphatic first stage (S1) to a meningo-encephalitic second stage (S2) due to the penetration of parasites into the CNS. In the present study, we investigated and compared the cerebrospinal fluid (CSF) from S2 patients affected by either T. b. gambiense or T. b. rhodesiense HAT, using a mass spectrometry quantitative proteomics approach. Gene ontology and pathway analyses on the 222 quantified human proteins revealed a predominant activation of the innate immune and the acute phase responses in rhodesiense HAT patients. These results were further confirmed through the verification of the over-expression of two proteins involved in these mechanisms, C-reactive protein (CRP) and orosomucoid 1 (ORM1), in 126 S2 HAT patients suffering from either the chronic or the acute form of HAT Both proteins were significantly increased (p < 0.0001) in the CSF of rhodesiense HAT patients. These findings contribute to a better understanding of the pathophysiological mechanisms of late stage HAT caused by T. b. gambiense or T. b. rhodesiense and pave the way for further investigations on the clinical significance of CRP and ORM1. Mass spectrometry data are available via ProteomeXchange (identifier PXD001082).


Departments of Neurology, Neuroradiology, Neuropathology and Infectious Diseases and Respiratory Medicine, Charite, Chariteplatz 1, 10117 Berlin, Germany; Bernhard
Human African trypanosomiasis (HAT), also referred to as "sleeping sickness", is caused by the parasite *Trypanosoma brucei*. Diagnosing imported HAT outside endemic areas is difficult and diagnosis is often delayed. We report a case of imported human African trypanosomiasis caused by *Trypanosoma brucei gambiense* with an unusually long incubation period of at least seven years. A 33-year-old male African patient, a former resident of Cameroon, presented with a four-month history of progressive personality changes. A few weeks before presentation, the patient had first been admitted to a psychiatric ward and received antidepressant treatment, until a lumbar puncture showed pleocytosis and then antibiotic treatment for suspected neuroborreliosis was initiated. The patient continued to deteriorate during antibiotic treatment and became increasingly lethargic. Under antiparasitic and anti-inflammatory treatment, the condition of the patient gradually improved over the following months and he recovered completely after 24 months of follow-up. This well-documented case illustrates typical difficulties in establishing the correct diagnosis outside endemic areas and provides an overview of typical clinical, neuropathological and neuroimaging findings in *T. b. gambiense* trypanosomiasis, guiding the clinician in establishing the correct diagnosis in this rare disease.

(c) TREATMENT

[See also 37: 17295, 17299, 17301, 17305]


Geneva University Hospitals, Geneva, Switzerland. [francois.chappuis@hcuge.ch].

Treatment of second-stage *gambiense* human African trypanosomiasis relied on toxic arsenic-based derivatives for over 50 years. The availability and subsequent use of efloinithine, initially in monotherapy and more recently in combination with nifurtimox (NECT), has drastically improved the prognosis of treated patients. However, NECT logistic and nursing requirements remain obstacles to its deployment and use in peripheral health structures in rural sub-Saharan Africa. Two oral compounds, fexinidazole and SCYX-7158, are currently in clinical development. The main scope of this article is to discuss the potential impact of new oral therapies to improve diagnosis-treatment algorithms and patients' access to treatment, and to contribute to reach the objectives of the recently launched *gambiense* human African trypanosomiasis elimination programme.


Unit for Pharmacokinetics and Drug Metabolism, Dept. Pharmacology, Sahlgrenska Academy, University of Gothenburg, Sweden; CVMD iMed DMPK, AstraZeneca
This study aimed to characterize the stereoselective pharmacokinetics of oral eflornithine in 25 patients with late-stage \textit{Trypanosoma brucei} (\textit{T. b.}) gambiense sleeping sickness. A secondary aim was to determine the concentration of L- and D - eflornithine required in plasma or CSF for an efficient eradication of the \textit{T. b. gambiense} parasites. Patients were randomly allocated to receive either 100 (Group I, \(n = 12\)) or 125 (Group II, \(n = 13\)) mg/kg every six h for 14 d. Concentrations of L- and D-eflornithine in plasma and CSF were measured using a stereospecific liquid chromatographic method. Non-linear mixed effects modelling was used to characterize the plasma pharmacokinetics. Plasma concentrations of L-eflornithine were on average 52 percent (95 percent CI: 51, 54, \(n = 321\)) of the D-enantiomer concentrations. The typical oral clearance of L- and D-eflornithine were 17.4 (95 percent CI: 15.5, 19.3) and 8.23 (95 percent CI: 7.36, 9.10) L/h, respectively. These differences were likely due to stereoselective intestinal absorption. The distribution of eflornithine enantiomers to the CSF was not stereoselective. A correlation was found between the probability of cure and plasma drug exposure although this was not more pronounced for the L-enantiomer compared with total eflornithine. This study may explain why an oral treatment of late-stage HAT patients with racemic eflornithine has previously failed; the more potent L-enantiomer is present at much lower concentrations in both plasma and CSF compared with the D-enantiomer. Eflornithine stereoselective pharmacokinetics needs to be considered if an oral dosage regimen is further explored.


Institute of Tropical Medicine, Antwerpen, Belgium; Faculty of Medicine, Kinshasa University, Kinshasa, Democratic Republic of the Congo; Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of Congo; International Health, Antwerp University, Antwerpen, Belgium. [pmitashi@yahoo.fr].

Control of human African trypanosomiasis (HAT) in the Democratic Republic of Congo (DRC) has always been a vertical programme, although attempts at integration in general health services were made in recent years. Now that HAT prevalence is declining, the integration question becomes even more crucial. We studied the level of attainment of integration of HAT case detection and management in primary care centres in two high-prevalence districts in the province of Bandundu, DRC. We visited all 43 first-line health centres of Mushie and Kwamouth districts, conducted structured interviews and inspected facilities using a standardized checklist. We focused on: availability of well trained staff - besides HAT; we also tested for knowledge on tuberculosis; availability of equipment, consumables and supplies; and utilization of the services. All health centres were operating but most were poorly equipped, and attendance rates were very low. We observed a median of 14 outpatient consultations per facility (interquartile range [IQR], 8-21) in the week prior to our visit, i.e. two patients per day. The staff had good knowledge on presenting symptoms, diagnosis and treatment of both HAT and tuberculosis. Nine centres were accredited by the national programme as HAT diagnosis and treatment centres, but the most sensitive
diagnostic confirmation test, the mini-anion exchange centrifugation technique (mAECT), was not present in any. Although all nine were performing the CATT screening test, only two had the required cold chain in working order. In these high-prevalence districts in DRC, staff is well acquainted with HAT but lack the tools required for an adequate diagnostic procedure. Attendance rates at these primary care centres are extremely low, making timely recognition of a resurgence of HAT unlikely in the current state of affairs.


Département de Parasitologie, Institut National de Recherche Biomédicale, Av. de la Démocratie, BP 1197, Kinshasa Gombe, Congo; Centre International de Recherche-Développement sur l’Élevage en zones Subhumides (CIRDES), Unité de Recherches sur les Bases Biologiques de la Lutte Intégrée, Institut de Recherche pour le Développement, Unité Mixte de Recherche IRD-CIRAD 177 INTERTRYP, 01 BP 454 Bobo-Dioulasso 01, Burkina Faso; Université Polytechnique de Bobo-Dioulasso, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso; Institut de Recherche pour le Développement, Unité Mixte de Recherche IRD-CIRAD 177 INTERTRYP, TA A-17/G, Campus International de Baillarguet, F-34398 Montpellier, France; Wellcome Centre for Molecular Parasitology, University of Glasgow; Biomedical Research Centre, 120 University Place, Glasgow G12 8TA, UK; Programme National de Lutte contre la Trypanosomiasi Humaine Africaine, Conakry BP 851, Guinea; Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. [thierry.demeeus@ird.fr].

Human African trypanosomiasis (HAT) in the Democratic Republic of the Congo (DRC) is caused by the protozoan *Trypanosoma brucei gambiense*. Until recently, all patients in the second or neurological stage of the disease were treated with melarsoprol. At the end of the past and the beginning of the present century, alarmingly high relapse rates in patients treated with melarsoprol were reported in isolated HAT foci. In the Mbuji-Mayi focus of DRC, a particular mutation that confers cross resistance for pentamidine and melarsoprol was recently found for all strains studied. Nevertheless, treatment successfully cured a significant proportion of patients. To check for the existence of other possible genetic factors of the parasites, we genotyped trypanosomes isolated from patients before and after treatment (relapsing patients) with eight microsatellite markers. We found no evidence of any genetic correlation between parasite genotype and treatment outcome and we concluded that relapse or cure probably depends more on patient factors such as disease progression, nutritional or immunological status or co-infections with other pathogens. The existence of a melarsoprol and pentamidine resistance associated mutation at such high rates highlights an increasing problem, even for other drugs, especially those using the same transporters as melarsoprol and pentamidine.

Sleeping sickness caused by *Trypanosoma brucei* (*T. b.*) *gambiense* constitutes a serious health problem in sub-Saharan Africa. In some foci, alarmingly high relapse rates were observed in patients treated with melarsoprol, which used to be the first line treatment for patients in the neurological disease stage. Particularly problematic was the situation in Mbuji-Mayi, East Kasai Province in the Democratic Republic of the Congo with a 57 percent relapse rate compared with a 5 percent relapse rate in Masi-Manimba, Bandundu Province. The present study aimed at investigating the mechanisms underlying the high relapse rate in Mbuji-Mayi using an extended collection of recently isolated *T. b. gambiense* strains from Mbuji-Mayi and from Masi-Manimba. Forty five *T. b. gambiense* strains were used. Forty one were isolated from patients who were cured or relapsed after melarsoprol treatment in Mbuji-Mayi. *In vivo* drug sensitivity tests provide evidence of reduced melarsoprol sensitivity in these strains. This reduced melarsoprol sensitivity was not attributable to mutations in *TbAT1*. However, in all these strains, irrespective of the patient treatment outcome, the two aquaglyceroporin (AQP) 2 and 3 genes are replaced by chimeric AQP2/3 genes that may be associated with resistance to pentamidine and melarsoprol. The four *T. b. gambiense* strains isolated in Masi-Manimba contain both wild-type AQP2 and a different chimeric AQP2/3. These findings suggest that the reduced *in vivo* melarsoprol sensitivity of the Mbuji-Mayi strains and the high relapse rates in that sleeping sickness focus are caused by mutations in the AQP2/AQP3 locus and not by mutations in *TbAT1*. We conclude that mutations in the *TbAQP2/3* locus of the local *T. b. gambiense* strains may explain the high melarsoprol relapse rates in the Mbuji-Mayi focus but other factors must also be involved in the treatment outcome of individual patients.

6. ANIMAL TRYPANOSOMOSIS

(a) SURVEY AND DISTRIBUTION

[See also 37: 17291, 17292, 17315]


Department of Preventive Veterinary Medicine and Animal Science, Faculty of Veterinary Medicine, University of São Paulo, SP, 05508-270, Brazil. [amarcili@usp.br].

*Trypanosoma* and *Leishmania* infections affect wild and domestic animals and human populations. The growing process of deforestation and urbanization of Atlantic Rainforest areas has given rise to introduction of humans and domestic animals to the sylvatic cycles of *Trypanosoma* and *Leishmania* species. Serological, parasitological, and molecular surveys among wild and domestic animals in the Corrego do Veado Biological Reserve, which is an Atlantic Rainforest fragment in the state of Espirito Santo, southeastern Brazil, were evaluated. In total, 154 wild animals of 25 species and 67 domestic animals (47 dogs and 20 horses) were sampled. All the domestic animals were serologically negative for anti-
Leishmania infantum chagasi antibodies and negative in parasitological approaches. Only the Order Chiroptera presented positive blood cultures and cryopreserved isolates. The phylogenetic trees based on SSU rDNA and gGAPDH genes confirmed the occurrence of Trypanosoma dionisii and provided the first record of Trypanosoma cruzi marinkellei in southeastern Brazil. The studies conducted in the remaining trees of the Atlantic Rainforest provide the knowledge of parasite diversity or detect parasites that can accelerate the loss of host diversity.


Facultad de Ciencias Pecuarias, Escuela Superior Politecnica del Chimbombo, Riobamba, Provincia del Chimborazo, Ecuador; Departments of Microbiology, Health Sciences and Animal Medicine and Surgery, Veterinary Faculty, University of Las Palmas de Gran Canaria, Canary Islands, Spain. [mtejedor@dcc.ulpgc.es].

Trypanosoma evansi was first identified in the Canary Islands in 1997, and is still present in a small area of the Archipelago. To date, the disease has exclusively affected camel herds, and has not been detected in any other animal hosts. However, potential vectors of Trypanosoma evansi must be identified. One Nzi trap was placed on a camel farm located in the infected area for a period of one year. Two thousand five hundred and five insects were trapped, of which Stomoxys calcitrans was the sole haematophagous vector captured. Stomoxys calcitrans could be exclusively responsible for the transmission of Trypanosoma evansi among camels in the surveyed area, as other species do not seem to be infected by S. calcitrans in the presence of camels.


Department of Veterinary Parasitology, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, 141004, Punjab, India. [ldsingla@gmail.com].

Duplex PCR consisting of two primer sets within a single mixture for the simultaneous detection of Anaplasma marginale and Trypanosoma evansi was standardized and employed on 219 blood samples collected from cattle (165) and buffaloes (54) from eastern Punjab to evaluate the status of concurrent infection and associated risk factors. The reaction produced 257- and 407-bp amplification products targeting the repetitive nucleotide sequence of T. evansi and the msp1beta gene of A. marginale, respectively. The nucleotide sequence analysis of individual amplicons expressed the fidelity of the primer pairs used; duplex PCR was 100 percent sensitive and 92.66 percent specific for the detection of mixed infections. Among the agro-climatic zones of interest, the undulating zone was at higher risk of T. evansi infection (odds ratio [OR] = 1.75, 95 percent confidence interval [CI] = 0.94-3.27), and the submountain zone (OR = 1.89, 95 percent CI = 1.11-3.33) for A. marginale. For the concurrent infection, the relative risk among the two zones was almost the same. The cross-
bred cattle population had the highest risk of infection, whether it be single infection of *T. evansi* (OR = infinity, 95 percent CI = 1.18-infinity)/*A. marginale* (OR = 6.39, 95 percent CI = 1.14-125.3) or dual infection (OR = infinity, 95 percent CI = 0.39-infinity) as the indigenous cattle are more resistant to infection. Cross-bred cattle had approximately a three times higher risk than buffaloes. For the dual infection, the cattle calves were at about 2.5 times higher risk than buffalo calves. Results indicate the endemic status of these infections in the region and highlight the animals at great risk and requiring better surveillance.


Mercy Hospital Research Laboratory (MHRL), Kulanda Town, Bo, Sierra Leone; Njala University, Njala, Sierra Leone; Department of Global and Community Health, George Mason University, Fairfax, VA, USA; U.S. Naval Research Laboratory, Washington, DC, USA. [rashidansumana@gmail.com].

The purpose of this study was to conduct syndromic surveillance for important veterinary diseases in Koinadugu district, Northern Province, Sierra Leone. The study examined all veterinary syndromic surveillance reports submitted to the district veterinary office from January 2011 through December 2012. In total, 5 679 case reports were submitted, including 2 394 fatalities. The most common syndrome reported was consistent with peste de petits ruminants (PPR) in goats (n = 1 649). PPR cases were reported from eight of 11 chiefdoms in the district, with a 42 per 1 000 reported incidence rate and a 48 percent case fatality rate. Other syndromes reported were consistent with trypanosomiasis in cattle (n = 1 402), Newcastle disease in poultry (n = 911), black quarter in cattle (n = 691), and haemorrhagic septicaemia in cattle (n = 542). Expanded use of the PPR virus vaccine may be required to help control the spread of the infection. Improved community-based prevention efforts may be effective for better control of trypanosomiasis and all these conditions.

(b) **PATHOLOGY AND IMMUNOLOGY**


School of Agriculture, Adama Science and Technology University, P.O. Box 193, Asella, Ethiopia; International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria; International Livestock Research Institute (ILRI), P.O. Box 5689, Addis Ababa, Ethiopia; International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi, Kenya. [endashawterefe1@gmail.com].

The study was conducted to characterize the morphological features of Mursi cattle breed and to identify the species of trypanosome infecting the cattle and its prevalence in these traditionally managed cattle in the Bodi and Mursi pastoral communities. Cattle body description and measurements were made on 201 mature animals. Blood samples were collected from 409 animals into heparin-treated capillary tubes and centrifuged at 12 000 rpm
for 5 min. to identify trypanosome species from the wet smeared buffy coat and to estimate the degree of anaemia (PCV). Tsetse flies were collected using phenol-treated biconical traps and the caught flies identified to species level. The breed possesses a variable coat colour pattern and coat colour type, and has small to medium humps on the thoracic vertebrae. Body measurement of Mursi cattle in the two locations did not show significant differences except chest girth, rump width, and horn length. Trypanosome prevalence in the Mursi cattle breed was 6.1 percent. The highest trypanosome infection was caused by *Trypanosoma congoense* (56 percent) followed by *Trypanosoma vivax* (40 percent) and *Trypanosoma brucei* (4 percent). Trypanosome prevalence varied significantly between the dry (2.0 percent) and late rainy (10.1 percent) seasons (p < 0.001) and between lean (11.9 percent) and medium (2.4 percent) body condition score (p < 0.01). The PCV value was 22.1 +/- 0.5 percent, which varied significantly with season (p < 0.01) and parasitism (p < 0.001). Parasitaemic cattle showed lower PCV values (20.4 +/- 1 percent) than aperasitaemic (23.7 +/- 0.3 percent) cattle, and cattle with lean BCS had the lowest (p < 0.0001) PCV values (20.4 +/- 0.6 percent). Tsetse fly species identified in the study area were *Glossina pallidipes*, *Glossina morsitans submorsitans*, and *Glossina fuscipes*. The number of flies captured during the late rainy season was higher than in the dry season (p < 0.01). Despite the existence of trypanosomes and high tsetse fly infestations in the areas, a large proportion of the Mursi cattle had medium BCS, low trypanosome prevalence, and higher PCV value.

(c) TRYPANOTOLERANCE

(d) TREATMENT

7. EXPERIMENTAL TRYPANOSOMIASIS

(a) DIAGNOSTICS

[See also 37: 17294]


Institute of Tropical Medicine, Department of Biomedical Sciences, Antwerp, Belgium; Coris BioConcept, Crealys Park, Gembloux, Belgium; Applied Technology and Production Unit, Antwerp, Belgium; Institut National de Recherche Biomédicale, Kinshasa Gombe, DR Congo; Department of Public Health, Antwerp, Belgium; Institut de Recherche pour le Développement, UMR 177, Montpellier, France. [pbuscher@itg.be].

Human African trypanosomiasis (HAT) is a life-threatening infection affecting rural populations in sub-Saharan Africa. Large-scale population screening by antibody detection with the card agglutination test for trypanosomiasis (CATT)/*Trypanosoma brucei (T. b.)* gambiense helped reduce the number of reported cases of gambiense HAT to fewer than 10 000 in 2011. Because low case numbers lead to decreased cost-effectiveness of such active screening, we aimed to assess the diagnostic accuracy of a rapid serodiagnostic test (HAT
Sero-K-SeT) applicable in primary healthcare centres. In our case-control study, we assessed participants older than 11 years who presented for HAT Sero-K-SeT and CATT/T. b. gambiense at primary care centres or to mobile teams (and existing patients with confirmed disease status at these centres) in Bandundu Province, DR Congo. We defined cases as patients with trypanosomes that had been identified in lymph node aspirate, blood, or cerebrospinal fluid. During screening, we recruited controls without previous history of HAT or detectable trypanosomes in blood or lymph who resided in the same area as the cases. We assessed diagnostic accuracy of three antibody detection tests for gambiense HAT: HAT Sero-K-SeT and CATT/T. b. gambiense (done with venous blood at the primary care centres) and immune trypanolysis (done with plasma at the Institute of Tropical Medicine, Antwerp, Belgium). Between June 6, 2012, and February 25, 2013, we included 134 cases and 356 controls. HAT Sero-K-SeT had a sensitivity of 0.985 (132 true positives, 95 percent CI 0.947-0.996) and a specificity of 0.986 (351 true negatives, 0.968-0.994), which did not differ significantly from CATT/T. b. gambiense (sensitivity 95 percent CI 0.955, 95 percent CI 0.906-0.979 [128 true positives] and specificity 0.972, 0.949-0.985 [346 true negatives]) or immune trypanolysis (sensitivity 0.985, 0.947-0.996 [132 true positives] and specificity 0.980, 0.960-0.990 [349 true negatives]). It is concluded that the diagnostic accuracy of HAT Sero-K-SeT is adequate for T. b. gambiense antibody detection in local health centres and could be used for active screening whenever a cold chain and electricity supply are unavailable and CATT/T. b. gambiense cannot be done.


Automated extraction of DNA for testing of laboratory samples is an attractive alternative to labour-intensive manual methods when higher throughput is required. However, it is important to maintain the maximum detection sensitivity possible to reduce the occurrence of type II errors (false negatives; failure to detect the target when it is present), especially in the biomedical field, where PCR is used for diagnosis. We used blood infected with known concentrations of *Trypanosoma copemani* to test the impact of analysis techniques on trypanosome detection sensitivity by PCR. We compared combinations of a manual and an automated DNA extraction method and two different PCR primer sets to investigate the impact of each on detection levels. Both extraction techniques and specificity of primer sets had a significant impact on detection sensitivity. Samples extracted using the same DNA extraction technique performed substantially differently for each of the separate primer sets. Type I errors (false positives; detection of the target when it is not present), produced by contaminants, were avoided with both extraction methods. This study highlights the importance of testing laboratory techniques with known samples to optimize accuracy of test results.

The present study aimed at comparing the trypanosome specific 18S-PCR-RFLP using samples stored either on Whatman filter papers (PCR-RFLP-fp) or in a commercial cell lysis and DNA protecting buffer (PCR-RFLP-pb) with the haematocrit centrifugation technique (HCT), a method widely used for the diagnosis of African animal trypanosomosis. Out of 411 head of cattle, 49 (11.92 percent) (CI = 8.95-15.45) scored positive for the presence of trypanosomes by HCT whereas 75 (18.25 percent, CI = 14.63-22.33) and 124 (30.17 percent, CI = 25.77-34.86) scored positive using PCR-RFLP-fp and PCR-RFLP-pb, respectively. Out of the 49 positives by HCT, 14 (28.57 percent, CI = 16.58-43.26) and 28 (57.14 percent, CI = 42.21-71.18) were concordant by PCR-RFLP-fp and PCR-RFLP-pb, respectively. None of the PCR techniques detected parasites from the Trypanozoon group. Although HCT detected more cases of *Trypanosoma vivax* (33), species identification using PCR-RFLP-fp and PCR-RFLP-pb were significantly different (p < 0.001) from the HCT technique. The use of DNA protective buffer is thus recommended as the output of the PCR-RFLP-pb is improved and the risk of contamination between samples is reduced.


A total of 231 serum samples were collected from sheep (n = 9), goats (n = 99) and cattle (n = 123) in northeastern KwaZulu-Natal, South Africa. Trypanosome infection was detected using *Trypanosoma brucei brucei* crude antigen (TbbCA) and *T. congolense* crude antigen (TcoCA) ELISA assays. Recombinant antigen (*T. evansi* GM6 which consisted of four repeat domains, TeGM6-4r) ELISA and an immunochromatographic test (ICT) were also used. Crude antigen ELISA, TeGM6-4r-ELISA and ICT detected 27.3 percent, 29 percent and 19.9 percent of trypanosome seropositive samples, respectively. Trypanosome infection prevalence in cattle and goats was 35.8-46.3 percent and 0-9.1 percent, respectively. Out of nine sheep serum samples, 2-4 sera (22.2-44.4 percent) were positive. The detection performance of crude and recombinant antigen ELISAs was relatively similar (K = 0.6-0.7); both are recommended for reference diagnosis and large scale epidemiological surveys. There
is potential application for ICT in on-site diagnosis, but its sensitivity should be improved.


Programa de pos-graduação em Pesquisa Clinica em Doenças Infecciosas, Instituto de Pesquisa Clinica Evandro Chagas, Fundacao Oswaldo Cruz, Av. Brasil 4365, 21040-900 Rio de Janeiro, RJ, Brazil; Laboratorio de Vigilancia em Leishmanioseis, Instituto de Pesquisa Clinica Evandro Chagas, Fundacao Oswaldo Cruz, Av. Brasil 4365, 21040-900 Rio de Janeiro, RJ, Brazil; Laboratorio de Diagnostico Molecular e Hematologia, Faculdade de Farmacia, Universidade Federal do Rio de Janeiro, Av. Carlos Chagas Filho 373, Rio de Janeiro, RJ, Brazil; Laboratorio de Pesquisa Clinica em Dermatozoonoses de Animais Domesticos, Instituto de Pesquisa Clinica Evandro Chagas, Fundacao Oswaldo Cruz, Av. Brasil 4365, 21040-900 Rio de Janeiro, RJ, Brazil. [fatima.madeira@ipec.fiocruz.br].

*Trypanosoma caninum* is a new species that has been recently identified in Brazil and infects domestic dogs. To date, no accurate diagnostic assays for this parasite have been established; thus, our aim was to evaluate more than one type of PCR for the diagnosis and molecular screening of *T. caninum* in 229 dogs living in Rio de Janeiro State. The tests were based on the amplification and sequencing of the 18S ribosomal DNA (rDNA) gene using healthy skin fragments. Additionally, PCR amplification of the kDNA minicircles region specific to the *Leishmania* genus was performed. The PCR results were compared with those of culture-based analysis performed with the same specimen. Using cultures, *T. caninum* and *Leishmania chagasi* were isolated from 11 and 12 dogs, respectively, whereas the 18S rDNA PCR assay detected parasitic infection in 35 dogs. Among these, 25 dogs showed an amplification pattern similar to *T. caninum* and 10 showed a pattern similar to *L. chagasi*; these results were confirmed by sequencing analysis. The kDNA PCR analysis showed that 14 dogs were positive for *Leishmania* infection. Of these, two dogs showed negative culture results and 12 were positive for *L. chagasi*, including four with negative 18S rDNA PCR results. Thus far, culture-based testing has been the only tool used successfully for *T. caninum* diagnosis. Our results demonstrate that the 18S rDNA PCR-based test should be a useful diagnostic tool, particularly for distinguishing between *T. caninum* and *L. chagasi* infections in areas where these two parasites co-exist.


Department of Biomedical Sciences, Unit of Parasite Diagnostics, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium; Laboratory for Molecular Plant Physiology and Biotechnology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium; Production and Applied Technology Unit, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium; Animal Health Service, Food and Agriculture Organization of the United Nations (FAO), Viale delle Terme di Caracalla, 10532 Rome, Italy; Institut de Recherche pour le Développement, Unité Mixte de Recherche UMR177 - Intertryp, Campus International de Baillarguet TA A-17/G, 34398 Montpellier, France. [sroge@itg.be].
Serodiagnosis of surra is commonly performed with the CATT/Trypanosoma evansi direct agglutination test. This antibody detection test is based on lyophilized bloodstream form trypanosomes propagated in rats and presenting the predominant variant surface glycoprotein (VSG) RoTat 1.2 on their surface. Recently, the N-terminal fragment of VSG RoTat 1.2 has been expressed as a recombinant protein in the yeast Pichia pastoris and showed diagnostic potential in ELISA. This recombinant antigen has now been incorporated in a latex agglutination test, the rLATEX/T. evansi. In this study, we compared the diagnostic accuracy of rLATEX/T. evansi and CATT/T. evansi with immune trypanolysis (TL) as reference test on a total of 1 717 sera from camels, horses, bovines, water buffaloes, dogs and sheep. The rLATEX/T. evansi displayed a slightly better agreement with TL than CATT/T. evansi (kappa respectively 0.84 and 0.72). The sensitivities of rLATEX/T. evansi (84.2 percent, 95 percent CI 80.8-87.1) and CATT/T. evansi (84.0 percent, 95 percent CI 80.6-87.0) were similar, but rLATEX/T. evansi was significantly more specific (97.7 percent, 95 percent CI 96.7-98.4) than CATT/T. evansi (89.4 percent; 95 percent CI 87.6-91.1). We consider the rLATEX/T. evansi an alternative to the CATT/T. evansi, with the advantage that the use of a purified recombinant antigen leads to a more standardized diagnostic test with an improved specificity. Moreover, it eliminates the use of laboratory animals and can be easily scaled-up, e.g. in biofermentors.


Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia. [nsheena@um.edu.my].

The quantitative buffy coat (QBC) technique and conventional Giemsa thin blood smear were compared to determine their sensitivity and specificity in detecting blood parasitic infection of the rodent populations from four urban cities in Peninsular Malaysia. A total of 432 blood samples from four rat species (Rattus norvegicus, Rattus rattus diardii, Rattus exulans and Rattus argentiventer) were screened using both techniques which successfully detected two blood protozoan species (Trypanosoma lewisi and Plasmodium sp.) with Trypanosoma lewisi predominantly infecting the population. Results showed that Giemsa-stained thin film (GTF) was the better detection method for blood parasitaemia (46.7 percent) compared with the QBC method (38.9 percent) with overall detection technique sensitivity and specificities of 83.2 percent and 74.8 percent respectively. The sensitivity in detecting Trypanosoma lewisi was 84.4 percent with the value slightly lower for Plasmodium sp. infections at 76.6 percent. Statistical analysis indicated that the GTF technique was significantly more sensitive in the detection of blood protozoan infections in the rodent population compared to QBC (p < 0.05).

Trypanosoma evansi, a haemoflagellate, causes "surra" an important chronic wasting disease in a wide range of wild and domestic herbivorous and carnivorous animals including cattle, buffaloes, camels, horses, etc. The untreated recovered animal can act as a carrier without exhibiting the disease symptoms and can be a source of infection to healthy animals. The diagnosis and subsequent treatment of carrier animals is helpful to curb the disease. As the parasitaemia in carrier animals is very scanty, the conventional blood smear examination, which is widely practised in the field, cannot detect such condition. For this purpose improved diagnostics using a mass screening test such as ELISA are very useful. In the present study, the VSG of T. evansi was expressed in a prokaryotic system (E. coli) and thereafter its immunoreactivity was evaluated in immunoblot and enzyme immuno assays. The expressed protein showed 95.6 percent sensitivity, 98.0 percent specificity and 0.93 Cohen's kappa value, when compared with standard antigens. The developed antigen was also validated with field serum samples from cattle, camels and horses collected from different states of India. The data showed that the recombinant antigen can be a diagnostic tool to detect carrier animals as well as control the disease.

manufacture, lower unit cost and assured reproducibility.

(b) PATHOLOGY AND IMMUNOLOGY

[See also 37: 17251, 17389, 17392, 17393, 17399, 17400, 17404, 17406, 17410, 17417, 17424]


Departments of Microbiology and Parasitology, Small Animal, Large Animal & Clinical & Toxicological Analysis, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil; Toxicological Biochemistry of Graduate Program, UFSM, Santa Maria, RS, Brazil; Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapeco, SC, Brazil. [aleksandro_ss@yahoo.com.br].

The aim of this study was to assess the effects of iron supplementation on oxidative stress and on the activity of adenosine deaminase (ADA) in rats experimentally infected with Trypanosoma evansi. For this purpose, 20 rats were divided into four experimental groups with five animals each as follows: groups A and B were composed of healthy animals, while animals from groups C and D were infected with T. evansi. Additionally, groups B and D received two subcutaneous doses of iron (60 mg kg⁻¹) within an interval of five d. Blood samples were drawn on d eight post infection in order to assess haematological and biochemical variables. Among the main results were: (i) animals from group C showed a reduced erythrogram (with tendency to anaemia); however the same results were not observed for group D; this might be a direct effect of free iron on trypanosomes which helped to reduce the parasitaemia and the damage to erythrocytes caused by the infection; (ii) iron supplementation reduced NOx levels by inhibiting iNOS, and thus providing an antioxidant action, and indirectly reducing the ALT levels in groups B and D; (iii) increased FRAP levels in group D; (iv) reduced ADA activity in serum and erythrocytes in group C; however, this supplementation increased the protein oxidation in groups B and D, as well as group C (positive control). Therefore, iron showed antioxidant and oxidant effects on animals that received supplementation, and it kept the activity of E-ADA stable in infected/supplemented animals.

There are few studies about the immune response during trypanosomosis in cattle. The objective of this research was to evaluate the effect of experimental infection with *Trypanosoma vivax* (*T. vivax*) on serum levels of TNF-alpha in bulls and its relationship to haematocrit, body temperature and parasitaemia. Two adult crossbred bulls were infected experimentally with *T. vivax* and two were used as controls. The bulls were evaluated during a 64 d period in terms of temperature, haematocrit, and parasitaemia. Serum TNF-alpha levels were determined by ELISA using an antibody specific for bovine. TNF-alpha in serum began rising on the seventh d after infection and reached a peak on d 40 of post-infection, then dropped. The lowest haematocrit levels corresponded to the upper levels of TNF-alpha for each animal. In conclusion, the experimental infection of cattle with *T. vivax* promotes the release of TNF-alpha, demonstrating a pro-inflammatory immune response to this haemotropic parasite. Moreover, the lowest haematocrit levels coincided with high concentrations of TNF-alpha, suggesting that this cytokine can be linked to the anaemia observed during the course of infection by *T. vivax* in cattle.


Elucidating the underlying genetic determinants of disease pathology is still in the early stages for many pathogenic parasites. There have, however, been a number of advances in which natural genetic diversity has been successfully utilized to untangle the often complex interactions between parasite and host. In this chapter we discuss various methods capable of exploiting this natural genetic variation to determine genes involved in phenotypes of interest, using virulence in the pathogenic parasite *Trypanosoma brucei* as a case study. This species is an ideal system to benefit from such an approach as there are several well-characterized laboratory strains, the parasite undergoes genetic exchange in both the field and the laboratory, and is amenable to efficient reverse genetics and RNAi.

Trypanosomosis is a vector-borne protozoan disease of animals and humans in sub-Saharan Africa. In Ethiopia, particularly the northwest region is affected by both tsetse and non-tsetse transmitted trypanosomosis. The aim of the present study was to determine the effects and compare differences in virulence of \textit{Trypanosoma vivax} infection between tsetse and non-tsetse infested areas of northwest Ethiopia on the basis of serum biochemical values in Zebu cattle. Eighteen cattle purchased from the trypanosome free area and aged between 9 and 12 months were assigned to three groups of six animals (Group TT = infected with \textit{T. vivax} from the tsetse infested area, Group NT = infected with \textit{T. vivax} from the non-tsetse infested area and Group C = non-infected control). Each experimental animal in Groups TT and NT was inoculated intravenously with 3 mL of blood from naturally infected cattle containing $10^6$ trypanosomes/mL. Blood samples were collected once a week for eight consecutive weeks for analysing serum biochemical values (glucose, total cholesterol, total protein, albumin, and enzymes including GOT, GPT and ALP) using a Humastar 80 clinical chemistry analyser. In both inoculated groups, \textit{T. vivax} parasites caused an acute infection with parasites appearing in the circulation on six and 12 d post-infection for NT and TT cattle, respectively. A significant reduction ($p < 0.001$) in glucose levels was observed in infected groups compared with the control, with mean values of 33.8$\pm$3.6 mg/dL for TT, 34.3$\pm$3.6 mg/dL for NT and 70.9$\pm$3.0 mg/dL recorded for the control group. A similar reduction was also seen in total cholesterol values ($p = 0.001$) with values of 70.4$\pm$10.6 mg/dL recorded for TT Group and 78.0$\pm$10.6 mg/dL for NT group compared with 139.5$\pm$8.7 mg/dL for the control group. No difference was observed in total serum protein levels between the three groups ($p = 0.260$) whereas the mean albumin levels were significantly ($p < 0.001$) decreased (3.5$\pm$0.1 g/dL and 2.9$\pm$0.1 g/dL in TT and NT groups respectively) compared with that for control cattle (4.5$\pm$0.1 g/dL). On the other hand, infected groups had higher ALP values compared with the control ($p = 0.007$), with mean values of 538.4$\pm$64.4 IU/L, 564.9$\pm$64.4 IU/L and 273.2$\pm$52.6 IU/L for TT, NT and control cattle, respectively. In conclusion, the two \textit{T. vivax} parasites caused significant biochemical changes indicative of pathological responses. However, there was no significant variation between the two parasites in initiating these changes despite the difference in the onset of parasitaemia.


The aim of this study was to evaluate the relationship between testicular lesions and hormone levels in rats experimentally infected with \textit{Trypanosoma evansi}. Measurements of reproductive hormones, histopathology and biomarkers of cellular injury were carried out on 24 animals, which were divided into two groups with 12 animals each. Group A was the negative control, or uninfected, while group B was composed of animals infected with \textit{T.
Both groups were divided again into two subgroups (n = 6), from which serum and testicular fragments were collected on d 5 (A1 and B1) and d 15 (A2 and B2) post-infection (PI). The morphological analysis showed increased alterations of sperm head and tail in infected rats when compared with those of the control group. Significant reductions (p < 0.01) in the levels of LH, FSH, testosterone and estradiol, associated with an increase in cortisol, were recorded in the serum of group B animals when compared with the negative controls. Additionally, NOx, lipid peroxidation and protein oxidation were enhanced in testicles, indicating the occurrence of cellular lesions. On histopathology, it was possible to observe testicular degeneration, among other disorders in infected animals. Based on these results, it is possible to conclude that experimental infection with *T. evansi* caused changes in the levels of the main hormones of male rats associated with cellular injury.


Center for Electron Microscopy, Faculty of Sciences, Central University of Venezuela, Caracas, Venezuela. [hector.finol@gmail.com].

The ultrastructural study in different tissues of mice experimentally infected with isolates of *Trypanosoma evansi*, *Trypanosoma cruzi*, and *Leishmania mexicana* revealed changes in cardiac myocytes, skeletal muscle fibres, and hepatic, adrenal, kidney, and spleen cells. Some of these changes were cytoarchitectural and others consisted of necrosis. Alterations in the microvasculature were also found. The mononuclear cell infiltrate included neutrophils, eosinophils, and macrophages. This work shows that diverse mice tissues are important targets for trypanosomatids.


Departments of Biochemistry and Crop Protection, Ahmadu Bello University, Zaria, Nigeria; Cancer Research and Molecular Biology Laboratories, Department of Biochemistry, University of Ibadan, Ibadan, Oyo State, Nigeria; Department of Biochemistry and Molecular Biology, School of Biomedical Sciences, Monash University, 106 Wellington Road, Clayton Campus, Victoria 3800, Australia. [nathanhabila@yahoo.com].

The emergence of bone marrow micronucleated polychromatic erythrocytes (MN-PCEs) in rats experimentally infected with *Trypanosoma brucei brucei* was examined in order to understand the bone marrow effects in trypanosomiasis. Bone marrow was collected for micronucleus assay while blood samples were collected from infected rats for haematological analysis. The results showed evidence of MN-PCE at 12.75 +/- 0.65 micronuclei/ 1 000 PCE and 9.60 +/- 2.95 micronuclei/1 000 PCE for rats infected respectively for 21 d and 14 d. The haematological examination revealed changes in packed cell volume, haemoglobin and red blood cells with concomitant increase in parasitaemia. This study revealed that the generation of MN-PCEs was induced by an acute infection of *T. b. brucei* in rats and this highlights an important phase in the pathogenesis of the disease that may indicate possible damage to genetic information.

Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State 810001, Nigeria; Department of Biochemistry and Molecular Biology, Monash University, Clayton, Vic. 3800, Australia; Department of Biochemistry, Ahmadu Bello University Zaria, Kaduna State 810001, Nigeria. [nathanhabila@gmail.com].

The clastogenic effect of mixed infection of Trypanosoma evansi and Trypanosoma brucei brucei in the bone marrow (BM) cells of Wistar albino rats was investigated. Clastogenic effects were observed in the BM cells using the micronucleus assay. The findings indicate that T. evansi, T. b. brucei and mixed infection with both parasites increased the formation of micronucleated polychromatric erythrocyte (MN-PCEs) in the BM cells by 60, 63 and 81 micronuclei/1 000 PCE respectively (p < 0.05). Mixed infection induced increase in the formation of MN-PCEs by about 1.33 - fold when compared with single infections of T. b. brucei and T. evansi. These data give preliminary evidence of possible genotoxic effects in trypanosomiasis.


Department of Veterinary Parasitology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, 37 K.B. Sarani, Kolkata, 700037 West Bengal, India. [bal_epi@yahoo.co.in].

Twenty adult Swiss albino mice, 20 rats and 10 rabbits were experimentally infected with Trypanosoma evansi and killed at the peak of parasitaemia to know the period of survivability of T. evansi and degenerative changes of the parasite after death of these hosts. Examination of Giemsa stained blood smears and wet blood smears revealed the presence of parasites and live trypanosomes along with motility in the heart blood of mice and rats up to 14 h and in rabbits up to 13 h post death. Mouse inoculation test (MIT) conducted with heart blood up to 13 h post death of mice and rabbits became positive. The MIT with both heart blood and portal blood of rats became positive up to 14 h post death. The liver and lung impression smears could detect the parasites up to 14 h after death of mice and rats, and up to 13 h after death of rabbits, whereas spleen impression smears revealed the presence of parasites up to 12 h after the death of these animals. It is confirmed that T. evansi infection in animals may be diagnosed by demonstration of parasites after post mortem examination of hosts.


National Research Centre on Equines, Sirsa Road, Hisar 125001, Haryana, India; Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125004, Haryana,
Trypanosoma evansi is the causative agent of surra, which is the most common and widespread trypanosomal disease. The infection is mainly restricted to animals, but it has also been documented in humans. Trypanosomes possess a thick immunogenic surface coat known as variant surface glycoprotein (VSG). The parasite modifies the VSG constantly resulting in continuous antigenic variations and thus evades the host immune response. Due to antigenic variation, vaccination against trypanosomosis is not useful. Therefore, alternate strategies to augment the immune response are required. CpG-ODN class-C has the combined immune effects of both A and B classes of CpG-ODN. In this study, we observed that CpG-ODN class-C stimulated horse peripheral blood mononuclear cells (PBMC) to induce the expression of interferon-alpha (IFN-alpha), tumour necrosis factor-alpha (TNF-alpha), IL-12 and nitric oxide (NO), indicating an enhanced innate immune response. We have for the first time demonstrated that co-culture of CpG-ODN with *T. evansi* antigen, by inducing lymphocyte responses has a synergistic effect in eliciting the immune response.


Department of Cellular and Molecular Immunology, Vrije Universiteit Brussel (VUB), Brussels, Belgium; Myeloid Cell Immunology Laboratory, Vlaams Instituut voor Biotechnologie, Brussels, Belgium; Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut, USA; Unit of Veterinary Protozoology, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium. [bstijlem@vub.ac.be].

African trypanosomiasis is a chronic debilitating disease affecting the health and economic well-being of many people in developing countries. The pathogenicity associated with this disease involves a persistent inflammatory response, whereby M1-type myeloid cells, including Ly6C (high) inflammatory monocytes, are centrally implicated. A comparative gene analysis between trypanosusceptible and trypanotolerant animals identified MIF (macrophage migration inhibitory factor) as an important pathogenic candidate molecule. Using MIF-deficient mice and anti-MIF antibody treated mice, we show that MIF mediates the pathogenic inflammatory immune response and increases the recruitment of inflammatory monocytes and neutrophils to contribute to liver injury in *Trypanosoma brucei* infected mice. Moreover, neutrophil-derived MIF contributed more significantly than monocyte-derived MIF to increased pathogenic liver TNF production and liver injury during trypanosome infection. MIF deficient animals also featured limited anaemia, coinciding with increased iron bio-availability, improved erythropoiesis and reduced RBC clearance during the chronic phase of infection. Our data suggest that MIF promotes the most prominent pathological features of experimental trypanosome infections (i.e. anaemia and liver injury), and that prompt consideration be given to MIF as a novel target for treatment of trypanosomiasis-associated immunopathogenicity.

ApolipoproteinL1 (APOL1) protects humans and some primates against several African trypanosomes. APOL1 genetic variants strongly associated with kidney disease in African Americans have additional trypanolytic activity against *Trypanosoma brucei rhodesiense*, the cause of acute African sleeping sickness. We combined genetic, physiological, and biochemical studies to explore coevolution between the APOL1 gene and trypanosomes. We analysed the APOL1 sequence in modern and archaic humans and baboons along with geographic distribution in present day Africa to understand how the kidney risk variants evolved. Then, we tested Old World monkey, human, and engineered APOL1 variants for their ability to kill human infective trypanosomes *in vivo* to identify the molecular mechanism whereby human trypanolytic APOL1 variants evade *T. brucei rhodesiense* virulence factor serum resistance-associated protein (SRA). For one APOL1 kidney risk variant, a two-residue deletion of amino acids 388 and 389 causes a shift in a single lysine residue that mimics the Old World monkey sequence, which augments trypanolytic activity by preventing SRA binding. A second human APOL1 kidney risk allele, with an amino acid substitution that also restores sequence alignment with Old World monkeys, protected against *T. brucei rhodesiense* due in part to reduced SRA binding. Both APOL1 risk variants induced tissue injury in murine livers, the site of transgenic gene expression. Our study shows that both genetic variants of human APOL1 that protect against *T. brucei rhodesiense* have recapitulated molecular signatures found in Old World monkeys and raises the possibility that APOL1 variants have broader innate immune activity that extends beyond trypanosomes.

(c) CHEMOTHERAPEUTICS

[See also 37: 17249, 17250, 17261, 17262, 17263, 17264, 17396, 17413, 17414, 17416, 17418, 17428]

We have previously reported that curcumin analogues with a C7 linker bearing a C4-C5 olefinic linker with a single keto group at C3 (enone linker) display mid-nanomolar activity against the bloodstream form of *Trypanosoma brucei*. However, no clear indication of their mechanism of action, or of their superior antiparasitic activity relative to analogues with the original di-ketone curcumin linker was apparent. In order to further investigate their utility as antiparasitic agents, we here compared the cellular effects of curcumin and the enone linker lead compound, AS-HK014. An AS-HK014-resistant line, TA014, was developed by *in vitro* exposure to the drug. Metabolomic analysis revealed that exposure to AS-HK014, but not curcumin, rapidly depleted glutathione and trypanothione in the wild-type line, although almost all other metabolites were unchanged relative to control. In TA014 cells thiol levels were similar to untreated wild-type cells, and were not significantly depleted by AS-HK014. Adducts of AS-HK014 with both glutathione and trypanothione were identified in AS-HK014-exposed wild-type cells, and reproduced by chemical reaction. However, adduct accumulation in sensitive cells was much lower than in resistant cells. TA014 cells did not exhibit any changes in sequence or protein levels of glutathione synthetase and gamma-glutamylcysteine synthetase relative to wild-type cells. We conclude that mono-enone curcuminoids have a different mode of action than curcumin, rapidly and specifically depleting thiol levels in trypanosomes by forming an adduct. This adduct may ultimately be responsible for the highly potent trypanocidal activity of the mono-enone curcuminoids.


Northeastern University, Department of Chemistry and Chemical Biology, 417 Egan Research Center, 360 Huntington Avenue, Boston, MA 02115, USA; Marine Biological Laboratory, Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, 7 MBL Street, Woods Hole, MA 02543, USA. [m.pollastri@neu.edu].

A medicinal chemistry exploration of the human phosphodiesterase 4 (hPDE4) inhibitor cilomilast (1) was undertaken in order to identify inhibitors of phosphodiesterase B1 of *Trypanosoma brucei* (TbrPDEB1). *T. brucei* is the parasite which causes African sleeping sickness, a neglected tropical disease that affects thousands each year, and TbrPDEB1 has been shown to be an essential target of therapeutic relevance. Noting that cilomilast (1) is a weak inhibitor of TbrPDEB1, we report the design and synthesis of analogues of this compound, culminating in 12b, a sub-µM inhibitor of TbrPDEB1 that shows modest inhibition of *T. brucei* proliferation.

Targeted delivery of therapeutics is an alternative approach for the selective treatment of infectious diseases. The surface of African trypanosomes, the causative agents of African trypanosomiasis, is covered by a surface coat consisting of a single variant surface glycoprotein, termed VSG. This coat is recycled by endocytosis at a very high speed, making the trypanosome surface an excellent target for the delivery of trypanocidal drugs. Here, we report the design of a drug nanocarrier based on poly ethylene glycol (PEG) covalently attached (PEGylated) to poly (D,L-lactide-co-glycolide acid) (PLGA) to generate PEGylated PLGA nanoparticles. This nanocarrier was coupled to a single domain heavy chain antibody fragment (nanobody) that specifically recognizes the surface of the protozoan pathogen *Trypanosoma brucei*. Nanoparticles were loaded with pentamidine, the first-line drug for *T. b. gambiense* acute infection. An *in vitro* effectiveness assay showed a seven-fold decrease in the half-inhibitory concentration (IC$_{50}$) of the formulation relative to free drug. Furthermore, *in vivo* therapy using a murine model of African trypanosomiasis demonstrated that the formulation cured all infected mice at a 10-fold lower dose than the minimal full curative dose of free pentamidine and 60 percent of mice at a 100-fold lower dose. This nanocarrier has been designed with components approved for use in humans and loaded with a drug that is currently in use to treat the disease. Moreover, this flexible nanobody-based system can be adapted to load any compound, opening a range of new potential therapies with application to other diseases.


The Trypanosomatidae family, composed of unicellular parasites, causes severe vector-borne diseases that afflict human populations worldwide. Chagas disease, sleeping sickness, as well as different sorts of leishmaniasis are amongst the most important infectious diseases produced by *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania* spp., respectively. All these infections are closely related to weak health care services in low-income populations of less developed and least economically developed countries. Search for new therapeutic targets in order to hit these pathogens is of paramount priority, as no effective vaccine is currently in use against any of these parasites. Furthermore, present day chemotherapy comprises old-fashioned drugs full of important side effects. Besides, they are prone to produce tolerance and resistance as a consequence of their continuous use for decades. DNA topoisomerases (Top) are ubiquitous enzymes responsible for solving the torsional tensions caused during replication and transcription processes, as well as in maintaining genomic stability during DNA recombination. As the inhibition of these enzymes produces cell arrest and triggers cell death, Top inhibitors are among the most effective and most widely used drugs in both cancer and antibacterial therapies. Top relaxation and decatenation activities, which are based on a common nicking-closing cycle involving one or both DNA strands, have been described as a promising drug target. Specific inhibitors that bind to the interface of DNA-Top complexes can stabilize Top-mediated transient DNA breaks. In addition, important structural differences have been found between Tops from the Trypanosomatidae
family members and Tops from the host. Such dissimilarities make these proteins very interesting for drug design and molecular intervention. The present review is a critical update of the latest findings regarding the Tops of trypanosomatids, their new structural features, their involvement both in the physiology and virulence of these parasites, as well as their use as promising targets for drug discovery.


Zentrum fur Molekulare Biologie der Universität Heidelberg, DKFZ-ZMBH Alliance, Heidelberg, Germany. [d.begolo@zmbh.uni-heidelberg].

Elucidation of molecular targets is very important for lead optimization during the drug development process. We describe a direct method to find targets of antitrypanosomal compounds against *Trypanosoma brucei* using a trypanosome overexpression library. As proof of concept, we treated the library with difluoromethylornithine and DDD85646 and identified their respective targets, ornithine decarboxylase and N-myristoyltransferase. The overexpression library could be a useful tool to study the modes of action of novel antitrypanosomal drug candidates.


Division of Medicinal Chemistry, Faculty of Sciences, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), VU University Amsterdam, Amsterdam, The Netherlands. [r.leurs@vu.nl].

Methods to discover biologically active small molecules include target-based and phenotypic screening approaches. One of the main difficulties in drug discovery is elucidating and exploiting the relationship between drug activity at the protein target and disease modification, a phenotypic endpoint. Fragment-based drug discovery is a target-based approach that typically involves the screening of a relatively small number of fragment-like (molecular weight < 300) molecules that efficiently cover chemical space. Here, we report a fragment screening on TbrPDEB1, an essential cyclic nucleotide phosphodiesterase (PDE) from *Trypanosoma brucei*, and human PDE4D, an off-target, in a workflow in which fragment hits and a series of close analogues are subsequently screened for antiparasitic activity in a phenotypic panel. The phenotypic panel contained *T. brucei*, *T. cruzi*, *L. infantum*, and *P. falciparum*, the causative agents of human African trypanosomiasis (sleeping sickness), Chagas disease, leishmaniasis, and malaria, respectively, as well as MRC-5 human lung cells. This hybrid screening workflow resulted in the discovery of various benzhydryl ethers with antiprotozoal activity and low toxicity, representing interesting starting points for further antiparasitic optimization.


Laboratorio de Síntesis Orgánica, Escuela de Química, Universidad Industrial de Santander, A.A. 678, Bucaramanga, Santander, Colombia. [apalma@uis.edu.co].


Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Sir James Black Centre, Dundee DD1 5EH, U.K.


Department of Immunology and Infection, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK. [theresa.ward@lshtm.ac.uk].

The objective of this study was to optimize the *Trypanosoma brucei brucei* GVR35 VSL-2 bioluminescent strain as an innovative drug evaluation model for late-stage human African trypanosomiasis. An IVIS® lumina II imaging system was used to detect bioluminescent *T. b. brucei* GVR35 parasites in mice to evaluate parasite localization and disease progression. Drug treatment was assessed using qualitative bioluminescence imaging and real-time quantitative PCR (qPCR). We have shown that drug dose-response can be evaluated using bioluminescence imaging and confirmed quantification of tissue parasite load using qPCR. The model was also able to detect drug relapse earlier than the traditional blood film detection and even in the absence of any detectable peripheral parasites. In conclusion, we have developed and optimized a new, efficient method to evaluate novel anti-trypanosomal drugs *in vivo* and reduce the current 180-d drug relapse experiment to a 90-d model. The non-invasive *in vivo* imaging model reduces the time required to assess preclinical efficacy of new anti-trypanosomal drugs.

To investigate the effect of diminazene aceturate (DA) alone or in combination with either levamisole and/or vitamin C in albino rats experimentally infected with Trypanosoma brucei brucei, 30 adult male albino rats, randomly assigned into 6 groups (A-F) of five rats each were used. They were either infected with 1x10⁶ trypanosomes intraperitoneally (groups A-E) or uninfected (group F). The different groups were treated respectively as follows: group A—with 3.5 mg/kg DA; group B—with 3.5 mg/kg DA and 7.5 mg/kg levamisole; group C—with 3.5 mg/kg DA and 100 mg/kg vitamin C; and group D—with 3.5 mg/kg DA and 7.5 mg/kg levamisole and 100 mg/kg vitamin C. Group E was left untreated. Parameters assessed included: rectal temperature, body weight changes, packed cell volume (PCV), haemoglobin concentration (Hb), total leucocyte count (TLC), differential leucocyte count (DLC), parasitaemia, clinical signs and survivability. An average pre-patent period of five d was recorded. Parasites in the blood were cleared in all treated groups (A-D) within 48 h post-treatment (PT). Untreated rats in group E died between 25 and 32 d post-infection (PI). Relapse was not recorded in all the treated groups (A-D). The initial reduction in PCV, Hb, TLC and increases in rectal temperature following infection were reversed by the treatments. The rats that received drug combinations (groups B, C and D) showed faster and higher recovery rates than the uninfected control and group A. It is concluded that levamisole and/or vitamin C in combination with DA was more effective in the treatment of rats infected with Trypanosoma brucei brucei.


tomography) after intravenous and oral administration in rats. A total of 60 male Sprague Dawley rats was examined. Each $^{[123]}$I-labelled substance ($n = 3$) was given to 12 rats ($n = 6$ i.v. and $n = 6$ orally). In two additional test series both $^{[123]}$I iodopentamidine ($n = 6$) and N,N'-bis(succinylxylo)-$^{[123]}$I iodopentamidine ($n = 6$) were administered orally together with the non-radioactive homologues. To evaluate the in vivo stability of the labelled compounds, $^{[123]}$I NaI solution was administered intravenously ($n = 6$) and orally ($n = 6$). In vivo SPECT images were acquired after 30 min, four h, and 24 h and blood samples were taken over 24 h. The SPECT images were fusioned with previously acquired magnetic resonance images. After the last SPECT the rats were perfused, sacrificed and the organ gamma-radiation levels were determined with a gamma counter. Analysis and quantification of the reconstructed SPECT images were performed using the region of interest technique. The data showed a highly improved oral bioavailability of the $^{[123]}$I-labelled pro-drugs compared with $^{[123]}$I-labelled pentamidine. While $^{[123]}$I iodopentamidine was mainly eliminated renally, the pro-drugs were metabolized primarily in the liver and underwent biliary elimination. Considering pentamidine's nephrotoxicity, this feature has to be seen as an advantage of the pro-drug principle. Moreover, a significantly higher concentration in the brain was detected after intravenous injection of N,N'-dihydroxy($^{[123]}$I) iodopentamidine compared with $^{[123]}$I iodopentamidine. The feasibility of an effective treatment of second stage African trypanosomiasis, in which the parasites already infect the brain, with the pro-drugs investigated here remains to be clarified with infected animals in additional in vivo studies.


School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland. [philippe.christen@unige.ch].


Instituto de Parasitologia y Biomedicina "Lopez-Neyra" Consejo Superior de Investigaciones Cientificas, Granada, Spain. [miguel.navarro@ipb.csic.es].

In the interest of identification of new kinase-targeting chemotypes for target and pathway analysis and drug discovery in Trypanosoma brucei, a high-throughput screen of 42 444 focused inhibitors from the GlaxoSmithKline screening collection was performed against parasite cell cultures and counter-screened against human hepatocarcinoma (HepG2) cells. In this way, we have identified 797 sub-µM inhibitors of T. brucei growth that are at least 100-fold selective over HepG2 cells. Importantly, 242 of these hit compounds acted rapidly in inhibiting cellular growth and 137 showed rapid cidality. A variety of in silico and in vitro physicochemical and drug metabolism properties were assessed, and human kinase selectivity data were obtained. Based on these data, we prioritized three compounds for pharmacokinetic assessment and demonstrated parasitological cure of a murine bloodstream infection of T.
brucei rhodesiense with one of these compounds (NEU-1053). This work represents the successful implementation of a unique industrial-academic collaboration model aimed at identification of high quality inhibitors that will provide the parasitology community with chemical materials that can be utilized to develop kinase-targeting compounds. Furthermore, these results are expected to provide rich starting points for discovery of kinase-targeting tool compounds for T. brucei, and new HAT therapeutics discovery programmes.


This study aimed to verify the effect of the treatment with A. satureioides essential oil (free and nanoencapsulated forms) and diminazene aceturate on the haematological and biochemical variables in rats infected by Trypanosoma evansi. The 56 rats were divided into seven groups with eight rats each. Groups A, C and D were composed of uninfected animals, and groups B, E, F and G were formed by rats infected with T. evansi. Rats from groups A and B were used as negative and positive controls, respectively. Rats from the groups C and E were treated with A. satureioides essential oil, and groups D and F were treated with A. satureioides nanoencapsulated essential oil. Groups C, D, E and F received one dose orally of oil (1.5 mL kg⁻¹) during five consecutive d. Group G was treated with one therapeutic dose of diminazene aceturate (DA) (3.5 mg kg⁻¹). Blood samples were collected on d 5 post infection for analyses of haematological (erythrocytes and leukocytes count, haemoglobin concentration, haematocrit, mean corpuscular and mean corpuscular haemoglobin concentration) and biochemical (glucose, triglycerides, cholesterol, alanine aminotransferase [ALT], aspartate aminotransferase [AST], albumin, urea and creatinine) variables. The administered A. satureioides was able to maintain low parasitaemia, mainly the nanoencapsulated form, at 5 d post infection. In the infected animals with T. evansi treated with A. satureioides essential oil (free and nanocapsules) the number of total leucocytes, lymphocytes and monocytes present was similar to uninfected rats, and different from infected and untreated animals (leukocytosis). Treatment with A. satureioides in the free form elevated levels of ALT and AST, demonstrating liver damage; however, treatment with the nanoencapsulated form did not cause elevation of these enzymes. Finally, treatments inhibited the increase in creatinine levels caused by infection for T. evansi. In summary, the nanoencapsulated form showed better activity on the trypanosome; it did not cause liver toxicity and prevented renal damage.

Chemical Theory & Computation, 10 (11): 5047-5056.

Department of Chemistry & Biochemistry, University of California San Diego, La Jolla, California 92093, USA; National Biomedical Computation Resource, Center for Research in Biological Systems, University of California San Diego, La Jolla, California 92093, USA.

Analysis of macromolecular/small-molecule binding pockets can provide important insights into molecular recognition and receptor dynamics. Since its release in 2011, the POVME (POcket Volume MEasurer) algorithm has been widely adopted as a simple-to-use tool for measuring and characterizing pocket volumes and shapes. We here present POVME 2.0, which is an order of magnitude faster, has improved accuracy, includes a graphical user interface, and can produce volumetric density maps for improved pocket analysis. To demonstrate the utility of the algorithm, we use it to analyse the binding pocket of RNA editing ligase 1 from the unicellular parasite Trypanosoma brucei, the aetiological agent of African sleeping sickness. The POVME analysis characterizes the full dynamics of a potentially druggable transient binding pocket and so may guide future antitrypanosomal drug discovery efforts. We are hopeful that this new version will be a useful tool for the computational and medicinal chemist communities.


Center for Neglected Diseases Drug Discovery (CND3), Institut Pasteur Korea, Seongnam-si, Gyeonggi-do, South Korea; Parasite Disease Group, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), Campinas-SP, Brazil. [lucio.freitasjunior@lnbio.cnpem.br].

Human African trypanosomiasis (HAT) is a vector-transmitted tropical disease caused by the protozoan parasite Trypanosoma brucei. High-throughput screening (HTS) of small molecule libraries in whole cell assays is one of the most frequently used approaches in drug discovery for infectious diseases. To aid in drug discovery efforts for HAT, the SYBR green assay was developed for T. brucei in a 384-well format. This semi-automated assay is cost- and time-effective, robust, and reproducible. The SYBR green assay was compared with the resazurin assay by screening a library of 4 000 putative kinase inhibitors, revealing a superior performance in terms of assay time, sensitivity, simplicity, and reproducibility, and resulting in a higher hit confirmation rate. Although the resazurin assay allows for comparatively improved detection of slow-killing compounds, it also has higher false positive rates that are likely to arise from the assay experimental conditions. The compounds with the most potent antitrypanosomal activity were selected in both screens and grouped into 13 structural clusters, with 11 new scaffolds as antitrypanosomal agents. Several of the identified compounds had IC_{50} values < 1 µM coupled with high selectivity toward the parasite. The core structures of the scaffolds are shown, providing promising new starting points for drug discovery for HAT.

Neglected tropical diseases remain a serious global health concern. Here, a series of novel bis-tetrahydropyran 1,4-triazole analogues based on the framework of chamuvarinin, a polyketide natural product isolated from the Annonaceae plant species are detailed. The analogues synthesized display low µM trypanocidal activities towards both bloodstream and insect forms of Trypanosoma brucei, the causative agent of African sleeping sickness, also known as human African trypanosomiasis (HAT). A divergent synthetic strategy was adopted for the synthesis of the key tetrahydropyran intermediates to enable rapid access to diastereochemical variation either side of the 1,4-triazole core. The resulting diastereomeric analogues displayed varying degrees of trypanocidal activity and selectivity in structure-activity relationship studies. Together, the biological potency and calculated lipophilicity values indicate that while there is room for improvement, these derivatives may represent a promising novel class of anti-HAT agents.

This study aimed to evaluate in vitro and in vivo trypanocidal activity of free and nanoencapsulated curcumin against Trypanosoma evansi. In vitro efficacy of free curcumin (CURC) and curcumin-loaded in lipid-core nanocapsules (C-LNCs) was evaluated to verify their lethal effect on T. evansi. To perform the in vivo tests, T. evansi-infected animals were treated with CURC (10 and 100 mg kg⁻¹, intraperitoneally [i.p.]) and C-LNCs (10 mg kg⁻¹, i.p.) during six d, with the results showing that these treatments significantly attenuated the parasitaemia. Infected untreated rats showed protein peroxidation and an increase of nitrites/nitrates, whereas animals treated with curcumin showed a reduction in these variables. As a result, the activity of antioxidant enzymes (superoxide dismutase and catalase) differed between groups (p < 0.05). Infected animals treated with CURC exhibited reductions in the levels of alanine aminotransferase and creatinine, when compared with the positive control group. The use of curcumin in vitro resulted in a better parasitaemia control, an antioxidant activity and a protective effect on liver and kidney functions of T. evansi-infected adult male Wistar rats.

A series of novel 4-thiazolidinone-pyrazoline conjugates was synthesized and tested for anti-*Trypanosoma brucei* activity. Screening data allowed us to identify five thiazolidinone-pyrazoline hybrids, which possess promising trypanocidal activity, with IC$_{50}$ values $\leq 1.2$ µM. The highest active thiazolidinone-pyrazoline conjugates 3c and 6b (IC$_{50}$ values of 0.6 µM and 0.7 µM, respectively) were six times more potent antitrypanosomal agents than nifurtimox. In addition, these compounds, as well as 6d and 6e had selectivity indices higher than 50, and were more selective than nifurtimox. SAR study included substituent variations at the pyrazoline moiety, modifications of the N3 position of the thiazolidinone portion, elongation of the linker between the heterocycles, as well as rhodanine-isorhodanine isomerism. It was also shown that methyl or aryl substitution at the thiazolidinone N3-position is crucial for trypanocidal activity.


Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halysky Lviv National Medical University, Ukraine, Pekarska 69, Lviv 79010, Ukraine. [roman.lesyk@gmail.com].

Treatment of trypanosomiasis urgently requires new effective and non-toxic drugs. This article covers some of the achievements in the search for new antitrypanosomal agents; also, the "validated" biological targets used in the design of antitrypanosomal agents are outlined. The major part of the manuscript focuses on synthetic small molecules, such as thiosemicarbazone and thiazole (as their cyclic analogues) derivatives, benzofuran derivatives, and heterocycles bearing nitro group, etc. Also, the attractiveness of metal complexes and well known drugs as sources for antitrypanosomal agent design is discussed.


Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India.


Instituto de Quimica Medica, IQM-CSIC, Madrid, Spain. [dardonville@iqm.csic.es]

Treatment of late-stage sleeping sickness requires drugs that can cross the blood-brain barrier (BBB) to reach the parasites located in the brain. We report here the synthesis and evaluation of four new N-hydroxy and twelve new N-alkoxy derivatives of bisimidazoline
leads as potential agents for the treatment of late-stage sleeping sickness. These compounds, which are less basic than the parent leads (i.e. are less ionized at physiological pH), were evaluated in vitro against T. brucei rhodesiense and in vivo in murine models of first- and second-stage sleeping sickness. Resistance profile, physicochemical parameters, in vitro BBB permeability, and microsomal stability were also determined. The N-hydroxy imidazoline analogues were the most effective in vivo with 4-((1-hydroxy-4,5-dihydro-1H-imidazol-2-yl)amino)-N-(4-((1-hydroxy-4,5-dihydro-1H-imidazol-2-yl)amino)phenyl)benzamide (14D:) showing 100 percent cures in the first-stage disease, whilst 15D:, 16D: and 17D: appeared to slightly improve survival. In addition, 14D: showed weak activity in the chronic model of CNS infection in mice. No evidence was found of reduction of this compound with hepatic microsomes and mitochondria in vitro suggesting that N-hydroxy imidazolines are metabolically stable and have intrinsic activity against T. brucei. In contrast to its unsubstituted parent compound, uptake of 14D: in T. brucei was independent of known drug transporters (i.e. TbAT1/P2 and HAPT) indicating a lower predisposition to cross-resistance with other diamidines and arsenical drugs. Hence, the N-hydroxy bisimidazolines (14D: in particular) represent a new class of promising antitrypanosomal agents.


Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

The effect of aqueous extracts of Acacia albida stem bark was investigated in Wistar albino rats infected with Trypanosoma evansi. The extracts showed highest reduction in parasitaemia at the dose of 600 mg/kg bw. A dose of 300 mg/kg bw improved packed cell volume the most (by 14.35 percent). The group treated with 150 and 600 mg/kg bw of the extracts showed significant decreases (p < 0.05) in alanine transaminase and aspartate transaminase levels which were lower than those of the group treated with diminazene aceturate. The group treated with 150 mg/kg bw extract showed the lowest urea, albumin and protein levels and the lowest relative organ weight. There were significant differences (p < 0.05) in the levels of catalase and thiobarbituric acid reactive substances in liver and kidney of the animals in the infected-untreated group and the extract-treated groups. The results of this study show that extracts of A. albida have antitrypanosomal activity against T. evansi infection.


Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Brazil. [camilabelmontevet@yahoo.com.br].

**Tsetse and Trypanosomosis Information**

*Journal of Medicinal Chemistry, 87: 79-88.*

NorthShore University HealthSystem, Evanston, IL, USA. [mpapadopoulou@northshore.org].


Laboratorium für Organische Chemie, ETH Zurich, Vladimir-Prelog-Weg 3, 8093 Zurich, Switzerland. [Reto.Brun@unibas.ch].


Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynska dolina CH-2, 842 15 Bratislava, Slovakia. [kubinec@fns.uniba.sk].


Laboratory of Molecular Physiology, Institute of Experimental Medicine, School of Medicine Luis Razetti, Faculty of Medicine, Universidad Central de Venezuela, Caracas, Venezuela. [aiponte@gmail.com].


Instituto de Quimica Medica, IQM-CSIC, Juan de la Cierva 3, E-28006 Madrid, Spain. [dardonville@iqm.csic.es].

Two series of N-alkyl, N-alkoxy, and N-hydroxy bisguanidines derived from the N-phenylbenzamide and 1,3-diphenylurea scaffolds were synthesized in three steps from the corresponding 4-amino-N-(4-aminophenyl)benzamide and 1,3-bis(4-aminophenyl)urea, respectively. All of the new compounds were evaluated in *vitro* against *T. b. rhodesiense* (STIB900) trypomastigotes and *Plasmodium falciparum* NF54 parasites (erythrocytic stage). N-alkoxy and N-hydroxy derivatives showed weak μM range IC₅₀ values against *T. b. rhodesiense* and *P. falciparum* whereas the N-alkyl analogues displayed sub μM and low nano M IC₅₀ values against *P. falciparum* and *Trypanosoma brucei*, respectively. Two compounds, 4-(2-ethylguanidino)-N-(4-(2-ethylguanidino)phenyl)benzamide dihydrochloride...
(7b) and 4-(2-isopropylguanidino)-N-(4-(2-isopropylguanidino)phenyl)benzamide dihydrochloride (7c), which showed favourable drug-like properties and in vivo efficacy (100 percent cures) in the STIB900 mouse model of acute human African trypanosomiasis represent interesting leads for further in vivo studies. The binding of these compounds to AT-rich DNA was confirmed by surface plasmon resonance (SPR) biosensor experiments.


Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow, G4 0NR, UK. [d.g.watson@strath.ac.uk].

Propolis is increasingly being explored as a source of biologically active compounds. Until now, there has been no study of Libyan propolis. Two samples were collected in North East Libya and tested for their activity against *Trypanosoma brucei*. Extracts from both samples had quite high activity. One of the samples was fractionated and yielded a number of active fractions. Three of the active fractions contained single compounds, which were found to be 13-epitorulosal, acetyl-13-epi-cupressic acid and 13-epi-cupressic acid, which have been described before in Mediterranean propolis. Two of the compounds had a minimum inhibitory concentration value of 1.56 µg/mL against *T. brucei*. The active fractions were also tested against macrophages infected with *Leishmania donovani*, and again moderate to strong activity was observed with the compounds having IC₅₀ values in the range 5.1-21.9 µg/mL.


Department of Pharmacy, University Gamal Abdel Nasser of Conakry, Guinea.

Based on an ethnobotanical survey, 41 Guinean plant species widely used in the traditional treatment of fever and/or malaria were collected. From these, 74 polar and apolar extracts were prepared and tested for their in vitro antiprotozoal activity along with their cytotoxicity on MRC-5 cells. A potent activity (IC₅₀ < 5 µg/mL) was observed for *Terminalia albida*, *Vismia guineensis*, *Spondias mombin*, and *Pavetta crassipes* against *Plasmodium falciparum*; for *Pavetta crassipes*, *Vismia guineensis*, *Guiiera senegalensis*, *Spondias mombin*, *Terminalia macroptera*, and for *Combretum glutinosum* against *Trypanosoma brucei*; for *Bridelia ferruginea*, *G. senegalensis*, *V. guineensis*, *P. crassipes*, and *C. glutinosum* against *Trypanosoma cruzi*. Only the extract of *Tetracera alnifolia* showed a good activity (IC₅₀ 8.1 µg/mL) against *Leishmania infantum*. The selectivity index of the active samples varied from 0.08 to > 100. These results may validate at least in part the traditional use of some of the plant species.


Faculdade de Ciencias Farmaceuticas, Universidade de Sao Paulo, Av. Lineu Prestes,
Tsetse and Trypanosomosis Information

580, 05508-000 Sao Paulo, Brazil. [trossini@usp.br].


Faculty of Pharmaceutical Sciences, Nagasaki International University, Sasebo, Nagasaki 859-3298, Japan.


Drug Discovery Unit, College of Life Sciences, University of Dundee, Sir James Black Centre, Dow Street, Dundee DD1 5EH, UK. [p.g.wyatt@dundee.ac.uk]

Glycogen synthase kinase 3 (GSK3) is a genetically validated drug target for human African trypanosomiasis (HAT), also called African sleeping sickness. We report the synthesis and biological evaluation of aminopyrazole derivatives as Trypanosoma brucei GSK3 short inhibitors. Low nM inhibitors, which had high selectivity over the off-target human CDK2 and good selectivity over human GSK3beta enzyme, were prepared. These potent kinase inhibitors demonstrated low µM levels of inhibition of the Trypanosoma brucei parasite grown in culture.


Laboratory of Medicinal Chemistry, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium. [Koen.Augustyns@uantwerpen.be].

The presence of a structural recognition motif for the nucleoside P2 transporter in a library of pyrimidine and triazine non-nucleoside HIV-1 reverse transcriptase inhibitors prompted the evaluation of antitrypanosomal activity. It was demonstrated that the structure-activity relationship for anti-HIV and antitrypanosomal activity was different. Optimization in the diaryl triazine series led to 6-(mesityloxy)-N2-phenyl-1,3,5-triazine-2,4-diamine (69), a compound with potent in vitro and moderate in vivo antitrypanosomal activity.


Medical Parasitology & Infection Biology, Swiss Tropical and Public Health
African sleeping sickness is a neglected tropical disease transmitted by tsetse flies. New and better drugs are still needed especially for its second stage, which is fatal if untreated. 28DAP010, a dipryridylbenzene analogue of DB829, is the second simple diamidine found to cure mice with central nervous system infections by a parenteral route of administration. 28DAP010 showed efficacy similar to that of DB829 in dose-response studies in mouse models of first- and second-stage African sleeping sickness. The in vitro time to kill, determined by microcalorimetry, and the parasite clearance time in mice were shorter for 28DAP010 than for DB829. No cross-resistance was observed between 28DAP010 and pentamidine on the tested Trypanosoma brucei gambiense isolates from melarsoprol-refractory patients. 28DAP010 is the second promising preclinical candidate among the diamidines for the treatment of second-stage African sleeping sickness.


Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH, USA. [werbovetz.1@osu.edu].

N1-benzylated dihydroquinolin-6-ols and their corresponding esters display exceptional activity against African trypanosomes in vitro, and administration of members of this class of compound to trypanosome-infected mice results in cures in a first-stage African trypanosomiasis model. Since a quinone imine intermediate has been implicated in the antiparasitic mechanism of action of these compounds, evaluation of the hepatotoxic, mutagenic, and methaemoglobin-promoting effects of these agents was performed. 1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride and 1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate showed outstanding in vitro selectivity for Trypanosoma brucei compared with the HepG2, Hep3B, Huh7, and PLC5 hepatocyte cell lines. 1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-ol hydrochloride and 1-(2-methoxybenzyl)-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate were not mutagenic when screened in the Ames assay, with or without metabolic activation. The latter two compounds promoted time- and dose-dependent formation of methaemoglobin when incubated in whole human blood, but such levels were below those typically required to produce symptoms of methaemoglobinemia in humans. Although compounds capable of quinone imine formation require careful evaluation, these in vitro studies indicate that antitrypanosomal dihydroquinolines merit further study as drug candidates against the neglected tropical disease human African trypanosomiasis.


Department of Pharmacognosy, Institute of Pharmaceutical Sciences, University of Graz, Universitatsplatz 4/1, A-8010 Graz, Austria. [abraham.wube@uni-graz.at].

A diverse array of 4-(1H)-quinolone derivatives bearing substituents at positions 1 and
2 were synthesized and evaluated for antiprotozoal activities against *Plasmodium falciparum* and *Trypanosoma brucei rhodesiense*, and cytotoxicity against L-6 cells *in vitro*. Furthermore, selectivity indices were also determined for both parasites. All compounds tested showed antimalarial activity at low µM concentrations, with varied degrees of selectivity against L-6 cells. Compound 5a was found to be the most active against *P. falciparum*, with an IC\textsubscript{50} value of 90 nM and good selectivity for the malarial parasite compared to the L-6 cells. Compound 10a, on the other hand, showed a strong antitrypanosomal effect with an IC\textsubscript{50} value of 1.25 µM. In this study side chain diversity was explored by varying the side chain length and substitution pattern on the aliphatic group at position-2 and a structure-antiprotozoal activity study revealed that the aromatic ring introduced at C-2 contributed significantly to the antiprotozoal activities.


Department of Pharmaceutical Chemistry, School of Pharmacy, The University of Kansas, Lawrence, Kansas, USA. [michael.wang@ku.edu].

Eflornithine (alpha-difluoromethylornithine) has been used to treat second-stage (or meningoencephalitic-stage) human African trypanosomiasis and currently is under clinical development for cancer prevention. In this study, a new ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS)-based assay was developed and validated for the quantification of eflornithine in rat brain. To improve chromatographic retention and MS detection, eflornithine was derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate for five min at room temperature prior to injection. Derivatized eflornithine was separated on a reverse-phase C18 UPLC column with a six-min gradient; elution occurred at approximately 1.5 min. Prior to derivatization, eflornithine was reproducibly extracted from rat brain homogenate by methanol protein precipitation (~70 percent recovery). Derivatized eflornithine was stable in the autosampler (6°C) for at least 24 h. This new assay had acceptable intra- and inter-day accuracy and precision over a wide dynamic range (5 000-fold) and excellent sensitivity with a lower limit of quantification of 0.1 µM (18 ng/mL) using only 10 µL of rat brain homogenate. The validated eflornithine assay was applied successfully to determine eflornithine distribution in different regions of rat brain in an *in situ* rat brain perfusion study.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES


Department of Laboratory Medicine, Delhi State Cancer Institute, New Delhi, India.

Parasite cultivation techniques constitute a substantial segment of present-day study of parasites, especially of protozoa. Success in establishing *in vitro* and *in vivo* culture of parasites not only allows their physiology, behaviour and metabolism to be studied dynamically, but also allows the nature of the antigenic molecules in the excretory and secretory products to be vigorously pursued and analysed. The complex life cycles of various
parasites having different stages and host species requirements, particularly in the case of parasitic helminths, often make parasite cultivation an uphill assignment. Culturing of parasites depends on the combined expertise of all types of microbiological cultures. Different parasites require different cultivation conditions such as nutrients, temperature and even incubation conditions. Cultivation is an important method for diagnosis of many clinically important parasites, for example, Entamoeba histolytica, Trichomonas vaginalis, Leishmania spp., Strongyloides stercoralis and free-living amoebae. Many commercial systems like InPouch TV for T. vaginalis, microaerophilous stationary phase culture for Babesia bovis and Harada-Mori culture technique for larval-stage nematodes have been developed for the rapid diagnosis of the parasitic infections. Cultivation also has immense utility in the production of vaccines, testing vaccine efficacy, and antigen production for obtaining serological reagents, detection of drug-resistance, screening of potential therapeutic agents and conducting epidemiological studies. Though in vitro cultivation techniques are used more often compared with in vivo techniques, the in vivo techniques are sometimes used for diagnosing some parasitic infections such as trypanosomiasis and toxoplasmosis. Parasite cultivation continues to be a challenging diagnostic option. This review provides an overview of the intricacies of parasitic culture and an update on popular methods used for cultivating parasites.

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

[See also 37: 17305, 17306]


Universidad Simon Bolivar, Departamento de Biologia Celular, Caracas, Venezuela. [jbubis@usb.ve].

Salivarian trypanosomes sequentially express only one variant surface glycoprotein (VSG) on their cell surface from a large repertoire of VSG genes. Seven cryopreserved animal trypanosome isolates known as TeAp-Elfrio01, TEVA1 (or TeAp-N/D1), TeGu-N/D1, TeAp-Mantecal01, TeGu-TerecayTrino, TeGu-Terecay03 and TeGu-Terecay323, which had been isolated from different hosts identified in several geographical areas of Venezuela were expanded using adult albino rats. Soluble forms of predominant VSGs expressed during the early infection stages were purified and corresponded to concanavalin A-binding proteins with molecular masses of 48-67 kDa by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and pI values between 6.1 and 7.5. The biochemical characterization of all purified soluble VSGs revealed that they were dimers in their native form and represented different gene products. Sequencing of some of these proteins yielded peptides homologous to VSGs from Trypanosoma (Trypanozoon) brucei and Trypanosoma (Trypanozoon) evansi and established that they most likely are mosaics generated by homologous recombination. Western blot analysis showed that all purified VSGs were cross-reacting antigens that were recognized by sera from animals infected with either T. evansi or Trypanosoma (Dutonella) vivax. The VSG glycosyl-phosphatidylinositol cross-reacting determinant epitope was only partially responsible for the cross-reactivity of the purified proteins, and antibodies appeared to recognize cross-reacting conformational epitopes from
the various soluble VSGs. ELISA experiments were performed using infected bovine sera collected from cattle in a Venezuelan trypanosome-endemic area. In particular, soluble VSGs from two trypanosome isolates, TeGu-N/D1 and TeGu-TeracayTrino, were recognized by 93.38 percent and 73.55 percent of naturally T. vivax-infected bovine sera, respectively. However, approximately 70 percent of the sera samples did not recognize all seven purified proteins. Hence, the use of a combination of various VSGs for the diagnosis of animal trypanosomosis is recommended.


Kenya Agricultural Research Institute - Trypanosomiasis Research Centre (KARI-TRC), Kikuyu, Kenya; Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland. [gmurilla@yahoo.co.uk].

The cryobank contains 2 347 stabilates, including 1 747 primary isolates, out of which there are 42 mixed infections and one miscellaneous Herpetomonas muscorum, and 600 derivatives, including six mixed infections. Primary isolates were collected mainly from countries in the eastern Africa region, including Kenya, Uganda, Tanzania, Sudan, and Ethiopia. However, collections or donations from countries outside the region, including Nigeria, Mozambique, Botswana, Germany, and South America, have been added as part of collaborations between KARI-TRC and other institutions around the world. The stabilates were isolated between 1934 and 2010. The majority of the stabilates were recovered between 1960 and 1970, the same period when some of the worst epidemics occurred, after which the numbers added have been on the decline. The period from 1940 to 1949 coincided with World War II, when the work on trypanosomiasis research and control stalled: the laboratories in eastern Africa that were the source of isolates were closed, only to resume after 1945 when the war came to an end. An electronic database has been developed for the existing data and can be accessed through the KARI website (www.kari.org), which is currently being updated, and the WIPO Re:Search website (www.wipo.int/research/en/partnership/). The data are categorized into human, animal, tsetse fly derived, and isolates characterized by drug sensitivity and molecular techniques. The data contained in the KETRI cryobank, including (i) history of isolates, (ii) diversity of localities and of sample sources, (iii) size, (iv) published and unpublished information on the stabilates, and (v) availability of T. b. gambiense stabilates infective for laboratory Swiss white mice, make it a unique reference research facility on trypanosomiasis. This collection has potential uses in the development and validation of drugs, vaccines, diagnostics, and interrogation of biological phenomenon such as treatment failures. Studies on the effect of storage on the characteristics of the trypanosomes collected over time have been initiated. Scientists wishing to collaborate and/or enter into partnership on the use of the biospecimens at the KETRI biobank should contact the Centre directly or through the WIPO Re:Search website (www.wipo.int/research/en/partnership/) for details. This data is published in anticipation that it will attract potential partners and collaborators to invest in this facility and make it self-
sustaining. It is anticipated that other institutions working in trypanosomiasis-endemic areas will be encouraged to isolate and cryopreserve parasites during regular surveillance and control of African trypanosomiasis for future research and to avoid loss of vital biological information.


Molecular Parasitology and Entomology Unit, Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon; Departments of Animal Biology and Physiology, and the Parasitology and Ecology Laboratory, Faculty of Science, University of Yaoundé 1, P.O. Box 812, Yaoundé, Cameroon; Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon; Faculty of Medicine and Biomedical Science, University of Yaoundé 1, P.O. Box 1364, Yaoundé, Cameroon. [gsimoca@yahoo.fr].

Genetic variation of microsatellite loci is a widely used method for the analysis of population genetic structure of several organisms. To improve our knowledge on the population genetics of trypanosomes, Trypanosoma congolense forest and savannah types were identified in the mid-guts of Glossina palpalis palpalis caught in five villages of Fontem in the South-West region of Cameroon. From the positive samples of Trypanosoma congolense forest, the genetic diversity and the population genetic structure of these parasites were evaluated. Microscopic examination revealed 25 (1.31 percent) mid-gut infections with trypanosomes while the PCR method identified 120 (6.3 percent) infections due to Trypanosoma congolense: 94 (78.33 percent) Trypanosoma congolense forest and 28 (21.77 percent) Trypanosoma congolense savannah. The trypanosome infection rates varied significantly between villages and years of capture. Menji recorded the highest infection rate (15.11 percent); and samples captured in 2009 were more infected (14.33 percent). The microsatellite markers revealed a genetic variability between the Trypanosoma congolense forest populations of Fontem villages and 6.38 percent of mixed infections due to different genotypes of T. congolense “forest type”. The results of this study have shown that the infection rates of T. congolense vary significantly between the populations of tsetse flies of different villages of Fontem as well as the years of capture. The microsatellite markers showed a low genetic variability within Fontem samples. Our results on the population genetics of T. congolense forest favour a predominant clonal reproduction of these parasites. No sub-structuring was observed between subpopulations of different villages of Fontem although with DRC samples, a perfect structuring was observed, certainly due to geographical distance between the two sampling areas. Our results also show that T. congolense forest caused higher rates of infection than T. congolense savannah. The low infection rate of T. congolense savannah is probably due to the level of pathogenicity of this trypanosome subspecies. Indeed, it has been shown that Zebu cattle infected by T. congolense savannah presented a severe syndrome, which led inexorably to death within four to seven weeks post-infection if no treatment was administered. This probably occurs in the Fontem area since the inhabitants of this area do not use trypanocidal drugs to prevent AAT. This would mean that the number of animals infected by T. congolense savannah and on which tsetse flies can become infected by this trypanosome subspecies would be considerably reduced. Moreover, the lack of trypanocidal drugs to prevent AAT could explain the
increasing trypanosome prevalence from one year to the next (T. congolense infection rates tripled from 2007 to 2009 and quadrupled from 2006 to 2009). These results indicate that for large scale breeding of animals in this area, control measures for AAT at Fontem need to be instituted.


Department of Ecology and Evolutionary Biology, Yale University, P.O. Box 208106, New Haven, CT, USA. [mark.sistrom@yale.edu].

The Trypanosoma brucei complex contains a number of subspecies with exceptionally variable life histories, including zoonotic subspecies, which are causative agents of human African trypanosomiasis (HAT) in sub-Saharan Africa. Paradoxically, genomic variation between taxa is extremely low. We analysed the whole-genome sequences of 39 isolates across the T. brucei complex from diverse hosts and regions, identifying 608 501 single nucleotide polymorphisms that represent 2.33 percent of the nuclear genome. We show that human pathogenicity occurs across a wide range of parasite genotypes, and taxonomic designation does not reflect genetic variation across the group, as previous studies have suggested based on a small number of genes. This genome-wide study allowed the identification of significant host and geographic location associations. Strong purifying selection was detected in genomic regions associated with cytoskeleton structure, and regulatory genes associated with antigenic variation, suggesting conservation of these regions in African trypanosomes. In agreement with expectations drawn from meiotic reciprocal recombination, differences in average linkage disequilibrium between chromosomes in T. brucei correlate positively with chromosome size. In addition to insights into the life history of a diverse group of eukaryotic parasites, the documentation of genomic variation across the T. brucei complex and its association with specific hosts and geographic localities will aid in the development of comprehensive monitoring tools crucial to the proposed elimination of HAT by 2020, and on a shorter term, for monitoring the feared merger between the two human infective parasites, T. brucei rhodesiense and T. b. gambiense, in northern Uganda.

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

[See also 37: 17298, 17323, 17337, 17338, 17339, 17347, 17378]


Wellcome Trust Centre of Molecular Parasitology and Glasgow Polyomics, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK. [michael.barrett@glasgow.ac.uk].

African trypanosomes cause devastating diseases in humans and domestic animals. The parasites evolved early in the eukaryotic lineage and have numerous biochemical peculiarities that distinguish them from other systems. These include unconventional mechanisms for expressing nuclear and mitochondrial genes as well as unusual subcellular localizations for a
variety of enzymes. Systems biology has arisen partly to allow contextualization of the massive datasets that describe individual chemical parts of biological systems. Here we describe recent efforts to collect and analyse data pertaining to all aspects of the trypanosome’s biochemical physiology that go some way to describing the parasite as an integrated system.


Department of Biological Sciences, Landmark University, Omu-Aran 370102, Nigeria.


Institute of Parasitology, McGill University, MacDonald Campus, 21,111 Lakeshore Ave., St-Anne-de-Bellevue, Quebec H9X 3V9, Canada.

Despite recent advances in medical technology and a global effort to improve public health and hygiene, parasitic infections remain a major health and economic burden worldwide. The World Health Organization estimates that about one third of the world’s population is currently infected with a soil-transmitted helminth, and millions more suffer from diseases caused by protozoan parasites including Plasmodium, Trypanosoma, and Leishmania species. Due to the selective pressure applied by parasitic and other infections, animals have evolved an intricate immune system; however, the current worldwide prevalence of parasitic infections clearly indicates that these pathogens have adapted equally well. Thus, developing a better understanding of the host-parasite relationship, particularly by focusing on the host immune response and the mechanisms by which parasites evade this response, is a critical first step in mitigating the detrimental effects of parasitic diseases. Macrophages are critical contributors during the host response to protozoan parasites, and the success or failure of these cells often tips the balance in favour of the host or parasite. Here, we briefly discuss macrophage biology and provide an update on our current understanding of how these cells recognize glycosylphosphatidylinositol anchors from protozoan parasites as well as malarial hemozoin.


Fakultat fur Biologie, Genetik, Ludwig-Maximilians-Universitat Munchen, Biozentrum, Martinsried, Germany. [boshart@lmu.de].

Trypanosomatids are unicellular protozoans of medical and economic relevance since they are the aetiologic agents of infectious diseases in humans as well as livestock. Whereas *Trypanosoma cruzi* and different species of *Leishmania* are obligate intracellular parasites, *Trypanosoma brucei* and other trypanosomatids develop extracellularly throughout their entire life cycle. After their genomes have been sequenced, various comparative genomic studies aimed at identifying sequences involved with host cell invasion and intracellular survival have been described. However, for only a handful of genes, mostly present in the *T. cruzi* or *Leishmania* genomes, has there been any experimental evidence associating them with intracellular parasitism. With the increasing number of published complete genome sequences of members of the trypanosomatid family, including not only different *Trypanosoma* and *Leishmania* strains and subspecies but also trypanosomatids that do not infect humans or other mammals, we may now be able to contemplate a slightly better picture regarding the specific set of parasite factors that define each organism’s mode of living and the associated disease phenotypes. Here, we review the studies concerning *T. cruzi* and *Leishmania* genes that have been implicated in cell invasion and intracellular parasitism and also summarize the wealth of new information regarding the mode of living of intracellular parasites that is resulting from comparative genome studies based on increasingly larger trypanosomatid genome datasets.


Department of Genetics and Biochemistry, Eukaryotic Pathogens Innovation Center, Clemson University, 130 McGinty Court, Clemson, SC 29634, USA. [mmorri3@clemson.edu].

*Trypanosoma brucei* is a kinetoplastid parasite that causes human African trypanosomiasis (HAT), or sleeping sickness, and a wasting disease, nagana, in cattle. The parasite alternates between the bloodstream of the mammalian host and the tsetse fly vector. The composition of many cellular organelles changes in response to these different extracellular conditions. Glycosomes are highly specialized peroxisomes in which many of the enzymes involved in glycolysis are compartmentalized. Glycosome composition changes in a developmental and environmentally regulated manner. Currently, the most common techniques used to study glycosome dynamics are electron and fluorescence microscopy, techniques that are expensive, time and labour intensive, and not easily adapted to high throughput analyses. To overcome these limitations, a fluorescent-glycosome reporter system has been established in which enhanced yellow fluorescent protein (eYFP) is fused to a peroxisome targeting sequence (PTS2), which directs the fusion protein to glycosomes. Upon import of the PTS2eYFP fusion protein, glycosomes become fluorescent. Organelle degradation and recycling results in the loss of fluorescence that can be measured by flow cytometry. Large numbers of cells (5 000 cells/sec) can be analysed in real-time without extensive sample preparation such as fixation and mounting. This method offers a rapid way of detecting changes in organelle composition in response to fluctuating environmental conditions.


Institute of Biochemistry, Justus Liebig University of Giessen, D-35392 Giessen, Germany. [albrecht.bindereif@chemie.bio.uni-giessen.de].

An efficient response to endoplasmic reticulum (ER) stress is essential for the viability of eukaryotic cells. The causative agent of African sleeping sickness, Trypanosoma brucei, responds to such stress by inducing spliced leader RNA silencing (SLS), resulting in shutdown of mRNA biogenesis. A new study elucidates the activation cascade and its molecular components, which are unique to the ER stress response in trypanosomes.


Department of Biological Sciences, Centre for BioImaging Sciences, National University of Singapore, 14 Science Drive 4, S1A #06-04, Singapore 117543, Singapore. [dbshyc@nus.edu.sg].


National Center for Advancing Translational Sciences, National Institutes of Health, 9800 Medical Center Drive, Rockville, Maryland 20850, USA. [boxerm@mail.nih.gov].


Seattle Biomedical Research Institute, Seattle, Washington 98109, USA. [ken.stuart@sbri.org].

Mitochondrial RNA processing in the kinetoplastid parasite Trypanosoma brucei involves numerous specialized catalytic activities that are incompletely understood. The mitochondrial genome consists of maxicircles that primarily encode rRNAs and mRNAs, and minicircles that encode a diverse array of guide RNAs (gRNAs). RNA editing uses these gRNAs as templates to recode mRNAs by insertion and deletion of uridine (U) residues. While the multiprotein complex that catalyzes RNA editing has been extensively studied, other players involved in mitochondrial RNA processing have remained enigmatic. The proteins required for processing mitochondrial polycistronic transcripts into mature species were essentially unknown until an RNase III endonuclease, called mRPN1, was reported to be involved in gRNA processing in procyclic form parasites. In this work, we examine the role of mRPN1 in gRNA processing in bloodstream form parasites, and show that complete elimination of mRPN1 by gene knockout does not alter gRNA maturation. These results indicate that another enzyme must be involved in gRNA processing.

Division of Cell and Molecular Biology, Sir Alexander Fleming Building, Imperial College London, South Kensington, London, SW7 2AZ, UK. [gloria.rudenko@imperial.ac.uk].

Chromatin remodelling is involved in the transcriptional regulation of the RNA polymerase I transcribed variant surface glycoprotein (VSG) expression sites (ESs) of *Trypanosoma brucei*. We show that the *T. brucei* FACT complex contains the Pob3 and Spt16 subunits, and plays a key role in ES silencing. We see an inverse correlation between transcription and condensed chromatin, whereby FACT knockdown results in ES derepression and more open chromatin around silent ES promoters. Derepressed ESs show increased sensitivity to micrococcal nuclease (MNase) digestion, and a decrease in histones at silent ES promoters but not in telomeres. In contrast, FACT knockdown results in more histones at the active ES, correlated with transcription shut-down. ES promoters are derepressed in cells stalled at the G2/M cell cycle stage after knockdown of FACT, but not in G2/M cells stalled after knockdown of cyclin 6. This argues that the observed ES derepression is a direct consequence of histone chaperone activity by FACT at the G2/M cell cycle stage which could affect transcription elongation, rather than an indirect consequence of a cell cycle checkpoint. These experiments highlight the role of the FACT complex in cell cycle-specific chromatin remodelling within VSG ESs.


Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30620, USA. [rdocampo@uga.edu].

Calcium ion (Ca$^{2+}$) is an important second messenger in trypanosomatids and essential for their survival although prolonged high intracellular Ca$^{2+}$ levels lead to cell death. As with other eukaryotic cells, trypanosomes use two sources of Ca$^{2+}$ for generating signals: Ca$^{2+}$ release from intracellular stores and Ca$^{2+}$ entry across the plasma membrane. Ca$^{2+}$ release from intracellular stores is controlled by the inositol 1,4,5-trisphosphate receptor (IP3R) that is located in acidocalcisomes, acidic organelles that are the primary Ca$^{2+}$ reservoir in these cells. A plasma membrane Ca$^{2+}$-ATPase controls the cytosolic Ca$^{2+}$ levels and a number of pumps and exchangers are responsible for Ca$^{2+}$ uptake and release from intracellular compartments. The trypanosomatid genomes contain a wide variety of signalling and regulatory proteins that bind Ca$^{2+}$ as well as many Ca$^{2+}$-binding proteins that await further characterization. The mitochondrial Ca$^{2+}$ transporters of trypanosomatids have an important role in the regulation of cell bioenergetics and flagellar Ca$^{2+}$ appears to have roles in sensing the environment. In trypanosomatids in which an intracellular life cycle is present, Ca$^{2+}$ signalling is important for host cell invasion.

Instituto de Parasitología y Biomedicina "Lopez-Neyra", IPBLN-CSIC, Armilla, Granada, Spain. [aestevez@ipb.csic.es].

We have characterized the RNA-binding protein RBP33 in *Trypanosoma brucei*, and found that it localizes to the nucleus and is essential for viability. The subset of RNAs bound to RBP33 was determined by immunoprecipitation of ribonucleoprotein complexes followed by deep sequencing. Most RBP33-bound transcripts are predicted to be non-coding. Among these, over one-third are located close to the end of transcriptional units (TUs) or have an antisense orientation within a TU. Depletion of RBP33 resulted in an increase in the level of RNAs derived from regions that are normally silenced, such as strand-switch regions, retroposon and repeat sequences. Our work provides the first example of an RNA-binding protein involved in the regulation of gene silencing in trypanosomes.


Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK. [d.horn@dundee.ac.uk].

The African trypanosome *Trypanosoma brucei* is a parasitic protozoan that achieves antigenic variation through DNA repair processes involving variant surface glycoprotein (VSG) gene rearrangements at subtelomeres. Subtelomeric suppression of DNA repair operates in eukaryotes but little is known about these controls in trypanosomes. Here, we identify a trypanosome histone acetyltransferase (HAT3) and a deacetylase (SIR2rp1) required for efficient RAD51-dependent homologous recombination. HAT3 and SIR2rp1 were required for RAD51-focus assembly and disassembly, respectively, at a chromosome-internal locus and a synthetic defect indicated distinct contributions to DNA repair. Although HAT3 promoted chromosome-internal recombination, it suppressed subtelomeric VSG recombination, and these locus-specific effects were mediated through differential production of ssDNA by DNA resection; HAT3 promoted chromosome-internal resection but suppressed subtelomeric resection. Consistent with the resection defect, HAT3 was specifically required for the G2-checkpoint response at a chromosome-internal locus. HAT3 also promoted resection at a second chromosome-internal locus comprising tandem-duplicated genes. We conclude that HAT3 and SIR2rp1 can facilitate temporally distinct steps in DNA repair. HAT3 promotes ssDNA formation and recombination at chromosome-internal sites but has the opposite effect at a subtelomeric VSG. These locus-specific controls reveal compartmentalization of the *T. brucei* genome in terms of the DNA damage response and suppression of antigenic variation by HAT3.


Department of Genetics and Developmental Biology, University of Connecticut School of Medicine, 400 Farmington Avenue, Farmington, CT 06030-6403, USA. [gunzl@uchc.edu].

*Trypanosoma brucei* is a vector-borne, lethal protistan parasite of humans and livestock in sub-Saharan Africa. Antigenic variation of its cell surface coat enables the parasite to
evade adaptive immune responses and to live freely in the blood of its mammalian hosts. The coat consists of ten million copies of variant surface glycoprotein (VSG) that is expressed from a single VSG gene, drawn from a large repertoire and located near the telomere at one of fifteen so-called bloodstream expression sites (BESs). Thus, antigenic variation is achieved by switching to the expression of a different VSG gene. A BES is a tandem array of expression site-associated genes and a terminal VSG gene. It is polycistronically transcribed by a multifunctional RNA polymerase I (RNAPI) from a short promoter that is located 45-60 kb upstream of the VSG gene. The mechanism(s) restricting VSG expression to a single BES are not well understood. There is convincing evidence that epigenetic silencing and transcription attenuation play important roles. Furthermore, recent data indicated that there is regulation at the level of transcription initiation and that, surprisingly, the VSG mRNA appears to have a role in restricting VSG expression to a single gene. Here, we review BES expression regulation and propose a model in which telomere-directed, epigenetic BES silencing is opposed by BES promoter-directed, activated RNAPI transcription.


Department of Biosciences, Integral University, Lucknow, 226026, India.


Department of Molecular Cell Physiology, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Faculty of Earth and Life Sciences, Vrije Universiteit Amsterdam, The Netherlands. [j.r.haanstra@vu.nl].

Trypanosomatids sequester large parts of glucose metabolism inside specialized peroxisomes, called glycosomes. Many studies have shown that correct glycosomal compartmentalization of glycolytic enzymes is essential for bloodstream-form *Trypanosoma brucei*. The recent finding of pore-forming activities in glycosomal membrane preparations and extensions of the trypanosome glycolysis computer model with size-selective pores sparked again an old debate on the extent of the (im) permeability of the glycosomal membrane and whether glycosomally located glycolytic enzymes could and should also be present with some activity in the cytosol. This review presents a critical discussion of the experimental and theoretical evidence for and against the different hypotheses.


Center for Chemical Biology and Department of Chemistry & Biochemistry, Texas Tech University, Lubbock, Texas 79409, USA; Department of Microbiology and Immunology, Meharry Medical College, Nashville, TN 37208, USA; Laboratoire de Parasitologie Moléculaire IBMM Université Libre de Bruxelles, B6041 Gosselies,
Ergosterol biosynthesis and homeostasis in the parasitic protozoan *Trypanosoma brucei* were analysed by RNAi silencing and inhibition of sterol C24beta-methyltransferase (TbSMT) and sterol 14alpha-demethylase (TbSDM = TbCYP51) to explore the functions of sterols in *T. brucei* growth. Inhibition of the amount or activity of these enzymes depletes ergosterol from cells at < 6 fg/cell for procyclic form cells (PCF) or < 0.01 fg/cell for bloodstream forms (BSF) and reduces infectivity in a mouse model of infection. Silencing of TbSMT expression by RNAi in PCF or BSF in combination with 25-azalanosterol inhibited parasite growth and this inhibition was restored completely by adding synergistic cholesterol (7.8 µM from lipid-depleted media) with small amounts of ergosterol (1.2 µM) to the medium. These observations are consistent with the proposed requirement for ergosterol as a signalling factor to spark cell proliferation while imported cholesterol or the endogenously formed cholesta-5,7,24-trienol act as bulk membrane components. To test the potential chemotherapeutic importance of disrupting ergosterol biosynthesis using pairs of mechanism-based inhibitors that block two enzymes in the post-squalene segment, parasites were treated with 25-azalanosterol and itraconazole at 1 µM each (ED_{50} values) resulting in parasite death. Taken together, our results demonstrate that the ergosterol pathway is a prime drug target for intervention in *T. brucei* infection.


The parasite *Trypanosoma brucei* is the causative agent of human African sleeping sickness. *T. brucei* genes are constitutively transcribed in polycistronic units that are processed by trans-splicing and polyadenylation. All mRNAs are trans-spliced to generate mRNAs with a common 5' exon derived from the spliced leader RNA (SL RNA). Persistent endoplasmic reticulum (ER) stress induces the spliced leader silencing (SLS) pathway, which inhibits trans-splicing by silencing SL RNA transcription, and correlates with increased programmed cell death. We found that during ER stress induced by SEC63 silencing or low pH, the serine-threonine kinase PK3 translocated from the ER to the nucleus, where it phosphorylated the TATA-binding protein TRF4, leading to the dissociation of the transcription preinitiation complex from the promoter of the SL RNA encoding gene. PK3 loss of function attenuated programmed cell death induced by ER stress, suggesting that SLS may contribute to the activation of programmed cell death.


Division of Biological Chemistry and Drug Discovery, The College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK. [m.a.j.ferguson@dundee.ac.uk].
Trypanosoma brucei causes human African trypanosomiasis and regularly switches its major surface antigen, VSG, in the bloodstream of its mammalian host to evade the host immune response. VSGs are expressed exclusively from subtelomeric loci, and we have previously shown that telomere proteins TbTIF2 and TbRAP1 play important roles in VSG switching and VSG silencing regulation, respectively. We now discover that the telomere duplex DNA-binding factor, TbTRF, also plays a critical role in VSG switching regulation, as a transient depletion of TbTRF leads to significantly more VSG switching events. We solved the NMR structure of the DNA-binding Myb domain of TbTRF, which folds into a canonical helix-loop-helix structure that is conserved to the Myb domains of mammalian TRF proteins. The TbTRF Myb domain tolerates well the bulky J base in T. brucei telomere DNA, and the DNA-binding affinity of TbTRF is not affected by the presence of J both in vitro and in vivo. In addition, we find that point mutations in TbTRF Myb that significantly reduced its in vivo telomere DNA-binding affinity also led to significantly increased VSG switching frequencies, indicating that the telomere DNA-binding activity is critical for TbTRF's role in VSG switching regulation.

Subtelomeres consist of sequences adjacent to telomeres and contain genes involved in important cellular functions, as subtelomere instability is associated with several human diseases. Balancing between subtelomere stability and plasticity is particularly important for Trypanosoma brucei, a protozoan parasite that causes human African trypanosomiasis. T. brucei regularly switches its major variant surface antigen, variant surface glycoprotein (VSG), to evade the host immune response, and VSGs are expressed exclusively from subtelomeres in a strictly monoallelic fashion. Telomere proteins are important for protecting chromosome ends from illegitimate DNA processes. However, whether they contribute to subtelomere integrity and stability has not been well studied. We have identified a novel T. brucei telomere protein, T. brucei TRF-interacting factor 2 (TbTIF2), as a functional homologue of mammalian TIN2. A transient depletion of TbTIF2 led to an elevated VSG switching frequency and an increased amount of DNA double-strand breaks (DSBs) in both active and silent subtelomeric bloodstream form expression sites (BESs). Therefore, TbTIF2 plays an important role in VSG switching regulation and is important for subtelomere integrity and stability. TbTIF2 depletion increased the association of TbRAD51 with the telomeric and subtelomeric chromatin, and TbRAD51 deletion further increased subtelomeric DSBs in TbTIF2-depleted cells, suggesting that TbRAD51-mediated DSB repair is the
underlying mechanism of subsequent VSG switching. Surprisingly, significantly more TbRAD51 associated with the active BES than with the silent BESs upon TbTIF2 depletion, and TbRAD51 deletion induced much more DSBs in the active BES than in the silent BESs in TbTIF2-depleted cells, suggesting that TbRAD51 preferentially repairs DSBs in the active BES.


Seattle Biomedical Research Institute, 307 Westlake Ave N., Seattle, WA 98109-5219, USA. [mparsons@u.washington.edu].

*Trypanosoma brucei* subspecies infect humans and animals in sub-Saharan Africa. This early diverging eukaryote shows many novel features in basic biological processes, including the use of polycistronic transcription to generate all protein-coding mRNAs. Therefore we hypothesized that translational control provides a means to tune gene expression during parasite development in mammalian and fly hosts. We used ribosome profiling to examine genome-wide protein synthesis in animal-derived slender bloodstream forms and cultured procyclic (insect midgut) forms. About one-third of all downstream coding sequences (CDSs) showed statistically significant regulation of protein production between the two stages. Of these, more than two thirds showed a change in translation efficiency, but few appeared to be controlled by this alone. Ribosomal proteins were translated poorly, especially in animal-derived parasites. A disproportionate number of metabolic enzymes were up-regulated at the mRNA level in procyclic forms, as were variant surface glycoproteins in bloodstream forms. Comparison with cultured bloodstream forms from another strain revealed stage-specific changes in gene expression that transcend strain and growth conditions. Genes with upstream ORFs had lower mean translation efficiency, but no evidence was found for involvement of uORFs in stage regulation. In conclusion, ribosome profiling revealed that differences in the production of specific proteins in *T. brucei* bloodstream and procyclic forms are more extensive than predicted by analysis of mRNA abundance. While *in vivo* and *in vitro* derived bloodstream forms from different strains are more similar to one another than to procyclic forms, they showed many differences at both the mRNA and protein production levels.


Department of Biochemistry, University of Washington, Seattle, WA 98195, USA. [wghol@u.washington.edu].


Department of Epidemiology of Microbial Diseases, School of Public Health, Yale University, New Haven, CT, USA. [nikolay.kolev@yale.edu].

High-throughput RNA sequencing (RNA-Seq) has quickly occupied centre stage in the repertoire of available tools for transcriptomics. Among many advantages, the single-
nucleotide resolution of this powerful approach allows mapping on a genome-wide scale of splice junctions and polyadenylation sites, and thus, the precise definition of mature transcript boundaries. This greatly facilitated the transcriptome annotation of the human pathogen *Trypanosoma brucei*, a protozoan organism in which all mRNA molecules are matured by spliced leader (SL) trans-splicing from longer polycistronic precursors. The protocols described here for the generation of three types of libraries for illumina RNA-Seq, 5'-SL enriched, 5'-triphosphate-end enriched, and 3'-poly(A) enriched, enabled the discovery of an unprecedented heterogeneity of pre-mRNA-processing sites, a large number of novel coding and noncoding transcripts from previously unannotated genes, and quantification of the cellular abundance of RNA molecules. The method for producing 5'-triphosphate-end-enriched libraries was instrumental in obtaining evidence that transcription initiation by RNA polymerase II in trypanosomes is bidirectional and biosynthesis of mRNA precursors is primed not only at the beginning of unidirectional gene clusters, but also at specific internal sites.


Department of Biochemistry, University of Oxford, Oxford, UK. [matthew.higgins@bioch.ox.ac.uk]

The haptoglobin-haemoglobin receptor (HpHbR) of African trypanosomes allows acquisition of haem and provides an uptake route for trypanolytic factor-1, a mediator of innate immunity against trypanosome infection. Here we report the structure of *Trypanosoma brucei* HpHbR in complex with human haptoglobin-haemoglobin (HpHb), revealing an elongated ligand-binding site that extends along its membrane distal half. This contacts haptoglobin and the beta-subunit of haemoglobin, showing how the receptor selectively binds HpHb over individual components. Lateral mobility of the glycosylphophatidylinositol-anchored HpHbR, and a ~50 degree kink in the receptor, allows two receptors to simultaneously bind one HpHb dimer. Indeed, trypanosomes take up dimeric HpHb at significantly lower concentrations than monomeric HpHb, due to increased ligand avidity that comes from bivalent binding. The structure therefore reveals the molecular basis for ligand and innate immunity factor uptake by trypanosomes, and identifies adaptations that allow efficient ligand uptake in the context of the complex trypanosome cell surface.


Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, California 90095, USA. [kenthill@mednet.ucla.edu].

*Trypanosoma brucei* is a pathogenic unicellular eukaryote that infects humans and other mammals in sub-Saharan Africa. A central feature of trypanosome biology is the single flagellum of the parasite, which is an essential and multifunctional organelle that facilitates cell propulsion, controls cell morphogenesis and directs cytokinesis. Moreover, the flagellar membrane is a specialized subdomain of the cell surface that mediates attachment to host tissues and harbours multiple virulence factors. In this review, we discuss the structure, assembly and function of the trypanosome flagellum, including canonical roles in cell motility as well as novel and emerging roles in cell morphogenesis and host-parasite
interactions.


Laboratory of Molecular Parasitology, Institute of Molecular Biology and Medicine, Université Libre de Bruxelles, 12 Rue des Professeurs Jeener et Brachet, B-6041, Gosselies, Belgium. [epays@ulb.ac.be].

Normal human serum (NHS) confers human resistance to infection by the parasite *Trypanosoma brucei* owing to the trypanolytic activity of apolipoprotein L1 (APOL1), present in two serum complexes termed trypanolytic factors (TLF-1 and -2). In order to identify parasite components involved in the intracellular trafficking and activity of TLFs, an inducible RNA interference (RNAi) genomic DNA library constructed in bloodstream form *T. brucei* was subjected to RNAi induction and selection for resistant parasites under NHS conditions favouring either TLF-1 or TLF-2 uptake. While TLF-1 conditions readily selected the haptoglobin-haemoglobin (HP-HB) surface receptor TbHpHbR as expected, given its known ability to bind TLF-1, under TLF-2 conditions no specific receptor for TLF-2 was identified. Instead, the screen allowed the identification of five distinct factors expected to be involved in the assembly of the vacuolar proton pump V-ATPase and consecutive endosomal acidification. These data confirm that lowering the pH during endocytosis is required for APOL1 toxic activity.


Department of Biological Sciences, National University of Singapore, Singapore. [dbshyc@nus.edu.sg].

Lysosomes play important roles in autophagy, not only in autophagosome degradation, but also in autophagy initiation. In *Trypanosoma brucei*, an early divergent protozoan parasite, we discovered a previously unappreciated function of the acidocalcisome, a lysosome-related organelle characterized by acidic pH and large content of Ca\(^{2+}\) and polyphosphates, in autophagy regulation. Starvation- and chemical-induced autophagy is accompanied by acidocalcisome acidification, and blocking the acidification completely inhibits autophagosome formation. Blocking acidocalcisome biogenesis by depleting the adaptor protein-3 complex, which does not affect lysosome biogenesis or function, also inhibits autophagy. Overall, our results support the role of the acidocalcisome, a conserved organelle from bacteria to human, as a relevant regulator in autophagy.


Environmental Toxicology Graduate Program, University of California, Riverside, CA, 92521, USA.
Sophisticated systems for cell-cell communication enable unicellular microbes to act as multicellular entities, capable of group-level behaviours that are not evident in individuals. These group behaviours influence microbe physiology and the underlying signalling pathways are considered potential drug targets in microbial pathogens. *Trypanosoma brucei* is a protozoan parasite that causes substantial human suffering and economic hardship in some of the most impoverished regions of the world. *T. brucei* lives on host tissue surfaces during transmission through its tsetse fly vector, and cultivation on surfaces causes the parasites to assemble into multicellular communities in which individual cells coordinate their movements in response to external signals. This behaviour is termed "social motility", based on similarities with surface-induced social motility in bacteria, and demonstrates that trypanosomes are capable of group-level behaviour. Mechanisms governing *T. brucei* social motility are unknown. Here we report that a subset of receptor-type adenylate cyclases (ACs) in the trypanosome flagellum regulates social motility. RNAi-mediated knockdown of adenylate cyclase 6 (AC6), or dual knockdown of AC1 and AC2, causes a hypersocial phenotype but has no discernible effect on individual cells in suspension culture. Mutation of the AC6 catalytic domain phenocopies AC6 knockdown, demonstrating loss of adenylate cyclase activity, is responsible for the phenotype. Notably, knockdown of other ACs did not affect social motility, indicating segregation of AC functions. These studies reveal interesting parallels in systems that control social behaviour in trypanosomes and bacteria, and provide insight into a feature of parasite biology that may be exploited for novel intervention strategies.

Bloodstream form trypanosomes avoid the host immune response by switching the expression of their surface proteins between variant surface glycoproteins (VSG), only one of which is expressed at any given time. Monoallelic transcription of the telomeric VSG expression site (ES) by RNA polymerase I (RNA pol I) localizes to a unique nuclear body named the ESB. Most work has focused on silencing mechanisms of inactive VSG-ESs, but the mechanisms involved in transcriptional activation of a single VSG-ES remain largely unknown. Here, we identify a highly SUMOylated focus (HSF) in the nucleus of the bloodstream form that partially colocalizes with the ESB and the active VSG-ES locus. SUMOylation of chromatin-associated proteins was enriched along the active VSG-ES transcriptional unit, in contrast to silent VSG-ES or rDNA, suggesting that it is a distinct feature of VSG-ES monoallelic expression. In addition, sequences upstream of the active VSG-ES promoter were highly enriched in SUMOylated proteins. We identified TbSIZ1/PIAS1 as the SUMO E3 ligase responsible for SUMOylation in the active VSG-ES.
chromatin. Reduction of SUMO-conjugated proteins by TbSIZ1 knockdown decreased the recruitment of RNA pol I to the VSG-ES and the VSG-ES-derived transcripts. Furthermore, cells depleted of SUMO conjugated proteins by TbUBC9 and TbSUMO knockdown confirmed the positive function of SUMO for VSG-ES expression. In addition, the largest subunit of RNA pol I TbRPA1 was SUMOylated in a TbSIZ-dependent manner. Our results show a positive mechanism associated with active VSG-ES expression via post-translational modification, and indicate that chromatin SUMOylation plays an important role in the regulation of VSG-ES. Thus, protein SUMOylation is linked to active gene expression in this protozoan parasite that diverged early in evolution.


Biology Centre, Institute of Parasitology, Czech Academy of Sciences and Faculty of Science, University of South Bohemia, 37005 Ceske Budejovice (Budweis), Czech Republic. [jula@paru.cas.cz].

*Trypanosoma brucei*, the causative agent of African sleeping sickness in humans, and other kinetoplastid flagellates belong to the eukaryotic supergroup Excavata. This early-branching model protist is known for a broad range of unique features. As it is amenable to most techniques of forward and reverse genetics, *T. brucei* was subject to several studies of its iron-sulphur (Fe/S) protein biogenesis and thus represents the best studied excavate eukaryote. Here we review what is known about the Fe/S protein biogenesis of *T. brucei*, and focus especially on the interesting comparative and evolutionary aspects. We also explore the connections between the well-known and quite conserved ISC and CIA machineries and the tRNA thiolation pathway. Moreover, the Fe/S cluster protein biogenesis is dissected in the procyclic stage of *T. brucei* which has an active mitochondrion, as well as in its pathogenic bloodstream stage with a metabolically repressed organelle. This article is part of a Special Issue entitled: Fe/S proteins: Analysis, structure, function, biogenesis and diseases.


Department of Microbiology and Immunology, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, New York, USA. [lread@buffalo.edu].

In kinetoplastid parasites, regulation of mitochondrial gene expression occurs posttranscriptionally via RNA stability and RNA editing. In addition to the 20S editosome that contains the enzymes required for RNA editing, a dynamic complex called the mitochondrial RNA binding 1 (MRB1) complex is also essential for editing. TbRGG3 was originally identified through its interaction with the guide RNA associated proteins 1 and 2 (GAP1/2) components of the MRB1 complex. Both the arginine-glycine rich character of TbRGG3, which suggests a function in RNA binding, and its interaction with MRB1 implicate TbRGG3 in mitochondrial gene regulation. Here, we report an *in vitro* and *in vivo* characterization of TbRGG3 function in *T. brucei* mitochondria. We show that *in vitro* TbRGG3 binds RNA with broad sequence specificity and has the capacity to modulate RNA-
RNA interactions. *In vivo*, inducible RNAi studies demonstrate that TbRGG3 is essential for proliferation of insect vector stage *T. brucei*. TbRGG3 ablation does not cause a defect in RNA editing, but rather specifically affects the abundance of two pre-edited transcripts as well as their edited counterparts. Protein-protein interaction studies show that TbRGG3 associates with GAP1/2 apart from the remainder of the MRB1 complex, as well as with several non-MRB1 proteins that are required for mitochondrial RNA editing and/or stability. Together, these studies demonstrate that TbRGG3 is an essential mitochondrial gene regulatory factor that impacts the stabilities of specific RNAs.


Department of Biochemistry, McGill University, Montreal, Quebec, Canada; Institute of Parasitology, McGill University, Montreal, Quebec, Canada. [reza.salavati@mcgill.ca].

Most mitochondrial messenger RNAs in trypanosomatid pathogens undergo a unique type of posttranscriptional modification involving insertion and/or deletion of uridylates. This process, RNA editing, is catalysed by a multiprotein complex (~1.6 MDa), the editosome. Knockdown of core editosome proteins compromises mitochondrial function and, ultimately, parasite viability. Hence, because the editosome is restricted to trypanosomatids, it serves as a unique drug target in these pathogens. Currently, there is a lack of editosome inhibitors for antitrypanosomatid drug development or that could serve as unique tools for perturbing and characterizing editosome interactions or RNA editing reaction stages. Here, we screened a library of pharmacologically active compounds (LOPAC1280) using high-throughput screening to identify RNA editing inhibitors. We report that aurintricarboxylic acid, mitoxantrone, PPND, and NF449 are potent inhibitors of deletion RNA editing (IC$_{50}$ range, 1-5 µM). However, none of these compounds could specifically inhibit the catalytic steps of RNA editing. Mitoxantrone blocked editing by inducing RNA-protein aggregates, whereas the other three compounds interfered with editosome-RNA interactions to varying extents. Furthermore, NF449, a suramin analogue, was effective in killing *Trypanosoma brucei* *in vitro*. Thus, new tools for editosome characterization and downstream RNA editing inhibition have been identified.


Department of Chemistry and Chemical Biology, Northeastern University, 417 Egan Research Center, 360 Huntington Avenue, Boston, MA, 02115, USA. [m.pollastri@neu.edu].

Improved therapies for the treatment of *Trypanosoma brucei*, the aetiological agent of the neglected tropical disease human African trypanosomiasis, are urgently needed. We targeted *T. brucei* methionyl-tRNA synthetase (MetRS), an aminoacyl-tRNA synthase (aaRS), which is considered an important drug target due to its role in protein synthesis, cell survival, and its significant differences in structure from its mammalian orthologue. Previous work using RNA interference of MetRS demonstrated growth inhibition of *T. brucei*, further validating it as an attractive target. We report the development and implementation of two orthogonal high-throughput screening assays to identify inhibitors of *T. brucei* MetRS. First, a chemiluminescence assay was implemented in a 1,536-well plate format and used to monitor adenosine triphosphate depletion during the aminoacylation reaction. Hit confirmation then used a counterscreen in which adenosine monophosphate production was assessed using fluorescence polarization technology. In addition, a miniaturized cell viability assay was used to triage cytotoxic compounds. Finally, lower throughput assays involving whole parasite growth inhibition of both human and parasite MetRS were used to analyse compound selectivity and efficacy. The outcome of this high-throughput screening campaign has led to the discovery of 19 potent and selective *T. brucei* MetRS inhibitors.

*Trypanosoma brucei* is a unicellular parasite that causes sleeping sickness in humans. Most of its transcription is constitutive and driven by RNA polymerase II. RNA polymerase I (Pol I) transcribes not only ribosomal RNA genes, but also protein-encoding genes, including variant surface glycoproteins (VSGs) and procyclins. In *T. brucei*, histone H1 (H1) is required for VSG silencing and chromatin condensation. However, whether H1 has a genome-wide role in transcription is unknown. Here, using RNA sequencing we show that H1 depletion changes the expression of a specific cohort of genes. Interestingly, the predominant effect is partial loss of silencing of Pol I loci, such as VSG and procyclin genes. Labelling of nascent transcripts with 4-thiouridine showed that H1 depletion does not alter the level of labelled Pol II transcripts. In contrast, the levels of 4sU-labelled Pol I transcripts
were increased by two- to six-fold, suggesting that H1 preferentially blocks transcription at Pol I loci. Finally, we observed that parasites depleted of H1 grow almost normally in culture but they have a reduced fitness in mice, suggesting that H1 is important for host-pathogen interactions.


IDIM, Combatientes de Malvinas 3150, (1427) Bs. As., Argentina. [cpereira@retina.ar].

Trypanosomatids parasites have complex life cycles which involve a wide diversity of milieus with very different physicochemical properties. Arginine kinase is one of the key enzymes, responsible for the parasites’ metabolic plasticity, which maintains the cell energy homeostasis during environment changes. Arginine kinase catalyses the reversible phosphorylation between phosphoarginine and ADP. The phosphagen phosphoarginine sustains high levels of cellular activity until metabolic events such as glycolysis and oxidative phosphorylation are switched on. In different unicellular and multicellular organisms including trypanosomatids, it was demonstrated that arginine kinase is an important component in resistance mechanisms to different stress factors, such as reactive oxygen species, trypanocidal drugs, pH and starvation. In addition, a few arginine kinase inhibitors were identified during the last years, some of them with trypanocidal activity, such as polyphenolic compounds. All these unique features, in addition to the fact that arginine kinase is completely absent in mammals, make this pathway a favourable starting point for rational drug design for the treatment of human trypanosomiasis.


Sir William Dunn School of Pathology, University of Oxford, Oxford, UK; Faculty of Veterinary Science, University of Sydney, Sydney, Australia. [neil.portman@sydney.edu.au].


Department of Biochemistry and Molecular Biology, University of Georgia, Davison Life Sciences Building, 120 Green Street, Athens, GA 30602-7229, USA. [rsabatini@bmb.uga.edu].


Seattle Biomedical Research Institute, 307 Westlake Avenue N., Seattle WA 98109-5219, USA. [peter.myler@seattlebiomed.org].

17422. **Shameer, S., Logan-Klumpler, F. J., Vinson, F., Cottret, L., Merlet, B., Achcar,**
The metabolic network of a cell represents the catabolic and anabolic reactions that interconvert small molecules (metabolites) through the activity of enzymes, transporters and non-catalysed chemical reactions. Our understanding of individual metabolic networks is increasing as we learn more about the enzymes that are active in particular cells under particular conditions and as technologies advance to allow detailed measurements of the cellular metabolome. Metabolic network databases are of increasing importance in allowing us to contextualise data sets emerging from transcriptomic, proteomic and metabolomic experiments. Here we present a dynamic database, TrypanoCyc (http://www.metexplore.fr/trypanocyc/), which describes the generic and condition-specific metabolic network of Trypanosoma brucei, a parasitic protozoan responsible for human and animal African trypanosomiasis. In addition to enabling navigation through the BioCyc-based TrypanoCyc interface, we have also implemented a network-based representation of the information through MetExplore, yielding a novel environment in which to visualize the metabolism of this important parasite.


Department of Chemistry, University of Basel, 4056 Basel, Switzerland. [Thomas.Pfohl@unibas.ch].

Unicellular parasites have developed sophisticated swimming mechanisms to survive in a wide range of environments. Cell motility of African trypanosomes, the parasites responsible for fatal illness in humans and animals, is crucial both in the insect vector and the mammalian host. Using millisecond-scale imaging in a microfluidics platform along with a custom made optical trap, we are able to confine single cells to study trypanosome motility. From the trapping characteristics of the cells, we determine the propulsion force generated by cells with a single flagellum as well as of dividing trypanosomes with two fully developed flagella. Estimates of the dissipative energy and the power generation of single cells obtained from the motility patterns of the trypanosomes within the optical trap indicate that specific motility characteristics, in addition to locomotion, may be required for antibody clearance. Introducing a steerable second optical trap we could further measure the force which is...
generated at the flagellar tip. Differences in the cellular structure of the trypanosomes are correlated with the trapping and motility characteristics and in consequence with their propulsion force, dissipative energy and power generation.


Department of Biomedicine, Aarhus University, Ole Worms Alle 3, DK-8000 Aarhus C, Denmark. [kst@biomed.au.dk].

Sleeping sickness is caused by trypanosome parasites, which infect humans and livestock in sub-Saharan Africa. Haem is an important growth factor for the parasites and is acquired from the host by receptor-mediated uptake of haptoglobin (Hp)-haemoglobin (Hb) complexes. The parasite Hp-Hb receptor (HpHbR) is also a target for a specialized innate immune defence executed by trypanosome-killing lipoprotein particles containing an Hp-related protein in complex with Hb. Here we report the structure of the multimeric complex between human Hp-Hb and Trypanosoma brucei brucei HpHbR. Two receptors forming kinked three-helical rods with small head regions bind to Hp and the beta-subunits of Hb (betaHb), with one receptor at each end of the dimeric Hp-Hb complex. The Hb beta-subunit haem group directly associates with the receptors, which allows for sensing of haem-containing Hp-Hb. The HpHbR-binding region of Hp is conserved in Hp-related protein, indicating an identical recognition of Hp-Hb and trypanolytic particles by HpHbR in human plasma.


Department of Biochemistry and Molecular Biology; University of Georgia; Athens, GA 30602, USA. [shajduk@bmb.uga.edu].

The alpha-ketoglutarate decarboxylase (alpha-KDE1) is a Krebs cycle enzyme found in the mitochondrion of the procyclic form (PF) Trypanosoma brucei. The bloodstream form (BF) of T. brucei lacks a functional Krebs cycle and relies exclusively on glycolysis for ATP production. Despite the lack of a functional Krebs cycle, alpha-KDE1 was expressed in BF T. brucei and RNAi knockdown of alpha-KDE1 mRNA resulted in rapid growth arrest and killing. Cell death was preceded by progressive swelling of the flagellar pocket as a consequence of recruitment of both flagella and plasma membranes into the pocket. Bloodstream form T. brucei expressing an epitope tagged copy of alpha-KDE1 showed localization to glycosomes and not the mitochondrion. We used a cell line transfected with a reporter construct containing the N-terminal sequence of alpha-KDE1 fused to GFP to examine the requirements for glycosome targeting. We found that the N-terminal 18 amino acids of alpha-KDE1 contained overlapping mitochondria and peroxisome targeting sequences and were sufficient to direct localization to the glycosome in BF T. brucei. These results suggest that alpha-KDE1 has a novel moonlighting function outside the mitochondrion in BF T. brucei.

Kinetoplastea such as trypanosomatid parasites contain specialized peroxisomes that uniquely contain enzymes of the glycolytic pathway and other parts of intermediary metabolism and hence are called glycosomes. Their specific enzyme content can vary strongly, quantitatively and qualitatively, between different species and during the parasites’ life cycle. The correct sequestering of enzymes has great importance for the regulation of the trypanosomatids’ metabolism and can, dependent on environmental conditions, even be essential. Glycosomes also play a pivotal role in life-cycle regulation of *Trypanosoma brucei*, as the translocation of a protein phosphatase from the cytosol forms part of a crucial developmental control switch. Many glycosomal proteins are differentially phosphorylated in different life-cycle stages, possibly indicative of unique forms of activity regulation, whereas many kinetic activity regulation mechanisms common for glycolytic enzymes are absent in these organisms. Glycosome turnover occurs by autophagic degradation of redundant organelles and assembly of new ones. This may provide the trypanosomatids with a manner to rapidly and efficiently adapt their metabolism to the sudden, major nutritional changes often encountered during the life cycle. This could also have helped facilitating successful adaptation of kinetoplastids, at multiple occasions during evolution, to their parasitic life style.


Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [jshorter@mail.med.upenn.edu].

Hsp104 is a hexameric AAA+ protein that utilizes energy from ATP hydrolysis to dissolve disordered protein aggregates as well as amyloid fibres. Interestingly, Hsp104 orthologues are found in all kingdoms of life except animals. Thus, Hsp104 could represent an interesting drug target. Specific inhibition of Hsp104 activity might antagonize non-metazoan parasites that depend on a potent heat shock response, while producing little or no side effects to the host. However, no small molecule inhibitors of Hsp104 are known except guanidinium chloride. Here, we screen over 16,000 small molecules and identify 16 novel inhibitors of Hsp104 ATPase activity. Excluding compounds that inhibited Hsp104 activity by non-specific colloidal effects, we defined Suramin as an inhibitor of Hsp104 ATPase activity. Suramin is a polysulphonated naphthylurea and is used as an antiprotozoal drug for African trypanosomiasis. Suramin also interfered with Hsp104 disaggregase, unfoldase, and translocase activities, and the inhibitory effect of Suramin was not rescued by Hsp70 and Hsp40. Suramin does not disrupt Hsp104 hexamers and does not effectively inhibit ClpB, the *E. coli* homologue of Hsp104, establishing yet another key difference between Hsp104 and ClpB behaviour. Intriguingly, a potentiated Hsp104 variant, Hsp104A503V, is more sensitive to Suramin than wild-type Hsp104. By contrast, Hsp104 variants bearing inactivating sensor-1 mutations in nucleotide-binding domain (NBD) 1 or 2 are more resistant to Suramin. Thus, Suramin depends upon ATPase events at both NBDs to exert its maximal effect. Suramin could develop into an important mechanistic probe to study Hsp104 structure and function.

17428. Van Reet, N., Van de Vyver, H., Pyana, P. P., Van der Linden, A. M. & Buscher,

Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium. [nvanreet@itg.be].

Genetic engineering with luciferase reporter genes allows monitoring Trypanosoma brucei (T. b.) infections in mice by *in vivo* bioluminescence imaging (BLI). Until recently, luminescent T. b. models were based on Renilla luciferase (RLuc) activity. Our study aimed at evaluating red-shifted luciferases for *in vivo* BLI in a set of diverse T. b. strains of all three subspecies, including some recently isolated from human patients. We transfected T. b. brucei, T. b. rhodesiense and T. b. gambiense strains with either RLuc, click beetle red (CBR) or *Photinus pyralis* RE9 (PpyRE9) luciferase and characterized their *in vitro* luciferase activity, growth profile and drug sensitivity, and their potential for *in vivo* BLI. Compared with RLuc, the red-shifted luciferases, CBR and PpyRE9, allow tracking of T. b. brucei AnTaR 1 trypanosomes with higher details on tissue distribution, and PpyRE9 allows detection of the parasites with a sensitivity of at least one order of magnitude higher than CBR luciferase. With CBR-tagged T. b. gambiense LiTaR1, T. b. rhodesiense RUMPHI and T. b. gambiense 348 BT in an acute, subacute and chronic infection model respectively, we observed differences in parasite tropism for murine tissues during *in vivo* BLI. *Ex vivo* BLI on the brain confirmed central nervous system infection by all luminescent strains of T. b. brucei AnTaR 1, T. b. rhodesiense RUMPHI and T. b. gambiense 348 BT. In conclusion, we established a genetically and phenotypically diverse collection of bioluminescent T. b. brucei, T. b. gambiense and T. b. rhodesiense strains, including drug resistant strains. For *in vivo* BLI monitoring of murine infections, we recommend trypanosome strains transfected with red-shifted luciferase reporter genes, such as CBR and PpyRE9. Red-shifted luciferases can be detected with a higher sensitivity *in vivo* and at the same time they improve the spatial resolution of the parasites in the entire body due to the better kinetics of their substrate D-luciferin.